

Monday, December 13

Panel Session

Imaging Reward in the Addicted Human Brain: What Have We Learned So Far?

Modeling Reward Processing: fMRI Validation and Psychopharmacological Application

Brian Knutson*

Psychology, Stanford University, Stanford, CA, USA

Background: Comparative brain stimulation studies have historically indicated that mesolimbic regions play a critical role in the reward processing (Panksepp, 1998). Recent functional magnetic resonance imaging (fMRI) studies of humans suggest that stages of reward processing can be decomposed — while nucleus accumbens (NAcc) shows activation during anticipation of monetary gains, mesial prefrontal cortex (MPFC) shows activation instead to gain outcomes (Knutson et al., 2003). Here, we validated a computational model of reward processing on an existing dataset of healthy volunteers, and then applied the model to a dataset of subjects treated either with dextroamphetamine (AMPH) or placebo (PLAC). **Methods:** Healthy volunteers played a monetary incentive delay (MID) task while undergoing fMRI as described in Knutson et al (2001). Data was modeled with AFNI software (Cox, 1996) using multiple regressions that included either traditional contrasts (i.e., gain versus nongain anticipation, gain versus nongain outcome); or dynamic regressors (i.e., gain prediction, gain prediction error). Both qualitative (i.e., focus location) and quantitative (i.e., peak Z-score) aspects of the traditional and dynamic models were compared. The dynamic model was then used to analyze a dataset in which subjects played the MID task while undergoing fMRI 60-180 min after oral administration of either AMPH (0.25 mg/kg) or PLAC in a counterbalanced order (cf. Knutson et al., 2004). **Results:** In the validation sample, the dynamic model yielded maximum activation foci in the NAcc for gain prediction and in the MPFC for gain prediction error, indicating qualitative similarity to traditional contrasts. However, peak activation Z-scores were uniformly larger, suggesting that the dynamic model accounted for more variance in the activation of these regions of interest. Application of the dynamic model to AMPH- vs PLAC treated subjects (n=8) and comparison via paired t-tests suggested both decreased activation of the NAcc during gain anticipation and increased activation of the MPFC in response to gain outcomes during AMPH vs PLAC treatment. **Discussion:** These findings suggest that a dynamic model of gain prediction and gain prediction error yields qualitatively similar activation foci as traditional contrast modeling techniques, but quantitatively more robust results. Application of the dynamic model to a dataset involving psychostimulant treatment suggested that relative to PLAC, low dose AMPH reduced gain prediction but increased gain prediction error. In the future, computational modeling may be combined with fMRI to better elucidate how pharmacology can modulate psychological function. **References:** Cox RW (1996) *Comput Biomed Res*, 29, 162-173; Knutson B et al (2001); *J Neurosci*, 21, RC159; Knutson B et al (2003); *NeuroImage*, 18, 263-272; Knutson B et al (2004). *Neuron*, 43, 261-269; Panksepp J (1998). *Affective Neuroscience*, Oxford: New York.

Mesolimbic Dopamine and the Choice for Cocaine in Humans

Diana Martinez*

Psychiatry, NYSPI/Columbia University, New York, NY, USA

Background: Previous PET studies using [¹¹C]raclopride have demonstrated a decrease in striatal D2 receptor availability in cocaine dependence as well as a reduction in methylphenidate-induced dopamine release (Volkow et al *Nature*, 1997). No imaging studies have been performed measuring D1 receptor availability in cocaine abusers compared to healthy controls. A laboratory model has been developed to investigate the effects of a priming dose of cocaine in human subjects. We previously demonstrated that baseline D2 receptor availability measured with PET did not predict the choice for cocaine in this setting (Martinez et al 2004). The goal of this study was to investigate the role of amphetamine-induced [¹¹C]raclopride displacement and D1 receptor availability (using [¹¹C]NNC112) in the functional subdivisions of the striatum and the choice to self-administer cocaine. **Methods:** Chronic cocaine dependent (CCD) subjects and matched healthy control (HC) subjects underwent PET scans with [¹¹C]NNC112 (n = 17 CCD and HC) and [¹¹C]raclopride under baseline conditions and following 0.3 mg/kg IV amphetamine (n = 24 CCD and HC). [¹¹C]Raclopride was administered as a bolus with constant infusion for determination of the equilibrium distribution volume. [¹¹C]NNC112 was administered as a bolus with arterial sampling for kinetic modeling. D1 and D2 receptor availability were estimated using the binding potential (BP) and specific to nonspecific equilibrium partition coefficient (V3''), whereas amphetamine-induced [¹¹C]raclopride displacement was expressed as the reduction in V3'' from baseline ($\Delta V3''$). Each outcome measure was calculated for the limbic (LST), associative (AST) and sensorimotor (SMST) functional subdivisions of the striatum. Following the PET scans, CCD subjects underwent cocaine self-administration sessions where they received a priming dose of cocaine followed by the choice to self-administer cocaine (0, 6, and 12 mg) or to receive money (\$5). **Results:** Cocaine dependence was associated with a blunted effect of amphetamine on [¹¹C]raclopride V3''. These values were $-1.3 \pm 7.3\%$ CCD vs $-12.4 \pm 9.0\%$ HC (p < 0.0001) in the LST. Similar results were seen in the AST ($-2.7 \pm 6.8\%$ CCD vs $-6.7 \pm 5.7\%$ HC, p = 0.03) and SMST ($-4.4 \pm 7.7\%$ CCD vs $14.1 \pm 7.8\%$ HC, p < 0.0001). A negative association was seen between amphetamine-induced [¹¹C]raclopride displacement and the choice for cocaine (LST: r = 0.59, p = 0.003; AST: r = 0.43, p = 0.04; SMST: r = 0.51, p = 0.01). No difference in BP or V3'' was seen between the two groups (RM ANOVA for BP: group factor: p = 0.49; group by region interaction: p = 0.11; RM ANOVA for V3'': group factor: p = 0.26; group by region interaction: p = 0.15). However, a negative association was seen between D1 receptor V3'' and the choice for the 6 mg dose of cocaine in the LST (LST: r = 0.48, p = 0.04). **Significance:** The results of this study show that cocaine dependence is associated with a loss of psychostimulant-induced dopamine release in the striatum, with no change in D1 receptor availability in this brain region. It also puts these changes in neurochemistry into perspective by correlating them with the choice for cocaine in the presence of an alternative reinforcer. The loss of dopamine release in the striatum was associated with the choice for cocaine, whereas high D1 receptor availability in the ventral striatum was found to protect against primed cocaine seeking behavior. Insofar as the sessions serve as a model of relapse, these data suggest that a deficit in presynaptic DA function is associated with higher risk of relapse, while preservation of the D1 receptor might be engaged in processing a satiety response.

Activation of the Rectal Gyrus by Intravenous Methylphenidate in Cocaine Addicted Subjects but not in Controls

Nora D Volkow*, Gene-Jack Wang, Jim Ma, Joanna S Fowler, Christopher Wong, Jean Logan and Yu-Shin Ding

National Institute on Drug Abuse, Bethesda, MD, USA; National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, USA

Drugs of abuse are reinforcing to addicted and non-addicted subjects but they trigger craving and compulsive intake only in addicted subjects. Here we used PET and FDG to compare the brain metabolic responses (a marker of brain function) to methylphenidate (a stimulant drug that cocaine abusers report to be similar to cocaine when administered intravenously), between cocaine addicted subjects (n=21) and controls (n=15). In parallel, we also measured the subjects' behavioral responses. Methylphenidate (MP) induced self-reports of "high" in both groups but the responses were significantly greater in controls than in cocaine addicted subjects. In contrast, in cocaine addicted subjects but not in controls, MP induced cocaine craving and desire for more MP. Regional brain metabolic responses to MP were similar between groups and differed only in the right rectal gyrus (including Brodmann areas 25 and 11) (p 0.0001). In the right rectal gyrus methylphenidate increased metabolism in addicted subjects but decreased it in controls and these changes were associated with "desire for MP" and also with "cocaine craving" in cocaine abusers. These findings provide evidence that enhanced sensitivity of the rectal gyrus (region involved with salience attribution and implicated in compulsive behaviors) may underlie the enhanced saliency value of the drug in addicted subjects and the compulsive drug intake that characterizes cocaine addiction.

Cognitive Sequelae of Chronic Drug Abuse: Complementary Studies in Humans and Animals

Trevor W Robbins*

Experimental Psychology, University of Cambridge, Cambridge, United Kingdom

Background: There is much evidence of profound cognitive sequelae of chronic abuse of psychomotor stimulant drugs such as cocaine and amphetamine, but establishing the neural correlates and causal basis of these is difficult because of interpretative problems of controlling drug exposure, and multivariate factors associated with addiction including polydrug abuse and premorbid traits. This paper reviews the evidence for decision-making cognition and extends it by describing the results of a recent unpublished study (Ersche et al) in which a group of stimulant abusers volunteered for a functional imaging study of decision-making cognition using PET. We probed causality by testing rats on a fronto-executive test of cognition before and after chronic i.v. self-administration of cocaine or amphetamine (Dalley et al submitted). **Method:** Human. We employed a modification of the decision-making task previously described by Rogers et al (1999 J. Neuroscience 20, 9029) and measured regional cerebral blood flow using PET. Four groups of subjects were tested, chronic stimulant abusers, chronic opiate abusers, (diagnosed according to DSM-IV criteria for stimulant or opiate dependence), 1 year abstinent, former chronic abusers, and an age- and IQ-matched control group. Data were analysed according to standard SPM methodology. Rats were pre-trained on the 5-choice serial reaction time task (5CSRTT) before receiving an i.v. catheter and the opportunity to self-administer cocaine (or amphetamine) for a one week period. Rats were then withdrawn from drug and re-tested for a week on the 5CSRTT, this cycle being repeated up to 7 times, before final tests after several weeks abstinence from drug. **Results:** Human. Decision-making performance was

equivalent across groups, but the current chronic abusers nevertheless exhibited marked changes in certain regions of interest engaged by the decision-making task relative to normal controls. There was increased activation in the left orbitofrontal cortex, and relative reductions in the right dorsolateral prefrontal cortex in chronic abusers. Rat. There were initially profound effects of acute stimulant withdrawal on several parameters of performance on the 5CSRTT (though not including reward collection latency), which however often recovered in most rats by the end of the test week. However, a proportion of the animals with a prior history of behavioural impulsivity on the 5CSRTT were affected in a more sustained, though not necessarily permanent, way. **Significance:** The human data show that chronic abusers have altered activation of the right dorsolateral cortex, a region implicated in the strategic control of behavior, even when their performance is at control levels. The greater activation of left orbitofrontal cortex may reflect the greater degree of inhibitory executive control required by drug abusers to maintain normal decision-making behavior. These data confirm abnormalities in stimulant and opiate abusers in neural, as well as behavioural, aspects of decision-making cognition. These data suggest an interaction of drug use with these prefrontal cortical brain regions engaged by decision-making tasks. The rat data address the possible causality of drug-induced changes on performance on an analogous test of response choice similarly dependent on the integrity of the prefrontal cortex. Detrimental effects on 5CSRTT performance were profound in early withdrawal, but tended to recover over time, except in a certain sub-group of animals who showed more long-lasting deficits. These data are consistent with the possibility that stimulant drug exposure leads to changes in fronto-executive function in vulnerable humans and rats and highlight an important role of animal models of cognitive sequelae of chronic drug abuse.

Panel Session

Drug Development: Non-nicotinic Therapies for Smoking Cessation

Central Nervous System Reinforcement Circuitry Suggests Non-Nicotinic Targets for Medication Development

William A Corrigan*

Corrigan Consulting, Thornhill, ON, Canada

Background: Intravenous self-administration of nicotine affords an animal model of the reinforcing effects of the drug as delivered to tobacco smokers by the inhalation of tobacco smoke. Research with this model has demonstrated that nicotine acts at several central nervous system loci to maintain self-administration behavior, and in so doing recruits neurochemical mechanisms in addition to dopaminergic ones in the midbrain system. This presentation will use several examples to show the range of neurochemical systems involved in the reinforcing effects of nicotine. In addition, this research will be used to describe how several funding initiatives at the National Institute on Drug Abuse (NIDA) have been used to advance target discovery and medication development effects relevant to tobacco addiction. **Methods:** Animals are prepared surgically with intravenous catheters and trained to press a lever to receive infusions of nicotine on either a fixed-ratio or a progressive ratio schedule or reinforcement. When responding is stable, several experimental tactics are employed: (i) for systemic manipulations, combinations of agents are delivered subcutaneously prior to self-administration sessions; (ii) for CNS studies, stereotaxic surgery is used to implant micro-cannulae into specific re-

gions of the brain, animals re-attain stable nicotine-maintained behavior, and neurochemical manipulations are made by micro-injections of agents into target nuclei. Manipulations in nicotine self-administration are compared to those in animals trained to self-administer cocaine on identical schedules of reinforcement. Anatomical data obtained through studies of c-Fos expression are used as an aid to the interpretation of behavioral experiments. **Results:** Previous studies have established that self-administered nicotine acts on nicotinic receptors in the vicinity of the ventral tegmental area; however, other neurochemical systems are involved. Detailed data will be presented that show the effects of microinjections of either GABA-A or GABA-B agonists into the VTA. Such experimental manipulations have a greater effect on nicotine than cocaine self-administration. In addition, the effects of GABA are prominent also within the mesopontine tegmentum, where it appears that nicotine acts on GABAergic neurons, and glutamatergic neurons, directly. Hence, one conclusion from this presentation is that the GABA system may be an appropriate medication development target for nicotine addiction, a drug against which it appears to show at least regionally selective effects. The presentation will situate these data in the context of an emerging body of preclinical data with respect to the reinforcing effects of nicotine. **Discussion:** As noted, there is an emerging body of evidence implicating several neurochemical systems in nicotine addiction. Other systems will be apparent from additional presentations in this panel. A main focus of the discussion in this particular presentation will be on the efforts at NIDA to begin to translate such neurochemical evidence to at least early stages of application.

Novel Pharmacological Agents Modulating GABA-B Receptor Function: Potential Therapeutics for Nicotine Dependence

Klemens Kaupmann*, Loic Lhuillier, Cedric Mombereau, David A Slattery, Delphine S Dupuis and John F Cryan

Neuroscience Research, Novartis Institutes for BioMedical Research, Novartis Pharma AG, Basel, Switzerland

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain where it acts on two receptor classes; ionotropic GABA-A/GABA-C receptors and metabotropic GABA-B receptors. The cloning of the GABA-B receptors in 1997/1998 revived interest in these receptors and their potential as therapeutic targets. With the availability of molecular tools rapid progress was made in our understanding of the GABA-B system. This led to the surprising discovery that GABA-B receptors are heterodimers of two subunits, GABA-B1 and GABA-B2, and provided exciting new insights into the structure of G-protein coupled receptors (GPCRs) in general. Baclofen, the prototypical GABA-B agonist, has been in clinical use for the treatment of spasticity for over 30 years. However, its muscle relaxing properties limits a potential widespread use in other indications. Nonetheless, as a pharmacological tool, baclofen has been invaluable in elucidating the role of GABA-B receptors in various CNS disorders including epilepsy, cognition, pain and addiction. There is strong evidence that GABA-B agonists can reduce the self-administration and craving for drugs of abuse such as cocaine, heroin and alcohol. GABA-B receptor agonists attenuate the reinforcing effects of abused drugs by influencing the mesolimbic dopamine system. Drugs of abuse increase extracellular dopamine levels in the nucleus accumbens, a brain region that is involved in reward and reinforcement neurocircuitry. Activation of GABA-B receptors in the nucleus accumbens reduces firing of dopaminergic cells and inhibits the release of dopamine, thus it appears that GABA-B activation can block the increase in dopamine release that is induced by drugs of abuse. Accumulating evidence also suggests that increased GABAergic transmission through the GABA-B receptors may be a suitable approach for aiding in smoking cessation. Systemic or intracerebral administration of the GABA-B receptor agonists baclofen or CGP 44532 into the nucleus accumbens shell, the

VTA or the peduncular pontine nucleus, decreased the reinforcing effects of nicotine. Baclofen also attenuated nicotine-induced increases in accumbal dopamine. Taken together, these results suggest that enhancement of GABA transmission through activation of GABA-B receptors blocks the reinforcing effects of various drugs of abuse, including nicotine. With positive allosteric modulators we recently identified a novel means to activate GABA-B receptors. GABA-B positive modulators such as GS39783 enhance the action of GABA at the GABA-B site, without having intrinsic agonistic efficacy. The concept of positive allosteric GABA-B modulators for the treatment of drug addiction offers a potential advantage over agonists such as baclofen. Positive allosteric modulators enhance the action of the endogenous transmitter only; therefore their effects are linked to physiological synaptic activity. In behavioral experiments in rodents, in contrast to baclofen, positive modulators did not produce muscle relaxation, sedation, hypothermia and were also devoid of the characteristic side effects reported for the GABA-A acting benzodiazepines such as ethanol interaction, cognitive dysfunction and tolerance. As has been demonstrated for full GABA-B receptor agonists, positive modulators have been shown to attenuate the acute behavioural effects of cocaine. Therefore, GABA-B receptor positive modulators may be useful therapeutic strategy for the development of novel anti-smoking compounds. Supported by National Institutes of Mental Health/ National Institute on drug abuse grant U01 MH60962.

Metabotropic Glutamate Receptor Antagonists for Nicotine Dependence

Athina Markou*, Paul J Kenny, Neil E Paterson and Svetlana Semenova

Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA, USA

Most available treatments for smoking cessation are nicotine replacement therapies that are only partially effective. Advances in our understanding of the neurobiology of nicotine dependence suggest novel therapeutic targets for smoking cessation, including the glutamate system and in particular metabotropic glutamate (mGlu) receptors. Two factors that lead to the tobacco smoking habit are the rewarding effects of nicotine, and the motivation to alleviate the negative affective aspects of nicotine withdrawal. Thus, targeting both of these two aspects of nicotine dependence may be needed to assist people in smoking cessation. Cholinergic-glutamatergic interactions in the ventral tegmental area (VTA) and other brain sites may be critically involved in mediating the rewarding effects of nicotine, and may exhibit adaptations with the development of nicotine dependence that contribute to nicotine withdrawal. Using the intravenous self-administration procedure in rats, we demonstrated that administration of the mGlu5 receptor antagonist MPEP (6-methyl-2-[phenylethynyl]-pyridine), the competitive NMDA receptor antagonist LY235959, or the AMPA/kainate receptor antagonist NBQX, either systemically or into the VTA, decreased intravenous nicotine self-administration. Many of these treatments had no effect on responding for food. Thus, the reinforcing effects of nicotine may be mediated at least partly by glutamatergic transmission through mGlu5, NMDA and AMPA/kainate receptors. In nicotine-dependent subjects, but not control rats, an agonist at inhibitory presynaptic mGlu2/3 receptors (LY314582), injected systemically or intra-VTA, precipitated withdrawal-like elevations in brain reward thresholds, a sensitive measure of reward function. Thus, there is increased activity of inhibitory mGlu2/3 receptors with the development of nicotine dependence, possibly to counteract the increased glutamate release induced by chronic nicotine administration. Accordingly, administration of the mGlu2/3 receptor antagonist LY341495 attenuated the threshold elevations observed in rats undergoing spontaneous nicotine withdrawal. Hence, adaptations in the function of

mGlu2/3 receptors during chronic nicotine exposure likely contribute to the expression of withdrawal during nicotine abstinence. In conclusion, the above data indicate that glutamate transmission through mGlu5, NMDA and AMPA/kainate receptors, in areas such as the ventral tegmental area, is critically involved in mediating the reinforcing effects of nicotine reflected in intravenous nicotine self-administration. Further, there appear to be adaptations in mGlu2/3 receptors with the development of nicotine dependence that probably lead to decreased glutamate transmission upon discontinuation of nicotine administration, possibly mediating the negative affective aspects of nicotine withdrawal. Thus, dual antagonism at mGlu5 and mGlu2/3 receptors may be an effective pharmacological strategy that will first assist people in decreasing tobacco smoking and subsequently alleviate the affective depression-like aspects of nicotine withdrawal.

Active Immunization Against Nicotine

Torgny H Svensson*, Sabina De Villiers, Nina Lindblom, Anette M Johansson, Sandra Gordon and Genadiy Kalayanov

Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Active immunization against nicotine may offer an interesting novel strategy in smoking cessation with potential to provide long-lasting relapse prevention. The mode of action is to generate antibodies that bind nicotine in plasma, preventing its entry to the brain and its rewarding action. To achieve this goal, nicotine is linked to a carrier protein to form an immunoconjugate, which should generate antibodies binding selectively to nicotine. Therefore, the hapten should be as similar to nicotine as possible, both in three dimensional structure and physical-chemical properties. Nicotine is metabolized in the pyrrolidine ring. Thus, binding the linker-protein part to the pyridine ring will expose that part of the nicotine molecule that is different from nicotine metabolites and, thereby, increase the possibility that the antibodies formed bind nicotine only. The binding of the linker-protein part to the rigid pyridine ring, as far as possible from the flexible pyrrolidine ring, has the additional advantage that it leaves its conformation intact and maximizes the three dimensional similarity with nicotine. Although no clinical results as yet show that nicotine vaccines actually work in smoking cessation, experimental data provide proof of principle. Our studies show, using active immunization of rats, that nicotine immunoconjugates constructed as above generate a sustained increase in nicotine antibodies that are highly selective for nicotine, as assessed by ELISA and competitive ELISA, and effectively prevent nicotine from reaching the brain and stimulate the dopaminergic reward pathway, as assessed by voltammetry *in vivo*. This work also shows that both the structure of the linker as well as its binding localization on the nicotine molecule influence the functional efficacy of the vaccines. The utility of one of these vaccines was tested in a long-term relapse model, measuring the reinstatement of nicotine-seeking behavior in rats trained to self-administer nicotine on a fixed ratio (FR3) schedule followed by extinction through removal of the nicotine regimen. Active immunization completely suppressed relapse induced by priming doses of nicotine, thus in principle providing proof of concept. Several studies show that active immunization indeed alters the distribution of nicotine, i.e. increases the plasma concentration and decreases the brain concentration of nicotine. Interestingly, our work indicates that despite the sustained nicotine binding by the antibodies, active immunization may not necessarily alter the metabolism of nicotine to cotinine, suggesting that the vaccine used does not increase the half-life of nicotine, in contrast to previous findings with other vaccines. Since KLH is not optimal for humans other proteins should be used, and several alternatives have been chosen by different groups active in the field. Clinical work shows that nicotine vaccines are indeed able to generate a sustained increase in nicotine antibodies in the blood lasting for several months, although whether these levels are therapeutically effective and the optimal

treatment regimen remain to be established. Clinical monitoring may involve e.g. measurements of antibodies, blood levels of CO or COHb and cotinine. Assessments of anabasine may also be of interest, since this metabolite is only formed from tobacco, but not from nicotine, e.g. by concomitant use of NRT, and since the immunization at least by some vaccines may alter the metabolism of nicotine. Although so far the use of nicotine vaccines for relapse prevention has been discussed, they may potentially be useful also for acute smoking cessation as well as combined with other smoking cessation therapies.

Panel Session

Behavioral and Neurophysiological Markers for Risk and Psychopathology in Adolescence

Three Generations at High Risk for Depression

Myrna M Weissman*, Priya Wickramaratne, Yoko Nomura and Virginia Warner

Psychiatry, NYSPI/Columbia, New York, NY, USA

Background: The familial nature of major depression (MDD) is well documented with about a three fold increased risk in the first degree relative of probands with MDD. None of the published studies have gone beyond two generations or have followed the families over many years. We report results of a high risk, longitudinal study of three generation at high and low risk for MDD. **Methods:** 161 grandchildren their parents and grandparents were systematically assessed blind to previous generations and previous interview on their psychiatric status and functioning. The first two generations were assessed over 20 years. **Results:** They were high rates of anxiety disorders in the grandchildren (mean age 12 years) with two generation of MDD. Grandchildren with both a depressed parent and grandparent as compared with those where the parent was not depressed had over a four fold increased risk of anxiety and over two fold increased rise of mood disorders. **Discussion:** Anxiety disorders are the early signs of psychopathology in young grandchildren at high risk for MDD. Grandchildren from two generations have the highest rates of anxiety and depression. Families with three generations affected with MDD or anxiety could be the target for neuroimaging, genetic and other biological studies. Supported in part by NIMH grant R01 MH036197 (MMW, PI).

Affective Influences on Decision Making in Adolescents

Ronald E Dahl*, Jennifer Lerner, Ahmad R Hariri, Cecile Ladouceur, Cameron Carter and Neal D Ryan

Psychiatry, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Background: Most measures of cognitive abilities approach adult levels of functioning by age 15; yet many adolescents reveal a sobering contrast in maturity for real-life decision making. Relevant to these discrepancies are a set of affective changes during pubertal maturation that contribute to adolescent tendencies toward risk-taking, sensation-seeking, and emotional turmoil. The neural underpinnings and maturational mechanisms of these changes are poorly understood, but represent an important dimension to a broad range of behavioral and emotional health problems emerging in adolescence. This presentation describes a conceptual model, and examples of investigative approaches, to understanding key aspects of these vulnerabilities. It focuses on motivational and emotional changes during pubertal maturation that may contribute to apparent deficiencies in responsible decision making in adolescents. Data are presented from two sets of ongoing lines of research

relevant to this model. **Methods (study one):** A large sample of normal control adolescents underwent mood inductions (viewing film clips depicting sad, disgusting, or neutral scenes) while participating in a study of economic decision making. The study design is derived from a well-established line of research in decision-making science using actual transactions, having subjects choose between receiving real objects and specific amounts of money. **Results (study one):** Sadness resulted in subjects setting lower selling prices and higher buying prices. In contrast, disgust caused subjects to set lower buying and selling prices. These emotion-specific effects on judgment were robust. When considered in a larger context of decision making science, these data raise provocative questions about mechanisms. **Methods (study two):** ERP (event related potential) measures were used to examine action monitoring and event-related negativity (ERN). Samples of normal youth and adolescents with anxiety and depression were studied. ERPs were recorded using 128-channel dense array EEG during an 840-trial arrow-flanker task. Amplitudes and latencies were scored in response-locked error trials for the ERN and the PE. **Results (study two):** The data in the normal control subjects showed a relatively late development of ERN, with significant maturational changes continuing through mid/late adolescence. In addition, analyses of variance conducted on the ERP in the clinical samples showed a significant diagnosis by response-type interaction, $F(1,14) = 4.83$, $p < .05$. Post hoc analyses revealed that the youth with anxiety disorders had significantly larger ERN amplitude relative to the control group, $t = -2.24$, $df = 14$, $p < .05$. There were no significant group differences in ERN latency or PE amplitude and latency. **Discussion:** The results of these studies are brought together in a discussion of the clinical significance of affective changes during pubertal development (occurring relatively early in adolescence) in relation to the slow and gradual maturation of cognitive control. These are discussed within a broader context of adolescent brain development and maturational periods of vulnerability for behavioral and emotional disorders.

Attention to Threat in Adolescents with Anxiety Disorders: Evidence for Abnormal Behavioral and Ventrolateral Prefrontal Cortex Function

Christopher S Monk*, Eric E Nelson, Erin B McClure, Karin Mogg, Brendan P Bradley, Ellen Leibenluft, James R Blair, Gang Cheng, Dennis S Charney, Monique Ernst and Daniel S Pine

NIMH, NIH, Bethesda, MD, USA

Background: While adolescent anxiety disorders represent prevalent, debilitating conditions, virtually no research explores their neurophysiology. A cognitive task sensitive to symptom-relevant behaviors, such as attention-to-threat, may effectively engage brain structures that underlie these disorders. Perturbed attention to threats represents a key symptom of both adult and pediatric anxiety disorders. The objective of the present study was to investigate attention function and brain activation in adolescents with anxiety disorders. **Methods:** Fourteen adolescents with anxiety disorders and 16 healthy adolescents participated in this study. We used event-related functional magnetic resonance imaging (fMRI) with a task assessing attention to threat. Subjects pressed a button to a probe preceded by a threat face paired with a neutral-face. Behavioral and fMRI analyses compared trials during which the probe and threat were on the same (congruent) side vs. opposite (incongruent) sides. **Results:** Relative to controls, patients show slower reaction time on trials where threat and probe were spatially congruent, compared to trials where they were incongruent, indicating an attention bias away from threat. Patients show greater right ventrolateral prefrontal cortex (VLPFC) engagement than controls in the fMRI contrast of these same trials. This difference reflects particularly robust engagement in patients to congruent threat trials. **Discussion:** Adolescents with anxiety disorders show altered attention bias to threat associated with abnormal

VLPFC function. Enhanced VLPFC engagement may reflect abnormal function directly in this region or in projecting afferents. Alternatively, VLPFC activation may reflect compensatory processes associated with redirecting of attention to threat locations.

MRI in Children at High Risk for Major Depression

Bradley Peterson*, Hongtu Zhu, Ravi Bansal and Myrna Weissman

Psychiatry, Columbia University, New York, NY, USA

Objective: The correlational nature of imaging data complicates attempts to distinguish findings associated with an illness that represent pathophysiological causes from those that are compensatory or epiphenomenal effects. Imaging studies of individuals at high risk for an illness before, during, and after illness onset may distinguish brain abnormalities that represent trait vulnerabilities from those that are state effects. **Methods:** An initial group of approximately 120 individuals, offspring recruited from an ongoing study of 241 probands in a 3-generation sample of children and adults at either high or low risk for developing major depression, have undergone structural and functional Magnetic Resonance Imaging (MRI). The fMRI scans have been acquired while the subjects perform the Simon spatial incompatibility task, a nonverbal analogue of the Stroop task. Like the Stroop, this task engages self-regulatory capacities that are based primarily in frontal, striatal, and anterior cingulate regions, dysfunction of which have been implicated in affective disorders. **Results:** Preliminary imaging data will be presented that include regional volumes of cortical gray matter, amygdala, hippocampus, and cerebral ventricles, as well as functional MRI measures of brain activity during performance of the self-regulatory task. Measures from individuals who are at high risk for developing the illness but who have not yet onset with it will be compared with individuals who are both at low risk for the illness and unaffected by it. **Conclusion:** Imaging studies of children at high risk for developing depression are feasible and may permit distinguishing trait vulnerabilities from compensatory and epiphenomenal effects.

Panel Session

Gamma Oscillations in Psychiatric Illness

Prefrontal Gamma Band Reductions and Impaired Cognitive Control in Schizophrenia

Cameron Carter*

Western Psychiatric Institute & Clinic, Pittsburgh, PA, USA

Functional neuroimaging studies using hemodynamic methods that have implicated reductions in activity in the dorsolateral prefrontal cortex in schizophrenia associated with impaired higher cognitive functions in the illness.

The Thalamo-Cortico-Thalamic Network and Oscillatory Activity

Edward G Jones*

Center for Neuroscience, University of California Davis, Davis, CA, USA

High frequency electrocortical rhythms in the 40Hz range are thought to form essential components of the normal "binding" process that underlies perception and cognition. New imaging and event related potential studies suggest that disruption of cognitive processing in disorders such as schizophrenia may be associated with disorganization of these normal brain rhythms. In this symposium, experts derived from several disciplines will consider the current status of these studies, along with basic anatomical and physiological

properties of the thalamocorticothalamic network that underlie normal and disturbed forebrain rhythms.

Content of a Gamma Cycle: Why Are Cortical Assemblies Formed? Gyorgy Buzsaki*

Rutgers University, Millburn, NJ, USA

Recent observations at the single cell and network levels that demonstrate how larger ensembles of cortical and thalamic cells can be formed to promote high frequency oscillations.

The Thalamocortical Dysrhythmia Syndrome: New Electrophysiological Insights

Rodolfo Llinas*

Physiology & Neuroscience, New York University Medical Center, New York, NY, USA

Magnetoencephalographic studies that demonstrate the spread of gamma oscillations across the cortex during cognitive events, their underlying cellular substrates, and the evidence that dysrhythmias of the thalamocortical network underlie many neuropsychiatric conditions. Accumulating data in this field suggest that developing drugs to target this aspect of cortical function might be a viable strategy for developing treatments for impaired cognition in schizophrenia.

Panel Session Modeling Complex Genetic and Environmental Influences on the Development of Cortico-Limbic Abnormalities in Mood Disorders

Genetic Modulation of Early Life Trauma and Neglect in Mice

Andrew Holmes*, Rachel A Millstein, Janel M Boyce-Rustay, Lisa Wiedholz and Alicia Izquierdo

Section on Behavioral Science and Genetics, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, USA

BACKGROUND: Childhood exposure to adversity affects later risk for emotional disorders and addiction. While there is evidence that genetic factors also exert a profound influence on risk for these disorders, the interplay between genes and early life stress remains poorly understood. There is also little understanding of how different types of early life stressors (e.g., neglect vs. abuse) can produce differing psychopathologies. **METHODS:** The genetic malleability of the mouse makes it an excellent model system to study gene x early life environment interactions shaping the development of brain systems mediating emotion and addiction. We assessed the effects of postnatal maternal separation (MS) on the development of emotional and reward-related phenotypes in adulthood. From postnatal days 0-13, mouse pups were separated from the dam and their littermates for either 3 hr/day (MS) or 15 min/day (handling). An additional control was undisturbed (facility reared). To study genetic modulation of postnatal MS, 8 separate inbred mouse strains (129S1/SvImJ, 129P3/J, A/J, BALB/cByJ, BALB/cJ, C57BL/6J, DBA/2J, FVB/NJ) were tested. On reaching adulthood, mice were tested for anxiety-related behaviors and stress-responsivity using the open field, elevated plus-maze, acoustic startle, dark-light emergence, and forced swim tests, and by measurement of plasma adrenocorticotropin and corticosterone responses to restraint stress. The effects of MS on voluntary ethanol consumption and acute behavioral responses to ethanol were assessed in an additional cohort of C57BL/6J mice. In a separate study in C57BL/6J mice, the effects of MS on emotional behavior were compared with those resulting from a more

direct psychological stressor to the pup (footshock). **RESULTS:** MS produced permanent abnormalities in anxiety-like behavior and stress-reactivity; however, these effects were strongly modified by genotype. Consistent with previous work in rats, these strain differences in responsivity to MS appeared to be, in part, mediated by genotypic influences on maternal behaviors. Suggesting that the form of early life stress is also an important determinant of later emotional abnormalities, the effects of MS differed from those of postnatal footshock. **CONCLUSIONS:** This research provides a basis for future mouse studies aimed at unraveling genetic modulation of early life trauma and neglect, with implications for identifying genetic factors affecting vulnerability and resilience to childhood trauma. Supported by the National Institute on Alcohol Abuse and Alcoholism Division of Intramural Clinical and Biological Research.

Interactive Effects of Monoamine Function and Genetic Variables on Regional Cerebral Glucose Utilization

Alexander Neumeister*, Inna Belfer, David Goldman, Wayne Drevets and Dennis Charney

Department of Psychiatry, Yale University, New Haven, CT, USA

Background: Considerable progress has been made recently in the identification of the brain circuitry being important in the pathogenesis of unipolar major depressive disorder (MDD) as well as the role of monoamines in the etiology of MDD. To enhance our understanding of the biological basis of MDD, it is important to characterize the interaction between neurotransmission and its genetic regulation, and how this interaction may affect behavior and neural circuits that are implicated in MDD. Herein, we will describe the role of a functional polymorphism in the promoter region of the 5-HT transporter, designated 5-HTTLPR in mediating the behavioral and neural responses to tryptophan depletion in people with a history of MDD. Moreover, positron emission tomography (PET) data will be presented showing how a recently identified in-frame deletion of the $\alpha 2C$ -adrenoreceptor subtype ($\alpha 2C$ DEL322-325) affects regional cerebral glucose utilization (rCMRglu). **Methods:** We studied unmedicated remitted patients with a history of MDD and controls during tryptophan depletion and under resting conditions. [F-18] FDG PET studies were performed using a GE Advance Scanner. 4.5 mCi of [F-18] FDG were administered. PET images were co-registered to whole brain MRIs and spatially normalized using SPM 99 after filtering with a 12x12x12 mm smoothing Kernel. **Results:** Reduced 5-HT function during tryptophan depletion unmasked a cortico-limbic circuit that appears to play a key role in MDD. Within the circuit, MDD carriers of the l/l 5-HTTLPR genotype had increased rCMRglu whereas carriers of the s/l genotype had decreased rCMRglu during tryptophan depletion. Carriers of the $\alpha 2C$ DEL322-325 polymorphism had increased rCMRglu in the orbitofrontal cortex and dorsolateral prefrontal cortex under resting conditions. **Discussion:** The present study identifies serotonin and noradrenaline systems-related genes and their polymorphisms that affect rCMRglu in people with MDD and healthy controls. We show how these genes differentially affect rCMRglu in distinct brain regions, and discuss whether these genes and their variants may represent vulnerability markers for MDD.

Genetic Influences on the Development of Cortico-Limbic Abnormalities in Bipolar Disorder

Hilary Blumberg*, John H Krystal, Ronald Duman and Joel Gelernter

Psychiatry, Yale University School of Medicine and VA CT Healthcare System, West Haven, CT, USA

Background: Interaction of multiple genes is thought to influence the expression of the bipolar disorder phenotype. Recent evidence supports roles for polymorphic variation in genes encoding proteins that influence expression of biogenic amines and neurot

rophic factors in bipolar disorder. These genes have already been implicated in behaviors that are abnormal in bipolar disorder, as well as in the development and plasticity of the cortico-limbic structures that subserve these behaviors. Thus, contributions to symptoms in bipolar disorder may be mediated, at least in part, by the same genes that have effects on cortico-limbic structure and function. We are investigating potential interactions between variation in these genes and their influence on cortico-limbic structure, function and connectivity in bipolar disorder. **Methods:** Genotyping and multimodality magnetic resonance imaging (MRI), including high resolution structural MRI, functional MRI and diffusion tensor MRI (DT-MRI) were performed in patients with bipolar disorder, and healthy comparison research participants. Participants were subgrouped by diagnosis (bipolar disorder vs. healthy comparison) and the subgroups further stratified by genotype. Regional brain volumes, fMRI signal changes and anisotropy measures served as dependent measures. **Results:** Preliminary findings support the presence of epistatic interactions between the aforementioned genes in their influence on phenomena related to bipolar affective disorder. For example, preliminary data suggest epistatic interactions between genes encoding a serotonin transporter promoter protein (SLC6A4) and brain-derived neurotrophic growth factor (BDNF) in their influence on hippocampal volume in bipolar disorder. **Discussion:** Preliminary evidence supports the presence of epistatic genetic influences on the development of cortico-limbic pathology in bipolar disorder. Implications and future directions of this work will be discussed.

5HTTLPR Affects Development and Function of Limbic Circuitry

Daniel Weinberger

Abstract not available.

Panel Session

Early Diagnostic Markers of Alzheimer's Disease

Neuropsychological Correlates of Brain Aging and Early Cognitive Markers of MCI and AD

Steven Ferris*

Psychiatry, NYU School of Medicine, New York, NY, USA

It is well documented that cognition declines over the life-span, and that age is the major risk factor for Alzheimer's disease (AD). Therefore, it is logical to expect that differential patterns of age-related cognitive decline might predict future accelerated decline associated with early AD. This presentation will review previous and current research that has examined the predictive utility of cognitive and functional deficits as clinically useful early markers of AD that may identify prodromal AD. As the field has moved toward treating AD as early as possible, the sensitive cognitive testing has increased in importance. There is great interest in studying treatments for "Mild Cognitive Impairment" (MCI), a heterogeneous syndrome that includes a high proportion of individuals with mild memory impairment associated with early AD pathology. Current evidence suggests that "MCI of the AD type" represents prodromal or incipient AD, since such individuals are at high risk to progress to a clinical diagnosis of AD within several years.[1,2] Sensitive memory tests have proven useful in selecting AD-type MCI subjects for clinical trials, and for enriching the selection with individuals at high risk of conversion to AD. Our longitudinal study results suggest that delayed paragraph recall predicts with high accuracy which MCI individuals will progress to AD within several years.[3] Results from an MCI trial conducted by the Alzheimer's Disease Cooperative Study (ADCS) demonstrate the pattern of cognitive deficit that distinguishes MCI from early AD.[4] More recently, sensitive tests have also shown

promise for identifying cognitively normal elderly individuals who are at risk for progressing to MCI and subsequently to AD. If successful, these methods may prove useful for enriching subject selection for primary prevention trials. Cognitive data from the ADCS MCI trial, when compared with results from a parallel cohort of normal elderly, suggest patterns of cognitive decline that differentiates normal elderly from MCI individuals.[4] Additional relevant data is available from the ongoing ADCS Prevention Instrument Project[5], a four-year, simulated primary prevention trial (no treatment) in 650 non-demented elderly (age 75 or older). The study is designed to evaluate the sensitivity to longitudinal change and to progression to MCI and AD of more efficient outcome measures. Cross-sectional cognitive differences at baseline between clinically normal (CDR=0) and mildly impaired (CDR=0.5) subjects, and differential longitudinal decline at 1-year follow-up provide initial evidence for cognitive markers that may predict future MCI and AD. 1. Flicker, C., Ferris, S. H., & Reisberg, B. (1991). Mild cognitive impairment in the elderly: Predictors of dementia. *Neurology*, 41, 1006-1009. 2. Golomb, J., Kluger, A., & Ferris, S. H. (2000). Mild Cognitive Impairment: Identifying and treating the earliest stages of Alzheimer's disease. *NeuroScience News*, 3, 46-53. 3. Kluger, A., Ferris, S. H., Golomb, J., Mittelman, M. S., & Reisberg, B. (1999). Neuropsychological prediction of decline to dementia in nondemented elderly. *Journal of Geriatric Psychiatry and Neurology*, 12, 168-179. 4. Grundman, M., Petersen, R. C., Ferris, S. H., et al, for the Alzheimer's Disease Cooperative Study (2004). Mild Cognitive Impairment can be distinguished from Alzheimer disease and normal aging for clinical trials. *Archives of Neurology*, 61, 59-66. 5. Ferris, S., Aisen, P., Galasko, D., Salmon, D., Schneider, L., Sano, M., & Whitehouse, P. (2004) ADCS Prevention Instrument Project: Overview and initial results. Presented at the International Congress of Alzheimer's Disease and Related Disorders, Philadelphia, July.

Hippocampal Atrophy and Olfactory Identification Deficits Predict Conversion to Alzheimer's Disease in Patients with Minimal to Mild Cognitive Impairment

Davangere P Devanand*, Matthis H Tabert, Gregory H Pelton, Gnani Pratap, Mony De Leon and Richard Doty

Biological Psychiatry, Columbia University, New York, NY, USA

Objective: To evaluate the predictive utility of apolipoprotein E e4 genotype, MRI-defined hippocampal and parahippocampal gyrus volumes, and olfactory identification deficits (UPSIT scores), for predicting conversion to AD in patients with minimal to mild cognitive impairment (MMCI). **Methods:** Patients with subjective memory complaints who presented to a Memory Disorders Clinic underwent extensive evaluation, and a consensus diagnosis was made. After exclusion of specific causes of cognitive deficits, 150 patients were identified with minimal to mild cognitive impairment (MMCI). The 150 patients were evaluated every 6 months, and 62 matched controls were evaluated annually. **Results:** Patients were followed an average of 46.9 (SD 25.1) months. Apo E e4 carrier status did not differ in frequency between patients and controls, and did not predict conversion to AD in patients, but reached significance ($p < .04$) in the older patient subgroup in Cox survival analyses after covarying for sex, education, and baseline cognitive scores. Baseline hippocampal volume was lower in patients compared to controls ($p < .01$). In Cox survival analyses, hippocampal volume predicted time to AD ($p < 0.04$) when age, sex, and education in years were included in the model, but the effect was reduced to trend level after baseline Mini Mental State Exam (MMSE) was also included in the model. Parahippocampal gyrus volume was not a significant predictor of time to AD in the corresponding Cox models. At baseline, patients had lower UPSIT scores (mean 31.3 SD 6.4) compared to controls (mean 34.8 SD 4.2; $t=4.1$, $p < .001$). UPSIT scores were lower in converters (mean 25.9 SD 8.2) compared to non-converters to AD (mean 32.9 SD 4.6; $t=6.5$, $p < .001$). In Cox survival analyses, the UPSIT score predicted time to

AD when age, sex, education in years, MMSE and Selective Reminding Test delayed recall scores were included in the model. Moderate to strong sensitivity and specificity were observed for UPSIT scores in the 27 to 32 range. **Conclusions:** Apo E e4 carrier status was not a significant predictor, hippocampal atrophy was a moderately significant predictor, and olfactory identification deficits strongly predicted conversion to AD in MMCI patients.

CSF Biomarkers in Alzheimer's Disease

Elaine R Peskind*

VA Northwest Network Mental Illness Research, Education, & Clinical Center, Seattle, WA, USA

There is a well-recognized need for biomarkers for Alzheimer's disease (AD) to improve clinical differential diagnosis, follow disease progression, evaluate potential therapies, and identify asymptomatic persons at risk. Cerebrospinal fluid (CSF) assays of specific proteins as well as protein signatures in CSF have potential utility as biomarkers. Of the proteins that undergo abnormal conformational changes that enhance their aggregation and deposition in brain in AD, Ab1-42, the ratio of Ab1-42:Ab1-40, and both total tau and specifically phosphorylated tau epitopes have all demonstrated significant differences when comparing groups of AD patients and controls; patients with Mild Cognitive Impairment have intermediate values. Other potential CSF biomarkers include isoprostanes, which are markers of lipid peroxidation, and sulfatide, a major component of cerebral white matter. CSF proteomics (recognition of complex protein patterns) is a promising approach that has proven effective in oncology applications. Although no single marker currently satisfies the requirements for a useful biomarker for AD, research on multiple candidate biomarkers may provide a panel or battery that proves superior to any single marker alone. Recently improved proteomics methods have revealed increasingly larger numbers of both identified and as yet unidentified proteins in human CSF. CSF biomarker research will be aided by the developing consensus regarding optimal methods for collection of CSF for studies of biomarkers. These methods maximize usefulness of samples while minimizing risk of adverse events.

Plaque and Tangle Brain Imaging Using [F-18]FDDNP PET Differentiates Alzheimer's Disease, Mild Cognitive Impairment and Older Controls

Gary Small*, Vladimir Kepe, S.C. Huang, Linda Ercoli, Prabha Siddarth, Karen Miller, Helen Lavretsky, Benjamin C Wright, Nagichettiar Satyamurthy, H.H. Wu, Kooresh Shoghi-Jadid, Andrej Petric, Michael E Phelps and Jorge R Barrio

Psychiatry & Biobehavioral Sciences & Molecular & Medical Pharmacology, UCLA Neuropsychiatric Institute, Los Angeles, CA, USA

Objective: [F-18]FDDNP-PET imaging is useful in detection of b-amyloid senile plaques and neurofibrillary tangles, the pathologic hallmarks of Alzheimer's disease (AD). In this report, we compare regional cerebral positron emission tomography (PET) assays of amyloid and tau burden for three subject groups of varying cognitive impairment to demonstrate [F-18]FDDNP diagnostic utility. **Methods:** [F-18]FDDNP-PET scans were performed on 14 AD patients (age: 74±9 years, MMSE: 19±6 [mean±SD]), 5 subjects with mild cognitive impairment (MCI; age: 73±12 years, MMSE: 27±2), and 12 controls (CTL; age: 63±10 years, MMSE: all 30). Medial temporal, parietal, frontal, and cerebellar regions of interest (ROIs) were drawn, and quantitation of regional binding for all ROIs was performed using relative standard uptake value (SUVRs) evaluated at equilibrium and relative distribution volumes (DVRs, Logan plot graphical analysis, cerebellum as reference region, 60-120 min). Nonparametric analyses of variance

were used for group comparisons. All subjects also received FDG-PET scans. **Results:** The global [F-18]FDDNP binding showed significant group differences (AD: 1.16±0.03; MCI: 1.10±0.05; CTL: 1.05±0.03, p<0.0001). The regional DVRs for medial temporal (AD: 1.23±0.06, MCI: 1.19±0.09; CTL: 1.12±0.06, p<0.001), parietal (AD: 1.17±0.07, MCI: 1.07±0.05; CTL: 1.05±0.04, p<0.001), and frontal (AD: 1.09±0.05; MCI: 1.01±0.08; CTL: 1.00±0.03, p<0.001) regions all demonstrated the expected pattern of AD pathology distribution. FDG-PET for AD patients showed parallel decreases in glucose metabolism in temporo-parietal regions and correlated well with the increased level of global [F-18]FDDNP binding. Independently or in combination with FDG, FDDNP effectively separated the AD subjects from controls. **Discussion:** These findings demonstrate the ability of [F-18]FDDNP binding to differentiate various degrees of cognitive decline in older populations. The [F-18]FDDNP regional binding patterns are consistent with known postmortem patterns of pathology distribution.

Panel Session

Glia & Astrocytes as Modulators of Synaptic Function

Thrombospondins are Astrocyte-Secreted Proteins that Promote CNS Synaptogenesis

Ben A Barres*

Neurobiology, Stanford University School of Medicine, Stanford, CA, USA

We have previously shown that the number of synapses between CNS neurons in culture is profoundly enhanced by a secreted signal from astrocytes. We now identify thrombospondins (TSP) as necessary and sufficient components of the synapse-promoting activity of astrocyte-conditioned medium. Interestingly, TSPs induce ultrastructurally normal synapses that are presynaptically active but postsynaptically inactive, indicating that it works in concert with other, as yet unidentified, astrocyte-derived signals to produce functional synapses. In vivo, TSP is concentrated in astrocytes and at synapses throughout the developing brain, and transgenic mice deficient in both TSP1 and TSP2 in mice have a significant decrease in synapse number. These studies identify TSPs as the first known non-neuronal synaptogenic proteins in the CNS, and add to the growing evidence that astrocytes are important promoters of synaptogenesis within the developing CNS.

Astrocyte Glutamate Transporters Regulate Metabotropic Glutamate Receptor-Mediated Excitation of Hippocampal Interneurons

Yanhua H Huang, Saurabh S Sinha, Tanaka Kohichi, Jeffrey D Rothstein and Dwight E Bergles*

Neuroscience, Johns Hopkins University, Baltimore, MD, USA

Background: Glutamate transporters prevent the accumulation of extracellular glutamate that would otherwise lead to tonic activation of receptors, disrupted signaling, and excitotoxic damage. These transporters are present at high densities near synapses and have a high affinity for glutamate. As a result, glutamate transporters can influence the number of receptors that bind transmitter and the length of time that receptors are activated following release. Transporters also restrict the diffusion of glutamate between synapses (cross talk), and thereby help to maintain the fidelity of signaling between discrete sets of neurons. Alterations in transporter expression have been described in many neurological diseases, and inappropriate reverse cycling of trans-

porters may contribute to the accumulation of extracellular glutamate during ischemia. Five distinct high affinity Na^+/K^+ dependent glutamate transporters are expressed in the CNS, termed GLAST (EAAT1), GLT-1 (EAAT2), EAAC1 (EAAT3), EAAT4, and EAAT5. These transporters are differentially expressed in the membranes of neurons and glial cells. Although much has been learned about the distribution of these transporters, little is known about their relative contributions to the clearance of glutamate away from receptors at synapses. To address this question, we examined the role of neuronal and glial glutamate transporters to the clearance of glutamate at synapses in the hippocampus. **Methods:** Hippocampal interneurons located at the oriens-alveus border (O-LM interneurons) interneurons express mGluR1 α , a metabotropic glutamate receptor that regulates excitability and synaptic plasticity. Activation of this receptor leads to the opening of cation-selective channels, providing a means to monitor mGluR activity using electrophysiological techniques. To determine if glutamate transporters are essential for removing glutamate at excitatory synapses formed with these interneurons, whole-cell voltage-clamp recordings were made from O-LM interneurons in acute hippocampal slices prepared from 2-3 week old rats and mice. mGluR-mediated excitatory postsynaptic currents (EPSCs) were elicited by applying a brief, high frequency train of stimuli (100 Hz, 10 pulses, 200 μ s, 30-35 μ A per pulse) to stratum oriens using a constant current stimulator. **Results and Discussion:** Stimulation of glutamatergic fibers in stratum oriens reliably elicited a slow mGluR1-mediated current in O-LM interneurons if $[\text{Ca}^{2+}]_i$ was elevated at the time of stimulation. Selective inhibition of the astrocyte transporter GLT-1 (EAAT2) with dihydrokainate (DHK, 300 μ M), increased the amplitude of these responses ~3-fold, indicating that these glial transporters compete with mGluRs for synaptically released glutamate. However, inhibition of all glutamate transporters with DL-threo-b-benzyloxyaspartic acid (TBOA, 100 μ M) increased mGluR EPSCs >15-fold, indicating that additional transporters also shape activation of these receptors. To identify these transporters we examined mGluR1 EPSCs in mice lacking GLAST (EAAT1) or EAAC1 (EAAT3). A comparison of responses recorded from wild-type and transporter knockout mice revealed that the astroglial glutamate transporters GLT-1 and GLAST, but not the neuronal transporter EAAC1, restrict activation of mGluRs in O-LM interneurons. Transporter-dependent potentiation of mGluR EPSCs led to a dramatic increase in interneuron firing and enhanced inhibition of CA1 pyramidal neurons, suggesting that acute or prolonged disruption of transporter activity could lead to changes in network activity as a result of enhanced interneuron excitability.

D-Serine, a Surprising Glial Neuromodulator Solomon Snyder*

Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Glutamate-NMDA receptors require a second agonist, long thought to be glycine, which is required for activation of the receptor. Upon learning of the existence of D-serine at levels about a third of L-serine in the brain, we wondered whether it might physiologically regulate NMDA receptors, as D-serine is more potent than glycine at these sites. Selective depletion of D-serine by D-amino acid oxidase (with no effect on glycine levels) greatly reduces NMDA neurotransmission. We purified and cloned serine racemase (SR) which converts L-serine to D-serine. SR and D-serine are selectively localized to glial astrocytes ensheathing synapses in NMDA receptor enriched brain areas. Activation of AMPA receptors on these astrocytes releases D-serine. Yeast two-hybrid analysis reveals intimate binding of SR to GRIP, a protein that binds AMPA receptors. GRIP transfection quintuples D-serine formation and release indicating that GRIP mediates AMPA receptor activation of the D-

serine system. D-serine also regulates neuronal migration during development. Thus, the migration of cerebellar granule cells along the D-serine enriched Bergmann glia is blocked by D-amino acid oxidase treatment or SR inhibitor drugs. As excess NMDA transmission is implicated in stroke, SR inhibitors offer a novel therapeutic strategy.

Role of Glial Cystine-Glutamate Transporters in Addiction

Peter Kalivas*, David Baker, Krista McFarland, Steven LaRowe and Robert Malcolm

Neurosciences, MUSC, Charleston, SC, USA

Withdrawal from repeated cocaine produces enduring adaptations in excitatory transmission in the nucleus accumbens that may contribute to the propensity of addicts to relapse. Among the changes in excitatory transmission observed in animal models of relapse is a large release of synaptic glutamate in response to a cocaine injection or a stressor that primes drug-seeking. This release of glutamate appears necessary for drug-seeking to go forward. Data will be presented demonstrating that the release of glutamate in the accumbens that is driving the reinstatement of drug-seeking results in part from down-regulation of the cystine-glutamate exchanger. The exchanger is a heterodimer that transports a cystine molecule into the cell in exchange for releasing an intracellular glutamate molecule. Restoring exchanger function by systemic treatment with the protcysteine drug N-acetylcysteine abolished cocaine-induced relapse in the reinstatement model of addiction. As a result of these preclinical data, a small clinical trial (n= 13) with cocaine addicts was conducted, and N-acetylcysteine reduced self-reported cocaine craving and withdrawal symptoms. The preclinical data, combined with the preliminary clinical findings, indicate that the cystine-glutamate exchanger may be a novel pharmacotherapeutic target in treating addiction.

Study Group Session

Identification of Candidate Genes Associated with Human Cognitive Variation: Perspectives, Problems and Pitfalls

Anil Malhotra*, Terry Goldberg, Dominique De Quervain, Bruce Pennington, Robert Plomin, Trey Sunderland, Raquel Gur and Michael Egan

Research, The Zucker Hillside Hospital, Glen Oaks, NY, USA

Recent work has identified several genes that appear to influence human cognition. For example, the gene for catechol-o-methyltransferase (COMT) has been implicated in working memory and executive function (Egan 2001, Malhotra 2002, Goldberg 2004). The BDNF gene may influence hippocampal morphology and function (Egan 2003, Malhotra 2004). The 5-HT2A receptor gene alters memory performance (de Quervain 2003). The succinate-semialdehyde dehydrogenase gene has been linked to general cognitive ability (Plomin 2004). Although these studies provide novel data towards dissecting the genetic heterogeneity of neurocognitive function, critical issues have emerged in the interpretation of these data: 1) Genetic design - Initial studies have utilized a variety of genetic designs including case control approaches, family based designs, extreme phenotypes, and linkage analyses. Each of these has distinct advantages and disadvantages. Each participant will discuss the rationale for their design decisions and their impact on study outcome. 2) Genomics - Most studies have been restricted to single SNP analyses. With comprehensive genomic information now available, haplotype or whole genome studies may be feasible. Data from studies utilizing haplotypes (Malhotra) and whole genome studies (Plomin) will be presented. 3) Subjects -

Healthy volunteers have commonly been assessed (Gur, Malhotra, de Quervain), but some studies have also included patients (Egan, Sunderland, Pennington). These approaches will be contrasted. 4) Cognitive assessment - Initial studies (Egan, Malhotra, de Quervain) focused on specific cognitive tasks. Other groups (Plomin, Pennington) have used assessments of general cognitive ability. The strengths and weaknesses of these approaches will be discussed. 5) Prospects for psychopharmacological intervention - Data from genetic studies has suggested potential pharmacological interventions for cognition. For example, studies of COMT in cognition have led to examination of the COMT inhibitor tolcapone. Results from this work will be presented (Egan, Goldberg) to facilitate discussion of this approach. 6) Ethics - The identification of genes associated with cognitive function may have considerable ethical implications. Ethical issues and their implication for future molecular work in cognition will be discussed (Sunderland). This study group should provide a synthesis of this rapidly emerging area and suggest new directions for investigations into the molecular substrates of human cognition. 1) What is the optimal study design for genetic studies of cognition? 2) Is the degree of replication observed in early studies sufficient to confirm genetic loci for neurocognitive phenotypes or do differences exist between studies that must be addressed? 3) Will genetic studies of cognition provide new targets for drug development for disorders in which cognitive impairment is common? 4) Is it feasible to consider whole genome studies for neurocognitive phenotypes? 5) What are the important ethical considerations in conducting this work?

Study Group Session

Is Consideration of a Wider Brain Circuitry Needed To Account for Drug Abuse? Implications for Psychiatry of Addiction and Addiction Therapy

Gary S Aston-Jones, Christelle Baunez, Steven J Grant, Thomas R Insel, T. Celeste Napier* and Philip Winn

Pharmacology and Exp. Therap., Loyola Univ Chicago Med CtrMed, Maywood, IL, USA

Understanding the brain circuits responsible for motivation is critical for understanding addiction and for the development of therapeutically efficacious treatments for this and related disorders. Focus has been on mesoaccumbal dopaminergic projections, and the ability of dopamine to modulate glutamatergic inputs from the prefrontal cortex. It has become evident that this model is too restrictive and cannot account for the complex psychological profile presented during drug addiction. This Study Group will provide a forum for re-considering the dogma that has arisen from this research focus and will explore new findings related to brain substrates that mediate various aspects of addiction psychiatry. To promote deliberation of substrates for reward-mediated learning, Dr. Christelle Baunez will present evidence that the subthalamic nucleus, typically considered a motor region, is critical for behavioral motivation. Dr. Celeste Napier (Chair) will overview reward-promoting transmitter-signaling within the ventral pallidum. Dr. Gary Aston-Jones will describe contributions of the amygdala, bed nucleus of the stria terminalis, and brainstem noradrenergic neurons to the mesoaccumbal circuit, and the influences these regions have on associative learning and altered motivational states that accompany repeated drug exposure. Dr. Thomas Insel (Moderator) will foster discussion relating to brain circuits that may mediate both ethologically relevant cues, such as social attachment, and addiction. Dr. Steve Grant (Moderator) will relate these preclinical findings to new human brain imaging results that reveal anatomical substrates engaged in various phases of drug addiction. Dialogue regarding the bearing that these findings have on the development of novel targets for addiction pharmacotherapy will be

encouraged. The following questions will be discussed: 1) What brain regions are critical for establishing the reinforcing properties of stimuli, and what type of information about reinforcers do they provide to the classical meso-cortical and limbic dopamine circuits? 2) Do these brain regions play a critical role in drug-seeking during protracted withdrawal and subsequent reinstatement? 3) Are these regions potential targets for deep brain stimulation for treatment of psychiatric disorders? 4) What is the role of neuropeptides receptors in these brain regions in mediating both ethologically relevant cues such as social attachment and addiction, and are they viable targets for addiction therapy? 5) Given that their size is at the resolution limits of current brain imaging technology, how can these regions be investigated in humans? We contend that exploring these new vistas will help to understand psychiatric components that comprise addiction, and will aid in devising creative, clinically efficacious therapies for this pathology.

Study Group Session

Potential of Antidepressant Effects with Thyroid Hormones: New Basic and Clinical Findings

Bernard Lerer*, Peter Whybrow, Michael E Newman, Rena Cooper-Kazaz, Bente Appelhof and Michael Bauer

Psychiatry - Biological Psychiatry Laboratory, Hadassah Hebrew University Medical Center, Jerusalem, Israel

Several lines of evidence support a relationship between thyroid gland function and affective illness. The thyroid hormones, triiodothyronine (T3) and L-thyroxine (L-T4) have been used to augment the action of antidepressant drugs in euthyroid patients. Meta-analyses support an effect of T3 to potentiate the therapeutic action of tricyclic antidepressants in treatment-refractory patients (Aronson et al, Arch Gen Psychiatry. 1996;53:842-8) and to accelerate the onset of antidepressant effects (Altshuler et al, Am J Psychiatry. 2001;158:1617-22). This study group (chaired by Bernard Lerer and moderated by Peter Whybrow) will focus on new basic, clinical and translational findings regarding the antidepressant effects of thyroid hormones and will open the following questions for discussion: 1) CAN THE REPORTED EFFECT OF T3 TO AUGMENT ANTIDEPRESSANTS BE EXPLAINED BY A MECHANISM IMPLICATING BRAIN SEROTONERGIC FUNCTION? - Michael Newman will present data from microdialysis studies in rats showing that chronically administered T3 increases synaptic levels of serotonin in a dose dependent fashion and reduces the sensitivity of 5-HT1A and 5-HT1B autoreceptors that control serotonin release. A genomic mechanism for these effects is suggested by mRNA studies that show reduced expression of the genes coding for these receptors in rats treated with T3. 2) DOES T3 AUGMENT THE ANTIDEPRESSANT ACTION OF SSRIS? - The results of two large, randomized controlled trials (RCT), with intriguingly different results, will be presented for discussion. These are the first controlled investigations of T3 augmentation of SSRIs following the algorithm-based, open-label study reported by Agid and Lerer (Int. J Neuropsychopharmacology 2003;6:41-49). Rena Cooper-Kazaz will present the results of the Jerusalem-Princeton study in which patients with unipolar, non-psychotic, major depression (MDD) were randomized to sertraline plus T3 (20-50mcg) or sertraline plus placebo for 8 weeks. The study is planned to encompass 110 patients. An interim analysis of data from the first 64 patients shows a strong effect of T3 to potentiate the antidepressant effect of sertraline and suggests an accelerated response. These findings differ from those of another RCT conducted in the Netherlands that will be presented by Bente Appelhof. In this study 113 patients with unipolar MDD were randomized to paroxetine plus T3 (25 or 50 mcg) or

paroxetine plus placebo. The results showed no advantage for the addition of either dose of T3. 3) WHICH BRAIN MECHANISMS MIGHT UNDERLIE THE REPORTED EFFICACY OF L-T4 AS AN ANTIDEPRESSANT AND PROPHYLACTIC AGENT? - Michael Bauer will present the results of FDG-glucose PET studies in euthyroid women with bipolar depression treated with L-T4. The findings suggest that L-T4 produces mood and anxiety improvement by actions on specific limbic and subcortical circuits that have been implicated in the pathophysiology of mood disorders. POINTS FOR FURTHER DISCUSSION: - A serotonergic mechanism for T3 potentiation of SSRIs is supported - relationship to the endocrine effects should be considered. - The first RCTs of T3 as an augmentor of SSRIs are presented. Two large studies show different results and raise intriguing questions regarding the reasons for this discrepancy. - A PET imaging study suggests brain mechanisms for the antidepressant effect of L-T4 in bipolar depression - how strong is the data for a clinical effect and which neurochemical mechanisms may be implicated?

Study Group Session

Radiotracer Development: Barriers and Solutions to Study Pathophysiology and Enhance Therapeutic Drug Development

Dean F Wong*, Mark Rohrbaugh, Richard Frank, Timothy McCarthy, Rikki Waterhouse, H. D Burns, Chester Mathis, Steven Paul, Linda Brady and Robert Innis

Radiology, Johns Hopkins University, Baltimore, MD, USA

Few biomarkers such as cholesterol or prostate specific antigen exist for neuro-psychiatric diseases, and none are surrogates of clinical outcome. Brain imaging should have the potential for understanding pathophysiology and aiding drug development. PET/SPECT measure the density/distribution of brain target proteins and are potential surrogate outcome measures. They are radiolabeled analogs of drugs themselves. Despite early successes, its full potential has been impeded by the limited development of appropriate radiotracers. The guiding principle of this study group is that a consortium of industry, academia, and government will be critically important to expand the development and use of radiotracers. We will explore the barriers to radiotracer development e.g., intellectual property (IP) concerns and FDA regulations and propose specific steps to overcome these problems. Legal issues regarding IP and radiotracers as research tools will be discussed. Specific examples of agreements between industry, academia and/or NIH will be outlined, highlighting their importance in tracer development. The relevant NIH Roadmap initiative to encourage collaborations will also be discussed. These unique radiotracers allow academic centers to study pathophysiology of disease and industry to enhance drug development. Dean Wong, Linda Brady and Robert Innis will moderate. Chet Mathis, (U Pitt) will discuss the new PET tracer for amyloid imaging that is available for academic research and also is licensed to Amersham-GE. Richard Frank (GE) and Don Burns (Merck) will review problems for industry providing radiotracers for academic research. Tim McCarthy (Pfizer) will describe recent academic/industry research PET centers. Steven Paul (Lilly) will discuss the availability of support for radiotracer collaborations. Mark Rohrbaugh (NIH Tech Transfer) will review IP issues on the development and use of radiotracers. Rikki Waterhouse (Columbia) will discuss advantages and limitations of NIH sponsored industry/academia collaborations. Dean Wong (Johns Hopkins) and Robert Innis (NIMH) will provide specific examples of the terms for collaborative research agreements with industry. A representative from the FDA will review regulatory approval issues. Linda Brady (NIMH) will describe extramural initiatives within the NIH Roadmap for radiotracer development. Unique Questions to Be Ad-

ressed 1. What are the most common means by which radioligands for PET/SPECT imaging have been shared amongst industry, academia, and government? 2. How can regulatory barriers be reduced for the first use of PET tracers in humans? 3. Under what circumstances have industrial innovators been willing to share their IP? 4. If there were no IP constraints what would be the implications for the initial sharing of radiotracers; optimization of chemical syntheses; toxicology management of the result and data base; development of methods to optimize image quantification; and research tools becoming clinically useful diagnostics? 5. How important is it to have FDA distinguishing differences between research versus diagnostic agents?

Study Group Session

Access to Clinical Trial Data: Public, Proprietary, or Limited? Research, Academic, and Industry Perspectives

Joel Braslow*, William T Carpenter, Donald Klein, Drummond Rennie, Ross J Baldessarini, Alan Breier, William Z Potter and John F Greden

Neuropsychiatric Institute, UCLA, Los Angeles, CA, USA; VA VISN 22 MIRECC, Veterans Administration, Los Angeles, CA, USA

The aim of our study group will be to examine the major issues involved in the funding and ownership of data and to suggest solutions to the problems the panelists raise. Individuals from academia and industry will present their perspectives: clinical researchers (Ross Baldessarini, John Greden); industry members (Alan Breier and William Potter); two medical journal editors (Drummond Rennie and Catherine DeAngelis); and an academic proposing potential solutions to identified problems (Donald Klein). Panelists will then participate in a discussion moderated by William Carpenter and Joel Braslow. This study group will address ethical, practical, and scientific issues surrounding the proprietary nature and availability of clinical trial data. With good reason, the pharmaceutical industry has argued that limited access to privately generated data is necessary for successful research and development. But while proprietary data may be critical to protect the interests of pharmaceutical companies, it can present serious problems for researchers, journals, clinicians, patients, government, and industry alike. Recent controversies over drug safety and study publication have focused more attention on the accessibility of clinical trial data, suggesting the need for interested parties to rethink our current approach. 1) What are the major issues involved in the funding and ownership of clinical trial data? 2) To what extent is publication bias an outcome of (a) the proprietary nature of most clinical data and (b) journal editorial policy? How can this problem best be addressed? 3) What are the pros and cons of a national registry, practically and ethically? Is it ethical to perform human experimentation when findings are suppressed - a suppression that is inevitable, whether intentionally or unintentionally, without a national registry? 4) What can be done to improve the open use of clinical trial data, especially through the development of collaborative relationships between industry and academia? 5) What safeguards can be developed to protect legitimate intellectual property rights while meeting the need for transparency in research?

Study Group Session

Do Mood Disorder Outcomes Improve When Practice Procedures Are Changed?

A. J Rush*, Madhukar Trivedi, Mazda Aldi, Mark Bauer and David Kupfer

Psychiatry, UT Southwestern Medical Center, Dallas, TX, USA

Both improving the availability of ongoing care and improving the quality of that care should profoundly improve clinical outcomes

in patients with chronic/recurrent mood disorders. Such improvements should also improve the cost efficiency of care and may well reduce the shunting of these patients to the legal/criminal or civil justice systems. This panel will provide some of the very first data to test these hypotheses. Adherence to treatment guidelines or algorithms is one potential indicator of the quality of care. Dr. Trivedi will present recent findings from the Texas Medication Algorithm Project (TMAP) which reveals that better algorithm adherence is associated with better clinical outcomes in patients with major depressive disorder (MDD) using a newly devised method to define algorithm adherence. Similarly, Dr. Aldi will present recent data from the German Treatment Algorithm Project that evaluates whether better adherence to this algorithm is associated with better clinical outcomes for inpatients with MDD. For bipolar disorder (BPD), Dr. Bauer will present data to address the question of whether improved delivery of care (by modification of the care system itself) improves clinical outcomes. This recently completed 5-year Veterans Administration Cooperative Study aimed at determining whether redesigning the delivery system changed clinical outcomes in patients with BPD. Dr. Rush will present descriptive data obtained in the first (nonrandomized) treatment step in STAR*D (Sequenced Treatment Alternatives to Relieve Depression). The types and frequencies of deviations from protocol recommendations for citalopram treatment of depressed outpatients will be described and compared for primary vs psychiatric practitioners. In sum, this Panel will provide a state-of-the-art snapshot, using recently acquired, prospective empirical evidence as to whether improvements in these two key elements (i.e., "process" parameters) - namely the improvements in the quality of care and in the delivery of care, actually are associated with improved short and longer-term clinical outcomes. Dr. Kupfer will discuss the weaknesses and strengths of the studies presented, and their implications for subsequent research, the psychiatric practice, and the care system administration. 1) Do adherence to guidelines improve outcomes? 2) Does changing the structure of the delivery system improve outcomes? 3) Do primary care and psychiatric practitioners differ in their use of SSRIs in a protocol driven study? 4) Given variation in practice procedures and site structures, how can one correctly analyze multi-site trial data?

Study Group Session

Substance Abuse in the 21st Century: What Problems Lie Ahead for the Baby Boomers?

Susan Weiss*, Timothy P Condon, Susan Resnick, David W Oslin and Frederic C Blow

NIDA, Bethesda, MD, USA

The baby boomer generation is America's largest thus far, and in thirty years will comprise 20% of the nation's population (~70 million people). While substance abuse typically declines with age, for a variety of social, cultural, and economic reasons, baby boomers appear to be using and abusing alcohol and illicit drugs at higher rates than previous cohorts. Questions therefore arise as to how their history of drug exposure might impact and interact with the aging process and how we can intervene to prevent the devastating consequences of drug abuse in this population. These questions have not yet been adequately addressed by research. The purpose of this symposium is to increase awareness, begin discussion, encourage interest, and help generate a research agenda in this area. Dr. Timothy Condon, Deputy Director of the National Institute on Drug Abuse, will provide an overview of the potential impact of substance abuse in the baby boomer generation and will highlight the unique issues that must be taken into consideration in understanding, preventing, and treating drug abuse in this population. Dr. Susan Resnick of the National In-

stitute on Aging will discuss the changes the brain undergoes across the lifespan and how drug abuse may impact these changes. Dr. David Oslin of the University of Pennsylvania will speak about the impact of substance abuse and psychiatric co-morbidity in older adults. Finally, Dr. Frederic Blow of the University of Michigan will discuss the misuse and abuse of prescription drugs and alcohol in elderly populations. A discussion of future research directions will follow to help frame a research agenda to prevent and/or ameliorate the medical, social, and financial consequences of continued or emerging substance abuse in aging baby boomers. 1) How does the brain change with aging? 2) How might drug abuse affect the aging processes of the brain? 3) What is the impact of drug abuse on co-morbid conditions in the elderly and vice versa? 4) What unique issues arise with the misuse of prescription drugs in the elderly?

Tuesday, December 14

Panel Session

ACNP Medication Development Task Force, Medication Development for Bipolar Disorder: Genetics, Endophenotypes, Animal Models, Target Validation and Clinical Trials

Cognitive Neuroscience and Bipolar Disorder: Mechanisms of Drug Action and Relevant Outcomes

Guy M Goodwin*

Oxford University, Oxford, United Kingdom

Background: Cognitive neuroscience offers new ways of understanding psychiatric disorder. Traditional clinical descriptions of both phenomena and outcome are atheoretical and have limited heuristic potential. Cognitive neuroscience offers, in contrast, a vibrant new synthesis of what we know about how the brain works in health and, more speculatively, in disease. The task is to identify semi-discrete components of cognition that provide insights into pathophysiology or drug action. This approach has worked in investigating the actions of antidepressants and is continuing into psychopathological studies of bipolar patients. **Methods:** In parallel group designs, the effects of antidepressants and tryptophan depletion were compared with appropriate control treatments. Volunteers have been tested on fear potentiated startle, memory for emotionally valenced words, recognition of facial expression processing: in separate experiments volunteers have been asked to make a series of choices between simultaneously presented gambles, differing in the magnitude of possible gains (i.e. reward), the magnitude of possible losses (i.e. punishment), and the probabilities with which these outcomes were delivered. Preliminary findings in patient groups compared to appropriate controls have also been obtained for a range of these tasks. **Results:** Citalopram and reboxetine after 7 days administration increased the relative recall of positive (versus negative) emotional material and reduced the identification of the negative facial expressions of anger and fear (1). Responses to fearful faces have now been recorded in the amygdala and inferior mesial orbital cortex using event-related fMRI. Processing was implicit, ensured by using backward masking with faces exhibiting neutral facial expressions. Citalopram attenuated the fMRI signal, in parallel with its behavioural effects. Citalopram, not reboxetine, also abolished a 'fear-potentiated' startle response found to white noise stimuli. Recovered patients with recurrent unipolar disorder show increased detection of fearful faces that is normalised by SSRI treatment (2). The gaming task has been shown with fMRI to tap specific areas of frontal cortex. In the decision phase, choices involving large gains were associated with increased BOLD responses in the pregenual ACC, paracingulate, and right orbitolateral cortex compared with choices involving small gains. In the outcome phase, good outcomes were associated with increased BOLD responses in

the posterior orbitomedial cortex, subcallosal ACC, and ventral striatum compared with negative outcomes. There was only limited overlap between responses in either phase illustrating the need to analyse separate components of complex tasks (3). Tryptophan depletion reduced the sensitivity to reward in the gaming task; hence serotonin may mediate decision-making in healthy volunteers within the orbitofrontal cortex (4). Preliminary findings suggest important differences between unmedicated Bipolar patients and control groups both in regard to decision making and sensitivity to losses and gains so providing a starting point for investigation of the action of mood stabilisers. **Discussion:** Facial expression, startle or gaming paradigms are all sensitive to monoamine manipulations in man and are starting points for an approach to experimental medicine in mood disorders with novel compounds. The specific relevance to bipolar disorder will be further clarified by ongoing studies of neuropsychological function in bipolar patients and their families. References: 1. Harmer et al. (2003) *American Journal of Psychiatry* 160, 990-2 2. Bhagwagar et al. (2004) *ibid* 161, 166-8 3. Rogers et al. (2004) *Biol. Psychiatry* 55, 594-602 4. Rogers et al. (2003) *Neuropsychopharmacology* 28, 153-62.

Animal Models for Bipolar Disorder: New Understanding and New Possibilities

Haim Einat*

College of Pharmacy, University of Minnesota-Duluth, Duluth, MN, USA; LMP, NIH/NIMH, Bethesda, MD, USA

Models of bipolar disorder (BPD) are usually considered flawed because none includes all the main facets of BPD. However, the growing understanding that BPD may not be a single disorder but rather a collection of related subtypes and the current attempts to break down BPD into its component parts, study the biology of the component parts and come up with treatments for them, supports further development of models that may reflect different facets of the disease. Whereas depression models do take on some of the main symptoms of the disorder such as reduced activity, despair, unhedonia, sleep disturbances and others, traditionally, the commonly used models for mania emphasized only hyperactivity. A number of approaches can be used in attempts to develop better animal models for the study of bipolar disorder: 1) It is now becoming more important to employ models that reflect the different components of mania such as increased risk taking, hedonistic behavior, aggression, reduced need for sleep and so on. Some such models are available but were previously used in different contexts and should therefore be validated properly for mania. Other models should be developed; 2) Increasingly, researchers are paying more attention to individual differences in animal behavior and attempt to develop specific models based on such differences. It may be possible that attention to individual differences may also be helpful in the development of models to bipolar disorder that have strong face validity and may be relevant to the exploration of endophenotypes of the disorder in humans. 3) The better understanding of mechanisms involved in BPD and its treatment, combined with the increasing ability to pharmacologically and genetically target specific molecules permit us to try and develop models based on mechanistic hypotheses. For example, targeting PKC, ERK, BCL-2 or GSK, all have been demonstrated in-vitro to be relevant to the disorder, may be the source for more precise and better serving new models. New approaches to modeling hold much promise and are necessary for further attempts to delineate the underlying pathophysiology of this devastating illness, and for the development of novel, improved therapeutics.

Molecular Correlates of Sleep Deprivation: A Clue to its Antidepressant Effects?

Giulio Tononi*

Psychiatry, UW Madison, Madison, WI, USA

Recent studies using high-density microarrays has revealed that a significant proportion of transcripts in the cerebral cortex are upregu-

lated during wakefulness and sleep deprivation and are downregulated during sleep (Cirelli et al., *Neuron* 41: 35-43, 2004). Waking-related transcripts are involved in energy metabolism, excitatory neurotransmission, transcriptional activation, synaptic potentiation and memory acquisition, and the response to cellular stress. Sleep-related transcripts are involved in brain protein synthesis, synaptic consolidation/depression, as well as membrane trafficking and maintenance, including cholesterol metabolism, myelin formation, and synaptic vesicle turnover. Further studies (Cirelli and Tononi, *J Neurosci* 24:5410-5419, 2004) have shown that a key factor controlling the modulation of gene expression by behavioral state is the activity of the noradrenergic system of the locus coeruleus, which is high during wakefulness and low during sleep. Specifically, high norepinephrine levels during wakefulness are required for the induction of transcripts involved in synaptic plasticity and in the cellular response to stress. One of the generalizations that can be drawn from such studies is that a few hours of wakefulness or sleep deprivation are sufficient to trigger the expression of many genes involved in synaptic plasticity and responses to cellular stress, and that a few hours of sleep reverse this process. This finding suggests that molecular changes induced by wakefulness may be related to the antidepressant effects of sleep deprivation, whereas the reversal of such changes by subsequent sleep may be responsible for their transitory nature. Possible implications concerning new pharmacological approaches will be discussed.

Molecular Mechanisms of Action of Antiepileptic Drugs in the Treatment of Bipolar Disorder: How Can More Effective Agents Be Developed?

Michael A Rogawski*

National Institute of Neurological Disorders & Stroke, Bethesda, MD, USA

Randomized controlled clinical trials have shown that the antiepileptic drugs (AEDs) carbamazepine, oxcarbazepine, and valproate are effective for treating bipolar mania and lamotrigine is effective for bipolar depression. I will review the cellular mechanisms of these AEDs to stimulate discussion of hypotheses as to how the drugs might exert beneficial actions in mood disorders. A key question for discussion is what characteristics confer lamotrigine with unique selectivity for bipolar depression. Carbamazepine and oxcarbazepine are believed to act by modulating the gating of brain voltage-gated sodium channels. These drugs bind selectively to inactivated conformations of the channels so as to block high-frequency repetitive spike firing (such as occurs during seizure activity) without affecting ordinary ongoing neural activity. As a result of the effect on sodium channels, the drugs inhibit the release of glutamate at synapses; this is believed to be the critical action responsible for anti-seizure activity. Lamotrigine also blocks sodium channels and has similar actions on glutamate release, but it may act differently on GABA release than the other sodium-channel blockers. Exactly how this occurs is not understood, but it does appear that lamotrigine may affect high-voltage activated calcium channels in addition to sodium channels. The mechanism of action of valproate is still poorly understood. There is evidence that it can also interact with sodium channels and possibly T-type (low voltage-activated) calcium channels. In addition, valproate seems to affect GABA systems; the best recognized action is an increase in GABA turnover. Interestingly, valproate also is an effective inhibitor of glutamate release at synapses. The delayed clinical efficacy of AEDs in mood disorders raises the possibility that the underlying mechanisms may be distinct from those that are relevant in epilepsy. Thus, it has been proposed that mood-stabilizing drugs can attenuate or reverse disease-related impairments in neuronal plasticity, neurogenesis or cell survival that are emerging as important hypotheses for the pathophysiology of mood disorders. Investigators have attempted to identify molecular actions that the AEDs share with lithium. Like lithium, valproate depletes inositol, which may result in stabilization of the structural integrity of neurons and enhancement of synaptic plasticity. Also like lithium, lamotrigine and

valproate inhibit GSK3 β , which may lead to antiapoptotic effects and improved cell structural stability. Another common action of lithium and valproate is to increase the activity of the ERK pathway, which is, like the other signaling pathways, involved in the differentiation, survival and structural and functional plasticity of neurons. Thus, there is emerging evidence that AEDs with mood-stabilizing activity can affect signaling pathways to reverse the pathology in cell survival and neuronal plasticity that is believed to be of key pathophysiological significance in bipolar disorder. Recent evidence indicates that multidrug resistance transporters (including P-glycoprotein and MRPs) limit the access of AEDs to the CNS and may contribute to pharmacoresistance. As in epilepsy, these transporters could influence clinical responsiveness when AEDs are used in the treatment of bipolar disorder. Greater understanding of the activity of these transporters in mood disorders may suggest strategies to optimize therapy.

Panel Session

Sexual Function and Dysfunction: A New Generation of Centrally Acting Therapies

Sexual Dysfunction and Depression

Robert M Hirschfeld*

Psychiatry, UTMB, Galveston, TX, USA

The prevalence of sexual dysfunction in the general population is 31% in men and 43% in women. The rate of sexual dysfunction in untreated depressed patients is approximately twice that in the general population. Prior to starting antidepressant treatment, 46% of men report impotence, and 50% of women report decreased sexual arousal. Antidepressants, including the SSRIs, produce treatment-emergent sexual dysfunction in addition to the already existing sexual dysfunction. Typically, such sexual side effects are reported by between 40 and 60 percent of depressed patients taking SSRIs. A variety of treatments for sexual dysfunction due to antidepressants have emerged over the last few years, with primarily antidotal evidence supporting their use. Most involve stimulation of the dopamine system. Bupropion has been shown to be an effective antidote to SSRI-induced sexual dysfunction, primarily through an increase in desire to engage in sexual activity. Sildenafil has also been shown to be effective in treating SSRI-induced sexual dysfunction. Erectile dysfunction, arousal, ejaculation, orgasm, and overall satisfaction improved in the sildenafil compared with placebo patients.

Central Neurophysiology and Pharmacology of Sexual Function

Francois Giuliano

Abstract not available.

Facilitation of Penile Erection by ABT-724, A Novel and Selective Dopamine D4 Receptor Agonist

Jorge D Brioni*

Neuroscience, Abbott Laboratories, Abbott Park, IL, USA

The physiological role of the dopamine D4 receptor remains unclear despite numerous reports indicating its participation in several CNS disorders including schizophrenia and cognitive deficits. Apomorphine, a non-selective dopamine receptor agonist, facilitates erection in patients with erectile dysfunction acting via a central dopaminergic mechanism but the dopamine receptor subtype/s responsible for the erectogenic effect of apomorphine are not known. The potential role of the D4 receptor on sexual function was investigated by means of the selective D4 agonist ABT-724. ABT-724 activates human dopamine D4 receptors with an EC₅₀ value of 12.4 nM

and 61% efficacy. It also activates rat and ferret dopamine D4 receptors (EC₅₀= 14.3 and 23.2 nM, with 70% and 64% efficacy, respectively). In contrast, the compound does not bind dopamine D1, D2, D3, or D5 receptors. In conscious rats, subcutaneous injections of ABT-724 facilitate penile erection at 0.03 μ mol/kg and this effect is blocked by haloperidol and clozapine but not by domperidone (a peripheral antagonist). A pro-erectile effect is also observed after intracerebroventricular administration of ABT-724 but not after intrathecal injections of the compound. Subcutaneous injections of ABT-724 increase intracavernosal pressure in awake freely-moving rats. In the presence of the PDE-5 inhibitor sildenafil, a 10-fold potentiation of the pro-erectile effect of ABT-724 is observed in conscious rats. ABT-724 does not induce nausea or emesis in ferrets and is devoid of cardiovascular effects in rats. The ability of the selective D4 receptor agonist ABT-724 to facilitate penile erection in preclinical in vivo models indicates that the dopamine D4 receptor plays a unique role in the regulation of penile function in mammals.

Development of PT-141, A Centrally Acting Melanocortin Agonist for the Treatment of Male Erectile Dysfunction

Dennis C Earle, Annette M Shadiack, Lisa E Diamond and Perry B Molinoff*

Office of the Vice Provost for Research, University of Pennsylvania, Philadelphia, PA, USA; Palatin Technologies, Cranbury, NJ, USA

Melanocortins, including α -MSH, have been implicated in the control of a variety of behaviors including food intake and sexual arousal. PT-141, a cyclic, heptapeptide, with potent erectogenic activity has high affinity for melanocortin receptors (MCRs), including MC3R and MC4R, which are found primarily in the hypothalamus. ICV administration of PT-141 to rats resulted in a dose dependent increase in the frequency of penile erections, requiring 100-1000-fold less drug than following its peripheral administration. Immunoreactivity of *c-fos*, used to determine neuronal activation following a single IN dose of PT-141 was localized to the paraventricular nucleus (PVN) and supraoptic nucleus of the hypothalamus. Using pseudorabies virus as a retrograde trans-synaptic marker, we confirmed that nerve endings in the corpus cavernosum of the rat penis are associated with the PVN. It is known that nitric oxide (NO) mediates in the vasodilation of blood vessels in the corpus cavernosum leading to PE. Pretreatment of rats with increasing doses of a NO synthase inhibitor blocked PT-141-induced PE's in a dose-dependent manner. These data suggest that PT-141 induces spontaneous erectile activity in rats by stimulating melanocortinergic neurons located in the hypothalamus and culminates in PE through the release of NO. The safety of PT-141 administered IN was demonstrated with doses of PT-141 up to 20 mg and the pharmacodynamic effects of doses of IN PT-141 of 7 and 20 mg was subsequently evaluated in a placebo-controlled, 3-way crossover in normal volunteers and in 24 Sildenafil-responsive patients with ED. Erectile responses were assessed by RigiScan[®] without visual sexual stimulation (VSS) in healthy subjects and with VSS in ED patients. A maximum tolerated dose (MTD) was not identified following the IN administration of PT-141. The most common adverse events (AEs) reported in both studies included flushing and nausea. In both studies, erectile responses induced by PT-141 administration were significantly greater than those induced by placebo at doses greater than 7 mg, with a mean onset time of first erection of approximately 30 minutes. A double-blind, placebo-controlled, study enrolled 271 ED patients responsive to Sildenafil. Patients with ED severity ranging from mild/moderate to severe were included. After an initial in-clinic dose to provide data on systemic exposure and tolerability, patients were sent home for 4 weeks with 10 single-dose units of placebo or 5, 10, 15, or 20 mg PT-141. Of the patients who completed at least 3 at home attempts (n = 203), the mean change in IIEF EF score relative to baseline was 1.4, 4.1, 5.7, 7.8 and 7.7 points (p < 0.05 for 10, 15, and 20 mg dose groups) for patients in the placebo, 5, 10, 15, and 20 mg groups respectively. Restoration to

normal erectile function (EF score ≥ 26) was achieved by 10, 30, 36, 53, and 50% of patients in the placebo, 5, 10, 15, and 20 mg groups respectively ($p < 0.05$ for all dose groups). There were no episodes of syncope or hypotension. The only serious AE reported was a prolonged erection that was painless and required no treatment. Gastrointestinal side-effects were the primary reasons for discontinuation in the two highest dose groups. In this at-home study, PT-141 was shown to be safe and highly effective in inducing high-quality erections in men with ED. ¹Current Address: University of Pennsylvania Office of the Vice Provost for Research 118 College Hall Philadelphia, PA 19104-6303

Panel Session

Mechanisms of Hippocampal Involvement in the Pathophysiology of Schizophrenia

The Hippocampal-VTA Loop: Regulation of the Entry of Information into Long-Term Memory

John E Lisman*

Biology Department, Brandeis University, Waltham, MA, USA

Progress in understanding hippocampal computations and the reciprocal flow of information with the VTA provides a detailed view of how entry of information into long-term memory is regulated. A key role of the hippocampus is in the episodic memory of sequences (e.g. the positions along a path). Recordings of hippocampal place cells reveal that the phase of spiking relative to ongoing theta oscillation changes systematically as the animal traverses a place field. This "phase precession" has been interpreted as cued sequence recall, a process that is predictive of what will happen next, and is generated in the dentate and CA3. The CA1 region receives both this prediction and current sensory information by a direct cortical input. This allows CA1 to compute whether current events are predicted or novel. In the latter case, a signal descends to the VTA (via relays in the subiculum, accumbens and pallidum), where it triggers novelty-dependent firing. Active VTA cells release dopamine in the hippocampus which enhances LTP and learning. The existence of this loop may be of importance for understanding schizophrenia. It has been shown that dopamine selectively reduces the cortical input to CA1. This would be expected to cause the system to generate "novelty" signals, which in turn generates more dopamine. There is thus the possibility that the hippocampal-VTA loop could go into positive feedback and that this feedback state would create a constitutive hyperdopaminergic s

Hippocampal Modulation of the Dopamine System and its Reciprocal Influence on Hippocampal Efferents

Anthony A Grace* and Yukiori Goto

Neuroscience, Psychiatry and Psychology, University of Pittsburgh, Pittsburgh, PA, USA

Introduction: The hippocampus and ventral tegmental dopamine (DA) neurons exhibit mutual interactions that affect information processing within the nucleus accumbens. We have shown previously that activation of the ventral hippocampus increases tonic DA neuron activity in the midbrain and tonic extracellular DA levels in the nucleus accumbens. In contrast, induction of phasic burst firing of DA neurons transiently increases synaptic DA levels in the accumbens (Floresco et al., *Nature-Neuroscience* 6: 968, 2003). We report here that tonic and phasic DA selectively modulate prefrontal cortical (PFC) and hippocampal afferents to the accumbens, and that modulation of these afferents mediate distinct behavioral processes. **Methods:** Phasic DA release was elicited by infusion of bicuculline into the pedunculo-pontine tegmentum, and tonic DA was evoked by infusion of the GABA agonists muscimol and baclofen into the ventral pallidum. Field potential recordings were made in the nucleus accumbens with stimulation of the PFC and ventral subiculum of the

hippocampus. Drugs were administered locally into the nucleus accumbens via reverse microdialysis. Behavioral studies examined learning in the plus maze task following afferent disconnection; this was done by infusing lidocaine into either the PFC or hippocampus unilaterally while administering D1 or D2 selective drugs into the contralateral nucleus accumbens. The maze task consisted of goal-directed behavior signaled by either a visual cue or by response direction, with switching of the salient cue after reaching criterion. **Results:** Increased tonic DA release into the accumbens resulted in attenuation of PFC-evoked field potentials without affecting the hippocampal input; this was blocked by local microdialysis infusion of the D2 antagonist sulpiride but not the D1 antagonist SCH23390. Decreasing tonic DA levels by activating the ventral pallidum or by local D2 antagonist infusion augmented the PFC evoked response above baseline, demonstrating that D2 receptors tonically regulate this afferent system. In contrast, increased phasic DA release caused a selective facilitation of the hippocampal input without affecting the PFC-evoked response; this effect was blocked by local infusion of the D1 antagonist but not the D2 antagonist. Inactivation of the pedunculo-pontine tegmentum or D1 antagonist infusion alone was ineffective at altering this response, suggesting that this system is not spontaneously active in the anesthetized rat. Disconnection of the PFC input by unilateral PFC inactivation and infusion of the D2 agonist quinpirole into the contralateral accumbens selectively disrupted set-shifting behavior in the plus maze, in that they acquired the task normally but made higher numbers of perseverative errors. In contrast, disconnection of the hippocampal input by unilateral hippocampal inactivation and D1 antagonist infusion into the contralateral accumbens disrupted acquisition of the task without affecting perseverative error rate. **Discussion:** These data show that tonic and phasic DA release can selectively modulate cortical and limbic input to the nucleus accumbens, respectively. In the anesthetized rat, PFC inputs are under tonic modulatory control by DA, whereas the hippocampal inputs are only modulated when the DA system is phasically activated. Moreover, each of these afferent inputs was found to exert regulation over distinct behavioral responses. Therefore, different activity states within the ventral tegmental DA system can shift the balance of information processing in the accumbens. In particular, hippocampal drive of tonic DA release coupled with glutamatergic activation of phasic DA neuron firing will result in a shift from PFC to hippocampal afferent drive of this structure.

Characteristics of Hippocampal Function in Schizophrenia

Carol Tamminga*, Ronald B Chin and Bin Thomas

Psychiatry, UT Southwestern Medical Center, Dallas, TX, USA

Considerable human data from in vivo imaging and post-mortem studies suggest an involvement of the hippocampus in schizophrenia. We as well as other investigators have shown that the performance of hippocampally-mediated tasks is abnormal in persons with the illness. Moreover, task-associated brain activation patterns in hippocampus also differ in schizophrenia, both at rest and during performance. Antipsychotic treatment alters activation patterns in hippocampus; and, ketamine-induced actions in hippocampus differ between volunteers with and without schizophrenia. This presentation will review new data in the field and will provide new behavioral and functional imaging results from hippocampal studies in humans. The rate of learning a transitive inference task is slower in persons with schizophrenia than for healthy volunteers (8.9+3.3 training trials for SZ patients vs 3.7+1.6 training trials for normals), and the schizophrenia volunteers fail to perform the transitive inference portion of the task normally (69%+/- 29% correct in schizophrenia vs 84%+/- 19% correct for normals). Antipsychotic treatment appears to modulate these performance parameters; we will report a comparison between medicated and non-medicated volunteers. During fMRI BOLD with event related acquisition, the schizophrenia volunteers fail to show hippocampal activation during a novelty detection

paradigm analyzing the successful memory events, as do the healthy volunteers. We will interpret these findings in light of several recent animal studies that suggest novel aspects of cognition mediated in hippocampus.

Imaging Hippocampal Dysfunction in Schizophrenia

Stephan Heckers*

Schizophrenia and Bipolar Disorder Program, McLean Hospital, Harvard Medical School, Belmont, MA, USA

Background: The study of hippocampal function in schizophrenia is of interest for two reasons: to establish the functional consequences of the well-known structural abnormalities and to explore a hippocampal contribution to the deficits of declarative memory in schizophrenia. We will review our ongoing studies of hippocampal memory function in patients with schizophrenia using functional neuroimaging techniques and propose a conceptual framework to explain the results. **Methods:** We studied hippocampal contribution to memory retrieval using positron emission tomography. In these studies we modulated encoding parameters (item repetition and task instruction) to test hippocampal function during subsequent retrieval. Then we studied three separate memory functions (semantic memory, episodic memory, and transitive inference) using functional magnetic resonance imaging to test for hippocampal dysfunction in schizophrenia and healthy control subjects. **Results:** We found increased activity and impaired recruitment of the right hippocampus during memory retrieval in schizophrenia. Although schizophrenia subjects demonstrated the normal depth-of-encoding effect during memory retrieval, they did not show the normal increase of right hippocampal blood flow. The study of semantic memory revealed that schizophrenia subjects were able to remember previously learned words. However, their ability to correctly identify new words was impaired and was associated with decreased right hippocampal recruitment. Episodic memory function was not impaired in our cohort of schizophrenia subjects and left hippocampal recruitment was intact. However, right hippocampal recruitment was significantly increased in schizophrenia. Finally, the ability to make transitive inference judgments on a previously learned sequence of overlapping stimulus pairs was preserved in schizophrenia subjects, but was associated with abnormal brain activation patterns. **Discussion:** Our results indicate that some, but not all, hippocampal memory functions are impaired in schizophrenia. Impaired memory function in schizophrenia subjects (i.e., retrieval after deep encoding and novelty detection) was associated with decreased hippocampal recruitment. Normal memory function (i.e., episodic memory and transitive inferences) was associated with either normal, decreased, or increased hippocampal recruitment. Our results indicate that several hippocampal memory functions are preserved despite a significant decrease of hippocampal volume. We will present a model of hippocampal dysfunction to explain these findings.

Panel Session

Complex Cortical-Basal Ganglia Neural Networks: 3-D Reconstructions in Rat and Monkey Help Understand the Network That Underlies Disease and Therapeutic Intervention

Cortical-Basal Ganglia Networks in Obsessive Compulsive Disorder: What is the Role of the Dorsal Striatum?

Scott L Rauch*

Psychiatry, Mass. General Hospital, Charlestown, MA, USA

Cortical-basal ganglia circuitry has been implicated in the pathophysiology of obsessive compulsive disorder (OCD). Neuroimaging studies of OCD, employing a variety of different experimental paradigms, have yielded convergent findings implicating or-

bitofrontal cortex (OFC), anterior cingulate cortex, caudate nucleus and thalamus. Interestingly, reviews and theoretical models of OCD have tended to focus on the more ventral subterritories of the striatum, in keeping with their purported receipt of direct projections from the relevant cortical regions implicated (i.e. OFC and anterior cingulate cortex). However, it is noteworthy that most early neuroimaging studies used region-of-interest-based analyses that did not enable a distinction between dorsal and ventral subterritories of striatum. Several more recent reports, using statistical parametric mapping methods have provided precise coordinates that do enable such distinctions. In the current presentation, those data will be critically reviewed. In addition, there is a growing body of relevant neuroimaging data derived from patients who have undergone neurosurgical treatment for their OCD. In one study of cingulotomy, a comparison of structural MRI measures from pre-operative vs. several months post-operative time points found volumetric reduction within a dorsal and posterior portion of the caudate (i.e. the body) rather than the head. Most recently, a PET blood flow study was performed in 6 OCD patients undergoing deep brain stimulation at a target within the ventral capsule and adjacent ventral striatum. During acute stimulation vs. control conditions, increases in blood flow were found within OFC, ventral anterior cingulate cortex, and dorsal, but not mid or ventral, striatum. These data will be discussed in the context of the overall theme of this symposium. An effort will be made to explain what role the dorsal striatum may play in OCD and/or its treatment.

Cortico-Striatal Connectivity Determines the Chronic Effects of Cocaine

Linda Porrino* and Michael Nader

Physiology and Pharmacology, Wake Forest University School of Medicine, Winston Salem, NC, USA

Although there are many studies investigating the structural and functional adaptations that occur in human cocaine addicts, the interpretation of reports in human abusers can be very difficult. Studies in nonhuman primates provide a way to systematically evaluate the structural and functional adaptations engendered by cocaine self-administration. We have used metabolic mapping methods along with in vitro receptor autoradiography to evaluate the topography of these changes within the dorsal and ventral striatum. Rhesus monkeys were trained to self-administer 0.3 mg/kg per injection cocaine for 5 days (initial stages; n=4), 100 days (chronic stages; n=4) days or 15-22 months (long-term; 0.03 mg/kg/injection) and compared with monkeys trained to respond under an identical schedule of food reinforcement (n=6). The effects of cocaine on functional activity, measured with metabolic mapping methods, within the striatum shifts dramatically over the course of drug exposure, expanding from only the ventral striatum in the initial stages of cocaine self-administration to include the dorsal striatum with longer durations of cocaine history. This expansion also spreads both rostrally and caudally to encompass almost all of the territories of the entire striatum. Alterations in dopamine transporter and receptor densities follow an identical path. These data suggest, first, that the influence of cocaine is augmented with continued exposure, and secondly, the progression follows known anatomical principles of organization of the striatum advancing from limbic to association to sensorimotor domains.

Reciprocal and Non-Reciprocal Components in the Striato-nigro-striatal Loop Circuits: A Three Dimensional Analysis in the Rat

Jean-Michel Deniau*, Philippe Maily, Stephane Charpier, Yvette C Van Dongen and Henk Groenewegen

Neuropharmacologie U114, Institut National de la Recherche Medicale (INSERM), Paris, France

The striatum provides a main integrative component of the basal ganglia (BG) through which cortical information is transmitted

to the BG output nuclei: the substantia nigra pars reticulata (SNR) and the internal segment of the globus pallidus. Growing evidence indicates that signals originating from functionally distinct cortical areas are processed in separate striatal territories and remain segregated in the striato-nigral and striato-pallidal pathways supporting the concept of parallel cortico-basal ganglia circuits. Integration of cortical information in the striatal circuits is under the neuromodulatory control of a dopaminergic innervation originating from the ventral mesencephalon including the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNC). The striatum and the dopaminergic nigro-striatal neurons are intimately interconnected via direct and indirect loop circuits involving projections of the GABAergic neurons of the SNR to the SNC. These loop circuits constitute potential mechanisms of integration across various components of the parallel cortico-BG circuits. The present communication will review recent progress on the organization of these striato-nigro-striatal loops using combination of tract tracing methods, single cell labeling and three dimensional reconstructions. The result show that (1) the various subdivisions of the cortico-striatal functional mosaic are orderly mapped in the SNR along longitudinal and curved lamina organized in an onion like manner; (2) each subdivision of the striatal mosaic is innervated by two subpopulations of nigro-striatal neurons, proximal and distal, located respectively in register and out of register from the corresponding striato-nigral projection fields in SNR; (3) the SNR cells located in the projection field of a given striatal region innervate mainly the proximal subpopulation of nigro-striatal neurons connected to the same striatal region but also provide some non reciprocal connections with the proximal neurons connected to other striatal regions; (4) the shell of the nucleus accumbens, a major component of the limbic striatum that receives input from the hippocampus innervates the VTA and SNC regions projecting to the shell and core subdivisions of the nucleus accumbens as well as to the distal subpopulation of nigro-striatal neurons innervating the sensorimotor territory of the dorsal striatum. These observations indicate that the various functional subdivisions of the striatum and their dopaminergic nigro-striatal afferent neurons are engaged in both closed and open loop circuits allowing interactions between segregated cortico-striatal circuits. Interactions between the ventral limbic striatum and the dorsal sensorimotor striatum has a definite ventro dorsal polarity and occurs via a subpopulation of nigro-striatal neurons that has no reciprocal connections with the sensorimotor striatum.

3D Reconstructions of Cortico-striatal Pathways Demonstrate a Complex Interface Between Functional Circuits

Suzanne Haber* and Roberta Calzavara

Dept. of Pharmacology and Physiology, University of Rochester, Rochester, ny, NY, USA

Emphasis has been placed on the segregation of function between the different cortico-basal ganglia loops; however, recent evidence demonstrates complex integrative networks between these circuits through the midbrain and thalamus. In the experiments described here we reexamined the topography of cortico-striatal pathways with modern tracing technique coupled with 3D computer analysis to reconstruct to the complexity of these pathways. While fibers from each functional region of cortex terminate densely in a topographically organized fashion, that topography differs somewhat from the conventionally held notion of these projections. Furthermore, there is extensive overlap between the dense terminal fields arising from widely separated frontal cortical areas. In addition, functionally different areas of the frontal cortex project densely in a center region of the striatum, but also have an extensive peripheral projection region with a sparser innervation. Thus, many fibers from each functional region terminate widely throughout the striatum. Therefore, while a partial separation between functional loops may exist in the striatum, there is also a complex interface between them.

Each cortical projection creates both a densely innervated striatal field (focal projection) and a wider less densely innervated area. Taken together, each striatal area receives a dense innervation from one or few cortical regions, and an equally dense innervation from a wide range of cortical areas, allowing complex integration between functional cortical areas in the striatum. These data support the idea that the striatum is an important site for further processing information between frontal regions and is and not simply a funneling station for separate functional cortical loops.

Panel Session

Drug Development: Therapeutic Role of D3 Receptor Drugs in Schizophrenia, Neuroprotection, and Drug Addiction

Cellular and Functional Actions Mediated by Dopamine D3 Receptors: Novel Insights and Focus on the Antagonist, S33138, A Potential Antipsychotic Agent

Mark J Millan*

Psychopharmacology, Servier, Croissy-Sur-Seine (Paris), France

Despite similarities to dopamine D2 receptors, D3 receptors show subtle differences in their coupling to intracellular transduction systems. Further, in contrast to D2 sites, D3 receptors are poorly represented as autoreceptors on dopaminergic neurones and they are enriched in limbic regions. Support for the contrasting significance of D3 vs D2 sites is provided by the distinctive phenotypes of mice lacking D3 vs D2 receptors. Moreover, while antiparkinson properties of D3(D2) agonists are mediated by D2 sites, activation of D3 receptors is involved in their neuroprotective properties. Interestingly, actions at D3/D2 heterodimers may also contribute to the functional profiles of D3(D2) receptor ligands. Selective D3 antagonists such as S33084 and SB277,011 do not suppress motor function indicating a low liability for extrapyramidal side-effects. Likewise in distinction to D2 antagonists, they enhance dialysis levels of acetylcholine in the frontal cortex and display procognitive effects in rats, both alone and in interaction with scopolamine. Interest in D3 receptor antagonists as antipsychotics is underpinned by clinical data showing that D3 receptor levels are altered in schizophrenics, a change normalized by antipsychotic treatment. Further, there is evidence for linkage between a D3 receptor polymorphism and the risk for schizophrenia. The above observations have prompted the generation of several classes of dopamine D3 receptor antagonist as potential antipsychotics. Of particular interest is the S33084 analogue, S33138, which revealed ca. 20-fold higher affinity for cloned, human (h)D3 vs hD2 sites. This profile suggests that at low and high doses, S33138 should act as a selective D3 antagonist and as a preferential D3 vs D2 antagonist, respectively. This was confirmed in primate studies of motor function. Employing measures of Gi/o, adenylyl cyclase and MAP-kinase activation, S33138 showed potent antagonist properties at hD3 and, at higher concentrations, hD2 sites. It was also a potent antagonist at D3/D2 heterodimers. In vivo, in neurochemical (dialysis), electrophysiological (dopaminergic neurone firing) and behavioural (drug discrimination) studies of rats, S33138 was a pure antagonist at central (pre and postsynaptic) D3 and D2 receptors. S33138 attenuated pro-psychotic actions of phencyclidine and amphetamine in behavioural paradigms. Without affecting extracellular levels of monoamines, it increased frontocortical cholinergic transmission and displayed procognitive actions in a social recognition test. In further models of cognitive-attentional function, S33138 elicited latent inhibition and blocked the disruption of pre-pulse inhibition by apomorphine and, at higher doses, phencyclidine. S33138 also enhanced social interaction in an anxiogenic environment and suppressed fear-associated ultrasonic vocalizations. At active doses, S33138 exerted only a modest influence upon motor function and

upon plasma levels of glucose and insulin. Phase I trials suggest acceptable tolerance, an appropriate duration of action and substantial entry into the CNS. In conclusion, there is evidence that dopamine D3 receptors are of functional importance in the CNS and that they fulfil a role distinct to their D2 counterparts. Dopamine D3 receptor antagonists may be of use in the management of psychotic states and other CNS disorders. However, only with the availability of robust Phase II/III efficacy data for S33138 and similar agents will the genuine therapeutic significance of dopamine D3 receptors become clear.

The Dopamine D3 Receptor and Drug Addiction: Studies with Preclinical Animal Models

Eliot Gardner*, Charles R Ashby, Z-X Xi, Arlene C Pak, Jeremy Gilbert, Amy H Newman and Christian A Heidbreder

Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD, USA

The dopamine (DA) D3 receptor is preferentially localized in mesolimbic brain areas, has the highest affinity for endogenous brain DA of any DA receptor, is significantly upregulated by cocaine use, and produces a modest overall activation of the mesolimbic DA system when pharmacologically blocked - leading some investigators to suggest that the D3 receptor may constitute a new and useful target for anti-addiction medication development. The recent development of SB277011A and NGB2904, two highly potent and highly selective D3 receptor antagonists with full D3 antagonism, high D3 affinity, high CNS penetration, and approximately 100-fold or better selectivity for the D3 receptor versus approximately 80 other CNS receptors, channels, or enzymes, now permits the exploration of highly-selective D3 receptor antagonists as potential anti-addiction compounds in preclinical animal models. We have carried out a number of such experiments, using a variety of preclinical animal models which are believed to have face- and/or predictive-validity to human drug addiction, craving, and relapse. In the electrical brain-stimulation reward (BSR) model, SB277011A and NGB2904 block both cocaine- and nicotine-enhanced BSR, without having any effects by themselves on BSR. SB277011A also blocks cannabinoid-enhanced BSR. In the conditioned place preference (CPP) paradigm, SB277011A blocks both acquisition and expression of cocaine-, heroin-, and nicotine-induced CPP, without having any effect by itself on CPP. In the intravenous self-administration (SA) paradigm, SB277011A does not alter cocaine or nicotine SA under low-response-cost (e.g., FR1) or high-payoff reinforcement schedules, but blocks cocaine or ethanol SA under high-response-cost or low-payoff reinforcement schedules. NGB2904 similarly blocks cocaine SA under low-payoff reinforcement. SB277011A dose-dependently blocks ethanol consumption in both ethanol-preferring and non-preferring genetic rat strains. In the progressive-ratio (PR) reinforcement paradigm, both SB277011A and NGB2904 dose-dependently lower the PR break-point for intravenous cocaine self-administration, reflecting a decreased desire to work for intravenous cocaine infusions. As measured by second-order reinforcement, SB277011A blocks cocaine-seeking behavior. In the reinstatement paradigm of relapse to drug-seeking behavior, SB277011A blocks drug-triggered relapse to nicotine-seeking and cocaine-seeking, blocks environmental-cue-triggered relapse to cocaine-seeking, blocks stress-triggered relapse to cocaine-seeking, and blocks relapse to ethanol-seeking. NGB2904 blocks drug-triggered and cue-triggered relapse to cocaine-seeking. In sum, high-potency high-selectivity D3 receptor antagonists show robust anti-addiction animal-model profiles, across a strikingly large number of animal models that are claimed to have face- and/or predictive-validity to human drug addiction. Thus, high-potency high-selectivity D3 receptor antagonists possess preclinical properties that appear to be predictive of anti-addiction, anti-craving, and anti-relapse potential at the human level.

Dopamine D3 Receptors May Regulate Dopaminergic Toxicity

Jeffrey M Witkin*, Beth Levant, Agustin Zapata, Rafal Kaminski, Durk Dijkstra, Hyacinth Akunne, Steve Peters, Harlan E Shannon and Maciej Gasior

Neuroscience Discovery, Eli Lilly and Company, Indianapolis, IN, USA

Cocaine and amphetamine abuse are serious public health concerns with seizures and death being one consequence of overdose. The mechanisms underlying these effects may involve dopamine D3 receptors. D3 receptor agonists can dampen dopaminergically-driven events controlling behavior (e.g., cocaine self-administration), sensitization to dopaminergic stimulants, and dopaminergic toxicity (e.g., models of Parkinson's disease). We demonstrate here that dopamine D3/D2 receptor agonists dose-dependently and completely prevent the convulsant effects of cocaine in male, Swiss-Webster mice. The D3-preferring agonists, (+)-PD 128,907, (+)-7-OH-DPAT, and the mixed D3/D2 agonists quinpirole and quinlorane were all effective against cocaine toxicity in mice. Effects of (+)-PD 128,907 were absent in mice lacking D3 receptors. The anticonvulsant effects of these compounds occurred at doses below those that produced motor impairment as assessed in the inverted screen test. The selectivity of the effects of (+)-PD 128,907 was demonstrated by its general lack of protective efficacy against a host of convulsants acting through other neural mechanisms. Direct and correlational evidence suggests that these effects were mediated by D3 receptors. Protection was stereospecific and reversible by an antagonist of D3 (PD 58491) but not D2 receptors (L-741,626). Anticonvulsant potencies were positively associated with potencies in an assay of D3 but not D2 receptor function. (+)-PD 128,907 also prevented the development and expression of cocaine-kindled seizures resulting from repeated daily dosing with 60 mg/kg cocaine. Repeated dosing with cocaine tended to increase D3 receptor number in ventral striatum, an effect enhanced by coadministration of (+)-PD 128,907. These data add to the body of literature that documents pharmacological effects that may be linked to D3 receptors *in vivo*. (+)-PD 128,907 reduces extracellular dopamine concentrations and until higher doses does so by through actions at D3 receptors. Reduction in dopamine overflow would reduce the intensity of the pharmacological stimulus for convulsions and lethality as reflected in potency shifts in the dose-effect functions in the presence of (+)-PD 128,907. (+)-PD 128,907 also facilitates the uptake of synaptic dopamine, an action that would also reduce the extracellular levels of dopamine engendered under these extreme toxic conditions. These findings have implications for disease states involving dopaminergic toxicity and sensitization.

D3 Receptor-mediated Intracellular Pathways for Neuroprotection Against the Parkinson Toxin 1-Methyl-4-Phenylpyridinium (MPP+)

Jeffrey N Joyce*, Steve Presgraves and Mark J Millan

Parkinson's Research Center, Sun Health Research Institute, Sun City, AZ, USA

Antiparkinsonian agents that are direct dopamine (DA) D3 preferring agonists, such pramipexole, have shown to be neuroprotective against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced damage to the DA system in mice and with measures of DA terminal function in Parkinson's disease (PD). The mechanisms by which direct DA agonists may provide neuroprotection are varied, however, *in vivo* and *in vitro* data support the role of DA receptors in their neuroprotective effects. Nonetheless, the signaling pathways involved in DA agonist protection have not been fully elucidated. To test if the D3 receptor preferring agonists, S32504 and pramipexole, act through D2 and/or D3 receptors and via brain derived neurotrophic factor (BDNF) dependent pathways, we utilized a terminally-differentiated neuroblastoma SH-SY5Y cell line exhibiting a dopaminergic phenotype (Presgraves et al, 2004; Exp Neurol.). This cell line, when differentiated with retinoic acid (RA) and 12-O-

tetradecanoyl-phorbol-13-acetate (TPA), exhibit high levels of tyrosine hydroxylase and dopamine transporter (DAT) but lower levels of vesicular monoamine transporter (Presgraves et al, Neurotoxicity Research, 5:579-598, 2004). The kinetics of [3H]DA uptake and [3H]MPP+ uptake to DAT in RA/TPA differentiated cells is similar to that of rat and mouse caudate-putamen synaptosomes. RA/TPA differentiated cells evidenced high sensitivity to the neurotoxic effects of MPP+ (0.03 to 3.0 mM), and the neurotoxic effects of MPP+ are blocked with the DAT inhibitor GBR12909. The cytotoxic effects of MPP+ (LD50 of 100uM) were stereospecifically antagonized by S32504 (EC50 = 2.0 uM) and, less potently, by pramipexole (EC50 = 64.3 uM), but not by their inactive stereoisomers, R(+) pramipexole and S32601, respectively. This is similar to their neuroprotective effects in vivo against MPTP, wherein S32504 is potent (Joyce et al, Exp. Neurol., 184/1 pp. 393-407, 2003). Neuroprotective effects afforded by EC50 doses of S32504 and pramipexole were antagonized by the selective D3 antagonists S33084, U99194A and SB269652 and by the D2/D3 antagonist raclopride. However, the preferential D2 receptor antagonist, LY741626 was ineffective as was the D1 antagonist, SCH23390. BDNF (1 nM) potently protected against MPP+-induced neurotoxicity. Antibody directed against BDNF concentration-dependently blocked both the neuroprotective effects of BDNF and those of pramipexole and S32504 against MPP+. The protection afforded by BDNF was blocked by the P3K-AKT pathway inhibitor, LY249002, and less so by the MEK/MAPKK pathway inhibitor, PD98059. LY249002 but not PD98059 blocked the neuroprotective effects of pramipexole and S32504 against MPP+ toxicity. In conclusion, S32504 and, less potently, pramipexole, show robust, stereospecific, and long-lasting neuroprotective effects against MPP+ toxicity that involve D3 receptors. Their actions also reflect downstream recruitment of BDNF and via a PK3-AKT pathway.

Panel Session

Cognition, Emotion, and the Prefrontal Cortex: Integrating Studies in Humans and Nonhuman Primates

The Prefrontal Cortex: Concepts, Rules, and Cognitive Control

Earl Miller*

Picower Center for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA, USA

What controls your thoughts? How do you focus attention? How do you know how to act while dining in a restaurant? This is cognitive control, the ability to organize thought and action around goals. Results from our laboratory have shown that PFC neurons have properties commensurate with a role in "executive" brain function. They are involved in directing attention, in recalling stored memories, predicting reward value, and they integrate the diverse information needed for a given goal. Perhaps most importantly, they transmit acquired knowledge. Their activity reflects learned task contingencies, concepts and rules. In short, they seem to underlie our internal representations of the "rules of the game". This may provide the foundation for the complex behavior of primates, in whom this structure is most elaborate.

Prefrontal-striatal-amygdala Dysfunction in Bipolar Disorder

Ellen Leibenluft* and Daniel Pine

Pediatrics & Developmental Neuropsychiatry Branch, National Institute of Mental Health, Bethesda, MD, USA

Research in human control subjects and animals indicates that the ability to adapt to changes in emotional stimuli is mediated by a circuit encompassing the prefrontal cortex, striatum, and amygdala.

We hypothesize that 1) the inability to modify one's behavior in response to emotional stimuli is a stable trait deficit in BPD; 2) this deficit in patients with BPD is mediated by ventral prefrontal-striatal-amygdala circuitry; and 3) the core symptoms of mania and depression occur when the underlying neural dysfunction causing this deficit is exacerbated. We present data collected in children with BPD supporting the first two hypotheses. Specifically, data from three reward-related tasks demonstrate that, compared to controls, children with BPD have decreased response flexibility. For example, we demonstrate that children with BPD have a deficit in reversal learning. In addition, fMRI data indicate that the striatum, and possibly the ventral PFC, may mediate the deficit observed on one reward-related task. Finally, we present preliminary data indicating that some abnormalities in response flexibility may differentiate children with BPD from those with anxiety disorders or with extreme irritability and ADHD.

Disambiguating the Roles of Human Amygdala and Orbitofrontal Cortex in Emotional Learning

Jay A Gottfried* and Ray Dolan

Cognitive Neurology and Alzheimer's Disease Center, Northwestern University, Chicago, IL, USA; Wellcome Dept. of Imaging Neuroscience, Functional Imaging Lab, London, United Kingdom

Background: In extinction, an animal learns that a previously conditioned stimulus (CS+) no longer predicts delivery of a salient reinforcer (unconditioned stimulus, UCS). Rodent studies indicate that extinction involves formation of memories that inhibit, without actually erasing, the original conditioning trace, and relies on amygdala-prefrontal interactions. By comparison, the neural substrates of extinction in humans are poorly understood, despite the possibility that a failure of extinction learning may provide an explanatory basis for specific psychopathologies such as phobias and post-traumatic stress disorder. **Methods:** We scanned 16 subjects in a functional magnetic resonance imaging (fMRI) paradigm of olfactory aversive conditioning, whereby neutrally valenced CS+ faces were repetitively paired with two different unpleasant UCS odors. One odor, destined for UCS inflation, was designated the target ("Tgt") UCS. The other odor underwent no incentive manipulation (non-target UCS, "nTgt"). Two additional faces, never paired with odor, served as non-conditioned controls (CS-). Immediately after conditioning, subjects underwent UCS inflation, whereby the Tgt UCS (at increased odor intensity) and nTgt UCS (at baseline intensity) were presented in the absence of the CS+. The aim of this session was to selectively enhance or "inflate" Tgt UCS aversiveness. The impact of this manipulation was assessed in extinction, whereby the Tgt CS+ and nTgt CS+ were delivered without the UCS. **Results:** Differential reaction times provided an objective index of conditioning. Subjects responded significantly faster to the CS+ (Tgt and nTgt) relative to CS- in the first half-session, though this effect later habituated. As another measure, post-hoc debriefing indicated that 13/16 subjects became aware of CS:UCS contingencies. The accompanying neural substrates of conditioning were identified in rostromedial orbitofrontal cortex (OFC) and dorsomedial amygdala. All 13 subjects reporting initial awareness of CS:UCS contingencies became aware that the CS+ items no longer predicted the UCS during the final scanning phase, providing an explicit measure of extinction learning. Extinction-related neural activity was observed in caudal OFC, ventromedial prefrontal cortex, and lateral amygdala. A direct contrast of [extinction - conditioning] indicated that neural responses in lateral amygdala and OFC were preferentially enhanced during extinction learning for both CS+ types. As a behavioral index of inflation, ratings of CS+ aversiveness were significantly higher for the Tgt (relative to nTgt) CS+. This effect was not contingent on explicit CS:UCS pairing, since after conditioning, subjects no longer experienced these items in combination. The most parsimonious explanation of UCS inflation is that at extinction, the

Tgt CS+ accessed a current, and updated, representation of UCS aversive value. The neural correlates of this behavioral effect were tested in the comparison of Tgt and nTgt CS+, revealing selective activation in lateral OFC. **Discussion:** Neural activity in human amygdala and OFC underpins conditioning, extinction, and the initial encoding of value representations. In contrast, maintenance of these representations relies preferentially on OFC. It is tempting to speculate that the amygdala is necessary for the formation of value representations in OFC, without itself being a repository of those traces. This interpretation is borne out by increasing evidence from rodent models of associative learning, which emphasize subtle but distinct roles for amygdala (acquisition) and OFC (maintenance) in the encoding of links between predictive cues and incentive values.

Role of Macaque Orbital Prefrontal Cortex in Affective Processing and Response Selection

Elisabeth A Murray*

Laboratory of Neuropsychology, NIMH, NIH, Bethesda, MD, USA

The orbital prefrontal cortex (PFO) in human and nonhuman primates operates as part of a network involved in reward-based learning and goal-directed behavior. Humans with damage to this region show poor decision making, especially in the area of social and emotional judgments. Surprisingly, there has been little systematic study of the role of macaque PFO in affective processing, broadly construed, and its influence on response selection. Accordingly, monkeys with bilateral removals of PFO were tested on a battery of tasks designed to evaluate stimulus-reward association abilities and emotional responses including: i) reinforcer devaluation, ii) object reversal learning, iii) emotional responses to a fake snake, and iv) emotional responses to an unfamiliar human. Monkeys with bilateral removals of PFO were significantly impaired on the test of reinforcer devaluation, which requires the association of objects with the particular value of a food reward, as well as on object-reversal learning, a test in which food reward value remains the same but reward contingencies are changed. The same operated monkeys learned visual discrimination problems at a normal rate, and had food preferences and satiety mechanisms that were indistinguishable from controls. The results suggest that PFO is critical for response selection based on predicted reward outcomes, regardless of whether the value of the outcome is predicted by affective signals (reinforcer devaluation) or by visual signals conveying reward contingency (object reversal learning). Relative to controls, monkeys with PFO removals exhibited attenuated responses to an emotionally-charged stimulus, a fake snake. Thus, PFO makes an essential contribution to both reward processing and emotion. The neural circuitry that interacts with PFO to support different types of affective processing will be discussed.

Panel Session

SSRI Medications and Cognitive-Behavioral Therapy in Children and Adolescents

The Pediatric OCD Treatment Study

John March*

Duke University Medical Center, Durham, NC, USA

Objective: To evaluate the efficacy of cognitive-behavior psychotherapy (CBT) and medication with a selective serotonin reuptake inhibitor (SSRI), sertraline, alone and in combination as initial treatment for children and adolescents with OCD. **Methods:** Balanced, masked randomized controlled trial conducted in three aca-

demically centers in the United States. A volunteer outpatient sample of 112 patients between the ages of 7-17 inclusive with a primary DSM-IV diagnosis of OCD and a Childrens Yale Brown Obsessive Scale (CY-BOCS) score of 16 or higher were randomly assigned to receive one of the three active treatments or pill placebo for 12 weeks. The main outcome measures were the change in CY-BOCS score over 12 weeks and the rate of excellent response (remission) defined as a CY-BOCS score < 10. **Results:** Intent-to-treat random regression analyses indicated a statistically significant advantage for combined treatment ($P = .001$), CBT ($p < .003$) and sertraline ($P = .007$) compared to placebo. Combined treatment also proved superior to CBT ($P = .008$) and to sertraline ($P = .006$), which did not differ. Site differences emerged for CBT and sertraline but not for combined treatment, suggesting that combined treatment is less susceptible to setting-specific variations in treatment. Rates of clinical remission were: COMB (53.6%; 95% CI 36-70%), CBT (39.3%; 95% CI 24-58), sertraline (21.4%; 95% CI 10-40) and placebo (3.6%; 95% CI 0 - 19). COMB did not differ from CBT ($p = .42$), but did differ from SER ($p = .026$) and from PBO ($p < .001$). CBT did not differ from SER ($p = .24$), but did differ from PBO ($p = .002$), whereas SER did not ($p = .10$). Treatments proved acceptable and tolerable. There were no serious adverse events or patients who developed increases in suicidal ideation or behaviors. **Conclusion:** Children and adolescents with obsessive-compulsive disorder should begin treatment with the combination of cognitive-behavior therapy plus a serotonin reuptake inhibitor or cognitive-behavior therapy alone.

The Treatment of Adolescents with Depression Study (TADS):

Efficacy Results

Benedetto Vitiello*

DSIR, NIMH, Bethesda, MD, USA

Objective: Both pharmacological treatment and psychotherapeutic interventions have been found to be efficacious for adolescents with major depressive disorder (MDD). The purpose of the Treatment for Adolescents with Depression Study (TADS) was to directly compare the effectiveness of different unimodal and combined treatment approaches to adolescent depression. **Methods:** A multi-site, randomized, controlled clinical trial with 4 parallel treatment conditions: medication management with fluoxetine, medication management with placebo, cognitive-behavior therapy (CBT), and a combination of CBT and fluoxetine treatment, each for 12 weeks, was conducted. Primary outcome measures were the Child Depression Rating Scale-Revised total score (CDRS-R) and the Clinical Global Impression-Improvement score (CGI-I). **Results:** A total of 439 adolescents (12-17 years of age) with DSM-IV MDD were randomized. On the CDRS-R, the combined treatment was superior to placebo ($p < 0.001$), fluoxetine alone ($p < 0.02$), and CBT alone ($P < 0.01$); fluoxetine alone was superior to CBT alone ($p < 0.01$). Responder rate was 71% for combined treatment, 61% for fluoxetine alone, 43% on CBT alone, and 35% on placebo. **Discussion:** The combination of fluoxetine and CBT showed the highest effectiveness. The results will be discussed in light of the strengths and limitations of the adopted design and methodology.

The Treatment for Adolescent With Adolescents With Depression Study (TADS): Safety Results

Graham Emslie*

Psychiatry, UT Southwestern Medical Center, Dallas, TX, USA

Objective: This presentation will report on how different treatment modalities for adolescents with major depressive disorder, including pharmacotherapy, cognitive-behavioral therapy (CBT), and their combination, compare with respect to tolerability and safety. **Methods:** TADS is a 12-week, multi-site, randomized, placebo-controlled clinical trial in outpatient adolescents with

major depressive disorder. A total of 439 adolescents were randomized, of whom about half to medication conditions (i.e., fluoxetine alone or in combination with CBT). Premature discontinuation rate was 10.5%. Emergence of possible adverse events was carefully monitored using both a broad, general elicitation method and a specific symptoms checklist. The rate of adverse events will be compared across treatment groups, with special attention to outcomes relevant to self-injurious behavior. **Results:** More adverse events were seen in the fluoxetine-treated groups than CBT or placebo. Psychiatric adverse events (e.g. agitation, anxiety, restlessness, etc.) were more common in subjects receiving fluoxetine than in those not receiving active medication. Twenty-three (5.2%) had a serious adverse event. Twenty-four (5.5%) had a suicide-related event: 8.3% (n=9) for fluoxetine alone, 5.6% (n=6) for combination treatment, 4.5% (n=5) for CBT, and 3.6% (n=4) for placebo. Based on a suicide severity measure (SIQ-Jr), the percent of subjects with a high level of suicidality (?31) decreased from 29% at baseline to 10.3% at endpoint. Incidence of actual suicide attempts was very low (n=7), with 4 in combination, 2 in fluoxetine, and 1 in CBT. **Conclusions:** Overall, the level of suicidality decreased in all treatment groups from baseline. Suicide-related events and suicide attempts were not statistically different between the four treatment groups. One limitation of studies comparing psychosocial and psychopharmacological treatments is the differing level of expectations regarding reporting of adverse events between CBT therapists and psychiatrists.

Antidepressants and Risk for Suicide-Related Behaviors in Youths

Paul Andreason

Abstract not available.

Panel Session

Drug Development: The Delayed Onset of Antipsychotics Action- Fact or Fiction

The Almost Immediate Onset of Anti“psychotic” Activity

Shitij Kapur*

CAMH - University of Toronto, Toronto, ON, Canada

Introduction: It is widely held that there is a delayed onset of antipsychotic action, and that any early effects represent non-specific behavioural sedation. Recently (Agid, 2003) it has been shown that antipsychotic action begins within the first week. We wanted to test the notion whether psychosis can improve within the first 24 hours. **Methods:** 311 patients were included in this multicentre, double-blind, placebo-controlled study of acute exacerbation of psychosis and were randomized to: IM olanzapine (10 mg), IM haloperidol (7.5 mg), or IM placebo. Subjects were rated using structured rating scales (PANSS and CGI) at baseline, 2 hours and 24 hours. **Results:** Olanzapine and haloperidol groups showed greater resolution of overall symptomatology than placebo, in the case of olanzapine this was evident at 2 hours. A factor analysis showed that an independent change in the psychosis (conceptual disorganization, hallucinatory behaviour, unusual thought content) was evident within the first 24 hours for both drugs. This improvement in core psychosis was not mediated or moderated by changes in other psychopathology, and a path analysis confirmed that this early psychosis improvement is not unidirectionally caused by changes in other factors. **Discussion:** These data reject the hypothesis of delayed onset of antipsychotic action, and instead suggest that the onset of antipsychotic action is early and its magnitude grows with time - both of these elements are critical for the clinical course of antipsychotics. These findings should provide an impetus to develop new animal models and a search for mecha-

nisms which faithfully reflect a fast onset and progressive accumulation of therapeutic effects.

The Early Onset of Antipsychotic Effect - A Hypothesis Tested, Confirmed and Extended

Stefan Leucht*

Psychiatry, University Medicine Berlin, Muenchen, Germany

Background: It had been stated in textbooks for decades that there is a delay of onset of action of antipsychotic drugs against the positive signs and symptoms of schizophrenia. This dogma was recently rejected by a meta-analysis by Agid and colleagues¹ who showed that a larger reduction of symptoms occurs during the first two weeks than during the second two weeks of treatment. This publication does not only have major scientific, but also clinical implications. However, meta-analyses are prone to a number of methodological problems. We, therefore, replicated this finding by a) using a large database of individual patient data rather than meta-analytic techniques, b) including another antipsychotic and c) extending the analysis from four weeks to one year. **Method:** We pooled the data of seven randomised controlled amisulpride trials with 1708 acutely ill patients with schizophrenia and a minimum of positive symptoms and examined the incremental percent Brief Psychiatric Rating Scale (BPRS) reduction over time with repeated measures analysis of variance. **Results:** The early onset of antipsychotic action hypothesis was confirmed, because the reduction of overall and of positive symptoms until week two was larger than the additional reduction until week four ($p < 0.0001$). Furthermore, in a long-term data subset (n=748) approximately 68% of the mean BPRS change after one year in the observed cases was already achieved after four weeks. Several sensitivity analyses corroborated the findings. **Conclusion:** It seems that the largest part of the antipsychotic drug effect occurs in the first weeks of treatment. Subsequent analyses need to establish how long an antipsychotic should be tried before it is considered ineffective and switched. **References:** 1. Agid O, Kapur S, Arenovich T, Zipursky RB. Delayed-onset hypothesis of antipsychotic action. A Hypothesis tested and rejected. Arch Gen Psychiatry. 2003;60:1228-1235.

Predicting and Interpreting Acute Response

John M Kane*

Psychiatry, The Zucker Hillside Hospital, Glen Oaks, NY, USA

Background: Clinical response of patients with schizophrenia to antipsychotic medication is heterogeneous, both in degree and time course. We were interested in examining the predictive value of early symptom changes in terms of subsequent response. We were also interested in exploring the clinical significance of typically used per cent reduction of BPRS ratings as a measure of response. **Method:** One hundred thirty one acutely ill patients with schizophrenia received four weeks of fluphenazine treatment. One hundred and twelve first-episode patients received four weeks of treatment with atypical antipsychotics. We examined the relationship between changes in psychopathology measures following 1 week of treatment and 4 weeks of treatment. We also examined a database of 1,979 patients participating in industry sponsored trials to examine the relationship between per cent changes in BPRS scores and CGI scores. **Results:** Among multi-episode, non-refractory patients (mean age 29) every patient who displayed an improvement of less than 20% in BPRS total score was classified as a non-responder at week four. The pattern among first episode patients is somewhat different. At the same time equipercentile equating of BPRS and CGI ratings in a sample of 1,979 patients suggests that minimal improvement according to a CGI score is associated with a percentage BPRS reduction of 24%, 27% and 30% at weeks 1, 2 and 4 respectively. **Discussion:** These data suggest that among multi-episode (non-treatment refractory) patients minimal improvement (<20% reduction

in total BPRS score) at 1 week is highly predictive of minimal response at 4 weeks. In addition, the 20% improvement criteria utilized in many trials may be too low to even reflect minimal improvement in terms of a global clinical impression. References: 1. Correll CU, Malhotra AK, Saurabh K, McMeniman M, Kane JM: Early prediction of antipsychotic response in schizophrenia. *Am J Psychiatry* 2003; 160:2063-2065 2. Leucht S, Kane JM, Kissling W, Hamann J, Etschel E, Engel R: What does the BPRS mean? Submitted for publication 2004

The Early Onset of Antipsychotic Action: Behavioral and Molecular Mechanisms

Richard J Beninger*

Psychology, Queen's University, Kingston, ON, Canada; Psychiatry, Queen's University, Kingston, ON, Canada

Background: It is part of the lore of psychopharmacology that dopamine receptor-blocking antipsychotic medications have a delayed onset of therapeutic action. Recent evidence shows that these medications act with minimal delay but that there is further improvement with continued treatment. This profile of action can be understood from basic research observations showing a role for dopamine in reward-related incentive learning. Dopamine may produce learning by modifying glutamatergic neurotransmission via a mechanism relying on a number of signaling pathways. **Methods:** The effects of dopamine receptor blocking drugs on incentive learning were studied in rats using operant learning tasks (e.g., lever pressing for food, conditioned avoidance), place conditioning or conditioned activity; drugs were given during task acquisition or during expression of previously learned responses. The role of signaling molecules was similarly studied but in most experiments pharmacological agents were administered centrally. **Results:** Dopamine receptor blocking drugs impaired acquisition of lever pressing for food reward, conditioned avoidance responding, place conditioning and conditioned activity. Animals that had learned these tasks showed a transient resistance to these agents but with repeated testing in the drug state, learned responding gradually diminished. Similarly, agents administered to the nucleus accumbens that blocked the signaling molecule cyclic adenosine monophosphate-dependent protein kinase (PKA) impaired acquisition but not expression of incentive learning. Other signaling molecules including protein kinase C (PKC) and the mitogen activated protein kinases (MAPKs) extracellular-signal regulated kinase (ERK) and p38 kinase have been implicated in acquisition; their effects on expression generally remain unknown. **Discussion:** Results implicate nucleus accumbens dopamine acting through several signaling cascades in the acquisition of reward-related incentive learning. Animals that have acquired the task prior to testing with drugs show a transient resistance to dopamine receptor blocking agents or nucleus accumbens injections of PKA inhibitors but, with repeated testing while in the drug state, responding gradually diminishes. These basic mechanisms of dopamine and signaling molecule function provide a basis for understanding the pattern of effects of antipsychotic medications in schizophrenia. Thus, schizophrenia can be understood as a disorder of excessive incentive learning that is a consequence of dopamine hyperfunction. Incentive learning is the acquisition by previously neutral stimuli of the ability to elicit approach and other responses. Delusions and other positive symptoms of schizophrenia may result from this excessive incentive learning. Once this learning has taken place, it is transiently resistant to the effects of dopamine receptor blocking medications but with continued treatment, the ability of previously acquired incentive learning to influence responding extinguishes and symptoms subside. According to this model, antipsychotic medications would begin to act immediately upon the initiation of treatment but their maximal effect would not be seen until some time after medication was begun, as has been reported previously.

Panel Session

New Insights into Panic Disorder: Basic and Clinical Studies

Recent Findings in the Central Regulation of Panic Response

Anantha Shekhar*

Psychiatry, Indiana University School of Medicine, Indianapolis, IN, USA

Background: The panic response is regulated by a CNS network that is exquisitely sensitive to perturbations by both internal physiological and external cues. An extensive inhibitory network maintains the response patterns in it. As long as these structures are normally regulated, such cues appear to result in appropriate physiological compensatory mechanisms and normal homeostasis is maintained. However, when there is some disruption of normal regulation (e.g., loss of inhibitory tone) at one of these sites, there could be hypersensitivity to these stimuli, resulting in a pathological activation of the panic-like reaction. These principles will be illustrated utilizing amygdala as a typical site in this network. The amygdala is thought to be critical in assigning emotional salience during behavioral responses. It is implicated in a number of human emotional disorders, particularly anxiety and depression, and appears to regulate the behavioral and autonomic responses associated with panic-like states. **Aims:** The purpose of this series of studies was to elucidate the role of the amygdala, one site that has been identified by our previous work, in regulating panic responses. **Methods:** A variety of behavioral, electrophysiological and anatomical studies were conducted. **Results:** Based on our experimental results, the following simplified circuit can be proposed for the function of the amygdala. Under basal conditions, the Basolateral/lateral (BLA) nucleus projection neurons (glutamatergic pyramidal cells) are tonically inhibited by local GABAergic interneurons. The stress peptide corticotropin releasing factor (CRF) is also excitatory, while the allostatic neuropeptide Y (NPY) is inhibitory in this network. The inhibitory tone in the BLA may also be facilitated by tonic serotonergic (5-HT) input or reduced by norepinephrine (NE) input coming from the brain stem. There are other key peptides that also seem to have specific modulatory functions. Processed sensory information is relayed to the BLA neurons via well-known excitatory inputs that activate both the glutamatergic pyramidal neurons and the local GABAergic cells. At the same time, information about the salience of that sensory input is also conveyed from the executive centers of the prefrontal circuit and this would determine whether the signal is propagated beyond the BLA to efferent targets, when it merits vigilance, or is inhibited when it is non-salient. If the information is deemed potentially dangerous, then the structures down-stream from the BLA will be activated via the efferent pathways such as the bed nucleus of the stria terminalis (BNST) for arousal/anxiety, the CE for fear and autonomic responses, hippocampus for conditioning, midbrain periaqueductal gray (PAG) and brain stem for flight responses, and thus modulate the panic-like behavior of the animal. The BLA is also an extremely plastic structure with potential for LTP following repeated excitatory stimuli. Thus under chronic stress, when afferent pathways are repeatedly activated with aversive inputs and stress peptides such as CRF, long-term plastic changes could occur in the BLA local circuit such that the tonic inhibition is reduced. Then seemingly non-salient stimuli could also elicit panic responses. **Conclusions:** These data support the notion that disruption of key amygdala pathways could result in abnormal panic responses and could be one potential site of pathology in panic disorder. (Supported by RO1MH52619 & RO1MH65702).

The Mysterious Role of Cortisol in Panic Disorder

Jack M Gorman*, Jose Martinez, Cindy Aaronson, Dorothy Reddy, Amir Garakani, Margaret Altemus, Emily Stern and David Silbersweig

Psychiatry, Mount Sinai School of Medicine, New York, NY, USA

Increased release of adrenal glucocorticoids in response to fear and stress is a nearly ubiquitous mammalian event, but it has been asserted that cortisol levels do not increase during panic attacks in patients with panic disorder. Given the overwhelming fear and anxiety experienced during a panic attack, lack of cortisol response would seem a strikingly unanticipated finding. Closer review of the literature, however, reveals that the belief that cortisol levels do not respond to panic attacks is based mainly on findings of lack of increase during laboratory-induced panic attacks following sodium lactate infusion or carbon dioxide inhalation. On the other hand, cortisol has been found increased in panic patients immediately prior to lactate- and carbon dioxide-induced attacks; in the laboratory during challenges with other substances (e.g. yohimbine, CRH, psychological stress); during spontaneously occurring attacks outside of the laboratory; and when measured over 24 hours in urine prior to treatment. Here, we report further evidence from three preliminary data sets of cortisol responsivity in panic disorder patients. First, compared to a baseline day, panic disorder patients showed higher salivary cortisol levels than control subjects immediately prior to an fMRI study. Second, we observed significant positive correlations between cortisol levels and BOLD signal increase in the hippocampus during an instructed fear conditioning task in panic disorder patients. Finally, we found that significant baseline elevations in salivary cortisol in panic patients compared to control subjects normalized with a course of cognitive behavioral therapy. Taken together, the bulk of the published data and these new preliminary data, strongly indicate that cortisol levels are increased in patients with panic disorder.

GABA Inhibitory Deficits in Panic Disorder: MRS and Clinical Studies

Andrew W Goddard*, Graeme Mason, Anantha Shekhar and John Krystal

Psychiatry, Indiana University, Indianapolis, IN, USA

Background: Accumulating evidence points to dysregulation in brain γ -aminobutyric acid (GABA) neuronal function in patients with panic disorder (PD). For example, preclinical studies have linked lowered brain GABA levels (Shekhar et al., 1996) and modulations in GABA_A receptor function (Crestani et al., 1999) with anxiety-like behaviors. Furthermore, deletions or reductions in the expression of the gene for the GABA synthetic enzyme, glutamate decarboxylase 65 (GAD65) in rodents, reduce basal cortical GABA levels (Kaneko and Obata, 2000)(Stork et al., 2000) or decrease stress-induced release of GABA in the cerebral cortex (Kash et al., 1999). GAD knockout animals exhibit increases in spontaneous fear behaviors, increased fear conditioning, and a blunted behavioral sensitivity to benzodiazepines in the absence of alterations in the density of post-synaptic GABA_A receptors. Recently, we generated clinical data that complement the above preclinical results since we observed an association between occipital cortex GABA deficits and the diagnosis of PD (Goddard et al., 2001). **Methods:** We utilized an ¹H-magnetic resonance spectroscopy (MRS) procedure (Rothman et al., 1993) to compare the occipital cortex total GABA levels (cortical GABA plus the GABA-containing dipeptide, homocarnosine) of unmedicated PD patients without clinical depression (n=14) to those of retrospectively age- and sex-matched controls (n=14). The imaging and spectroscopy work was conducted with a 2.1 Tesla Oxford Magnet Technologies 1 m bore magnet with a Bruker Avance Biospec spectrometer (Bruker Instruments), and actively shielded magnetic field gradients (Oxford Magnetic Technologies). A 13.5 cm³

(1.5x3x3 cm) volume of interest in the occipital cortex was chosen for GABA spectroscopy. We also determined the effects of acute and chronic benzodiazepine (clonazepam) exposure on occipital cortex GABA levels in a subgroup of our original sample of PD patients (n=10), and compared their results with those obtained from a prospectively recruited sample of control subjects (n=9)(studied pre and post acute benzodiazepine administration). **Results:** In the baseline assessment phase of this project we observed that total occipital cortex GABA levels were reduced by 35% in PD patients compared to age- and sex-matched controls (1.08±0.31 mmol/kg vs 1.66±0.32 mmol/kg; W=-105, p<0.0001)(Goddard et al., 2001, 2004). This effect was substantially greater than the test-retest variability of this MRS method (approximately 10%). Moreover, this finding appeared to be independent of state anxiety, depressive symptoms, years of illness, and severity of panic symptomatology. We also observed ongoing robust cortical GABA deficits (p<0.05) in a small subgroup of patients (n=8) with a positive family history of anxiety disorder (Goddard et al., 2004). Occipital cortex GABA levels remained abnormally low in PD patients following acute (post 90 mins) and chronic (post 4 weeks) clonazepam exposure. In contrast, healthy controls exhibited a 24% decrease in occipital cortex GABA concentrations from baseline following acute clonazepam administration (Goddard et al., in press). **Discussion:** Low occipital cortex GABA appears to be a trait-like marker associated with the diagnosis of PD. These results further implicate GABA neuronal dysfunction in the pathophysiology of PD. The magnitude of this abnormality may be influenced by family history of anxiety disorder. We speculate that dysfunction in the GAD₆₅ synthetic enzyme in PD patients could account for the above pattern of results.

Insights into Panic Disorder from Fear Conditioning Models

Nesha S Burghardt*, Gregory M Sullivan, Bruce S McEwen, Jack M Gorman and Joseph E LeDoux

Center for Neural Science, New York University, New York, NY, USA

Selective serotonin reuptake inhibitors (SSRIs) are efficacious in the treatment of a variety of anxiety disorders, including panic disorder. Although their pharmacological effects are immediate, their therapeutic effects are delayed for weeks. To gain insight into how these drugs alter the fear system, we evaluated the effects of acute (1 day) and chronic (22 days) treatment with the SSRI citalopram on the acquisition of fear memories in rats using auditory fear conditioning. To further understand the role of serotonin in modulating fear circuits, we compared these effects with those following acute and chronic treatment with tianeptine, a purported serotonin reuptake enhancer. Fear conditioning involved 2 presentations of a tone (20 sec, 10 kHz) that co-terminated with a footshock (0.7 mA, 0.5sec). Rats were treated systemically with drug before fear conditioning and tested drug-free 24 hours later to presentations of the tone alone. Freezing responses to the tone were taken as a measure of fear memory. We found that a single injection of citalopram (10mg/kg, i.p.) increased conditioned freezing responses, indicating an enhancement in the acquisition of freezing responses. In contrast, chronic treatment with citalopram (10mg/kg, i.p.) led to a significant decrease in freezing, revealing an impairment in fear acquisition. These effects are consistent with those found clinically, in which anxiety patients often experience an exacerbation of symptoms during early stages of treatment, and a relief from symptoms as treatment continues. In comparison, tianeptine (10mg/kg, i.p.) had no effect acutely, but also reduced acquisition of tone conditioning when administered chronically. The similarity of our results to clinical findings indicate that auditory fear conditioning can be a useful tool in understanding differences in the effects of short-term and long-term treatment with serotonergic medications. Understanding how these

drugs ultimately mediate their anxiolytic effects by effecting fear circuits may provide insight into the neural substrates that underlie panic pathophysiology.

Panel Session Cytokines and Psychopathology

Molecular Approach of the CNS Response to Interferon-Alpha

Jianping Wang* and Iain L Campbell

Pharmacology, University of Missouri-Kansas City, Kansas City, MO, USA

Chronic interferon-alpha (IFN- α) therapy causes severe neuropsychiatric side effects in humans, such as depression. In addition, while several neuromodulatory actions of IFN- α have been identified, it remains unresolved whether peripheral IFN- α acts directly on the brain to produce CNS effects. In order to investigate the mechanism for neuropsychiatric effects by systemic IFN- α treatment in human, a mouse model was adopted and a molecular approach was undertaken to directly examine expression of IFN- α signaling and IFN- α -regulated genes both *in vitro* and *in vivo*. *In vitro*, global gene profiling revealed that primary cultured neurons were highly responsive to IFN- α showing a transcription profile similar to those found previously in peripheral cells. Several highly expressed genes included ISG15 (IFN-induced 15 kDa protein) and USP18 (ubiquitin-specific proteinase 18), glucocorticoid attenuated response genes (GARGs), IFN-induced GTPases, CXCL10 and STAT1 (signal transducer and activator of transcription 1). *In vivo*, intraperitoneal injection of mouse IFN- α , but not human IFN- α markedly increased the expression of IFN- α -regulated genes in the brain, in particular STAT1, ISG15 and USP18. A time course study revealed that expression of IFN- α -regulated genes in the brain was significantly upregulated at 2 hours, peaked at 8 hours and returned to baseline after 24 hours following IFN- α administration. In conclusion, significantly enhanced expression of the genes directly involved in post-receptor IFN- α signaling in both cultured neurons and brain following IFN- α treatment indicates a direct action of this cytokine on the CNS, and provides the basis for further exploration of this anti-viral/immunoregulatory cytokine in human mental disorders.

Cytokine and Psychopathology: Lessons from Interferon-Alpha Treatment in Medically Ill Patients

Lucile Capuron*

Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA, USA

Interferon (IFN)-alpha is a potent inducer of the cytokine network and is notorious for causing behavioral alterations. Studies on IFN-alpha-treated patients reveal at least two distinct syndromes: 1) a mood/cognitive syndrome that appears late during IFN-alpha therapy, is responsive to antidepressants, and is associated with activation of neuroendocrine pathways and altered serotonin metabolism and 2) a neurovegetative syndrome characterized by psychomotor slowing, fatigue and anorexia that appears early during IFN-alpha treatment, is antidepressant non-responsive, and may be mediated by alterations in basal ganglia dopamine metabolism. Findings from IFN-alpha may provide important clues regarding the pathophysiology and treatment of cytokine-induced psychopathology in medically ill patients.

Receptor Mechanisms of IL-1-Induced Sickness Behavior

Robert Dantzer*, A. Nadjar, R.M. Bluthe, P. Parnet and J.P. Konsman

Integrative Neurology Unit, INSERM, Bordeaux, Cedex, France

The signaling pathways that mediate the behavioral effects of interleukin-1 (IL-1) during the acute phase reaction have not yet been studied. The behavioral effects of IL-1 are mediated by the interaction of this cytokine with the type I IL-1 receptor (IL-1RI) and its accessory protein (IL-1RacP) as demonstrated by the use of mice in which the gene coding for IL-1RI or for IL-1RacP has been deleted. Immunoneutralization experiments with an antibody specific of IL-1RI confirm the role of this receptor. IL-1 receptors are expressed at low abundance in the central nervous system of the rat and mouse brain. Immunohistochemistry techniques reveal a predominant expression of IL-1RI at the level of the blood-brain interface in the rat brain. Double immunolabeling techniques show that this expression is restricted to endothelial cells of venules within the brain parenchyma and circumventricular organs. Activation of IL-1 receptors is associated with nuclear factor kappa B (NF-kappaB) nuclear translocation and a robust transcriptional activation of inhibitor of kappa B alpha (IkappaB). NF-kappaB translocation is a better marker of IL-1R activation than IkappaB. Prevention of NF-kappaB activation by intracerebroventricular administration of a NEMO-binding domain peptide that selectively inhibits the IKKgamma/IKKbeta interaction abrogated sickness behavior induced by intraperitoneal but not by intracerebroventricular injection of IL-1, confirming the predominant role of IL-1 receptors at the blood-brain interface in the neural transduction of the peripheral immune message.

Nocturnal Proinflammatory Cytokine-Associated Sleep Disturbances: Human Studies

Michael R Irwin*

Cousins Center for Psychoneuroimmunology, UCLA Neuropsychiatric Institute, Los Angeles, CA, USA

Background: Basic observations demonstrate that cytokines play a key role in the regulation of sleep, with both somnogenic and inhibitory effects on sleep depending on the cytokine, plasma level, and circadian phase. Nocturnal levels of proinflammatory cytokines are hypothesized to be associated with sleep in healthy humans and with abnormalities of sleep continuity in clinical populations. **Methods:** All-night polysomnography and serial blood sampling for assay of circulating levels of proinflammatory cytokines (e.g., interleukin-6, IL-6, and tumor necrosis factor α , TNF) were conducted in healthy volunteers and in three clinical populations: patients with primary insomnia, patients with major depression, and abstinent alcohol-dependent persons. Partial night sleep deprivation (PSD) was used as a naturalistic probe to examine the effects of sleep loss and sleep recovery on the homeostatic regulation of sleep and cytokine expression. **Results:** In healthy volunteers (n=31), nocturnal increases of IL-6 occur following sleep onset ($P < 0.05$) with greater elevations of IL-6 during Stages 1-2 and rapid eye movement (REM) sleep as compared to delta sleep ($P < 0.01$). In patients with chronic insomnia (n=12) and with major depression (n=22), nocturnal levels of IL-6 are higher as compared to matched controls ($P < 0.01$), and difficulties with sleep initiation mediate the association between depression status and elevations of IL-6 ($\beta = 0.34$, $P < 0.05$). In alcoholics who show a defect in the recovery of delta sleep following sleep loss, PSD induces an exaggerated release of IL-6 and TNF (P 's < 0.05) that persists following recovery sleep (P 's < 0.01). Finally, across three separate nights, pre-sleep levels of IL-6 were found to correlate with prolonged sleep latency independent of confounding factors (e.g., alcohol consumption) and cytokine levels obtained later in the night (P 's < 0.05). **Conclusions:** Dysregulation of the bi-directional relationship between sleep and cytokines may result in a feed-forward loop in which

disrupted sleep and elevated proinflammatory cytokines create a vicious cycle exacerbating difficulties with sleep initiation and possibly contributing to other behavioral symptoms such as fatigue and depressed mood in psychiatric populations and in persons with chronic inflammatory disorders. Supported in part by grants AA10215, AA13239, DA16541, M01-RR0865, T32-MH19925, the General Clinical Research Center and the Cousins Center for Psychoneuroimmunology, UCLA Neuropsychiatric Institute.

Panel Session

PET Imaging of Transfected and Endogenous Gene Expression

Instrumentation Considerations in Small Animal PET

Michael Green*

Tident Imaging, Inc., Rockville, MD, USA

Imaging technologies originally developed for the diagnosis of disease in human subjects, e.g. CT, MRI, Ultrasound, etc. have been adapted to the study of basic biochemical, genetic and physiological phenomena in small laboratory animals. Positron emission tomography (PET) permits visualization and quantification in vivo of the transport of pharmaceuticals labeled with positron-emitting radioisotopes. This technology is non-invasive and can be repeated over time thereby allowing longitudinal studies of evolving biochemical processes in both health and disease. PET is useful experimentally in assessing the effects of therapeutic interventions, in assessing the amount and location of drugs active in the brain and other body organs, in visualizing the time-dependent transport of radiolabeled cells within the body and in imaging the amount and location of gene expression in vivo. These findings, at least in principle, can be directly translated into equivalent human imaging studies by virtue of a rapidly growing worldwide human PET infrastructure. In order for PET to be useful in studying small animals, e.g. mice and rats, PET scanners with properties substantially better than human PET imaging systems are required. The difference in size between humans and rodents places stringent requirements on spatial resolution, sensitivity and other imaging parameters that have required system designers to incorporate the most advanced scintillator/detector and electronics technologies currently available into these machines. Moreover, advanced computational methods, e.g. iterative image reconstruction methods, have been introduced that through detailed modeling of the physics of the imaging process yield images of unprecedented quality. This combination of state-of-the-art hardware and these "resolution recovery" reconstruction algorithms has produced a generation of machines with imaging characteristics that only a decade ago would have been considered impossible to achieve. In this presentation, the theory of operation of these contemporary small animal PET scanners will be described and applications of this technology presented that illustrate the state-of-the-art of this evolving methodology.

PET Imaging of Rodents to Examine Treatment with Stem Cells and Traditional Medications

Robert B Innis*

Molecular Imaging Branch, NIMH, Bethesda, MD, USA

Neuroimaging with PET (positron emission tomography) has in the past been restricted largely to human and nonhuman primates, because of its limited anatomic resolution (6-15 mm). New PET cameras have much better resolution (1-3 mm) and higher sensitivity so that imaging of rodent brain is feasible. Robert Innis will present representative uses of "molecular PET imaging" in rodents to examine models of patho-

physiology and drug mechanism. Initial studies of the rodent PET device at NIH (developed by Michael Green) assessed its accuracy for in vivo quantitation. Hiroshi Toyama and Masanori Ichise (NIMH) injected with mice both [18F]FDG and [14C]2-DG. Cerebral metabolism was varied among animals by using different anesthetics. After the in vivo [18F]FDG scan, the animals were sacrificed, [14C]2-DG autoradiography was performed in collaboration with Louis Sokoloff (NIMH) as the "gold standard." The PET images provided accurate quantitation of glucose metabolism but at a lower resolution than ex vivo autoradiography. PET imaging of the dopamine transporter (DAT) can be used as a marker of transplanted stem cells. Ron McKay (NINDS) has described the use of rodent stem cells that are genetically modified such that a large percentage develop into dopaminergic (DA) neurons. When transplanted into hemi-Parkinsonian rats, these stem cells reverse the parkinsonian symptoms. In collaboration with Dr. McKay, we have developed a PET method to image DAT as a marker of the survival and growth of the transplanted stem cells, in comparison to sham surgical animals. [11C]Rolipram binds to phosphodiesterase IV, an enzyme that metabolizes cAMP. As a marker of the cAMP signaling pathway, phosphodiesterase may play role in the mechanism of action of antidepressant medications. Masahiro Fujita (NIMH) developed a method to quantify this target protein in rat brain with serial PET imaging and concurrent arterial blood sampling. This kinetic method is now being applied to animals chronically treated with fluoxetine to examine the in vivo status of this enzyme.

Multi-Modality (PET, CT, MR, Bioluminescence, Fluorescence) Reporter Gene Imaging in Mice

Ronald G Blasberg*

Neurology & Radiology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Multi-modality imaging is increasingly being used in molecular-genetic studies in small animals. The coupling of nuclear and optical reporter genes represents the beginning of a far wider application of this technology. Optical imaging and optical reporter systems are cost-effective and time-efficient; they require less resources and space than PET or MRI, and they are particularly well suited for small animal imaging and for in vitro assays to validate different reporter systems. However, optical imaging techniques are limited by depth of light penetration and scatter, and do not yet provide optimal quantitative or tomographic information. These issues are not limiting for PET- or MRI-based reporter systems, and PET- and MRI-based animal studies are more easily generalized to human applications. Many of the shortcomings of each modality alone can be overcome by the use of dual- or triple-modality reporter constructs that incorporate the opportunity for PET, fluorescence and bioluminescence imaging. Tomographic microPET imaging can now be registered with anatomical imaging modalities, including microCT for precise skeletal and bone marrow localization and to MR images for precise soft tissue and organ localization of radioactivity in corresponding co-registered microPET images. We optimistically expect that tomographic optical imaging will be developed in the near future, and that this will provide the opportunity for the co-localization of optical signals to tomographic anatomical images provided by CT and MR.

Imaging Gene Expression: Preclinical Studies in a Primate Model of Parkinson's Disease

Jamie Eberling* and Krys S Bankiewicz

Lawrence Berkeley National Laboratory, Berkeley, CA, USA

The ability to determine the level and duration of gene expression in-vivo is critical for the clinical use of gene therapy. Positron emission tomography (PET) can be used to monitor gene expression using tracers that image the spatial distribution of the therapeutic transgene product. We have used PET to monitor gene expression in

a primate model of Parkinson's disease (PD). Our therapeutic strategy is aimed at increasing striatal aromatic L-amino acid decarboxylase (AADC) levels. Striatal neurons infected with the AADC gene by an adeno-associated viral (AAV) vector can convert low doses of systemically administered L-dopa to dopamine resulting in clinical improvement without the side effects typically associated with higher doses of L-dopa. We have shown that PET measures of striatal uptake of the AADC tracer, 6-[18F]fluoro-L-m-tyrosine (FMT), is substantially increased following the AAV delivery of AADC gene in parkinsonian monkeys, and striatal FMT uptake correlates with histological measures of the extent of gene expression. We have also performed longitudinal PET studies and have demonstrated sustained gene expression and clinical improvement for over 4 years. These findings have led to the development of a human clinical trial which will begin within the year.

Panel Session Molecular Genetics of Addiction Vulnerability and Treatment Responses

Molecular Genetics of Addiction Vulnerability and Treatment Responses

George R Uhl*, Ming D Li, Joel Gelertner, Wade Berrettini and Jonathan Pollock

Molecular Neurobiology, NIDA/Johns Hopkins, Baltimore, MD, USA

Overwhelming evidence from adoption and twin studies supports substantial genetic components for vulnerability to human addictions. This panel will present recent findings that identify genetic loci that confer susceptibility to drug addiction and nicotine dependence. The polygenic contributions to human addiction vulnerabilities that these studies provide can serve as a model for complex genetic studies. Converging results in addiction vulnerability may suggest more limited heterogeneity than that which may underlie the smaller amounts of convergence identified in studies of several other psychiatric disorders. The panel will present published and a large amount of unpublished observations from association genome scanning and linkage (TDT) studies of illegal substance dependence and nicotine dependence. Fine mapping results that implicate specific loci will be supplemented by knockout mouse data for the implicated genes. Berrettini will focus on genetics underlying individual differences in treatment responses. The NIDA director, Dr Volkow, and the head of the extramural genetics program, Dr Pollock, have both strongly encouraged this submission and will help discuss the panel. Currently unpublished data from genome scans for nicotine in two populations (Gelertner and Li). Currently unpublished data from association genome scans from 12,000 and 120,000 SNP marker sets (Uhl). Currently unpublished data from mu opiate receptor polymorphisms in nicotine dependence treatment responses (Berrettini). These sets of observations will allow the ACNP to view the explosive progress in this area, and the remarkable convergence of results from: a number of different genetic approaches (association and linkage), a number of different substances (nicotine, illegal drugs) and a number of different populations (European-American, African-American, Asian). The convergence will open the appropriate questions about the use of this data for treatment matching and prevention, with the data from Berrettini providing a striking example.

Mapping Susceptibility Loci/Genes for Nicotine Dependence Using a Combined Linkage and Association Analysis Approach

Ming D Li*, Jennie Z Ma, Joke Beuten, Thomas J Payne, Karen M Crews, Randolph T Dupont, Nancy J Williams and Robert C Elston

University of Texas Health Science Center, San Antonio, TX, USA

Background: Epidemiological studies have strongly indicated that genetics play a significant role in the determination of nicotine

dependence and other smoking-related behaviors. However, the susceptibility genes for these phenotypes remain largely unknown. **Methods:** We have been using a combined linkage and association analysis approach to identify susceptibility loci/genes for nicotine dependence. Populations investigated in our studies have included the Framingham Heart Study cohort, as well as the Caucasian and African-American populations recruited primarily from the Mid-South states of the USA. **Results:** Several genomic regions located on different chromosomes have been mapped by linkage analysis and some of them have been replicated in two independent populations. This indicates that some of these regions are likely to harbor susceptibility genes for nicotine dependence. Furthermore, several candidate genes selected from these positive genomic regions for nicotine dependence have been confirmed independently by using SNP-based association analysis. **Discussion:** Although genomic research for susceptibility genes on nicotine dependence is still in the early stages, recent findings from us and others demonstrate that such a systematic approach will eventually lead to the identification of genes associated with nicotine dependence and other smoking related behaviors. (Supported by NIH grant DA-12844).

Recent Findings from a Genomewide Linkage Scan for Cocaine Dependence

Joel Gelernter*, Carolien Panhuysen, Roger Weiss, Kathleen Brady, Victor Hesselbrock, Bruce Rounsaville, James Poling, Marsha Wilcox, Lindsay Farrer and Henry R Kranzler

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

Background: Cocaine dependence is associated with high rates of morbidity and has major economic consequences in the US. Risk for cocaine dependence is genetically influenced. **Methods:** We recruited a sample of 371 small nuclear families at four sites in the Eastern US. The sample was classified (by empirical clustering) as 44% European American (EA) and 56% African American (AA) (Hispanics allocated into either EA or AA groups). Assessment was via the Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA), which allows for detailed evaluation of substance use and dependence-related traits, and other major psychiatric diagnoses. In an effort to define subgroups with increased genetic homogeneity, we used cluster analytic methods to identify cocaine-related symptom clusters. The resultant six clusters were shown to be significantly heritable. We then completed a genomewide linkage scan for the cocaine dependence diagnosis, cocaine-induced paranoia (which occurs in some cocaine users and can be considered a pharmacogenetic trait), and the six heritable cocaine symptom clusters. **Results:** We observed four "suggestive" linkage signals for the trait of cocaine dependence, as defined in DSM-IV, in the full sample. We observed a genomewide-significant lod score of 3.65 for the trait of cocaine-induced paranoia on chromosome 9, in the AA part of the sample only. Our strongest results were observed for the cluster-derived traits; we observed a lod score of 4.66 for one of the cocaine clusters on chromosome 12 (in EAs only), a lod score of 3.35 for a second cocaine cluster on chromosome 18, and numerous additional lod scores between 2 and 3 for these derived traits. **Discussion:** We conclude that we have mapped loci increasing risk for a cocaine dependence-related trait represented by a symptom cluster to chromosome 12, and a locus increasing risk for cocaine-induced paranoia to chromosome 9. To our knowledge, this is the first linkage study using a sample ascertained for illicit substance dependence.

Pharmacogenetics of Nicotine Dependence

Wade Berrettini* and Caryn Lerman

Psychiatry, U. of Pennsylvania, Phila., PA, USA

Background: Although bupropion and nicotine replacement therapy (NRT) are effective smoking cessation treatments, there is

substantial inter-individual variability in therapeutic response and most smokers relapse to their former smoking practices. Pharmacogenetics research may improve treatment outcomes by allowing practitioners to individualize pharmacotherapy based on genotype. **Objective:** To investigate the roles of two functional genetic variants in the dopamine D2 receptor (DRD2) gene in response to pharmacotherapy for tobacco dependence. **Design:** Two randomized clinical trials, with a 6-month follow-up period: a double blind placebo-controlled trial of bupropion and an open label trial of transdermal nicotine versus nicotine nasal spray. **Setting:** Two university-based smoking cessation research programs. **Participants:** Treatment-seeking smokers of European ancestry (n=414 for bupropion trial, n=368 for NRT trial). **Intervention:** Behavioral group counseling and 10 weeks of bupropion or placebo (bupropion trial) or 8 weeks of NRT (NRT trial). **Measurements:** Demographic characteristics, smoking history, and genotype for two single nucleotide polymorphisms (SNPs) in DRD2 (-141C Ins/Del and C957T) were measured at baseline. Smoking practices were biochemically verified at the end of treatment and at 6 months after the target quit date. **Results:** At the end of the treatment phase, a statistically significant ($p = .01$) interaction between the DRD2 -141C Ins/Del genotype and treatment indicated a more favorable response to bupropion among smokers homozygous for the Ins C allele compared to those carrying a Del C allele. By contrast, smokers carrying the Del C allele had statistically significantly ($p = .006$) higher quit rates on NRT compared to those homozygous for the Ins C allele, independent of NRT type. The C957T variant was also associated ($p = .03$) with abstinence following NRT. **Limitations:** Small numbers of DRD2 -141 Del C homozygotes limit interpretation of response in this group. Study findings require confirmation in additional large studies before they are routinely applied. **Conclusions:** Bupropion may be the treatment of choice for smokers homozygous for the DRD2 -141C Ins C allele, while NRT may be more beneficial for those who carry the Del C allele.

Panel Session

Predictors of Treatment Response and Relapse: Neurobiological Markers

Neural Activation Patterns of Methamphetamine Dependent Subjects During Decision-making Predict Relapse

Martin P Paulus*, Marc A Schuckit and Susan F Tapert

Psychiatry, UCSD, La Jolla, CA, USA; Psychiatry Service, San Diego Veterans Affairs Healthcare System, San Diego, CA, USA

Background: Relapse is a common clinical problem in individuals with substance dependence. Previous studies have implicated a multifactorial process underlying relapse; however, the contribution of specific neural substrates has not yet been examined. This investigation tested the hypothesis that brain activation patterns during a simple decision-making task early in treatment can be used to predict those at highest risk for relapse among methamphetamine dependent individuals. **Methods:** Treatment seeking methamphetamine dependent males (N = 32) underwent functional magnetic resonance imaging (fMRI) about 3-4 weeks after cessation of drug use. Of the 26 subjects who were followed up to 966 days, 10 relapsed and 16 did not. **Results:** The fMRI activation pattern in the left superior temporal gyrus and right inferior parietal lobule obtained early in recovery was able to correctly predict 15/16 subjects who did not relapse and 10/10 subjects who did. A Cox regression analysis revealed that the right middle temporal gyrus and right insula activation patterns best predicted the time to relapse. **Conclusions:** This is the first investigation to show that fMRI can be used to predict a clinically important outcome in substance dependent individuals. Although the sample size of this investigation is relatively small, compared to other epi-

demiological studies, the precision with which fMRI patterns predicted relapse supports the view that neuroimaging will play a critical role to determine the course of drug addiction.

Brain Activity of Opiate Addicts Predicts Subsequent Treatment Retention

Steven D Forman*, George G Dougherty, Mary E Kelley, V A Stenger, Liubomir A Pizarov and Charlene Wick-Hull

Psychiatry, Dept of Veterans Affairs, Pittsburgh, PA, USA; Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA

BACKGROUND: To achieve any goal, humans must engage in goal-based action selection and they must monitor behavior for deviations from the task goals. The critical role for an error monitoring system in the control of behavior was first proposed by Rabbitt (1966). We recently showed that error-associated activation in medial prefrontal cortex correlates with a specific aspect of task performance, namely discriminative sensitivity. While discriminative sensitivity is frequently considered merely a measure of sensory discrimination, it actually reflects each individual's ability to separate extraneous from relevant information in the process of a decision. Addicts produce less error-associated activation than non-addicts in the medial frontal cortex and discriminate targets from nontargets more poorly. We suspected that more complex behaviors, including those relevant clinically, might also depend upon the ability to separate extraneous from relevant information (e.g., upon error-associated brain activation). One such clinically relevant behavior is the decision by an opiate addict whether or not to remain in methadone maintenance treatment (MMT). **METHODS:** To test our hypothesis that error-associated brain activation would predict measures of treatment retention, we recruited and obtained written informed consent from eighteen (10male) individuals at entry to MMT [mean(sd) years opiate use = 13.7(10.8)]. For comparison we also recruited thirty demographically similar control participants without history of substance addiction. All participants performed a response inhibition task (Go/NoGo) while undergoing blood oxygen level dependent functional MRI of the brain. We compared error-associated activation in each voxel in addicts and controls using a random effects model. Those contiguous voxels (cluster size > 100mm³) exceeding a statistical threshold ($p < 0.01$) in a contrast of error-associated activation (controls) — error-associated activation (opiate addicts) comprised candidate regions of interest. **RESULTS:** Error-associated activation in superior medial prefrontal cortex significantly predicted retention in treatment at one year (Likelihood-ratio Chi-square = 9.70, $p < 0.002$ by logistic regression) and time in MMT (L-R Chi-square = 5.82, $p = 0.016$ by proportional hazards model). None of a variety of possible alternative explanatory variables (e.g., age, gender, WAIS vocabulary score [as indicator of verbal IQ], education, comorbid cocaine dependence, years of opiate use, methadone dose) significantly predicted time in MMT. Two behavioral measures of task performance did show trends as predictors of time in MMT (e.g., Discriminative sensitivity: L-R Chi-square = 3.17, $p = 0.075$; log Response Bias: L-R Chi-square = 3.31, $p = 0.069$). **DISCUSSION:** Leaving MMT increases all-cause mortality in opiate addicts by up to 5-fold, yet half of all opiate addicts entering MMT stay in treatment less than a year. Thus, while providing recognizable long-term benefits, MMT generally requires a considerable immediate cost and lifestyle commitment from patients that often dissuade them from pursuing continued treatment. Indeed, the cognitive basis for addictive behavior may involve deficits in frontal cortical processes supporting evaluation of short-term versus long-term gains, specifically decreasing the likelihood of successful long-term treatment adherence. Neuroimaging-based markers of such cognitive deficits have potential value both in identifying populations at risk for treatment dropout and as indicators of neurobiological pathways to target for pharmacological intervention.

Imaging of Cocaine Self-Administration in Non-Human Primates Can Predict Effects in Human Cocaine Abusers

Linda Porrino*, Thomas Beveridge, Hilary Smith, Michael Nader, Anthony Liguori and Lynn Flowers

Wake Forest University School of Medicine, Winston Salem, NC, USA

Animal models of cocaine self-administration can provide important insights into the effects of chronic drug exposure, in contrast to many investigations of human cocaine addicts which are confounded by problems of different histories of drug use, polydrug abuse, and the presence of pre-existing psychiatric conditions. Can animal models, however, predict the brain circuitry associated with various aspects of abuse in humans? Rhesus monkeys were trained to self-administer either 0.03 mg/kg per injection cocaine (low dose; N=4) days or 0.3 mg/kg per injection (high dose; N=4) daily for 100 days and compared with monkeys trained to respond under an identical schedule of food reinforcement (N=6). The effects of cocaine on functional brain activity were measured with metabolic mapping methods. Chronic self-administration of high dose cocaine produced a different functional pattern of metabolic changes in the response to cocaine when compared to animals self-administering low dose cocaine for a similar duration. These differences were focused in frontal and temporal cortex. Within frontal cortex, monkeys self-administering high doses had larger decreases in glucose utilization within orbital and medial prefrontal cortex than those self-administering the lower dose. In the temporal cortex, the largest differences, also decreases, in metabolic activity were localized to parahippocampal and hippocampal areas. Rates of glucose utilization in two groups of cocaine abusers, current cocaine users (N=14) and cocaine users seeking treatment (N=13), were compared to non-using controls (N=12) with PET imaging. Rates of glucose utilization in both groups of cocaine users significantly differed from controls. However, when the differences in metabolic activity between controls and each of the two groups of cocaine users were compared, there was considerable dissimilarity in the patterns of functional activation. The differences between groups were greatest within the temporal lobe, specifically within those portions of the temporal lobe including the inferior gyrus and the parahippocampal area with those seeking treatment have lower rates of glucose utilization than current users. Taken together, it appears that portions of the temporal lobe are associated with the effects of cocaine exposure in both humans and animal models. This convergence suggests that animal models of cocaine self-administration may have considerable predictive validity. Furthermore, because there were considerable behavioral differences between the two groups of monkeys, as well as between the two groups of cocaine users, these studies may provide clues to the neurobiological markers associated with craving and relapse.

Cognitive Functioning in Cocaine Abusers: Predictor of Treatment Retention

Efrat Aharonovich*, Edward Nunes, Adam Brooks, Xinhua Liu, Adam Bisaga and Deborah Hasin

Psychiatry, Columbia University Medical Center, New York, NY, USA; New York State Psychiatric Institute, New York, NY, USA

Background: Various neuropsychological assessments and imaging techniques converge to show that cocaine abusers have cognitive impairments including deficits in attention, verbal and non-verbal memory, visuo-spatial ability and decision-making. Although cognitive functioning is of direct relevance to participation in many common psychological interventions, surprisingly little attention has been given to the effects of clinically significant cognitive deficits on substance abuse treatment. To date, for example, no studies have addressed the effects of cognitive deficits on the outcome of cognitive behavioral therapy in treating cocaine

abusers. **Method:** Fifty-six cocaine dependent patients who participated in an outpatient controlled clinical trial of cognitive behavioral therapy (CBT) plus medication or placebo consented for the study. The MicroCog computerized neuropsychological assessment battery and the Wisconsin Card Sort Test were used to assess cognitive performance at treatment entry. We hypothesized that lower cognitive functioning at treatment entry would predict premature treatment dropout and decrease overall treatment retention. The data were analyzed with logistic regression models that controlled for demographic and clinical variables. **Results:** lower attention, memory, spatial ability and general cognitive functioning scores significantly increased the odds of treatment dropout ($p < .05$). Better general cognitive functioning was positively correlated with increased weeks in treatment (Spearman correlation = 0.26, $p < 0.05$). None of the WCST scores were associated with reduced odds of treatment dropout. **Discussion:** Results suggest that lower cognitive functioning at treatment entry predicts dropout in cocaine dependent patients participating in an outpatient CBT. The MicroCog may be a sensitive and useful screening tool for detecting cognitive impairments in treatment seeking cocaine abusers. The negative findings on the WCST are consistent with other studies of substance abusers. They partly support the hypothesis that impaired decision-making abilities in cocaine abusers may not be associated with damage to the dorsolateral region of the prefrontal cortex. These findings have important implications for treatment modifications for the cognitively impaired substance abuser.

Panel Session Neuroendocrine Signaling in Adolescence: Relevance to Mental Health

Pubertal Brain Maturation: A Period of Vulnerabilities and Opportunities

Ronald E Dahl*

Psychiatry, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Adolescent development includes several component processes that create a period of special opportunities as well as vulnerabilities with respect to a wide range of behavioral and emotional problems in youth. Affective changes during pubertal maturation represent a central focus to these clinically-relevant questions. More specifically, changes in arousal and affect regulation at this point of the lifespan are a focal point for several research questions relevant to these vulnerabilities. This presentation provides an overview of normal maturational changes during adolescent development as a framework for considering these clinical research questions in greater depth. A model is then described that focuses on neurobehavioral changes at puberty that lead to an increase tendency toward risk-taking and sensation-seeking in adolescence. These developmental changes are considered in relation to a broad range of difficulties, especially youth violence and aggression. Key aspects of the model include: 1) Biologically-based increases in some drives, emotions and motivational tendencies that contribute to increased sensation-seeking and risk-taking that emerge early in adolescence; 2) The gradual and relatively prolonged maturation of self-regulatory skills and mature judgment that continues to develop through late adolescence; 3) Adolescent social contexts that create particular challenges to the slowly emerging skills in self-control. An additional factor to be considered is the recent historical change in the timing of biologic maturation. It appears that the relatively earlier activation of some biologic maturational processes (pubertal changes in brains, bodies, and social experience occurring at earlier ages) may create relative asynchronies in the development of cognitive-emotional integration, particularly in

some high-risk individuals and in some high-risk social contexts. Finally, the model is used to examine a specific set of changes in sleep/arousal regulation during adolescent development as a way to illustrate several key principles. The clinical and social policy implications of this model are discussed.

Adolescent Remodeling of the Brain and Social Behaviors

Cheryl L Sisk*

Neuroscience Program and Dept. Psychology, Michigan State University, East Lansing, MI, USA

The classical view of steroid-dependent organization of brain and behavior holds that steroids act during an early critical period of development to cause permanent changes in nervous system structure, which in turn determine neural and behavioral responses to steroids in adulthood. Revisions to the classical view over the past 40 years include the recognition that organizational effects of steroids can occur outside of the established perinatal critical period, and that multiple critical periods may exist during development. Using the Syrian hamster as a model for understanding mechanisms of behavioral maturation during adolescence, experiments in this laboratory show that remodeling of neural circuits mediating male and female social behaviors occurs during adolescence. Initial studies found that adult-typical reproductive and agonistic behaviors cannot be activated by gonadal steroids in prepubertal males, indicative of a developmentally timed process occurring during puberty that renders the nervous system responsive to activating effects of gonadal steroids. Subsequent experiments demonstrated that the presence or absence of gonadal hormones during puberty determines the degree to which steroid hormones are able to activate reproductive behavior in adult males and females. Thus, during adolescence, gonadal hormones appear to exert long-lasting changes in neural circuits and program activation responses to steroids later in adulthood. Recent experiments provide evidence for adolescent changes in dendritic branching of neurons in the medial amygdala, an area involved in the integration of sensory and steroidal stimuli important for social behaviors. Changes in connectivity and synaptic organization within behavioral circuits during adolescence are therefore likely to be among the neural mechanisms of behavioral maturation. Collectively, our studies demonstrate that interactions between gonadal hormones and the developing adolescent brain are key for the maturation of adult social behaviors, and that perturbation of these interactions has consequences for adult behavior. We propose that variations in the timing of interactions between steroid hormones and the human adolescent brain, such as those that occur with precocious or delayed puberty, contribute to individual differences in adult behavior and risk for sex-biased psychopathologies that emerge during adolescence.

Dynamic Role of Neuropeptides in the Regulation of the HPA Stress Response During Puberty

Mary Dallman

Abstract not available.

Neurochemical Changes in the Dopamine System During Puberty

Susan L Andersen* and Martin H Teicher

Psychiatry, McLean Hospital, Belmont, MA, USA

The marked changes in mood and cognition that occur during adolescence reflect more than just the pubertal surge of hormones. Our laboratory has studied male and female (during diestrus) Sprague-Dawley rats the ages of 25, 40 (the approximate onset of puberty in our hands), 60 (young adults), 80, and 100

days. Changes in neuroanatomy and brain function occur in both males and females, either separately, as in D1 and D2 receptor density, or together, as in dopamine synthesis modulation. For example, the overproduction and subsequent pruning of approximately 40% of synaptic connections and receptors is one of the most dramatic processes to occur during adolescence. Overproduction of D1 and D2 receptors is observed in the striatum of male rats, but not female rats. This D1 and D2 receptor overproduction is not dependent on the pubertal rise in gonadal hormones, nor is it dependent on glutamate, which often drives activity-dependent pruning in other systems. Remarkably, females, but not males, appear to compensate for reduced receptor numbers during puberty by increasing second messenger activity. Cyclic adenosine monophosphate activity is 40% higher in the striatum of females relative to males before it declines with further maturation. The prefrontal cortex has a unique profile of adolescent transitions that may serve to modulate its own maturation. Dopamine receptors are overproduced, but the peak occurs later (at 80 days of age) than in the striatum (40 days of age). Cyclic AMP activity does not change with age. However, transient regulation of dopamine synthesis is observed in the pre-pubertal cortex and wanes with the onset of puberty. In vivo and in vitro studies show that dopamine agonists inhibit L-DOPA accumulation in an autoreceptor-like fashion before puberty. As autoreceptor-like activity wanes, cortical dopamine assumes its adult-like pharmacology and becomes an important regulator in executive function, attention, and stress responsiveness (indicated by c-fos activation following FG-7142). Before puberty, c-fos activation is elevated in the accumbens. Taken together, these data suggest that the maturation of the adolescent dopamine system occurs in a regional dependent manner. Some regulatory processes, but not all, occur in both sexes.

Wednesday, December 15

Panel Session

Neurobiology of Obesity: Relations to Addiction

Neurobiology of Obesity: Relations to Addiction

Nora D Volkow, Joseph Frascella, Jeffrey Friedman*, Clifford B Saper, Brian Baldo, Edmund T Rolls, Julie A Mennella, Mary F Dallman, Gene-Jack Wang and Gerard LeFur

Department of Molecular Genetics, Rockefeller University, New York, NY, USA

This session will elucidate the neurobiology of obesity and describe commonalities between obesity and addiction. Given that the survival of an organism is dependent on consumption of adequate amounts of food for energy production, specialized brain systems have evolved to regulate homeostasis and hedonic control of feeding. Feeding and drug administration share behavioral and neurobiological processes; both involve motivational and appetitive systems, that when perturbed, can lead to obesity and addiction, respectively. The goal of this panel will be to summarize current research findings on the neurobiological control of feeding, to provide insights into obesity, and to highlight commonalities between obesity and addiction. The session will begin with a description of genetic influences on obesity by Dr. Friedman. Dr. Saper will present an overview of neuropeptide systems regulating feeding and describe the hedonic mechanisms controlling feeding. He will touch on how these systems important to feeding overlap with reward systems in drug abuse. Dr. Baldo will characterize the neurobiology of food reward systems and will continue discussions by Dr. Saper showing linkages between food and drug reward. Dr. Rolls will discuss cortical involvement in processing

specific aspects of food as well as cortical representation of reward. Dr. Mennella will then discuss recent studies on how food preferences may be programmed very early in infancy. Stress has been shown to be an extremely important contributor to drug abuse and addiction, and Dr. Dallman will describe the relationship between chronic stress, obesity, and glucocorticoids, drawing parallels to drug-seeking and addiction. Dr. Wang will present human PET results revealing how the brain dopamine system that is heavily implicated in drug abuse and addiction is similarly affected in obese individuals; he will also discuss the differences. Finally, Dr. LeFur will discuss some very recent work on the cannabinoid CB1 antagonist and its effects on feeding behavior. This work will be discussed in light of possible novel treatment approaches for obesity. Obesity is a major public health problem. As the number of overweight individuals continues to increase, it is imperative to understand the mechanisms that lead to obesity. Many parallels exist between obesity and addiction, and obesity, much like drug addiction, is a chronic, relapsing disorder. Like addiction, craving, bingeing, and compulsivity are characteristics of obesity, suggesting a dysregulation in motivational, appetitive and reward systems. While some aspects of feeding entail brain systems involved in energy production necessary for survival, the hedonic aspects of feeding are mediated by brain systems that have been implicated in drug abuse and addiction. The presentations will discuss current research findings on the neurobiological systems involved in the control of feeding as they relate to obesity. Commonalities between obesity and addiction also will be included, and a major emphasis in the discussion will be on the neurobiological similarities between obesity and addiction.

Dissecting the Neurobiology of Reward Liking and Wanting: Obesity and Addiction Issues

Kent C Berridge*

Department of Psychology, University of Michigan, Ann Arbor, MI, USA

Brain reward systems might contribute to binge eating and addictive drug taking behavior, if affected individuals have reward abnormalities. Alternatively, if brain reward systems function normally, then causation of the problem must lie in other factors (satiety deficits or metabolism; cognitive, psychodynamic or social factors, etc.). On the possibility that brain reward abnormalities might exist in some drug addicts or binge eaters, it is worth noting several ways in which abnormalities have been suggested to potentially cause excessive consumption of drugs or foods. Abnormal liking hypotheses posit that anhedonia, dysphoria, withdrawal, or anxiety prompts attempts to self-medicate hedonic mood by ingesting pleasurable drugs or food. Abnormal learning hypotheses posit excessive habit learning, distorted prediction errors, or excessive stamping-in of associations in brain reward systems creates overly strong consumption habits. Abnormal wanting hypotheses posit sensitization of brain reward systems that mediate incentive motivation to cause excessive attribution of incentive salience to reward stimuli. Excessive incentive salience would cause compulsive wanting to pursue and consume drugs or food (possibly exacerbated by separate weakening of cortical cognitive inhibition). All these hypotheses of dysfunction are based on competing affective neuroscience concepts of how mesocorticolimbic brain systems work in natural reward. Different concepts of how brain reward systems work lead to different explanations for how abnormalities cause excessive or compulsive consumption. Thus in order to understand obesity, binge eating or drug addiction, it will be helpful to understand how brain systems of natural reward really work. Some recent results will be presented from basic affective neuroscience studies on the neurobiology of sweetness liking and wanting functions. Insights from animal studies of reward in hyperdopaminergic mutants, and of reward functions in nucleus accumbens and ventral pallidum systems, may carry implications for understanding human appetitive dysfunctions.

Binge-type Eating in Rats: Relevance to Substance Abuse

Rebecca L Corwin* and Francis H Wojnicki

Nutritional Sciences, The Pennsylvania State University, University Park, PA, USA

Neurological mechanisms relevant to intermittent episodes of behavioral excess are not well understood. These mechanisms bear investigation due to the contribution of this kind of behavior to health problems such as obesity and substance abuse. Binge eating is characterized by the intermittent overconsumption of food in brief periods of time. Bingeing is associated with health problems directly related to overeating, as well as other co-morbidities, such as substance abuse. We have developed a behavioral model in which repeated intermittent episodes of excessive fat intake (binge-type eating) are induced in non-food-deprived rats. In this model, access to hydrogenated vegetable shortening (fat) is limited to 2-hr sessions, while access to a standard nutritionally complete rat chow is unlimited. Rats consume large amounts of the shortening when it is provided, with intakes approaching what control rats consume in 24-h. Establishing these elevated intakes takes about 4 weeks. However, once the binge intakes are established, they are easily maintained. Our studies typically last 4-8 weeks, and intakes remain reliably high. The phenomenon is not only robust, but is also quite reliable, as we have demonstrated it in different strains and ages of rats, in males and in females. The model has relevance to human bingeing, because binge foods consumed by humans typically consist of restricted high-fat items such as snacks and desserts. Two peptides proposed to be involved in the regulation of fat intake, galanin and enterostatin, have been tested under our conditions. Galanin, as well as the galanin antagonist M40, were microinjected bilaterally into the paraventricular nucleus (PVN) of the hypothalamus. Neither galanin nor M40 affected fat intake in our protocol. Likewise, enterostatin had no effect on fat intake under limited access conditions. These data indicate that bingeing, as induced in our protocol, is very different from eating induced by non-binge protocols. In contrast, recent data suggest that binge-type eating may be more closely aligned with substance abuse. For instance, rats maintained on our protocol demonstrated increased motivation to obtain access to shortening, as assessed by progressive ratio responding. In addition, GABA-B receptor activation selectively reduced fat intake, while having no effect on, or stimulating, chow intake in our model. Others have reported that GABA-B receptor activation reduces drug self-administration in animals and has shown clinical promise in the treatment of substance abuse disorders in humans. These data suggest that binge-type eating, as modeled in our protocol, is very different from non-binge eating, and may share neurological similarities with substance abuse.

Neurofunctional Imaging Studies of Obesity and Addiction

Gene-Jack Wang*, Nora D Volkow and Joanna S Fowler

Brookhaven National Laboratory, Upton, NY, USA

The incidence of obesity in the US and in the world has reached epidemic proportions and continues to rise adds urgency to understand the mechanisms underlying pathological overeating. Studies using Positron Emission Tomography (PET) implicate the involvement of brain dopamine in normal and pathological food intake in humans. In normal body weight fasting subjects, food presentation (visual, olfactory and gustatory display of food) that could not be consumed was associated with increases in striatal extracellular dopamine, which provides evidence of an involvement of dopamine in non-hedonic motivational properties of food intake. The food presentation also activated metabolic activity in orbitofrontal cortex, which might reflect downstream effects from dopamine stimulation. Dopamine involves in the drive for food consumption in human subjects, which is in part mediated by its effects in the orbitofrontal cortex. Overeating in obese individuals shares similarities with the loss of control and compulsive drug taking behavior observed in drug-addicted subjects. In morbidly obese subjects, we found reductions in striatal dopamine D2 receptors similar to that in drug-addicted sub-

jects. We postulated that decreased levels of dopamine receptors predisposed subjects to search for reinforcers; in the case of drug-addicted subjects for the drug and in the case of the obese subjects for food as a means to temporarily compensate for a decreased sensitivity of dopamine regulated reward circuit. Different from drug-addicted subjects we found increased metabolism in somatosensory cortex. In the case of obesity the reduction in receptors coupled with the enhanced sensitivity to food palatability makes them at risk for food over-consumption as their most salient reinforcer. (Supported by DOE/OBER, NIDA, and ONDCP).

Panel Session

Deep Brain Stimulation as a Putative Treatment for Refractory Major Depression - Neuroanatomy, Neurocircuitry, Neuroimaging and First Clinical Findings

Forebrain Structures Affected by Deep Brain Stimulation in the Anterior Internal Capsules and Ventral Striatum

Suzanne Haber*

Dept. of Pharmacology and Physiology, University of Rochester, Rochester, NY, USA

Forebrain structures affected by deep brain stimulation in the anterior internal capsule and ventral striatum. Neuroimaging studies support the central role of the frontal-basal ganglia-thalamic circuit in the pathophysiology of several psychiatric disorders including depression. Converging lines of evidence have pointed to abnormalities in this circuit, specifically involving the anterior cingulate cortex as well as the striatum and thalamus. Deep brain stimulation is an accepted treatment for movement disorders and recently been demonstrated to be effective in Psychiatric disorders including depression. However, the electrode targets for depression are often in the rostral white matter and little is known about which fibers actually pass through the different regions of this area. The studies presented here use tracing techniques and 3D reconstruction of pathways delineate the neural network underlying the effects of deep brain stimulation for depression. We find that Fibers that pass through the anterior internal capsule include: reciprocal medial and ventral prefrontal-thalamic fibers, cortico-striatal fibers, and reciprocal cortico-amygdala fibers. Axons leave the cortex and take up different positions in the anterior internal capsule. Descending fibers travel in both the capsule and in fiber bundles ventral to the ventral striatum to terminate in the amygdala, thalamus, hypothalamus, and brainstem. Axons from the subgenual cingulate enter the subcallosal area, travel ventral and lateral, and join medial orbital fibers. Fibers that terminate in the ventral striatum leave the bundle at the appropriate level to enter the striatum directly. Descending fibers enter the capsule while others remain in the more ventrally located fiber bundles. Fibers traveling in the capsule terminate in different regions of the thalamus, while those in the ventral white matter to terminate in the temporal lobe and amygdala. Cortical fibers travel in different parts of these fiber bundles to reach the different subcortical regions. As new fibers enter the bundle at more caudal levels, the axons that entered at rostral levels move to a more dorsal position in the bundle. Therefore, depending on the dorsoventral and rostrocaudal placement of the electrodes for DBS, different groups of fibers, and therefore structures, can be affected.

Development of DBS Targets for Treatment Resistant Depression using Functional Neuroimaging

Helen S Mayberg*, Andres Lozano, Sidney Kennedy, Valerie Voon and David Seminowicz

Psychiatry, Emory University, Atlanta, GA, USA; Rotman Research Institute, University of Toronto, Toronto, ON, Canada

Background: Advances in surgical technique and an evolving understanding of brain systems mediating normal and abnormal

mood states has led to a renewed interest in surgical approaches to treat patients with treatment resistant depression (TRD). Historically, several ablation procedures—cingulotomy, anterior capsulotomy, and subcaudate tractotomy—have provided clinical benefit for certain TRD patients. However the critical neural networks impacted by these surgeries are largely unknown. Of importance to the development of new surgical methods such as high frequency deep brain stimulation (DBS) for TRD, is the systematic characterization of neural substrates mediating chronic depressive symptoms considered in context of brain changes necessary for successful antidepressant response with other interventions. Such studies will potentially provide foundation for (1) developing DBS targets, (2) optimizing stimulation parameters and (3) guiding selection of patients for specific procedures. **Methods:** Towards these goals, baseline patterns of regional brain blood flow and metabolism measured using PET were examined in groups of depressed patients with known clinical response and non-response to different antidepressant treatments. Groups differences were first compared using Partial Least Squares (PLS). Structural Equation Modeling was then conducted to estimate the strength and direction of effective connections between 7 selected regions identified using PLS. Path Analyses were confined to a pre-defined model structure informed by known anatomical and physiological pathways and with theoretical relevance to potential new surgical targets. **Results:** Network differences among patient subgroups as well as specific targets of different treatments were identified. Pre-treatment differences involving interactions between subgenual cingulate (BA25) with rostral anterior cingulate (BA24), medial frontal (BA10), orbital frontal (BA11), lateral frontal (BA9), anterior thalamus and hippocampus distinguished depressed patients who later responded to SSRI pharmacotherapy or cognitive behavioral therapy, respectively. Differential path patterns involving these same regions again distinguished CBT responders from medication responders as well as multiple medication failures further confirming treatment-specific subgroup differences. **Discussion:** Multivariate imaging methods provide new insights into mechanisms mediating classic antidepressant treatments and lay foundation for testing specific targets using novel interventions such as DBS.

Neuroimaging the Effects of Deep Brain Stimulation: What Can We See?

Scott L Rauch*

Psychiatry, Mass. General Hospital, Charlestown, MA, USA

Neuroimaging tools offer powerful means for studying brain structure and function in vivo. Such methods have already had a major impact on neurobiological models of psychiatric diseases and have provided graphic illustrations of brain changes associated with pharmacological and cognitive-behavioral therapies. With a resurgence of interest in neurosurgical treatments for psychiatric diseases, including the emergence of deep brain stimulation (DBS), it is timely to consider how neuroimaging techniques can be applied to facilitate optimal progress in this domain. In the current presentation, examples will be provided to illustrate what can be "seen". Structural imaging studies will be reviewed indicating that volumetric changes from pre- to post-cingulotomy implicate circuitry effected by these lesions, and that assessment of the relationship between the volume or placement of capsulotomy lesions and subsequent outcome provides guidance regarding lesion optimization. PET-FDG experiments will be presented that have identified potential predictors of cingulotomy treatment response for obsessive compulsive disorder (OCD) as well as major depression (MD). This approach foreshadows the use of such tests to select among candidates for surgical treatment, or among treatment options for a given candidate. Finally, the imaging protocols being used in ongoing multisite trials of DBS will be explained. Pretreatment and posttreatment PET-FDG data will provide an opportunity to test for neuroimaging predictors of response, as well as changes in regional brain metabolic profiles associated with

beneficial and adverse effects. Finally, PET blood flow measures taken during acute stimulation vs. control conditions can demonstrate the neurocircuitry modulated by DBS. New data from such experiments will be presented showing that acute DBS at the ventral capsular target for OCD is associated with activation of orbitofrontal cortex and striatum. These findings are in accord with current neurocircuitry models of OCD and established associations between orbitofrontal-striatal modulation and therapeutic response to other antiobsessional treatments.

Deep Brain Stimulation: Clinical Findings in Intractable Depression and OCD

Ben Greenberg*, Gerhard Friehs, Linda Carpenter, Audrey Tyrka, Donald Malone, Ali Rezai, Nathan Shapira, Kelly Foote, Michael Okun, Wayne Goodman, Steven Rasmussen and Lawrence Price

Psychiatry/Clinical Neurosciences, Butler Hospital/Brown Medical School, Providence, RI, USA

Background: In contrast to lesion procedures sometimes used for intractable depression (MDD) or OCD, deep brain stimulation (DBS) is nonablative, reversible, and adjustable. More than 25,000 patients worldwide have undergone DBS for FDA-approved uses in movement disorders. We began studying the safety and efficacy of DBS of the ventral portion of the anterior limb of the internal capsule and adjacent dorsal ventral striatum ("VC/VS") in severe and highly refractory obsessive-compulsive disorder (OCD) in early 2001. This stimulation site was based on the target for the anterior capsulotomy lesion procedure, which overlaps that of subcaudate tractotomy (used in more than 1000 patients with highly refractory depression). Our collaborative OCD trial (Butler Hospital/Brown University, the Cleveland Clinic, and the University of Florida) has consistently found improvement in comorbid depressive symptoms in OCD patients undergoing DBS at this target. Improvement in affect and mood appeared to precede that in OCD itself. We therefore began FDA- and IRB-approved studies of VC/VS DBS in patients with severe and disabling depression, refractory to multiple adequate trials of medications, psychotherapy, and to bilateral ECT, which we term "intractable" MDD. We implanted the first patient in January 2003. **Method:** After multidisciplinary assessment and independent review of diagnosis, prior treatment adequacy, and consent capacity, patients had DBS leads implanted bilaterally in the VC/VS. Six MDD patients have begun stimulation, and 5 have been chronically stimulated. Patients and raters were blind to stimulation condition for the first 3 months. **Results:** As in the OCD patients, acute and chronic improvement in depressive symptoms was seen. Improvement in affect and daily functioning has tended to precede maximal improvement in depression ratings. All of the first 5 intractable MDD patients have shown improvement: 3 of 5 had > 50% improvement on the HDRS-28, one had a 23%, and the other a 17% reduction. HDRS-28 severity decreased from 31.4 +/- 3.42 (mean +/- SEM) to 15.8 +/- 2.91 overall after 3 months of DBS. Functional status on the Social and Occupational Functioning Assessment Scale (SOFAS) improved from 41.2 +/- 4.58 at baseline to 57.6 +/- 2.02 at 3 months. Most of the gain in SOFAS scores occurred during the first month of DBS. All 5 patients are undergoing continuing open stimulation. The first 3 patients have experienced stimulator battery failure, accompanied by symptom worsening. Symptoms improved when DBS resumed. **Discussion:** We view these results as encouraging. This research is demanding, requiring considerable commitment by a highly trained psychiatric neurosurgery team and very close patient followup. Persistent adverse effects have been infrequent in both OCD and depression patients. Induction of transient, reversible hypomania has been the most significant adverse effect of stimulation. Hypomania has become much less frequent with changes in stimulation technique. Additional efficacy and safety data will be presented.

Panel Session

Myelinating Glial Cells in Psychiatric Disorders

Myelin-related Gene Expression Abnormalities in 17 cortical and Subcortical Regions in Schizophrenia

Vahram Haroutunian*, Pavel Katsel, Stella Dracheva, Christina Copland, Jack M Gorman and Kenneth L Davis

Psychiatry Research, Mount Sinai School of Medicine, New York, NY, USA; Psychiatry, Bronx VA Medical Center, Bronx, NY, USA

Background: A number of different high-throughput approaches, such as microarray studies, as well as more selective gene expression studies have documented abnormalities in myelin-related gene expression in schizophrenia (SZ). These myelin-related gene expression deficits have been in close agreement with neuroanatomical, ultrastructural and protein based studies. Despite this broad agreement, knowledge of the regional specificity of these myelin-related abnormalities is lacking. It is not only important to identify brain regions of particular susceptibility, but also to understand better whether myelin-related gene expression deficits in one region are reflected in gene expression abnormalities in other regions. **Methods:** Gene expression in postmortem brain specimens from the frontal superior frontal gyrus (BA8), frontal pole BA10), insular cortex (BA44), dorsolateral prefrontal cortex (BA46), anterior cingulate (BA24/32), posterior cingulate (BA23/31), inferior parietal lobule (BA7), inferior, middle and superior temporal gyri (BA20, 21,22), parahippocampal gyrus/entorhinal cortex (BA36/28) and primary visual cortex (BA17), and the caudate, hippocampus and putamen of antemortem diagnosed and assessed elderly SZ patients (Ns=8-16) were compared to control subjects (Ns=8-16) using the Affymetrix HG-U133 microarray platform. **Results:** Overall gene expression changes between control and SZ subjects under both high (>1.7 fold change) and low (>1.4 fold change) stringency filtering conditions were greatest in the superior temporal gyrus (BA22) followed by the anterior cingulate cortex (BA23/32). The superior frontal gyrus (BA8), the parahippocampal and entorhinal regions (BA36/28), caudate, hippocampus and putamen showed intermediate numbers of differentially expressed genes. The dorsolateral prefrontal cortex (BA46), frontal cortical regions BA10 and BA44, which are frequent targets of SZ-associated studies as well as the inferior (BA20) and middle (BA21) temporal gyri and the primary visual cortex (BA17) showed the lowest number of altered transcripts. Consistent with previous findings, genes associated with oligodendrocyte function and myelination were among the significantly down-regulated genes in all of the analyzed brain regions. In the anterior cingulate, middle temporal gyrus and hippocampus, even more oligodendrocyte-related genes appeared in the differentially down-regulated set than in the frontal, parietal, occipital and subcortical regions studied. The oligodendrocyte associated gene expression deficits were confirmed by quantitative RT-PCR in selected regions (e.g., hippocampus, anterior cingulate, caudate, putamen). Factor analysis showed that myelin-associated genes clustered into two well differentiated factors, especially in the hippocampus, with within-factor correlations exceeding $r=0.6$ for pairs of genes. **Discussion:** The quantitative and enumerative non-uniform downregulation of oligodendrocyte-related genes across the brain regions studied suggests that the degree of myelin-related abnormalities varies from brain region to brain region. These regional variations in myelin-associated gene expression deficits correspond closely to brain regions implicated in the pathophysiology of schizophrenia and its associated behavioral and cognitive impairments. Regional analysis of gene expression provides a roadmap for identifying brain regions that may be at particular risk in schizophrenia and suggest that the cingulate and superior temporal cortices represent regions of special vulnerability.

Functional Genomics Studies of Schizophrenia and Bipolar Disorder

Sabine Bahn*

University of Cambridge, Cambridge, United Kingdom

Results of microarray studies on human post-mortem tissue have suggested abnormalities in lipid- and myelin-related genes in the prefrontal cortex of schizophrenia¹⁻³. Recently we found that key oligodendrocyte and myelination genes, including transcription factors known to regulate these genes, were altered not only in schizophrenia, but also in bipolar disorder⁴. Although the notion that these apparently diverse disorders may be related and may share common genetic and/or epigenetic pathways has been considered for the last 100 years, the molecular evidence supporting this assumption was still surprising. Thus, we now undertook an extensive microarray investigation coupled with proteomics (fluorescent 2-D gels with biological variation analysis) and metabolomics (high-resolution NMR) studies comparing the molecular signatures of a total of 150 post-mortem brains (50 schizophrenia, 50 bipolar and 50 control samples from the Stanley brain collection; all prefrontal cortex) to further examine the shared and disease-specific alterations at the gene, protein and metabolite level. We will present results from the different functional genomics tiers outlining both shared and distinct alterations in schizophrenia and bipolar disorder. 1. Hakak Y, Walker JR, Li C, et al. Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. *Proc Natl Acad Sci U S A* 2001; 98:4746-51. 2. Pongrac J, Middleton FA, Lewis DA, Levitt P, Mirnics K. Gene expression profiling with DNA microarrays: advancing our understanding of psychiatric disorders. *Neurochem Res* 2002; 27:1049-63. 3. Mimmack ML, Ryan M, Baba H, et al. Gene expression analysis in schizophrenia: reproducible up-regulation of several members of the apolipoprotein L family located in a high-susceptibility locus for schizophrenia on chromosome 22. *Proc Natl Acad Sci U S A* 2002; 99:4680-5. 4. Tkachev D, Mimmack ML, Ryan MM, et al. Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. *Lancet* 2003; 362:798-805. The authors gratefully acknowledge support from the Stanley Medical Research Institute.

Damage and Loss of Oligodendrocytes Are Crucial in the Pathogenesis of Schizophrenia and Mood Disorders (Findings From Postmortem Studies)

Natalya Uranova*

Laboratory of Clinical Neuropathology, Mental Health Research Center, Moscow, Russian Federation

Background: There is increasing evidence for glial cell deficits in the brain of persons with schizophrenia (SCH), bipolar disorder (BPD) and major depression (MDD). No direct evidence for reduction of astrocytes and microglia number has been found in SCH and in mood disorders. A prominent reduction of oligodendrocytes in SCH and MDD, downregulation of key myelination genes in SCH and BPD have been reported recently. A previous electron microscopic study revealed dystrophy, necrosis and apoptosis of oligodendrocytes and damage to myelin sheath lamellae in the prefrontal cortex (PFC) and caudate nucleus (CN) in SCH and BPD. Oligodendrocytes were the most severely affected among all cell types in SCH and BPD. Damage and loss of oligodendrocytes might occur in brain in SCH and mood disorders. **Methods:** Quantitative stereological and two-dimensional methods were used to estimate the numerical density of oligodendrocytes in Nissl-stained sections in layer VI of the PFC and in adjacent white matter; of the number of oligodendroglial satellites of pyramidal neurons in layer III of the PFC in SCH, BPD and MDD; and of the ultrastructure of oligodendrocytes in layer VI and of myelinated fibers in layer V of the PFC and in the CN in SCH and normal controls. We used brain samples from the Mental Health Research Center brain collection and from the Stanley Neuropathology Consortium brain collection. **Results:** A reduction of the numerical density of oligodendrocytes was detected in the PFC (area 9, layer VI) in SCH (-25%), BPD (-29%) and

MDD (-19%) as compared to controls. The number of oligodendroglial satellites of pyramidal neurons in layer III of the PFC, area 9 was lower in SCH (-29%), BPD (-40%) and MDD (-18%) than in the control group. Numerical density of oligodendrocytes was decreased in area 10, layer VI (-31%) and in adjacent white matter (-12%) in SCH compared to control cases. Electron microscopic morphometric study demonstrated a significant decrease in area of the nucleus, volume density and number of mitochondria in oligodendrocytes in the PFC and CN and ultrastructural alterations of myelinated fibers in SCH as compared to controls. The percentage of pathological myelinated fibers with atrophy of the inner axon and balloon swelling of periaxonal oligodendroglial processes was dramatically increased (10-fold in the PFC and 5.7-fold in the CN) in SCH relative to the control group. **Conclusions:** The glial cell reduction in the brain in SCH and mood disorders might be primarily due to oligodendrocytes. Dysfunction and deficit of oligodendrocytes in the brain are common and key pathology in SCH, BPD and MDD. Possible role of combination of genetic, environmental and stress factors during brain development and in acute state of severe mental illness is proposed. Support from the Stanley Medical Research Institute is appreciated.

Genetic Analysis of the Hypothesis of Altered Oligodendrocyte Function in Schizophrenia

Michael O'Donovan*

Psychological Medicine, University of Wales, College of Medicine, Cardiff, United Kingdom

Background: Oligodendrocyte abnormalities have been implicated in schizophrenia by a diverse range of experimental approaches including gene expression analysis, neuropathology, and neuroimaging. With the aim of establishing whether such abnormalities are of primary aetiological relevance to schizophrenia pathogenesis, we are currently examining genes relevant to myelination and oligodendrocyte function for association with schizophrenia. **Methods:** Candidate genes are being examined in large case control and family based association samples using a combination of direct association analysis based upon de novo mutation detection and also by indirect association analysis based upon dense maps of database markers. **Results:** Nominally significant evidence ($p < 0.05$) for association has been found for genes encoding 2',3' - Cyclic Nucleotide 3' - Phosphodiesterase, Neuregulin1, and its receptor ErbB4. Preliminary data also suggest association between the genes encoding the myelin and lymphocyte protein, and the neuregulin receptor ErbB3. **Discussion:** So far, with the exception of neuregulin, the evidence for association is modest and requires replication in independent laboratories. Nevertheless, our data provide support for the hypothesis that oligodendrocyte function is relevant to schizophrenia pathogenesis.

Panel Session

Drug Development: Developing Drugs for Cognitive Pathology in Schizophrenia

From Genes to Drugs for Cognitive Dysfunction

Tim Tully*

Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA

Behavioral and biochemical properties of simple forms of (Pavlovian) learning in *Drosophila* show properties conserved across the animal kingdom. Given this observation, we designed automated "Robotrainers" and screened 6700 randomly generated mutants for defects in long-term memory of an odor-shock association. From this "forward"-genetic strategy, we have identified 60 new memory mutants. Using a "reverse"-genetic strategy, we also have begun to

identify genes that are transcriptionally regulated during long-term memory formation - using DNA microarray technology. We have developed a novel statistical approach to analyze DNA chip data, yielding more than 3900 candidate memory genes (CMGs) genome-wide. This method has been validated by establishing significant overlap between CMGs from the DNA chip and molecular lesions associated with the memory mutants. These results suggest new biological pathways involved in memory formation, one of which has been biologically validated *in vivo*. Given the well-established molecular homology in neuronal function among invertebrates and vertebrates, such gene discovery leads to drug discovery ultimately to develop effective therapies for human cognitive dysfunction. A high-throughput cell-based screen for modulators of the CREB pathway has yielded several novel drug compounds. The most advanced of these, a phosphodiesterase inhibitor, has been shown in animal models to facilitate several forms of memory loss, including a genetic model of mental retardation.

Enhancement of Memory Storage Proteins as a Therapeutic Strategy for Cognitive Disorders

Kazumi Shiosaki*

MPM Capital, Boston, MA, USA

C105 is the single enantiomer of a racemic drug that has a well-defined safety profile in man. Long considered to be an inactive stereoisomer with no therapeutic utility, C105 was found to be more potent than the pure isomer of the marketed drug in enhancing performance of intact or lesioned rodents evaluated in various learning and memory assays. Extensive *in vitro* receptor binding and gene expression studies indicate that C105 possesses a unique pharmacological profile relative to its opposite enantiomer. Furthermore, C105 produced fewer side effects and was less toxic than the marketed isomer in preclinical safety studies. Although the specific molecular targets of C105 responsible for improving cognitive performance are not well understood at this time, data from others suggest that the ability to differentially modulate levels of dopamine and norepinephrine in relevant brain regions may contribute to its effects versus the known drug. In Phase I trials, C105 was well-tolerated in both young and elderly volunteers. Using the RAVLT (Rey Auditory Verbal Learning Test), a well-established and validated memory performance test, dose-related improvements in free recall scores were observed 30 minutes and 24 hours following administration of single oral doses. Additional indications of efficacy were observed in preliminary Phase II trials in patients suffering memory deficits related to aneurysms of the anterior communicating artery (ACoA) following both acute and chronic dosing (28 days) of C105. When examined in subjects with mild cognitive impairment (MCI), C105 significantly improved learning, memory and executive function following 28-day dosing. In addition to ACoA and MCI, C105 will be evaluated as treatment for cognitive deficits in multiple sclerosis (MS) patients. Deficits in memory, attention and processing speed are associated with nearly 50% of patients suffering from MS. Thus, C105 represents a potential agent for broadly treating the cognitive deficits that are associated with a wide-range of CNS disorders.

Positive Modulation of Excitatory Transmission as a Strategy for Treating the Cognitive Component of Schizophrenia

Gary Lynch*

Psychiatry, University of California, Irvine, CA, USA

Given the general assumption that higher mental functions arise in the cortical telencephalon, it not surprising that many investigators look to disturbances in that region as potential contributors to the cognitive pathology that accompanies schizophrenia. Particular forms of this argument posit that perturbations in communication within and between cortical regions, or between cortex and striatum, are involved. If so, then it is possible that compounds that enhance transmission at these connections will have positive therapeutic ef-

fects with regard to the cognitive aspect of the disease. Since the pertinent synapses are glutamatergic, enhancement would functionally translate into either increasing release of glutamate or in some way positively modulating the post-synaptic AMPA- and/or NMDA-type glutamate receptors. Either manipulation could have two additional, and potentially very important, benefits. First, induction of long-term potentiation (LTP), the now generally accepted substrate for everyday forms of memory, requires intense activation of glutamate receptors; increasing the current flow through the receptors would be expected to relax this requirement and thereby make it easier to form LTP and, by inference, encode memory. Recent work indicates that LTP is depressed in animal models of schizophrenia. Second, enhancing glutamatergic transmission could increase expression of those genes that are responsive to levels of excitatory (glutamatergic) drive. Included in this category is the gene that encodes Brain-Derived Neurotrophic Factor (BDNF), a neurotrophin that supports diverse neuronal types including the dopaminergic cells that innervate the striatum. This is of particular relevance to schizophrenia because of evidence that BDNF levels are depressed, at least in some areas, in patients suffering from the disease. Prompted by these arguments, we have developed a new class of drugs ('ampakines') that enhance synaptic currents gated by AMPA receptors. Using these compounds we have confirmed that such an effect improves the flow of activity through complex brain networks, reduces the amount of theta activity needed to generate LTP, and up-regulates the production of BDNF. Interestingly, subcategories of ampakine with different biophysical properties differ markedly in their efficacy with regard to the second and third of these effects. Other groups using subsequently developed, structurally distinct positive modulators of AMPA receptors have obtained similar results. Preclinical work by various groups showed that the drugs improve diverse forms of memory with minimal side effects and generate certain of the therapeutic benefits obtained with exogenous BDNF. Animal experiments also showed that the compounds act synergistically with neuroleptics in suppressing amphetamine-induced hyperactivity. Because there is no precedent for drugs of this type, clinical trials with ampakines have been limited to a drug with a short half-life and modest potency. Despite this, positive results were obtained in two small studies on memory; moreover, improvements in cognition were reported for a 30-day study in which the compound was used in combination with a neuroleptic in treating schizophrenia. A much more powerful compound with an excellent safety profile and pharmacological properties is now finishing Phase I testing. When this drug becomes available, it will be possible to carry out more compelling tests of whether enhancing excitatory transmission reduces the cognitive dysfunction aspect of schizophrenia. Beyond this lies the question of which subtype of positive modulator is most appropriate for treating the disease.

Rational Approaches to Drug Development for Cognitive Disorders

H. Christian Fibiger

Abstract not available.

Panel Session Imaging Neonatal Brain Development

Modification of Early Brain Development: Effects of Prematurity and Intrauterine Growth Restriction

Petra S Hüppi*, Cristina Tolsa, Gregory Lodygensky, Simon Warfield, Slava Zimine and Francois Lazeyras

Pediatrics, University of Geneva, Geneva, Switzerland; Neurology, Harvard Medical School, Boston, MA, USA

With the progress in reproductive medicine and neonatal intensive care, we are confronted with an increasing number of newborns

at high risk for modification of early brain development. Advanced Magnetic Resonance Imaging (MRI) techniques have recently provided us with new data on fine structural alteration of the brain in these high risk newborns. Volumetric analysis of 3D-MR imaging data sets are achieved by segmentation of the imaged volume into the different tissue types with 3-dimensional renderings. Applying these techniques to the study of the high risk newborn new insights into the modification of cortical development have been gained [1]. Preterm infants with perinatal white matter injury were found to have not only reduced myelination but also modification of cortical development at term [2]. Preterm infants exposed to postnatal corticosteroid treatment for chronic lung disease were found to have a 30% reduction in cortical gray matter development [3],[4]. A similar reduction in cortical development was found in preterm infants after intrauterine growth restriction (IUGR)[5]. The hippocampus is known for its crucial role in cognitive function such as memory and learning. It is sensitive to hypoxia, and to stress hormones. The total volume of both hippocampal formations was found to be significantly smaller in IUGR preterm infants than in the control group. These modifications of cortical development were also found in preterm infants studied at 8 years and were correlated with neuro-functional deficits. Diffusion weighted imaging further allows assessment of microstructural development of the white matter structures [6]. Factors influencing diffusion in the developing brain are related to anisotropy, which describes the preferential direction of water diffusivity. The geometric nature of the diffusion tensor can be used to display the fiber architecture of the brain white matter and its alteration due to modification of brain development. IUGR preterm infants showed microstructural alterations in parieto-occipital and frontal cortex with IUGR infants showing more immature cortical microstructure than control infants at term. To assess brain functioning early on, measurement tools of neurobehavioral functioning have been developed. The Assessment of preterm infant behavior (APIB) as a newborn behavioral assessment methodology provides an integrated subsystem profile of the infants current ability to process environmental input and assesses the level of brain functioning. Significant correlation of behavioral maturation and cortical brain development has been shown using APIB to assess brain function in newborns. Advanced MR-techniques such as 3D-MRI, diffusion tensor imaging combined with specific neurobehavioral testing allow us to study normal brain development in the newborn and assess effects of endogenous or exogenous insults with implications for longterm neuropsychological development. Reference List: [1] Huppi P, Warfield S, Kikinis R et al. *Ann Neurol* 1998; 43(2): 224-235.[2] Inder T, Huppi P, Warfield S et al. *Ann Neurol* 1999; 46: 755-760.[3] Murphy B, Inder T, Huppi P et al. *Pediatrics* 2001; 107: 217-221.[4] Modi N, Lewis H, Al Naqeeb N et al. *Pediatr. Res.* 2001; 50: 581-585.[5] Tolsa CB, Zimine S, Warfield SK, et al *Pediatr. Res.* 2004; 56: 132-138.[6] Huppi P, Murphy B, Maier S et al *Pediatrics* 2001; 107: 455-460.

MR Imaging of the Developing Brain and its Response to Injury

Robert C McKinstry*

Neuroradiology, Mallinckrodt Institute of Radiology, St. Louis, MO, USA; St. Louis Children's Hospital, Washington University, St. Louis, MO, USA

Background: Advances in the speed of acquisition and image contrast have presented an opportunity to reevaluate human brain development and maturation using quantitative MRI methods. **Methods/Results:** In a landmark multicenter study "The NIH MRI Study of Normal Brain Development," we seek to establish a quantitative brain development database from birth to age 18. For a subset of the children, serial assessments are made of brain structure, image contrast, relaxation time, MR spectra, DTI and behavioral measures from birth to age 4. The study will establish the standard for future imaging studies of newborns, infants and toddlers. In a separate in-

vestigation, we applied DTI to the evaluation of cortical development in human infants ranging from 26 to 41 weeks gestational age (GA). Diffusion in cortex was maximally anisotropic at 26 weeks GA and anisotropy values approached zero by 36 weeks GA. During this period, the major eigenvector of the diffusion tensor in cerebral cortex is oriented radially across the cortical plate. Because of its correlation with tissue microstructure and non-invasive nature, DTI offers unique insight into cortical development in preterm human newborns and, potentially, detection of derangements of its basic cytoarchitecture. In term neonates, we used DTI to study the mechanism and timing of newborn brain injury. DTI was performed on 3 occasions during the 1st week of life of 10 newborns at high risk for perinatal brain injury. DTI obtained on the 1st day after injury did not necessarily show the full extent of ultimate injury in newborn infants. Images obtained between the 2nd and 4th days of life reliably indicated the extent of injury. By the 7th day, diffusion MR was less sensitive to perinatal brain injury than conventional MRI. This study established that DTI may not be suitable as a gold standard for detection of brain injury during the first day after injury in newborn infants. We also used DTI to benchmark the maturational changes within central gray matter nuclei and central white matter pathways of the human brain. DTI was performed in 153 subjects (age range, 1 day to 11 years). Diffusion exhibited biexponential decay with age in most gray and white matter regions. There was a steep nonlinear increase of anisotropy in white matter tracts that paralleled the time course of the decline in diffusion. These results suggest that quantitative scalar parameters derived from DTI may provide clinically useful developmental milestones for brain maturity. In another study, we investigated Wallerian degeneration (WD), the secondary degeneration of axons from cortical and subcortical injury. Since DWI is sensitive to early changes of cytotoxic edema, we hypothesized that DWI may depict the acute injury to descending white matter tracts that precedes WD. We analyzed MR images in six children (aged 3 days to 5 months) with DWI findings consistent with acute territorial anterior or middle cerebral artery infarction. In all six patients, DWI performed 2-8 days after the onset of ischemia depicted injury to the descending white matter tract ipsilateral to the territorial infarct. In all five patients for which follow-up results were available, the presence of DWI changes was correlated with persistent neurologic disability. These DWI findings precede the development of WD, and they may portend a poor clinical outcome. **Discussion:** From the studies above, it is evident that MRI is useful tool for characterizing brain development and maturation, and a probe for understanding the timing, extent and mechanism of injury in the developing brain. Future studies will examine which developmental stimuli, insults, and therapies affect the normal brain maturational process.

Neonatal Brain Structure in High Risk Infants

John Gilmore*, Weili Lin, Keith Smith, Robert Hamer, Jeffrey Lieberman and Guido Gerig

Psychiatry, UNC Chapel Hill, Chapel Hill, NC, USA

Schizophrenia is currently conceptualized as a disorder of early brain development, though there is little direct evidence to support this idea. To study early brain development in children at risk for schizophrenia, we have developed methodology to obtain and analyze high resolution structural and diffusion tensor images using a 3T MR scanner in unsedated, outpatient neonates. In a pilot study of 20 neonates we found that the left ventricle is greater than the right ventricle, suggesting that the brain is lateralized even at birth. We also found that females have larger ventricles than males. In this age group we found that diffusion tensor parameters of the corpus callosum were related to gestational age at MRI. This methodology is being applied to study ventricle volume, gray and white matter volumes, and white matter development in a prospective study of two groups of high risk children and normal controls. One group is the children of

mothers with schizophrenia who are at genetic high risk for schizophrenia. The other group, fetuses with isolated mild ventriculomegaly, are considered a brain structure high risk group. To date we have enrolled 15 pregnant mothers with schizophrenia, 18 children with mild ventriculomegaly, and 95 normal controls. We have been able to get quality MR scans on approximately 70% of attempted scans and have scans on over 50 neonates. Preliminary results from this ongoing study will be presented.

Neonatal Brain Development Assessed by new Quantitative Analysis of High-field 3Tesla MRI and DTI

Guido Gerig*, Weili Lin, Marcel Prastawa, Isabelle Corouge and John Gilmore

Computer Science, UNC Chapel Hill, Chapel Hill, NC, USA; Psychiatry, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Background: Imaging of newborns and children is a challenging task in regard to scanning of subjects and computer-assisted processing of image data. High-field 3T MRI allows multiple high quality scan sequences in the short time period (less than 15 minutes) required when scanning unседated subjects for research purposes. Image analysis methodology developed for adult brain MRI fails due to the extremely low contrast, small size of anatomical objects, high level of noise, and intensity variations in non-myelinated white matter. **Methods:** We present new quantitative analysis of 3Tesla multi-contrast MRI and diffusion tensor MRI (DTI) to study brain structure and white matter development in unседated newborns. A new brain tissue segmentation technique uses a probabilistic brain atlas to separate brain from surrounding non-brain structure and to segment gray, white matter and fluid. As a novelty, we can quantify regions of non-myelinated and myelinated white matter tissue, a feature that will give us valuable information about timing and rate of early myelination. We have developed a probabilistic atlas of the neonate brain to support automatic segmentation. Lateral ventricles, cavum and intracranial cavity volumes are obtained by a reliable and efficient user-guided 3D segmentation tool. Limitations of conventional DTI analysis by manual ROI analysis is overcome by a novel method that extracts major fiber bundles by tractography and that provides a statistical analysis of diffusion properties along fiber bundles. Local diffusion properties in white matter are associated with axon density, degree of myelination and density of fluid. Population statistics of DTI requires reliable measurement of regions at corresponding anatomical locations across subjects, which is challenging due to the complexity of thin white matter bundles as presented in DTI of newborns. We have developed a new set of tools to track white matter bundles between well-defined source and target regions. These bundles serve as complex regions of interest (ROIs) to measure white matter tract properties along tracts and within cross-sections. This gives us the ability to study a tract as a whole and assess research questions in regard to brain connectivity. **Results:** The new methods were used in structural MRI to quantify regions 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. DTI tractography is applied to assess diffusion tensor properties of the commissural bundles of the corpus callosum, and to the projection bundles of the cortico-spinal tract. A comparison between neonates, 2-years old subjects and adults demonstrates the significant change of diffusion properties of brain tissue in early life. The quantitative analysis of ventricular size and ventricle lateralization reveals interesting gender differences and discrimination between controls and offsprings of schizophrenics. **Discussion:** This study on over 40 neonates indicates that 3Tesla MRI can be used to evaluate brain structure and white matter development in unседated newborns and will likely provide a vastly improved understanding of early brain development, changes due to delayed development or pathology, and its relationship to neuropsychiatric disorders.

Panel Session

Of Mice and Men: Serotonin Genes from the Cage to the Clinic

Pet-1 Dependent Genetic Program Controlling Development of Serotonin Neurons

Evan Deneris*, Michael Scott, Timothy Hendricks, Jeffery Erickson and Dmitry Fyodorov

Neurosciences, Case Western Reserve, Cleveland, OH, USA

Introduction: Serotonin neurons are generated in the ventral embryonic hindbrain in response to a sonic hedgehog-induced transcriptional program. My laboratory has combined molecular genetic and behavioral approaches to investigate this program and to determine its impact on behavior. A focus of our recent work is the Pet-1 ETS factor whose expression in the brain is restricted to developing and adult serotonin neurons. Pet-1 expression is initiated in post-mitotic serotonin neuron precursors just before serotonin appears in the ventral hindbrain. Thus, Pet-1 is likely to be a late acting component of the transcriptional cascade that generates serotonin neurons. **Results:** Our findings support this view by showing that in Pet-1 deficient mouse embryos serotonin neuron precursors were generated in normal numbers but most of them failed to synthesize serotonin. The deficiency of serotonin positive neurons and gene expression defects in the remaining ones resulted in severely reduced levels of transmitter throughout the developing and adult brain. Behavioral analyses indicated that the defective embryonic development of serotonin neurons was followed by heightened aggressive and anxiety-like behavior in adults. Further studies showed that Pet-1 deficient neonates displayed abnormal breathing patterns, which is consistent with the role of serotonin in respiratory control. Interestingly, there appeared to be no gross defects in overall morphology of the Pet-1 deficient brain outside of the raphe nuclei. However, it remains to be determined whether subtle defects are present. Because of their small numbers and scattered distribution in the midbrain and hindbrain it has been difficult to access serotonin neurons for molecular genetic studies. However, the highly restricted Pet-1 expression pattern suggested a potential solution. A crucial step was to find the Pet-1 transcriptional control region that directs its expression exclusively to serotonin neurons. We have identified such elements in a mouse BAC genomic fragment that drives strong and highly reproducible serotonin neuron-specific (SNS) reporter expression between different transgenic lines with little or no ectopic expression elsewhere in the developing and adult brain. This genomic fragment can therefore be used as a reliable genetic tool to drive transgene expression specifically in serotonin neurons. Indeed, we have used this fragment to generate two transgenic lines in which cre recombinase and yellow fluorescent protein are expressed specifically in serotonin neurons. Our cre lines are being used for conditional loss of function studies in serotonin neurons. Our YFP line is being used to facilitate electrophysiological characterization of serotonin neurons. This line is also being used to fluorescently sort serotonin neurons, which will allow us to prepare SNS microarray probes and cDNA libraries. **Discussion:** Our findings demonstrate that Pet-1 is a critical determinant of serotonin neuron identity. Moreover, they suggest the existence of a Pet-1-dependent genetic program that selectively couples early steps in serotonin neuron differentiation to serotonergic modulation of behavior in the adult and homeostatic functions in the neonate. It will be important to determine whether the Pet-1-dependent genetic program operates in humans. The identification of Pet-1 SNS transcriptional elements now provides a rich assortment of approaches with which to determine the factors that operate in the Pet-1-dependent genetic program and to determine the importance of these factors for serotonergic modulation of behavior. The tools we have developed are likely to have significant impact on molecular psychiatry research.

Serotonin Transporter Regulation: The Inside Story

Randy D Blakely*

Pharmacology, Vanderbilt University, Nashville, TN, USA

Background: The presynaptic serotonin (5-HT) transporter (SERT, 5HTT, SLC6A4) is a critical determinant of 5-HT inactivation and a major antidepressant target. Studies over the past several years have revealed multiple intracellular regulatory pathways and associated proteins that converge on SERT to regulate cell surface distribution, transport capacity and catalytic activity. **Methods:** We have pursued an examination of SERT regulation in multiple contexts-e.g. hSERT-transfected cells, natively expressing cell lines, platelets and primary brain preparations, examining activity, response to kinase/phosphatase activators, and associations with candidate modulators including PP2A and Hic-5. Additionally, we have examined hSERT variants derived from single nucleotide polymorphism (SNP) discovery efforts, investigating impact on basal activity as well as transporter regulation. **Results:** We have found evidence for pathways supporting SERT 1) exocytosis, 2) endocytosis and 3) catalytic activity. Each of these pathways appears governed in a complementary way by specific kinases and interacting proteins, orchestrating appropriate levels of transport function that can also be modulated by extracellular 5-HT through a non-receptor linked pathway. In my presentation, I will emphasize recent findings that reveal distinct PKG and p38MAPK linked pathways supporting surface expression and catalytic function of the transporter, respectively. I will describe our studies with human SERT variants derived from SNP discovery efforts where we identify multiple variants lacking appropriate regulation. **Discussion:** Our studies reveal multiple pathways impacting SERT surface expression and catalytic activity. These pathways, in turn, become novel areas to search for candidate genes governing disrupted 5-HT signaling in disease states and may represent novel targets for therapeutics.

A Dual Role for the Serotonin 1A Receptor in the Modulation of Anxiety and Depression Related Behaviors: Developmental Versus Adult Functions

Rene Hen*

Center for Neurobiology & Behavior, Columbia University, New York, NY, USA

Background: Serotonin is implicated in mood regulation, and drugs acting via the serotonergic system are effective in treating anxiety and depression. Specifically, agonists of the serotonin1A receptor have anxiolytic properties, and knockout mice lacking this receptor show increased anxiety-like behavior. **Methods:** Conditional knockout of 5-HT1A receptor, Hippocampal neurogenesis and hippocampal irradiation, Behavioral response to chronic antidepressant administration. **Results:** We have used a tissue-specific, conditional rescue strategy to show that expression of the serotonin1A receptor primarily in the hippocampus and cortex, but not in the raphe nuclei, is sufficient to rescue the behavioral phenotype of the knockout mice. Furthermore, using the conditional nature of these transgenic mice, we suggest that receptor expression during the early postnatal period, but not in the adult, is necessary for this behavioral rescue. We have also investigated whether the 5-HT1A receptor is involved in the antidepressant and anxiolytic effects of selective serotonin reuptake inhibitors (SSRI). These drugs, which are the most commonly prescribed antidepressants and anxiolytics, have a delayed onset of action (4-6 weeks), which has led to the suggestion that their effects are mediated by growth related events. Chronic antidepressant treatments have been shown to stimulate neurogenesis in the dentate gyrus of the hippocampus and to decrease certain anxiety-related behavioral responses. We have shown that the 5-HT1A knockout mice are insensitive to both the behavioral effects of chronic fluoxetine and the effects of chronic fluoxetine on hippocampal neurogenesis. In order to establish a causal relation between these two phenomena we have established a focal irradiation procedure that selectively disrupts hippocampal neurogenesis. We show that mice whose hippocampus

has been treated with X-ray, become insensitive to fluoxetine and imipramine treatment. **Discussion:** These results suggest that hippocampal neurogenesis is required to mediate the behavioral effects of antidepressants and point to a new role for the hippocampus in mood control. Our results show also that postnatal developmental processes help to establish adult anxiety-like behavior. In conclusion, the normal role of the serotonin1A receptor during development may be different from its function when this receptor is activated by therapeutic intervention in adulthood.

Gene Regulation at the C(-1019)G Serotonin1A Receptor Promoter Polymorphism and its Association with Major Depression and Suicide

Paul R Albert*, Sylvie Lemonde, Margaret Czesak and Erica Peterson

OHRI (Neuroscience, University of Ottawa, Ottawa, ON, Canada)

Background: Reduced serotonergic tone is implicated in major depression and is negatively regulated by somatodendritic raphe 5-HT1A autoreceptors. We characterized the human 5-HT1A receptor promoter to identify an alteration that could lead to dys-regulation of the 5-HT1A autoreceptor and examined its association with major depression and completed suicide. **Methods:** Blood samples from 129 unrelated DSMIV defined (minimum score 18, 17 items) unipolar depressed patients (43+/-11 years, 53 males, 95% Caucasian) and 134 HV (36+/-12 years, 55 males, 96% Caucasian) or 102 suicide completers (98 males) and 116 normal controls (116 males) matched for age, sex and ethnicity were amplified and sequenced to identify the C(-1019)G polymorphism. Depressed patients (N=118) were treated for 4 weeks with fluoxetine or nefazodone combined with pindolol or flibanserin alone. Binding of nuclear proteins to this site was identified by electrophoretic mobility shift assay. Proteins binding to the C(-1019)G site (NUDR and Hes5) were cloned by yeast one-hybrid approach. Repressor activity of these proteins was analyzed using 5-HT1A gene-luciferase reporter constructs to assay transcriptional activity, and repression of 5-HT1A receptors was confirmed by reverse transcription polymerase chain reaction (RNA), Western blot (protein) and specific [3H]-DPAT binding in rat raphe RN46A cells. NUDR was localized in rat brain using a specific antibody. **Results:** Depressed patients were at least twice as likely to have the homozygous G (-1019) genotype (P=0.0017**) and completed suicide cases were 4-fold as likely (P = 0.002) (Lemonde et al., 2003). The homozygous G(-1019) genotype was associated with decreased response to 5-HT1A agonist flibanserin (P=0.039, N=41) and in pooled antidepressant treatment groups (P=0.0497, N=118) (Lemonde et al. (2004) IJNP 7: in press). The C(-1019)G 5-HT1A promoter polymorphism is located in a palindrome sequence that binds transcriptional repressors NUDR/Deaf-1 and Hes5 at the C-allele but not the G-allele. However Hes5 is expressed mainly in embryonic tissues, whereas NUDR was colocalized with 5-HT1A receptors and 5-HT in raphe nuclei, as well as with post-synaptic 5-HT1A staining in hippocampus, cortex and septum. Two-three fold over-expression of NUDR in raphe RN46A cells reduced 5-HT1A transcription, and decreased 5-HT1A RNA, protein and binding levels. Oppositely, in septal SN48 cells NUDR enhanced 5-HT1A transcription at the C-allele. **Discussion:** The homozygous 5-HT1A G(-1019)G genotype inhibits the binding and repression of the gene by NUDR, leading to over-expression of raphe 5-HT1A autoreceptors, although post-synaptic receptors (e.g., in septum) may be oppositely reduced. This polymorphism is associated with major depression and completed suicide, consistent with observed increase in raphe 5-HT1A binding in post-mortem brains of depressed suicides (Stockmeier et al., 1998). The G-allele has been associated with panic disorder (with agoraphobia) (Rothe et al., 2004) and the depression/anxiety components of NEO neuroticism scales in normals (Strobel et al., 2003), implicating a general role in predisposition to mental illness. The association with reduced antidepressant response suggests a role for NUDR in down-regulation of the 5-HT1A

autoreceptor. Further studies are required to determine whether the G(-1019) genotype is associated with other mental illnesses, and correlates with alterations in 5-HT_{1A} binding in vivo.

Panel Session

Non-5-HT Components of the Midbrain Raphe Nuclei: Implications for Stress and Psychotherapy

Functional Organization of the Rodent Dorsal Raphe Nucleus: Efferent Topography, Neurochemical Organization, and Physiological Specificity

Barry Waterhouse*, Chuma Okere, Kimberly Simpson, Rick Lin and Hyun Lee

Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA, USA

The brainstem dorsal raphe (DR) nucleus is best known as the major source of serotonergic fibers that project broadly and diffusely throughout the forebrain and elsewhere along the neuraxis. However, an increasing number of studies in recent years have defined organizational features of this nucleus that were previously under appreciated, and have likewise revealed new information about the non-serotonergic components of the DR that emphasize the heterogeneous neurochemical composition of this monoaminergic circuit. For example, in the coronal plane the rat DR is divided into dorsomedial, ventromedial and lateral wing sub-regions that have differential projections to cortical and sub-cortical sensory targets. A large fraction of the projection neurons in these sub-divisions contain serotonin (5HT). There is also a well-defined bi-lateral cluster of 5HT-containing cells within the dorsomedial sub-division of the nucleus that project to the ventricular system. The sensory sub-regions of DR also contain substantial numbers of projection neurons that stain positively for nitric oxide (NO). NO is co-localized with 5HT in midline cells but is independent of 5HT in lateral wing cells. Afferents to these sub-regions include medial prefrontal cortex, hypothalamus, parabrachial and periaqueductal areas, laterodorsal tegmental nucleus, and preoptic area. In addition to these intrinsic anatomical and neurochemical patterns of organization, 5HT- and NO-containing neurons in sensory projecting sub-regions of DR respond differentially to experimentally-induced stress (capsaicin injection or immobilization). For example, following exposure to stressors, cFOS is expressed in a majority of midline 5HT + NO cells whereas lateral wing neurons that express cFOS after stress are neither 5HT- nor NO-positive. Glial cells in DR sub-regions also show marked morphological changes in response to capsaicin injection or immobilization. Thus, both serotonergic and non-serotonergic elements of the DR circuitry as well as its glial scaffolding exhibit dynamic, yet differential responses to stress. Taken together these observations underscore the need to consider the contribution of both 5HT and non-5HT components of the DR network in the normal operation of the nucleus and, in particular, in regulating the activity of its efferent targets through neurochemically specific DR outputs.

GABA Neurones in the Dorsal Raphe Nucleus-Where Are They, What Do They Look Like, and How Are They Regulated?

Kelly A Allers* and Trevor Sharp

Pharmacology, University of Oxford, Oxford, United Kingdom

Background: Of the non-5-HT neurones in the DRN, GABAergic neurones are of special interest because a wealth of anatomical physiological evidence suggests that they play a key role in 5-HT regulation. We have utilised newly developed juxtacellular labelling techniques chemically and morphologically characterise these neu-

rones, paving the way for future pharmacological studies. **Methods:** Adult male Sprague-Dawley rats were anaesthetized with urethane, placed in a stereotaxic apparatus and craniotomy performed. A glass microelectrode containing 1-5% neurobiotin was lowered into the DRN and advanced slowly until a neurone was encountered. Following detection of a unit, a baseline recording was obtained, then the electrode was advanced to a close proximity of the neuronal membrane and alternating current was applied to eject neurobiotin into the cell for 5-20 min. After a 2-7 hr survival time, animals were perfused and tissue processed for neurobiotin visualisation and immunohistochemistry. In another group of animals, the characteristics that were identified as belonging to GABA neurones were used to isolate these cells for the analysis of 5-HT_{1A} and 5-HT₇ receptor activation. **Results:** A total of 34 DRN neurones were juxtacellularly labelled. Of these 8 were immunoreactive for glutamic acid decarboxylase. The electrophysiological characteristics that defined this population of neurones were fast firing rates (mean 12.11 ± 1.95 , range 5.98-23.23 Hz) relative to 5-HT neurones, narrow action potential waveform widths (mean 1.08 ± 0.06 ms), and overall had regular firing patterns (coefficient of variation < 0.05) but 5/8 demonstrated low frequency oscillatory activity (1-2 Hz). Anatomically, these neurones had small soma diameters (mean $12.61 \pm 0.73 \mu\text{m}$) and the majority were located in the lateral regions of the DRN. Morphological analyses indicated that these neurones could be spiny or aspiny, extended dendrites great distances across the mediolateral plane and fine axon terminals displayed varicose swellings that were closely apposed to neurones. In a separate group of animals we used the above characteristics to identify GABA neurones in vivo, and explore the effects of 5-HT_{1A} and 5-HT₇ receptor stimulation on these neurones in urethane anaesthetised rats. The 5-HT_{1A/7} agonist 8-OH-DPAT was administered either alone or followed by selective antagonists for 5-HT_{1A} (WAY100635) or 5-HT₇ (SB269970) receptors. The agonist 8-OH-DPAT alone increased the firing rate of 6 neurones, decreased the firing rate of 13 neurones, and did not change firing rate in 6 neurones. 8-OH-DPAT combined with a 5-HT₇ antagonist decreased the firing rate in 6/6 neurones ($p < 0.01$) and also tended to increase COV (mean 0.8, $p < 0.09$). 8-OH-DPAT combined with a 5-HT_{1A} antagonist increased firing rates in 9/10 neurones ($p < 0.005$) and also decreased COV (mean = 0.5, $p < 0.05$). **Discussion:** Our data suggest that GABA neurones in the DRN have distinct electrophysiological characteristics that make them identifiable in vivo studies. The morphological features suggest they have the ability to have local influences in the DRN and could potentially receive information arising from functionally diverse DRN afferents. In addition, we have demonstrated that 5-HT_{1A} receptor activation decreases firing rates and regularity, and 5-HT₇ receptor activation increases firing rates and regularity. These data indicate that 5-HT_{1A} and 5-HT₇ receptors have opposing effects on DRN GABA neurones.

GABAergic and Glutamatergic Input to Median and Dorsal Raphe Sheryl Beck*

Pediatrics and Anesthesiology, Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA, USA

The dorsal (DR) and median raphe (MR) provide 5-HT input to forebrain areas that have been implicated in mediating stress responses and in the etiology and treatment of stress related mood disorders. Previous research has primarily focused on the 5-HT neurotransmitter system within the raphe. A point that is not frequently recognized is that 50% of the neurons in the DR and MR are non-5-HT in nature. Both glutamate and GABA neurons are found in the raphe. Glutamate and GABA are known to provide primary excitatory and inhibitory synaptic input to many brain areas. We propose that the non-5-HT containing neurons in the raphe may also be important, in concert with the 5-HT containing neurons, in understanding how the raphe regulates stress. Therefore, we examined both glutamatergic and GABAergic synaptic activity in 5-HT and non-5-

HT containing neurons of the MR and DR in a brain slice preparation. Visualized whole cell voltage-clamp techniques were used to record spontaneous postsynaptic currents (sPSCs) and TTX sensitive miniature PSCs (mPSCs). 5-HT and non-5-HT containing neurons were identified by fluorescence immunohistochemistry. The frequency of glutamatergic sEPSCs and mEPSCs were different in the 5-HT and non-5-HT containing neurons of the DR and the non-5-HT neurons in the MR; in contrast EPSC frequencies were the same and significantly lower in 5-HT containing neurons of the MR. Immunohistochemical analysis of glutamate vesicular transporters revealed a homogeneous distribution in DR, but not in MR in concert with the electrophysiological data. This differential glutamatergic input may be influential in directing the firing rate of raphe neurons and therefore be a mechanism underlying the difference in spontaneous activity between the MR and DR as well as a mechanism underlying the differential regulation of forebrain areas by these raphe nuclei. 5-HT_{1B} receptor activation selectively decreased mEPSC activity in the DR with no effect in the MR neurons. The results mean that the 5-HT_{1B} receptor, previously known primarily as an autoreceptor inhibiting the release of 5-HT in the raphe, also has significant disparate modulatory effects on glutamatergic activity. We examined the effect of swim stress on EPSC activity. The frequency of EPSCs in the DR was significantly enhanced in 5-HT containing neurons and reduced in non-5-HT neurons as compared to those recorded from handled rats. Swim stress induced changes in glutamatergic synaptic activity may underlie the behavior changes produced by this stressor, i.e., immobility. In contrast, the frequency of GABAergic IPSCs were not different between cell types or regions. Preliminary data indicate that the amplitude of the IPSCs were much greater in the MR than DR. Also, in DR neurons from WKY rats, that are hyperresponsive to stress, the decay time of the IPSC was significantly less. Changes in the amplitude and decay time indicate that the composition of GABA receptor subunits may not be the same. mRNA analysis of dissected DR tissue indicated that there is a difference in GABA α receptor subunits between control and WKY rats. Also, preliminary evidence indicates that the stress hormone CRF enhanced the frequency of mIPSCs in the DR in control rats; in WKY rats, the CRF response was substantially reduced. These results may be attributed to a down regulation of the CRF receptor in the WKY rats. These results disclose new mechanisms for the differential regulation of DR and MR raphe neuronal activity by both glutamatergic and GABAergic synaptic input. These mechanisms may underlie differences seen in DR and MR regulation of behaviors and stress responses and the etiology and treatment of mood disorders such as anxiety and depression.

Why Selectively Non-selective Drugs Are Superior to Selective Ones For Treating Mood and Anxiety Disorders

Bryan Roth* and Anushree Bhatnagar

CWRU Medical School, Cleveland, OH, USA

INTRODUCTION: Mood and anxiety disorders appear to be polygenic in origin, and the most effective medications for mood disorders have exceedingly complex actions (e.g. lithium, electroconvulsive therapy). Attempts to develop more effective treatments by developing drugs selective for single molecular targets (that is, 'magic bullets') have been largely unsuccessful. We have recently proposed that designing selectively non-selective drugs (that is, 'magic shotguns') that interact with several molecular targets will lead to more effective medications for a variety of central nervous system disorders including depression and anxiety (Roth et al Nature Rev Drug Discov, 2004). We have begun to search for potential signal transduction pathways which might yield molecular targets for drug discovery efforts for mood disorders and anxiety. We have focused in particular on pathways which are likely to yield pleiotypic responses. For decades, alterations in serum cholesterol levels have been linked by some to increased risks of suicide, de-

pression and anxiety, although the mechanism(s) responsible are unknown. **METHODS:** Standard proteomics- and genomics-based approaches were used to discover novel G-protein coupled receptor (GPCR)-interacting partners. RNAi-mediated 'knock-down' and gene-targeting approaches ('knock-out') were used to delete the interacting partner. **RESULTS:** We have recently discovered (Bhatnagar et al, J Biol Chem, 2004 and unpublished observations) that caveolin-1 (Cav-1) is a GPCR-interacting protein which exerts a profound and selectively non-selective action on G α q-coupled signaling. Cav-1 is a membrane protein involved in the creation of cholesterol-rich membrane microdomains called 'lipid rafts'. The RNAi-mediated knock-down of Cav-1 leads to a generalized down-regulation of cholesterol biosynthesis and a subsequent attenuation of signal transduction. We have obtained preliminary data that treatment with selected mood stabilizers reverses this decrement in signaling (Bhatnagar and Roth, manuscript in preparation). **CONCLUSIONS:** These results suggest that targeting signal transduction networks via selectively non-selective drugs will yield improved treatments for mood and anxiety disorders. Supported by grants from NIMH and the NIMH Psychoactive Drug Screening Program.

Panel Session

Drug Development: Novel Approaches to CNS Drug Discovery: Allosterism, Interfaces and Receptoromics

Allosteric or Orthosteric— How Best to Modulate GPCR Signaling Arthur Christopoulos*

Pharmacology, University of Melbourne, Parkville, VIC, Australia

G protein-coupled receptors (GPCRs) account for 1 - 3% of the human genome, are abundantly expressed in the CNS, and represent the major targets for approximately 50% of all medicines on the world market. Traditionally, optimizing the interaction of lead molecules with the binding site for the endogenous agonist (orthosteric site) has been viewed as the best means for obtaining selectivity of receptor action at GPCRs. This approach to drug discovery has led to the identification and classification of most receptor-based drugs as agonists, antagonists or inverse agonists. Nonetheless, current CNS-based GPCR drug discovery has a higher than average attrition rate with respect to translating fundamental research to the clinic [1]; this suggests that classic orthosteric drug-based approaches are likely sub-optimal in many cases. It is now becoming recognized that many GPCRs possess additional, allosteric binding sites that modulate receptor activity through conformational changes transmitted to the orthosteric site or directly to effector coupling sites; allosteric sites may offer advantages over orthosteric sites for some receptors, including a greater potential for receptor selectivity and a higher safety in overdose due to a ceiling level to their effect [2]. This is particularly pertinent to the CNS, where often the preferred pharmacologic approach is to tune-up or tune-down endogenous neurotransmission rather than using surrogate agonists or antagonists. Because allosteric modulators do not bind to the same site on the receptor as orthosteric agonists or antagonists, however, the detection, quantification and validation of allosteric drug effects represents a significant challenge for drug discovery. For example, we have found that allosteric modulators of muscarinic acetylcholine receptors are able to profoundly affect the kinetics of orthosteric drug binding and unbinding at these receptors. Although this phenomenon is a clear manifestation of an allosteric effect on receptor conformation, the resulting binding curves can be complex and difficult to interpret without a basic mechanistic framework that incorporates kinetic information [3]. Clearly, this is not an approach that is readily amenable to high throughput drug screening. In more recent studies, we have identified

the first allosteric modulators of cannabinoid CB₁ receptors, and found a striking dissimilitude between their effects on agonist binding, where they act as allosteric enhancers, and their effects on agonist-mediated signaling, where they act as allosteric antagonists! This difference between binding and function represents another challenge for allosteric modulator-based drug discovery, and the optimal detection of novel allosteric ligands thus requires the combination of both standard functional and modulator-optimized binding assays. Despite the difficulties associated with screening for allosteric modulators, the potential advantages of such drugs are also great, particularly in situations where the development of orthosteric ligands with sufficient selectivity and/or clinical safety is not tractable. There is now one GPCR allosteric modulator on the market, and a number in clinical trials. Provided that drug discovery programs recognize and accommodate the nuances involved in detecting allosteric effects, the search for allosteric modulators of GPCRs could become a routine feature of drug discovery in the new millennium. [1] Hopkins and Groom (2002) *Nature Rev. Drug Discover.* 1:727 [2] Christopoulos (2002) *Nature Rev. Drug Discover.* 1:198 [3] Avlani et al (2004) *J. Pharmacol. Exp. Ther.* 308:1062

Allosteric Ligands as Novel Treatments for Parkinson's Disease and Schizophrenia

Jeff Conn*

Department of Pharmacology, Vanderbilt Medical Center, Nashville, TN, USA

BACKGROUND: Metabotropic glutamate (mGlu) receptors are family 3 G protein-coupled receptors (GPCRs) that include eight subtypes termed mGlu1 - mGlu8. Anatomical, cellular, molecular, and behavioral studies suggest that agonists of the mGlu4 and mGlu5 receptors could provide a novel approach to treatment of Parkinson's disease and schizophrenia respectively. Activators of these receptors also have potential utility in treatment of cognitive dysfunction, epilepsy, and neurodegeneration. It has been difficult to develop highly selective agonists of mGlu4 and mGlu5 that have suitable properties for use as drugs. The glutamate binding site is highly conserved across mGlu receptor subtypes, making it difficult to develop highly selective ligands. Also, glutamate site agonists are analogs of glutamate and do not possess pharmacokinetic properties needed to allow them to be useful as drugs. In addition, there are a number of problems associated with the use of agonists as drugs, including adverse effects of excessive receptor activation, profound receptor desensitization, and loss of activity dependence of receptor activation. Another approach that has been successful for ion channels, such as the GABA-A receptor, has been the use of allosteric potentiators of the specific receptor subtypes. We have now performed studies aimed at discovery of allosteric potentiators for mGlu4 and mGlu5 as well as other GPCRs. **RESULTS:** Using a fluorescence-based assay of mGlu4 function, we have discovered PHCCC as a novel allosteric potentiator of mGlu4. PHCCC alone had no effect on mGlu4. However, increasing concentrations of PHCCC induced a progressive shift in the concentration-response curve of L-AP4-induced activation of mGlu4 and increased the maximal response. The EC₅₀ values for L-AP4-induced activation of mGlu4 were 484 + 45 nM in the absence of PHCCC and 71.4 + 2.9 nM with 10 μM PHCCC. The maximal effect of L-AP4 was increased greater than two-fold in the presence of 10 μM PHCCC. PHCCC is highly selective and does not potentiate responses to mGlu1b, mGlu2, mGlu5a, mGlu7 or mGlu8. Studies in midbrain slices revealed that PHCCC potentiates mGlu4-mediated inhibition of transmission at the striato-pallidal synapse, a synapse that is thought to be critical for antiparkinsonian effects of mGlu4 receptor agonists. Furthermore, we have shown that PHCCC induces behavioral effects in rodent models of Parkinson's disease that are similar to those of the group III mGlu receptor agonist L-AP4. These exciting findings suggest that allosteric potentiators of mGluRs can have behavioral effects similar to those of direct acting agonists. We have also made significant progress with discovery of multiple classes of allosteric potentiators of mGlu5.

As with the mGlu4 potentiator, these compounds are highly selective for mGlu5 and have predicted allosteric potentiator effects in native systems. For mGlu5 potentiators, we have identified multiple compounds that interact with distinct sites and have developed multiple compounds that interact with a single site with a range of activities from antagonist to potentiator and that include neutral compounds that selectively block the effects of allosteric potentiators and antagonists. **DISCUSSION:** These studies provide an exciting advance and support view that allosteric potentiators of may provide a viable novel approach to developing agents that regulate GPCRs. We and others have also performed similar studies with GPCRs belonging to family 1. Particularly exciting have been findings that allosteric potentiators induce behavioral effects similar to those of traditional agonists. Supported by NIH, NIMH, NARSAD, The Stanley Foundation, and Merck & Co.

Computational Structural Genomics and GPCRs

Ruben Abagyan*

Molecular Biology, The Scripps Research Institute, La Jolla, CA, USA

Advanced modeling by homology techniques can be employed to generate 3D models for most of the interesting new gene family members. This opens many new opportunities for structure-based functional annotation and quick identification of lead compounds via flexible docking and virtual ligand screening. Pocket identification. We have developed two new techniques to assign rational drug design using crystallographic structures or models by homology. The binding pockets can be automatically identified even if no native ligand is known. This algorithm has been tested on over 10,000 complexes. We have also built a comprehensive database of protein pockets and clustered them into families. The algorithm identifies the ligand binding pocket in Rhodopsin and Bacteriorhodopsin unambiguously. Receptor flexible docking. A deformed ligand binding pocket in a model by homology can be refined by explicit global optimization of one or several known ligands and surrounding receptor side-chains. Using the Rhodopsin structure as a GPCR model we demonstrate that even after complete randomization of the pocket side chains, the new receptor-flexible docking procedure restores the correct conformation of the pocket as well as accurately predicts the conformation of the retinal. De-orphanization. The ICM docking and scoring procedure was applied to find the native ligand from a collection of all known biological substrates. For bovine Rhodopsin, our automated computer screening of 7000 substrates from the KEGG database scored the ligand within 1.5% of the best docking energies, and for Bacteriorhodopsin within 0.2%. This virtual screening procedure in conjunction with pocket identification and modeling may be used to predict substrates of orphan GPCRs.

Screening the Receptorome: A Highly Efficient Approach for CNS Drug Discovery

Bryan Roth*

CWRU Medical School, Cleveland, OH, USA

Introduction: With the recently completed sequencing and annotation of the human genome it has become clear that a significant portion of the genome is devoted to encoding signal-transduction molecules. Indeed, one estimate is that approximately 20% of the human genome is devoted to signal transduction and that many of these signal transducing molecules represent receptors of various types. Of the many classes of receptors, G-protein coupled receptors (GPCRs) represent the largest family with estimates ranging from a low of 616 to a high of 950, of which 500 are putative odorant GPCRs (Kroeze et al, 2003). Using the upper limit of 950 and the lower estimates of ~26,000 bona-fide open reading frames in the human genome, one can place the upper boundaries of GPCRs as representing at most 3.7% of the human genome. Ion channels and transporters, which frequently function as receptors for drugs and neuro-

transmitters, represent another 3% of the genome while non-GPCR receptors represent at least 1.5% of the genome. Taken together we have estimated that the receptorome, which we have defined as that portion of the proteome encoding receptors, represents more than 8% of the human genome. **Methods:** In vitro and in silico technologies for the massively parallel screening of the receptorome will be introduced. Newly developed, freely available tools for datamining will be highlighted. **Results and Discussion:** Recent discoveries using receptoromics based approaches will be presented including: (1) Discovery that many commonly prescribed CNS medications are likely to induce valvular heart disease of the 'fen/phen'-type because they activate 5-HT_{2B} serotonin receptors. These results have also yielded the prediction that anorectic (appetite-suppressant) medications which do not target the 5-HT_{2B} serotonin receptor will be devoid of 'fen/phen'-like side-effects. (2) Discovery that the molecular target responsible for a severe neuropsychiatric complication of HIV infection is a GPCR. We have completed a comprehensive screen of available, generic medications and have found a large number which antagonize this GPCR and are predicted to be therapeutic. **Conclusion:** Taken together these results demonstrate that receptoromics-based approaches for CNS-drug discovery yield results which have immediate significance for patient care. Support: Grants from NIMH and NIDA and the NIMH Psychoactive Drug Screening Program.

Panel Session

Functional Organization of Cortical Activities During Visual Processing in Schizophrenia

NMDA Receptors and Visual Processing Deficits in Schizophrenia

Daniel Javitt*, Pamela Butler, Isaac Schecter and John J Foxe

CNS Division, Nathan Kline Institute, Orangeburg, NY, USA; Psychiatry, NYU School of Medicine, New York, NY, USA

Although deficits in visual processing have been documented for over 20 years, the pattern of deficit is only now becoming sufficiently clear to permit underlying mechanisms to be evaluated. Deficits in visual backward masking in schizophrenia were originally interpreted as showing impaired interplay between transient and sustained visual "channels." Subsequently, these psychophysically defined channels were shown to correspond to the operation of discrete but interrelated neuroanatomic pathways - the magnocellular and parvocellular visual pathways - with the transient channel corresponding roughly to the magnocellular pathway and the sustained channel corresponding roughly to the parvocellular pathway. The magnocellular system is optimized for the detection of large, low contrast and low luminance stimuli, and shows large, nonlinear increases in response to near-threshold stimuli. In contrast, the parvocellular system is optimized for characterization of stimulus detail, and shows linearly increasing response with increasing stimulus contrast. The magnocellular system projects preferentially to the "where" system (dorsal stream) and participates in motion detection and perceptual organization, whereas the parvocellular system projects preferentially to the "what" system and participates in object recognition. Interaction of both streams is required for complex object identification. NMDA receptors are present in both the dorsal and ventral stream, but mediate primarily motion detection and high-gain, non-linear response amplification. The present studies evaluated functioning of the magnocellular/parvocellular systems and dorsal/ventral stream pathways using a combination of behavioral, neurophysiological and neuroimaging-based approaches. Overall, patients showed deficits at all stages of magnocellular/dorsal stream processing, whereas processing within the parvocellular system and within the early ventral stream was intact. Thus, patients showed particular deficits in detection of low spatial frequency stimuli, but showed intact recognition of high

spatial frequencies when stimuli were manipulated to insure engagement of the parvocellular visual system. Further, patients showed reduced generation of steady-state visual evoked potentials elicited by magnocellular-biased but not parvocellular-biased stimuli, with the deficits in ssVEP generation predicting deficits in visual behavioral performance. In order to investigate potential mechanisms, magnocellular functions that do and do not require significant NMDA processing were probed psychophysically. Functions requiring NMDA involvement, such as motion detection or non-linear gain were particularly impaired, whereas other functions were relatively intact. Further, under specific stimulus conditions, parvocellular deficits could be demonstrated as well. These data suggest that visual processing deficits in schizophrenia are driven significantly by NMDA dysfunction at the level of visual cortex, and that, because of differential involvement of NMDA receptors in magnocellular vs. parvocellular processing, deficits affect the magnocellular system to a greater degree than the parvocellular system. Mechanisms by which low level visual deficits may affect higher level processing will be discussed.

Visual Masking in Schizophrenia: Applications to Neuroimaging and Electrophysiology

Michael F Green* and Jonathan K Wynn

Psychiatry and Biobehavioral Sciences, UCLA - Neuropsychiatric Institute, Los Angeles, CA, USA; Psychiatry, VA Greater Los Angeles Healthcare System, Los Angeles, CA, USA

Background: In visual backward masking, the visibility of a briefly-presented visual target is disrupted by a mask that is presented shortly thereafter. Schizophrenic patients demonstrate consistent deficits in visual masking. Our goal was to better isolate the neural substrates of masking deficits in schizophrenia by adapting visual masking methods for use in functional neuroimaging (for spatial resolution) and electrophysiology (for temporal resolution). **Methods:** We conducted two studies: one that applied visual masking to functional neuroimaging, and one that applied it to electrophysiology. For neuroimaging, 13 healthy subjects performed a backward masking task during functional MRI. The target and mask durations were kept constant and only the masking interval was manipulated. Two sets of regions of interest were identified: one set was cortical visual areas (i.e., human MT, LO, and retinotopic areas) that were activated with previously validated low-level visual stimuli. The other set of regions was selected in a data-driven approach based on a region's pattern of activity during the masking task. For each set, we determined the regions in which activation increased at longer masking intervals (reduced masking effects). In the electrophysiology study, we were interested in the role of gamma range cortical activity. EEG activity was recorded while 32 schizophrenia patients and 15 control subjects first viewed unmasked targets, and then performed a backward masking task at various intervals. Event-related gamma activity (30-40 Hz) to the target was examined at different masking intervals for correct vs. incorrect responses, and by selected cortical regions. **Results:** For the neuroimaging study, several regions showed reliable differences in activity with degree of masking. Among the visual regions, the lateral occipital (LO) region was sensitive to the strength of the mask (more activity with reduced masking). For the data-driven approach, the predicted regional activation effects were seen in thalamus, inferior parietal, and anterior cingulate. For the electrophysiology study, patients and controls did not differ in the amount of gamma activity produced to unmasked targets. However, in the masking task, normal controls showed significantly more event-related gamma activity overall, and the group difference was especially pronounced in the right hemisphere. There were no interaction effects for masking interval or whether the response was correct or not. **Discussion:** The results of the neuroimaging study isolated several regions (i.e., LO, inferior parietal, anterior cingulate, and thalamus) that showed a predictable relationships to the visibility of the target and may form

the neural substrate of backward masking. These will be the regions of interest for ongoing studies with schizophrenic patients. Results from the electrophysiology study revealed that patients show reduced gamma activity to targets (especially in the right hemisphere) compared to controls. These studies demonstrate that visual masking procedures can be used fruitfully with neuroimaging and electrophysiology to help identify the neural substrates of masking deficits in schizophrenia.

Cortical Activations During Motion Processing Are Reorganized in Schizophrenia

Yue Chen*, Emily Grossman, L Cinnamon Bidwell, Deborah Yurgelun-Todd, Staci Gruber, Deborah Levy, Ken Nakayama and Philip Holzman

Department of Psychiatry, Harvard Medical School/McLean Hospital, Belmont, MA, USA

Background: Schizophrenia patients exhibit many complex behavioral abnormalities that differ from those seen in patients with localized brain damage. In order to understand underlying neural mechanisms, it is important to identify the brain systems that play a role in an abnormal behavioral response in schizophrenia patients. These brain systems include those that are normally linked to a behavioral response and those that are normally not. We previously showed that schizophrenia patients performed poorly in motion discrimination, a sensory visual task that is normally mediated in the posterior extrastriate cortex. In this study, we examined functional activation during performance of motion and non-motion visual discrimination tasks to determine whether the posterior cortical system is responsible for the motion discrimination deficit, and what other cortical systems may also be involved. **Methods:** We used two motion tasks, direction discrimination and velocity discrimination, and a non-motion visual task, contrast discrimination. We employed fMRI methods to examine the pattern of cortical activation while schizophrenia patients (n=10) and normal controls (n=8) performed the three tasks. Psychophysical methods were used to assess behavioral performance before and during the scans. **Results:** In the posterior area MT, cortical activation of schizophrenia patients, measured with BOLD signal changes, was significantly reduced during the two motion discrimination tasks ($p < 0.02$), but not during the contrast discrimination task. In contrast, cortical activation of the patients was significantly increased in the inferior convexity of the prefrontal cortex (ICPFC) during velocity and direction discrimination ($p < 0.01$) but not during contrast discrimination. **Conclusion:** These results indicate that neural processing of visual motion signals, normally mediated in the posterior cortex, is shifted towards the anterior cortex, implicating altered neural processing at both sensory and cognitive levels in the degraded behavioral performance in patients. The recruitment of cognitive cortical areas to compensate for deficient sensory processing is one manifestation of neural reorganization in schizophrenia.

Disturbances of Attentional Control in Schizophrenia

John A Sweeney*, James Reilly, Margret S Harris, Robert W Marvin, Sarah Keedy and Matcheri S Keshavan

Psychiatry, University of Illinois at Chicago, Chicago, IL, USA; Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA

Background: Disturbances in attentional control are a cardinal feature of schizophrenia. Recent psychological models of attention have differentiated exogenous and endogenous attentional processes and their underlying neural systems. Exogenous attention refers to automatic orienting responses while endogenous attention regulated by executive control processes refers to voluntary controlled shifts of attention. Disturbances in visual attentional systems can be examined via psychophysical, ERP and fMRI studies, and by an analysis of oculomo-

tor responses to attention tasks placing demands on these two attentional systems. **Methods:** 35 antipsychotic-naïve first episode patients with schizophrenia performed exogenous and endogenous attention tasks in the laboratory before and after 6 weeks of controlled treatment. 15 of these patients participated in fMRI studies before treatment using similar paradigms. Matched healthy subjects performed the same tasks. **Results:** During the exogenous attention task in which subjects shifted attention and gaze to unpredictable peripheral targets, visual orienting responses were speeded relative to healthy subjects. Patients also performed poorly on an antisaccade task, which requires endogenous shifts of attention based on internal plans that over-ride exogenous orienting responses (i.e. subjects make saccades away from rather than toward targets). Treatment, especially with an atypical antipsychotic, significantly reduced the exogenous attention deficits. Endogenous deficits, though improved by treatment, remained robust after clinical stabilization. Deficits in the exogenous and endogenous tasks were significantly correlated in patients only prior to treatment. This observation suggests that the pronounced distractibility seen during acute psychosis may be related to the joint presence of an acute disruption of exogenous visual orienting systems coupled with exaggerated disturbances in the cognitive control of attentional systems. These deficits suggest disturbances in the top-down regulation of attention systems, which is consistent with findings from parallel fMRI studies. **Discussion:** These attention studies suggest a reduction in visual orienting systems regulated by exogenous attention systems that appears to be a state related deficit in schizophrenia. Deficits in endogenous attentional systems, while improved with treatment, are more persistent features of the illness.

Panel Session

ECNP Panel: Brain GABA-A Receptors - 25 Years of Progress

Molecular Genetics of GABA_A Receptors: Consequences for Drug Development

Uwe Rudolph*, Florence Crestani, Ruth Keist, Rachel Jurd, Karin Loew, Dietmar Benke, Jean-Marc Fritschy, Margarete Arras, Kaspar E Vogt, Horst Bluethmann and Hanns Mohler

Institute of Pharmacology, University of Zurich, Zurich, Switzerland

Based on a point mutation strategy, GABA_A receptor subtypes were identified as selective targets for behavioral actions of benzodiazepines and intravenous anesthetics. $\alpha 1$ -containing GABA_A receptors mediate the sedative action of diazepam as shown by the lack of sedation in $\alpha 1$ (H101R) mice containing diazepam-insensitive $\alpha 1$ -containing GABA_A receptors (Rudolph et al., 1999, Nature 401, 796-800; McKernan et al., 2000, Nature Neurosci. 3, 587-592). The anxiolytic-like action of diazepam was absent in $\alpha 2$ (H101R) mice but present in $\alpha 3$ (H126R) and $\alpha 5$ (H105R) mice, indicating that $\alpha 2$ GABA_A receptors are major mediators of this action (Loew et al., 2000, Science 290, 131-134). In $\alpha 5$ (H105R) mice, the expression of the $\alpha 5$ subunit in the hippocampal pyramidal cells was decreased and trace fear conditioning was facilitated (Crestani et al., 2002, Proc. Natl. Acad. Sci. USA 99, 8980-8985). These results implicate the largely extrasynaptic $\alpha 5$ GABA_A receptors in hippocampal pyramidal cells as control elements of associative aversive learning in trace fear conditioning. In $\beta 3$ (N265M) mice carrying propofol- and etomidate-insensitive $\beta 3$ -containing GABA_A receptors the immobilizing action of propofol and etomidate, as measured by the loss of the hindlimb withdrawal reflex, was completely absent. Thus, these receptors mediate this essential action of these intravenous anesthetic drugs (Jurd et al., 2003, FASEB J. 17, 250-252). Our results indicate that $\alpha 2$ -, $\alpha 5$ - and $\beta 3$ -containing GABA_A receptors may represent selective targets for the development of anxiolytics, memory enhancing agents, and intravenous general

anesthetics, respectively. Supported by the Swiss National Science Foundation.

GABA-A Receptor Subtypes as Novel Drug Targets

Ruth McKernan*, David Reynolds, Gerard Dawson, Keith Wafford, Paul Whiting, John Atack and Bjarke Ebert

Neuroscience Research Centre, Merck Research Laboratories, Harlow, Essex, United Kingdom

The structure and CNS distribution of many members of the GABA-A receptor family is now well described. Selective compounds and transgenic animals have together aided our understanding of which receptors mediate the hypnotic, anxiolytic, muscle-relaxant and anesthetic effects of GABA receptor activators and/or modulators. Recent advances in drug discovery are moving closer to improved drugs by targeting specific subtypes. For anxiety, potentiators of the GABA-A alpha2/3 subtype have an improved anxiolytic profile. For sleep disorders, an agonist at the alpha4/delta subtype, Gaboxadol, has an improved profile compared with non-selective benzodiazepines and the alpha1-selective compounds, Zolpidem and In-diplon. The potential utility of other subtypes will also be discussed.

Imaging Benzodiazepine Subtypes and Receptor Function

Anne Lingford-Hughes*

Psychopharmacology Unit, University of Bristol, Bristol, United Kingdom; MRC Clinical Sciences Centre, Imperial College, London, United Kingdom

Background: The GABA-benzodiazepine receptor [GBzR] can be visualised in vivo in man using positron emission tomography [PET] and 11C-flumazenil and single photon emission tomography [SPET] with 123I-iomazenil. Such techniques are widely used to measure GBzR levels in neuropsychiatric disorders such as anxiety, alcoholism and epilepsy. Newer developments include a paradigm to directly relate GBzR occupancy to function and characterization of which GBzR subtypes 11C-flumazenil and 11C-Ro15 4513 label in vivo with PET. These imaging strategies will be illustrated with studies in alcohol dependence. **Methods:** 123I-iomazenil SPET, 11C-flumazenil PET and 11C-Ro15 4513 PET scans were undertaken on brain dedicated scanners in male control populations and healthy abstinent (>4 weeks) alcohol dependent patients. A volume of distribution image of each tracer was generated, with an arterial input function in the PET studies, and comparisons were undertaken between the two groups. For subtype characterization of 11C-Ro15 4513 and 11C-flumazenil, small animal PET and competition studies were used. To measure the pharmacokinetics and pharmacodynamics of the GBzR, midazolam [a benzodiazepine agonist] was given during a 11C-flumazenil PET scan and the EEG response was measured [Malizia et al 1996 *Neuropharm* 35,1483-9]. **Results:** Reduced GBzR levels, particularly in the frontal cortex of up to 35%, were seen in the alcohol dependent group [Lingford-Hughes et al 1998 *B J Psych* 173:116-122]. No difference was seen between alcohol dependent and control groups in occupancy of the GBzR by midazolam or in the resulting increase in EEG beta-power. However in the alcohol dependent group, midazolam induced less sleep time compared to the control group [15.7 +/- 4.3 mins vs 33.5 +/- 3.8 mins]. 11C-flumazenil labels GBzR widely throughout the cortex, cerebellum, and subcortical regions such as the thalamus and basal ganglia. By contrast, 11C-Ro15 4513 uptake in rat and in man was predominantly in the limbic system [Lingford-Hughes et al 2002 *J Cereb Blood Flow Metab* 22:878-89]. The distribution in rat and man was broadly similar with the highest levels of uptake in the hippocampus and anterior cingulate and low levels in the cerebellum and occipital cortex. In man 11C-Ro15 4513 uptake was higher in the ventral compared with dorsal striatum whilst in the rat, uptake was at background levels throughout the striatum. Competi-

tion studies in the rat confirmed the relative selectivity of 11C-Ro15 4513 for the alpha5 subtype since only compounds with high affinity for the alpha5-containing subtype [RY80, L655,708] reduced radiolabelled Ro15 4513 uptake to non-specific levels. By contrast, 11C-flumazenil appears to be a less selective tracer since compounds active at the alpha5 subtype or at the alpha1 subtype [zolpidem] both reduced 11C-flumazenil uptake by ~50%. **Discussion:** We have exploited SPET and PET to show that alcohol dependence is associated with reduced GBzR levels primarily in the frontal cortex in man and that some aspects of GBzR function are preserved whilst others show reduced benzodiazepine sensitivity. Currently it is not clear whether these changes are caused by or underlie vulnerability to alcohol abuse. 11C-Ro15 4513 PET allows us to explore the role of the alpha5 subtype in man. Such a development is timely given our increasing understanding about which subtypes mediate specific effects of benzodiazepines. Further development of radioligands with known subtype selectivity is required to define the role of GBzR subtypes in neuropsychiatric disorders and to inform drug development in vivo in man. This work was funded by the Wellcome Trust Foundation and an MRC Programme grant.

Ethanol Exposure and Withdrawal Differentially Affect the GABA-A Receptor Plasticity and Function in Two Different Neuronal Populations

Giovanni Biggio*, Paolo Follera, Maria Cristina Mostallino and Enrico Sanna

Dept. of Experimental Biology, University of Cagliari, 09042 Monserrato (CA), Italy; Center of Excellence - Neurobiology of Dependence - University of Cagliari, 09042 Monserrato (CA), Italy

Gamma-Aminobutyric acid type A (GABA-A) receptors have been implicated as major target sites for the acute and chronic actions of ethanol (EtOH). Prolonged exposure to and withdrawal from EtOH are associated with alterations in GABA-A receptor subunit gene expression as well as in receptor function and pharmacological sensitivity in different in vivo and in vitro experimental models. Here, we focus on the effects of chronic EtOH exposure and withdrawal on the expression of the delta subunit of the GABA-A receptor in cultured rat hippocampal neurons (HP) and cerebellar granule (CG) neurons. GABA-A receptors containing the delta subunit are preferentially extrasynaptic, are responsible for the tonic inhibition, and possess an enhanced sensitivity to the agonist THIP, to the neurosteroid allopregnanolone as well as to low concentrations of EtOH. Immunocytochemical and confocal microscopy studies showed that delta subunit is preferentially expressed on the soma of CG cells, whereas it is mainly dendritic in HP neurons. Long-term EtOH exposure increased delta subunit mRNA and peptide levels in HP neurons, while it did not significantly modify its expression in CG cells. EtOH withdrawal was associated to a rapid (6 h) marked reduction of delta subunit expression in CG cells, and to a persistent enhancement in HP neurons during the first 6h from withdrawal compared to untreated control. Patch clamp recordings revealed that these EtOH induced changes in delta subunit expression are associated, in HP and CG cells, to an opposite effect, increase and decrease, respectively, of the efficacy of THIP and allopregnanolone to potentiate the chloride currents. Our data demonstrate that the changes in gene expression and function of GABA-A receptor containing the delta subunit induced by long-term EtOH exposure and withdrawal are opposite in HP and CG cells, suggesting a putative different role of these extrasynaptic receptors in controlling tonic inhibition in these two different cell types. The results indicate also a strict association existing between the GABA-A receptors subunit diversity and their differential sensitivity to drugs and endogenous modulators. This evidence together with the different

cellular localization of GABA-A receptors may explain the different threshold of excitability of selective neuronal populations in specific brain areas. It is well established that ethanol increases plasma and brain levels of GABA-A receptor active neurosteroids by activating the HPA axis. We now show that, in isolated rat hippocampal slices, EtOH dose dependently increased the concentration of allopregnanolone as well as the amplitude of GABA-A receptor-mediated inhibitory postsynaptic currents recorded from CA1 pyramidal neurons. This latter effect appears biphasic, consisting of a rapid, direct modulatory effect, insensitive to the 5 α reductase blocker finasteride, and a delayed, allopregnanolone-mediated action reversed by finasteride. These observations suggest that EtOH may modulate GABA-A receptor function through an increase in de novo neurosteroid synthesis in brain that is independent from the HPA axis. Given that neurosteroids play a major role in the physiological modulation of GABA-A receptor plasticity and function this novel mechanism may be important in mediating some effects of ethanol in physiological and pathological conditions (menstrual cycle, pregnancy, menopause, premenstrual syndrome) and a variety of psychiatric and neurological disorders in which the steroidogenesis undergoes dramatic functional changes.

Panel Session

New Vistas in Corticotropin-Releasing Factor (CRF) Neurobiology

The Molecular Neurobiology of CRF₁, Urocortin and Its Receptors

Wylie Vale

Abstract not available.

The Expanding Potential Clinical Utility of CRF₁ Receptor Antagonists: From Mood and Anxiety Disorders to Drug Withdrawal

Charles B Nemeroff*, Michael J Owens, Christine Heim and D. J Newport

Psychiatry, Emory University, Atlanta, GA, USA

A burgeoning literature, both preclinical and clinical, has provided evidence that increased availability of corticotropin-releasing factor (CRF) occurs in patients with depression and certain anxiety disorders, including post-traumatic stress disorder (PTSD). This presentation will summarize several novel findings concerning the role of CRF in the pathophysiology of mood disorders with a focus on the sizeable subpopulation of depressed patients with early life trauma, e.g. child abuse and/or neglect. In addition, recent studies from our laboratory and others have revealed an increase in CRF concentrations in CSF after tryptophan depletion in normal volunteers, and an increase in CRF concentration and decreased CRF₁ receptor density in postmortem tissue of depressed patients. In addition, the data supporting a preeminent role for CRF in the pathophysiology of drug withdrawal states will be described with a focus on benzodiazepine (BZ) studies. Abrupt BZ withdrawal in rats is, for example, associated with a marked increase in CRF mRNA expression in the cerebral cortex, and CRF receptor antagonists reduce withdrawal severity after abrupt discontinuation of a variety of agents. These data, taken together, suggest that CRF₁ receptor antagonists may have a broad spectrum of therapeutic activity. Supported by NIH MH-42088 and MH-52899.

The Role of CRF R2 Receptors in Mediating Anxiety and Behavior

Ned H Kalin*

Psychiatry, Univ of Wisconsin, Madison, WI, USA

CRF R2 receptors have a brain distribution that is distinct from CRF R1 receptors and important differences exist in the distribution of R2 between primate and non primate species. While numerous studies have demonstrated a role for R1 in mediating stress and anxiety-related responses, less is known regarding the role of R2 receptors. Data from rodent studies will be presented that demonstrate that the lateral septum is a site at which R2 activation results in anxiogenic behavioral effects. In contrast, the amygdala appears to be a site at which selective R1 plays a prominent role in mediating anxiety. Data from human post mortem studies regarding alterations in R1 and R2 in relation to psychopathology will also be presented. Finally, molecular mechanisms underlying the expression of the R2 alpha gene will be discussed in relation to stress and psychopathology.

CRH Receptors - The Most Promising Target Emerging from Hypothesis - Driven Research in Mood Disorders

Florian Holsboer*

Max Planck Institute of Psychiatry, Munich, Germany

CRH type 1 receptors had been deleted in mice completely and also in specific brain-areas and shown to be of key importance for mediating stress-related psychopathology. Recently, we complemented that view by generating a mouse mutant which conditionally overexpresses CRH and is well suited to study effects of novel small-molecule CRH-R1 antagonists. We also showed that in the brain CRH-CRHR1 signaling activates different signaling pathways beyond receptor raising the possibility of developing site-directed intervention strategies. Using cDNA-chip technology, the genes regulated by the CRHR1 antagonist NBI 30775 were shown to induce changes after long-term administration, that were very similar to the changes seen after CRHR1 gene deletion. On the behavioral level, all three technologies, namely CRHR1 antagonist, interference RNA blocking CRHR1 synthesis and CRHR1 knock-out, produce a phenotype that shows less anxiety-like behavior in the related tests.

Panel Session

The Hippocampus in Fear and Anxiety: A Neglected Portion of the Neural Fear Circuitry

The Role of Hippocampus in Fear Memory, Behavior & Extinction

Emmanuel Landau

Abstract not available.

What Role Does The Hippocampus Play In Fear, Mood And Depression?

Bruce McEwen*

Lab Neuroendocrinology, The Rockefeller University, New York, NY, USA

The hippocampus plays an important role in memory and in some vegetative functions of the body. These functions are distributed among the dorsal and ventral poles of the hippocampus (designated posterior and anterior in the human brain). The hippocampus receives input from the amygdala and its function in spatial memory is altered by amygdala activity. Repeated stress in the rat suppresses dentate gyrus neurogenesis and causes dendrites of hippocampal and medial prefrontal cortical neurons to shrink and amygdala neurons to grow. Repeated stress also increases fear and aggression, reduces spatial memory, and alters contextual fear conditioning. Antidepressants have diverse effects on these processes. Short-term and long-

term effects of two different types of antidepressants, citalopram and the novel tricyclic antidepressant, tianeptine, will be compared on fear conditioning, SSRI's and tianeptine also exert effects on structural plasticity in hippocampus and amygdala and a good case can be made that actions of antidepressants on neurogenesis in hippocampus is involved, at least in part, in antidepressant effects. In addition, the remodeling of dendrites in hippocampus and amygdala caused by chronic stress may also make a contribution to symptoms of depression, and the ability of antidepressants to alter this type of structural remodeling may also be important in their long-term effects. Therefore, because of the anatomical and functional connectivity of hippocampus with amygdala and with the prefrontal cortex, the hippocampus is likely to play an important role in the symptoms of depression and in fear memory and mood alterations associated with chronic stress. Supported by MH41256 and 5P50 MH58911.

Contextual Memory: Assessment of Hippocampal Dysfunction in Panic Disorder

Elizabeth Phelps

Abstract not available.

Hippocampal Activity in Panic Disorder and Posttraumatic Stress Disorder

Jack M Gorman*, Elizabeth Phelps, Marylene Cloitre, Bruce McEwen, Joseph LeDoux, Emily Stern and David Silbersweig

Department of Psychiatry, Mount Sinai School of Medicine, New York, NY, USA

Abundant preclinical evidence has linked the contextual portion of fear conditioning to activity in the hippocampus. It is now known that specific proteins are synthesized enabling both the acquisition and extinction of contextual fear memory. These findings have important implications for understanding fear-related disorders in humans. For example, phobic patients specifically avoid contexts associated with anxiety attacks and patients with posttraumatic stress disorder (PTSD) avoid situations that cue memories of the precipitating traumatic event. We therefore predicted that the hippocampus would be specifically involved in situations that evoke contextual memory in patients with panic disorder and patients with PTSD. Accordingly, patients with these diagnoses and matched normal volunteers underwent functional magnetic resonance imaging (fMRI) during two activation probes, one related to fear conditioning and one using linguistic anxiety probes relevant to the two disorders. We found that traumatic reminders indeed activated the hippocampus in patients with PTSD and that increased hippocampal activity is associated with CAPS scores for reexperiencing symptoms. In panic disorder patients, increased hippocampal activity during instructed fear conditioning correlated positively with agoraphobia scores on the PDSS. These findings are consistent with the prediction of a role for the hippocampus in the contextual aspects of anxiety disorders. They provide further support for the view that the fear conditioning paradigm in experimental animals is a valid replica for human fear-related psychopathology.

Panel Session

Novel Serotonin Transporter Gene Promoter Variants and Psychiatric Illness

Early Life Disruption of Serotonin Transporter Function Alters Affective and Anxiety-Like Behaviors in Adulthood

Mark Ansorge and Jay Gingrich*

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

Serotonin reuptake inhibitors (SSRIs) are used as a first-line treatment for psychiatric conditions such as major depression, gener-

alized anxiety disorder, panic disorder, and obsessive compulsive disorder. These agents act by inhibiting serotonin transporter (5-HTT) function and enhancing serotonin neurotransmission. Although SSRIs are increasingly used to treat children and pregnant mothers, little is known about their effects on the developing nervous system. We have used a strategy combining pharmacological and genetic manipulation to investigate whether early postnatal inhibition of 5-HTT function alters emotional behavior later in life. Here we provide evidence that a paradoxical depression-related phenotype is produced in adult mice by inhibition of 5-HTT function during early development. Specifically, inhibition of serotonin transporter function from postnatal day 4 until postnatal day 21 reduces exploratory behavior in the open field and in the elevated plus maze. Additionally, it increases time to feed in the novelty suppressed feeding test and latency to escape in the shock avoidance test. These observations support the notion that pharmacological inhibition of 5-HTT reuptake during brain development may predispose an exposed fetus or young child to increased vulnerability for psychiatric symptoms later in life. In addition, these findings provide a possible developmental mechanism explaining how low-expressing 5-HTT promoter alleles may increase vulnerability to affective disorders.

Novel Serotonin Transporter Gene Promoter Variants and Psychiatric Illness

Jay Gingrich, David Goldman*, J John Mann and Ahmad Hariri

Laboratory of Neurogenetics, NIH, NIAA, Rockville, MD, USA

Much convergent data has identified the serotonin transporter gene as an important susceptibility gene for anxiety and mood disorders. David Goldman's group has recently reported that there is a low expressing variant in the long form of the serotonin transporter promoter region known as HTTLPR. This finding has the potential for removing some of the noise from association and linkage studies with this gene. This panel will present new reanalyses of recent findings with regard to the role of the serotonin transporter gene in mood and anxiety disorders after a re-genotyping of the samples. Another set of studies will re-examine the relationship of levels of expression of the transporter gene to intermediate biological phenotypes that may underlie the psychopathologic associations. This panel will begin with Jay Gingrich reviewing the serotonin transporter knockout mouse as a model for mood disorders and related psychopathologies. Then David Goldman will report on the new polymorphism he has found in the long allele of the serotonin transporter promoter which is associated with lower expression and how a consideration of this variant has strengthened the association he and colleagues reported with OCD as well as its impact on other association studies. John Mann will describe the relationship between this variant and transporter binding in living subjects using PET scanning and a voxel based analysis to define the biological intermediate phenotype, and then report how the lower expressing variants appear to amplify the effect of recent life events on severity of major depression in his studies. Finally, Ahmad Hariri will relate the reclassified genotype in relation to amygdala responsiveness to threatening faces. This is a mechanism whereby the transporter may influence development of the serotonin system and result in an enduring change in functional capacity that may underlie the diathesis for anxiety disorders, recurrent mood disorders and behavioral traits. A new variant in the promoter of the serotonin transporter that includes a low expressing variant of the long allele has been recently reported at conferences. Data sets reporting associations of low expression of the transporter gene with psychopathology or morphological changes in the serotonin system and with responses in key brain regions such as the amygdala, will be re-examined in the light of this new variant. The genotype will be linked to intermediate biological endophenotypes that may explain its effects on psychopathology. Evidence will be presented for a potential effect on brain structure and function that may translate into a vulnerable

phenotype in terms of susceptibility to the stress of life events, anxiety disorders and recurrent mood disorders.

Effect of the HTTLPR Polymorphism on Serotonin Transporter Binding *In Vivo* and on Effect of Life Events on Major Depression

J John Mann*, David Goldman, Ramin V Parsey, Yung-yu Huang, Gil Zalsman, Maria A Oquendo and Victoria Arango

Dept. of Psychiatry, Columbia University, New York, NY, USA; Dept. of Neuroscience, New York State Psychiatric Institute, New York, NY, USA

Low serotonin transporter binding is associated with both major depression and with suicide. In postmortem studies we have previously reported a localized deficiency in serotonin transporter binding confined to the ventromedial prefrontal cortex. In contrast, low serotonin transporter binding was observed throughout the dorsal, ventral and medial prefrontal cortices. In addition, we have found evidence that depressed suicides have low serotonin transporter binding in the brainstem when one looks at binding throughout the dorsal raphe nucleus. Further, our post-mortem studies have demonstrated a deficiency in serotonin transporter gene expression in the dorsal raphe nucleus. More recently, we have conducted PET imaging studies to quantify serotonin transporter binding in drug-free subjects with major depression using ¹¹C-McN 5652. Those studies have identified deficiencies in serotonin transporter binding in several brain regions, although the specific to non-specific binding ratio for this PET tracer is insufficient to allow reliable quantification of serotonin transporter binding in the prefrontal cortex. However, we have been able to reliably quantify other brain regions, including the amygdala, thalamus, hippocampus and anterior cingulate and midbrain. In order to determine whether these binding changes in postmortem brain samples and in the studies with PET are related to differential expression of the serotonin transporter gene found with the HTTLPR variant, we genotyped our subjects and postmortem brain samples using the reported variants from Goldman et al. Postmortem brain tissue analyses did not reveal a clear relationship between genotype and transporter binding in the prefrontal cortex. PET imaging analyses indicated a similar absence of a relationship between transporter binding in the prefrontal cortex and other terminal field regions with genotype in both the healthy volunteer subjects and the depressed subjects analyzed separately or together. There was a suggestion of a weak signal in the brainstem that needs to be confirmed and replicated. If there is indeed such an effect in the brainstem, then that would be consistent with the postmortem finding of reduced gene expression using *in situ* hybridization histochemistry that we have reported previously.

Modulatory Effects of the 5-HTTLPR Long Allele Promoter SNP on Amygdala Reactivity

Ahmad R Hariri*

Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA

The 5-HTTLPR may contribute to individual differences in mood and temperament as well as risk for psychiatric illness by biasing the functional response dynamics of the amygdala and prefrontal cortex, brain regions critical for mediating and integrating arousal in response to environmental challenge. Recent data suggest that a single nucleotide polymorphism in the 5-HTTLPR long promoter variant (16A allele) can further influence 5-HTT availability and function. In the current study, we explored the modulatory effects of this SNP on 5-HTTLPR driven functional reactivity of the human amygdala and prefrontal cortex. Preliminary results will be presented and discussed in context of the 5-HTTLPR as a classic susceptibility factor for psychiatric illness.

Panel Session

Modeling Schizophrenia Vulnerability in Mice: Genes and Mechanisms

22q11 Deletion and Schizophrenia Vulnerability: A Model for Multigenic, Dosage-dependent Pathogenesis

Anthony S LaManita*, Thomas M Maynard, Daniel Meechan, Amanda Z Peters and Jeffrey A Lieberman

Cell & Molecular Physiology/UNC Neuroscience Center, The University of North Carolina School of Medicine, Chapel Hill, NC, USA; Psychiatry, The University of North Carolina School of Medicine, Chapel Hill, NC, USA

Background: Microdeletion at chromosome 22, position q11 in humans (the 22q11 Deletion Syndrome: 22q11DS) is one of the two most significant genetic mutations currently known to be associated with schizophrenia vulnerability. This microdeletion results in a spectrum of phenotypes that include limb, heart and craniofacial anomalies as well as a 25 to 50 fold increase in the incidence of schizophrenic spectrum disorders. Our analysis of the expression, regulation and function of 22q11 genes suggests that this vulnerability reflects the concerted and re-entrant function of a majority of the genes found in the deleted region of chromosome 22 from early embryogenesis through adulthood. The consequences of altered dosage likely begin when the shared mechanism of induction in the forebrain, face, heart, and limbs, is altered by diminished dosage of multiple 22q11 genes, and continues, in the forebrain, through the periods of cell migration, process growth and synapse formation. Finally, altered dosage of 22q11 genes can also compromise adult function, perhaps acutely, or perhaps reflecting the accumulated consequences of developmental disruptions. **Methods:** We have used a combination of PCR based arrays, antibody-based detection, and a variety of cellular and physiological methods to characterize the expression and function of the full set of mouse 22q11 orthologues in embryonic, juvenile, and mature normal mice as well as those deleted heterozygously at the chromosomal region syntenic with that deleted in human 22q11DS patients. **Results:** We have found that the majority (27/32) 22q11 orthologues are expressed either specifically or selectively at each embryonic site phenotypically compromised in 22q11DS: the forebrain, the branchial arches (face), aortic arches (heart) and limb buds. Most of these genes remain expressed in the developing brain, and their spatial or temporal expression patterns suggest contributions to cell migration, process outgrowth, and synaptogenesis. In 22q11 syntenic deleted mice (LgDel mice), all 22q11 genes thus far analyzed are diminished in their expression by at least 50%, and sometimes more, from early development through adulthood. We have found no evidence for dosage compensation or allelic biasing that might modify these effects. The diminished dosage of 22q11 genes apparently contributes to altered patterning and development of cerebral vasculature, decreased expression of proteins associated with GABAergic neurons, and altered mitochondrial function. **Discussion:** Our results indicate that heterozygous deletion of 22q11 genes in mice leads to widespread haploinsufficiency of multiple 22q11 genes. The temporal continuity of expression of these genes in the brain from embryonic stages through adulthood, as well as their mosaic expression in distinct cell classes suggests that 22q11 pathogenesis represents a unique "contiguous gene" syndrome where widespread expression of adjacent genes, plus re-entrant function leads to accumulation of phenotypes, perhaps reaching a disease threshold. Thus, schizophrenia vulnerability in 22q11DS patients may reflect the quantitative dysregulation of the set of 22q11 genes in the developing, maturing and adult nervous system.

Receptor Tyrosine Kinase ErbB4-neuregulin Interactions Modulate Neuroblast Migration and Placement in the Adult Forebrain

Eva S Anton*

Neuroscience Center, UNC, Chapel Hill, NC, USA

Neural progenitor proliferation, differentiation and migration are continually active processes in the rostral migratory stream (RMS) of the adult brain. Here, we show that the receptor tyrosine kinase ErbB4 is expressed prominently by the neuroblasts present in the subventricular zone (SVZ) and the RMS. The Neuregulins (NRGs 1,2,3), which have been identified as ErbB4 ligands, are detected either within the stream or in adjacent regions. Mice deficient in ErbB4 expression in either nestin or hGFAP promoter-active cells exhibit altered neuroblast chain organization, migration, and deficits in the placement and differentiation of olfactory interneurons. Together, these findings suggest that ErbB4 activation helps to regulate the organization of neural chains that form the RMS and influences the differentiation of olfactory interneuronal precursors.

Genetic and Environmental Factors that Disrupt the Developmental Trajectory of Circuit Formation and Function

Pat Levitt*, Philip Ebert, Daniel Campbell, J P Card, Linda Rinaman, Kyoko Koshibu and Eric Ahrens

Vanderbilt Kennedy Center, Vanderbilt University, Nashville, TN, USA

Background: Schizophrenia is a disorder that affects ~ 1% of the population worldwide. Genetic and environmental factors are thought to interact to disrupt the development of brain circuitry that is involved in regulating the endophenotypes classified as positive and negative. Recent studies suggest that deficits at the synapse may underlie the pathogenesis and pathophysiology of schizophrenia. To test the idea that altered prepubertal development, due to a combination of genetic susceptibility and early negative experience, can lead to changes in the integrity of brain development, as well as disrupted behavior, we have utilized two experimental strategies. **Methods:** Genetic models in mice are used to probe developmental and behavioral disruption. Transgenic mice misexpressing genes identified as reasonable susceptibility candidates, based on microarray expression and human genetic studies, were examined for alterations in synaptic development. We have focused on Regulator of G-Protein Signaling 4 (RGS4) and cytoplasmic malate dehydrogenase 1 (MAD-1). BAC-transgenic mice were produced that overexpress RGS4, and immunostaining for synaptic markers were used to assess changes during development in the cerebral cortex. Reconstitution of a mouse line that is hypomorphic for MAD-1 has been done in our laboratory for similar analysis. Using high resolution magnetic resonance microscopy (MRM) and behavioral assays, we have analyzed a third line, hypomorphic for transforming growth factor alpha (TGF- α), for changes in brain structure and function. Finally, an environmental perturbation, stress-induced handling during postnatal development, was performed and pseudorabies virus (PRV) tracing was utilized to monitor possible changes in forebrain connectivity with autonomic brainstem regions that mediate part of the central stress responsiveness. **Results:** Preliminary analyses of the genetic models demonstrate RGS4 overexpression and MAD-1 decreased expression, respectively. There is no evidence of major changes in brain structure. Staining with synaptogyrin indicates modest alterations in the density of synapses in developing cortical regions in the RGS4 transgenic lines. TGF- α hypomorphic mice are normal prior to puberty, but based on MRM analyses, exhibit sex-specific postpubertal changes in the size and shape of periventricular brain regions (hippocampus, amygdala, striatum) and deficiencies in learning. PRV studies on the impact of early stress on synapse formation demonstrate dramatic differences in the establishment of amygdala and bed nucleus of the stria

terminalis connections with the brainstem during the first postnatal week. **Discussion:** The data indicate that a combinatorial approach to genetic and environmental factors that disrupt neurodevelopment and ultimately, function, can be used to establish mechanisms that may underlie the early susceptibility and post-adolescent onset of schizophrenia. Detection of developmental changes requires detailed neuroanatomical analyses, consistent with known, highly targeted changes in synaptic organization in schizophrenia. The challenge will be to combine genetic model systems with early experiences to establish an experimental framework for determining the factors that mediate the development of circuits involved in higher cortical function. Supported by: NARSAD Young Investigator award (PE), McKnight Neuroscience of Brain Disorders award and NIMH P50MH45156 (PL), and the MacArthur Foundation Network on Early Experience and Brain Development (JPC).

Evidence for Involvement of Altered Calcineurin Function in Schizophrenia

David J Gerber*, Tsuyoshi Miyakawa, Diana Hall, Lorene Leiter, Sandra Demars, Hongqui Zeng, Raul R Gainetdinov, Tatyana D Sotnikova, Marc G Caron, Joseph A Gogos, Maria Karayiorgou and Susumu Tonegawa

The Picower Center for Learning and Memory, MIT, Cambridge, MA, USA

Forebrain-specific calcineurin knockout mice were previously shown to have impaired bi-directional synaptic plasticity and specific impairments in working memory. We have performed a comprehensive behavioral analysis of these mice and found that they have a spectrum of behavioral abnormalities similar to that observed in schizophrenia patients. In particular, in addition to the working memory deficit, these mice have impaired social interaction, impaired attentional function, increased sensitivity to NMDA receptor blockade and are hyperactive. To investigate the potential involvement of calcineurin dysfunction in schizophrenia etiology, we have performed genetic association studies of a set of calcineurin related genes in a large sample of schizophrenia affected families. These studies have provided evidence for association of the *PPP3CC* gene, encoding the calcineurin gamma catalytic subunit, with disease. RT-PCR and northern analysis indicate that *PPP3CC* is expressed in multiple regions of human brain. *In situ* hybridization analysis detects low levels of *PPP3CC* expression in the mouse forebrain. Our results identify *PPP3CC* as a potential schizophrenia susceptibility gene and support the proposal that altered calcineurin signaling contributes to schizophrenia pathogenesis.

Study Group Session

Promoter Polymorphism of the Serotonin Transporter Gene and Behavior: Untangling the Black Box Between Gene and Behavior

Margit Burmeister*, J D Higley, David Goldman, Andrew Holmes and Jon-Kar Zubieta

Mental Health Research Institute, University of Michigan, Ann Arbor, MI, USA

With some confirmed genetic variants at hand that affect risk for psychiatric disorders and behavior identified, our next challenge is even greater: How can we explain the steps from a genetic variant to a small, often also environmentally modulated, behavioral difference? A functional length polymorphism in the promoter for the serotonin transporter gene, often abbreviated 5-HTTLPR, has been the subject of >400 association studies in the literature. The effect of this repeat on expression in reporter constructs and cell lines has been established. Several Meta-analyses recently all confirmed association of the

S allele with higher scores on the anxiety-depression related personality trait Neuroticism. Several studies have suggested an interaction with stress or stressful life events in humans. Similarly, primate studies show that the phenotypic expression of the short allele genotype is dependent on rearing environment and gender, suggesting gene X environment interactions must be considered to understand the effects of this complex genotype. Recent work in 5-HTT null mutant mice also supports a link between genetically-driven 5-HTT deficiency and increased stress-reactivity. In addition, the story is complicated due to the presence of a common variant that makes some L allele functionally more like the less active S allele. In addition, comparison of L/L and S/S postmortem brains shows that the brain compensates for the difference in promoter activity, resulting in near equal mRNA levels. Microarray analysis shows differential gene expression between S/S and L/L brains that is modified by depression status. FMRI and PET data demonstrate more pronounced responses to affective stimuli at the level of synaptic activity and neurotransmitter systems implicated in the stress response in individuals presenting with the S allele. With ample audience participation and presenters on animal models and functional brain imaging, we will explore how these molecular genetic and expression differences may affect behavior in an environmentally modulated fashion. 1) How much will a newly discovered additional variation within the same repeat affect results including previously reported studies? 2) What are the downstream effects of these genetic variants on other genes expression, and how are they modulated by depression? 3) What functional differences between genetically different brains can be detected by imaging? 4) What do knock-out mice and monkeys with different promoter variants tell about the mechanism of low/high functioning serotonin transporter promoter? 5) Are there other approaches not addressed by the speakers that will help to bridge the gap between knowing a functional genetic variant and resulting behavioral differences?

Study Group Session

Therapeutic Modulation of Glutamate Neurotransmission: Where Do We Go From Here?

Darryle D Schoepp*, Gary Lynch, Dennis Choi and John H Krystal

Neuroscience Research, Eli Lilly and Company, Indianapolis, IN, USA

Drugs that modulate the actions of glutamate represent therapeutic approaches for a wide variety of psychiatric and neurological conditions. Glutamate, the major excitatory neurotransmitter in the mammalian central nervous system is a fundamental regulator of CNS excitability and plasticity. The actions of glutamate are mediated via a variety of pre- and post-synaptic receptors and transporters that are differentially distributed throughout the CNS and various peripheral tissues. As glutamate neurotransmission is essential to normal functions, the targeting of pathological processes involving glutamate are needed to provide a safe as well as effective therapy. In the past decade a variety of clinical candidates have now been investigated, including competitive and non-competitive NMDA receptor antagonists, glycine site NMDA antagonist, AMPA/kainate receptor antagonists, AMPA receptor potentiators and antagonists, and mGlu receptor agonists. However, to date there has been very limited drug approval success, with the exception of the recent introduction of the low affinity NMDA receptor antagonist memantine for Alzheimers disease. In general, progress in this area has been hampered by a combination of issues including short and long-term toxicities associated with on- or off target activities, lack of efficacy, less than optimal potency/selectivity, and drug-like properties that hamper interpretation of clinical results. Overall, the clinical investigation of these agents consistently across a variety of disorders has also not generally occurred, limiting knowledge for the design of more selective and

safer agents for clinically validated indications. This study group will address status of preclinical and therapeutic investigations involving drugs that target glutamate neurotransmission. Emphasis will be on issues that hamper clinical progress in this area, gaps in knowledge as a result of the strategic approaches that have been used to date, and future approaches being considered to progress this field. Specific subjects and related questions for discussion include: 1: Metabotropic glutamate receptor modulators for psychiatric disorders: What have we learned and what is next? (D. Schoepp, Eli Lilly and Company); 2: AMPA receptor potentiators: What issues need to be addressed for future success? (G. Lynch, Univ. California); 3: Glutamatergic agents as neuroprotectants: Is excitotoxicity a tested clinical hypothesis? (D. Choi, Merck); 4: What are the missed opportunities in the clinical investigation of glutamatergic agents? (J. Krystal, Yale Univ. School of Medicine)

Study Group Session

Onset of Clinical Actions of Antidepressants: When Does It Occur and What Does It Tell Us?

Alan Frazer*

Pharmacology, University of Texas Health Science Center, San Antonio, TN, USA

Current clinical discourse on the time course of clinical effects of antidepressants (ADs) demonstrates that the field still accepts the idea that there is a lag of several weeks before the onset of clinical actions. This is in spite of the fact that evidence has accumulated since the late 1980's against this view, i.e., AD-induced behavioral effects in drug-responsive patients being evident within 7-10 days. The evidence relative to onset has been the subject of reviews regarding the definition of "onset", the appropriate clinical methodology and statistics to apply in such studies, and the requirements of a design that would provide definitive results. This study group will discuss these issues and the results from such studies. Dr. S. Montgomery will discuss the validity of various measures and statistical approaches used to measure onset. He will present results relevant to onset from clinical trials of various ADs that demonstrate that large numbers are required to identify reliable drug/placebo differences in onset. Dr. H. H. Stassen will present new data on the onset of improvement and response to mirtazapine in a very large cohort of patients. Significant improvement within the first two weeks of treatment was highly predictive of final response. He will also show data on genetic factors that correlate highly with the time point for onset of improvement. Dr. M. Katz will present his recent results comparing onset, time course and early behavioral changes in depressed patients caused by either an SSRI, selective NRI or placebo. Improvement in specific behavioral components was detected within 7-10 days in drug-responsive patients but not in those who responded to placebo. New analyses relating early behavioral changes to the concentrations of drug in serum will be presented. Dr. I. Lucki will not only discuss the implications that the time course of clinical response has for basic studies of mechanisms of action of ADs but also present data differentiating fast behavioral effects (forced swim test) from ones that develop more slowly (chronic mild stress). Neurochemical effects that might be responsible for the differences in rapidity of response will be presented. This study group will highlight the importance of differentiating between the time it takes for ADs to elicit maximal improvement and the time when they begin to elicit improvement in drug-responsive patients and the importance of early improvement in predicting subsequent response to these drugs. Questions that will be addressed: 1) How should "clinical action" be defined, i.e., how do we distinguish, operationally, "improvement" and "full response"?; 2) What is the current evidence for the time of onset of clinical actions of antide-

pressants?; 3) How does the timing of clinical actions of antidepressants impact the capacity to predict outcome of treatment in individual patients?; 4) Do genetic factors play a role in the timing of a patient's response?; 5) What are the implications of the time of onset of clinical actions for research on basic neural mechanisms of antidepressants and the development of more rapidly acting drugs?

Study Group Session

Early Phase Drug Discovery in the 21st Century: Are Academic, Government, and Industrial Partnerships Possible?

Christian C Felder*, Glen R Hanson, Linda Brady and P. J Conn

Neuroscience, Eli Lilly & Co., Indianapolis, IN, USA

The cost of drug discovery and development has increased to such high levels (estimates reach \$1.2B/drug) that NCEs must reach blockbuster market status in order to sustain the pharmaceutical research enterprise. Moreover, the one pill/one target approach appears to be an inadequate strategy to treating major neuropsychiatric illnesses, which are most likely a collection of well-defined neuropathological states affecting smaller patient populations. Clearly, delivering innovative treatments for chronic and severe neuropsychiatric diseases challenges the biomedical community to explore creative research partnerships to support discovery biology and drug development. For example, genetic linkage studies in schizophrenia, depression, and bipolar disease are beginning to reveal potential novel drug targets such as GPCRs (glutamate and muscarinic receptors) growth factors (BDNF) and regulatory proteins (RGS4) that would benefit from more complete validation as to their role in currently ill defined neuropsychiatric diseases. Small molecule pharmacological validation significantly improves the confidence of continued research into the mechanism of a particular target approach and can potentially lead to a new chemical entity (NCE) having attractive properties for full drug development. The discovery of NCEs requires the creation and maintenance of large chemical libraries, high throughput screening, and lead optimization supported by teams of in vitro and in vivo biologists, medicinal chemists, drug disposition experts, toxicologists, biopharmaceutical formulation experts to reach the point of a viable clinical candidate. Although these expertises have historically been focused in large pharmaceutical firms, a new partnership is forming with academic centers to improve the success of small molecule discovery. The federal government has recently announced an RFA for academic centers to submit proposals for small molecule discovery centers to help support the need for pharmacological tools for target discovery and validation leading potentially to NCEs with further development potential. The focus of this panel will be to further explore the role of the NIH funded Molecular Libraries Screening Centers Network (MLSCN) as a way to foster increased industrial and academic scientific interactions and to address the following aspects: 1) The technology and expertise requirements to establishing a successful Center; 2) Medicinal chemistry support to generate high quality pharmacological tools; 3) The process and intellectual property aspects of sharing of ligands across centers; 4) The basic requirements for lead molecules to be adequately evaluated in preclinical in vivo studies; 5) Measuring how this approach will change and solve current drug discovery challenges.

Study Group Session

Increasing Ecological Validity in Neuropharmacological & Brain Imaging Studies Using Virtual Reality

Godfrey D Pearson*

Psychiatry, Yale University, Hartford, CT, USA

Virtual Reality (VR) and simulated scenarios are increasingly being used by psychopharmacologists investigating the efficacy of

behavior-altering and/or therapeutic drugs in diverse contexts. These latter include pharmacologic treatment of specific phobias, ADHD, PTSD and eating disorders. In addition, researchers wish to study behavioral changes due to abused substances on a variety of everyday behaviors, eg driving, in well-defined, ecologically valid contexts. VR provides one means to accomplish this. Pearlson will provide a general conceptual introduction to clinical applications of virtual reality and simulation used in psychopathologic assessment, monitoring of drug effects, and as neuro-imaging probes in a psychopharmacologic context. Each of the remaining four presenters, as outlined below will present novel data, providing examples of studies fitting into this overall context, including VR as a therapeutic tool in exposure therapy. The goal of this study group is to provide an introduction and update covering clinical uses of VR in a psychopharmacologic context. This panel will review the use of standardized collections of well-explored VR scenarios capable of being customized for individual research designs. Characteristics of these scenarios include ecological validity, reproducibility, safety, convenience and cost-effectiveness. Rizzo will discuss use of a realistically detailed virtual classroom for use with children suffering from ADHD, to assess both symptomatic severity and effects of putative pharmacologic interventions. Calhoun will show how brain activation associated with a complex behavior (automobile driving) can be assessed on a driving simulator in an fMRI scanner, and provide examples of dose-response relationships of alcohol and THC on behavioral impairment in the simulated driving scenario and their associated brain activation changes. Rothbaum will demonstrate how effects of a pharmacologic intervention, the NMDA partial agonist d-cycloserine (DCS) for phobic disorders were integrated with a cybertherapy (VR) intervention for acrophobia. She will demonstrate that acute treatment with DCS given shortly before each VR exposure therapy session enhanced the rate of fear extinction vs. placebo. These data support the use of cognitive enhancers to accelerate fear extinction in VR therapy. Astur will discuss the use of virtual cognitive tasks designed to reproduce well-characterized animal experiments, eg the use of maze tasks based on similar tests used to assess hippocampal damage in rodents. He will demonstrate normal sex-differences, effects of illness (schizophrenia, Alzheimer's disease) and of an abused substance (THC) both on task performance and on task-associated brain activation measured using fMRI. Questions that will be addressed are: 1) What are the practical benefits of using Virtual Reality (VR) methods in psychopharmacologic research?; 2) What are some of the barriers faced in importing VR scenarios into functional MRI?; 3) How have researchers interfaced drug delivery systems into VR-based research looking at abused substances?; 4) Are there particular constraints dealing with children and the elderly?; 5) What are future trends likely to be in the use of VR methods in psychopharmacologic research?

Study Group Session

New Animal Models and Biomarkers of Depression

Lisa Monteggia*

Psychiatry, UT Southwestern Medical Center, Dallas, TX, USA

The premise underlying the pathophysiology of depression is that both genetic and non-genetic factors produce alterations in gene expression that result in functional changes in the brain. However, there is little data on specific genes that play a role in the pathophysiology of depression or its treatment. Recent studies have suggested that brain-derived neurotrophic factor (BDNF) may contribute to the pathophysiology of depression or antidepressant efficacy. Here, we use complementary genetic approaches to delete

BDNF selectively in the brain to more closely examine the role of this growth factor in depression related behavior and antidepressant efficacy. One approach is the development of a novel inducible knockout system to delete BDNF in broad forebrain regions of mice in a temporal dependent manner. We have characterized animals in which BDNF is deleted in three-month old animals (Adult-KOs) and in embryonic mice (Early-KOs) and find that the loss of BDNF during earlier stages of development (Early-KOs) produces a differing behavioral profile than that observed with the Adult-KOs. We are now examining whether these BDNF Early-KOs or Adult-KOs have a differing behavioral profile in depression related behavior and antidepressant responses depending on the timing of the BDNF deletion. We are also using a viral mediated approach (AAV-Cre) to delete BDNF selectively in the hippocampus and observe whether the loss of this growth factor in this specific brain region contributes to animals being more-depressed or having altered response to antidepressants. In addition, we will discuss how serotonergic modulation of hippocampal neuronal function contributes to changes in gene expression. These studies highlight new and innovative approaches to more closely examine the role of BDNF in depression, and may have implications for its treatment. 1) What are the candidate genes that may play a role in the pathophysiology of depression and antidepressant treatment? 2) What is the functional role of BDNF in depression and antidepressant treatment? 3) What is the significance of alterations in growth factor signaling observed in both animal models and in human depressed subjects? How do antidepressants influence these growth factor pathways? 4) What are the roles of interferons and interleukins in mediating processes related to depression in animal models as well as in humans?

Study Group Session

Medication Development for Cannabis Dependence

Herbert D Kleber*, Francis Vocci, Alex Makriyannis, Aaron Litchman, Margaret Haney and Jack Cornelius

Psychiatry, Division on Substance Abuse, Columbia University College of Physicians & Surgeons, New York, NY, USA

1) What is the evidence for cannabis producing withdrawal and physical dependence? 2) What are the potential roles for cannabis exogenous agonists; inverse agonists and neutral antagonists in medication development for treating cannabis dependence? 3) Contrast the animal models and the human laboratory models for developing medications for cannabis withdrawal/dependence. 4) Describe the current state of knowledge regarding the anabolic and catabolic pathways of the endocannabinoid system and the endogenous ligands and receptors. 5) What have clinical trials shown regarding treatment of conditions comorbid with cannabis dependence?

Thursday, December 16

Panel Session

Drug Development: Serotonin's Role in Modulating Drug Reward and Relapse

DAT Knockout Mice and the Serotonin Switch

Sara R Jones*, Yolanda Mateo and Evgeny A Budygin

Physiology and Pharmacology, Wake Forest University Health Sciences, Winston-Salem, NC, USA

Background: It is generally accepted that the ability of cocaine (COC) and amphetamine (AMPH) to inhibit the dopamine transporter (DAT) is directly related to their reinforcing actions. However, mice with a genetic deletion of the DAT (DAT-KO mice)

still experience the rewarding effects of these stimulants as measured by conditioned place preference (CPP) and self-administration. These findings suggest that there is an alternate site for COC reinforcement. **Methods:** Homozygote DAT-KO and wild-type (WT) mice were used. For microdialysis, guide cannulas were stereotaxically implanted, one in the nucleus accumbens (NAc) and one in the ipsilateral ventral tegmental area (VTA). Probes (1mm) were implanted during recovery from anesthesia and experiments began 24h after surgery. 20-min samples were analyzed for dopamine (DA) by HPLC-EC. For fast scan cyclic voltammetry (FSCV), coronal mouse brain slices (400 μ m thick) were perfused at 1 ml/min with 34 deg C Krebs buffer. During FSCV DA recording, the carbon fiber electrode potential was scanned from -400 to 1200 mV and back at 300 V/sec, every 100 ms. DA release was evoked every 10 min by either 1 or 30 pulse, 30 Hz stimulations (350 μ A, 4 ms). The CPP apparatus had white and black chambers (21x28 cm) connected by an anteroom (21x12 cm) with guillotine doors. For pre-conditioning, mice were allowed access for 15 min to both chambers. For conditioning, mice received an i.p. dose (in mg/kg) of COC (20), AMPH (5) fluoxetine (15), WAY 100635 (2) or saline and were confined to one chamber for 15 min. Pairing was randomized across groups. This process was repeated for 4 days. On day 5, mice were placed in the anteroom and the doors opened. CPP was assessed by the amount of time spent in each chamber over a 15-min period. **Results:** In DAT-KO mice, systemic administration of COC or AMPH increased extracellular DA in the NAc as measured by microdialysis, and the increase was not due to blockade of DA uptake in the NAc but rather to effects in the VTA. Local infusion of COC and AMPH into the VTA, but not the NAc, elevated DA in the NAc. In addition, using FSCV in NAc slices, we found that neither COC nor AMPH decreased the rate of DA clearance. These drugs produced CPP, and in fact DAT-KO mice exhibited AMPH-induced CPP for many weeks after extinction occurred in WT mice. AMPH-induced CPP was abolished by pretreatment with WAY 100635, a serotonin 5-HT 1A receptor antagonist, in DAT-KO mice, but was without effect in WT mice. Fluoxetine and citalopram, selective serotonin reuptake inhibitors, also increased DA in the NAc of DAT-KO mice, and fluoxetine was shown to produce CPP. Finally, chronic treatment (10 daily i.p. injections) with either PTT (a high-affinity DAT inhibitor, 2 mg/kg) or Ritalin (DAT inhibitor, 5 mg/kg) produced the same switch in serotonin-DA interactions; i.e., fluoxetine administration elevated NAc DA levels following chronic treatment. **Conclusions:** Therefore, despite the absence of the DAT, COC and AMPH display rewarding effects and cause an increase in extracellular DA in the NAc of DAT-KO mice, acting indirectly in this case. The 5-HT system appears to be involved in the rewarding effects of stimulants in these mice. In addition, chronic treatment with DAT inhibitors causes changes in the serotonin system that may lead to greater activation of the DA system. We demonstrate here that modulation of the serotonergic system in the VTA, where the mesolimbic DA system originates, is involved in a novel target of COC and AMPH action. This novel mechanism may provide a feed-forward enhancement of DA levels in the NAc beyond that normally achieved by COC and AMPH, and may represent a crucial switch in the brain, leading towards increased motivation to take drug.

Serotonergic Control of Mesoaccumbal dopamine Pathway Activity and Implication for Drug Abuse

Philippe De Deurwaerdere*, Sylvia Navailles, Delphine Moison and Umberto Spampinato

CNRS 5541, University of Bordeaux 2, Bordeaux, France

Background : Several studies have focused on the role of serotonin (5-HT) receptors in the regulation of forebrain dopaminergic (DA) function and pointed out their potential as a target for im-

proved treatments of addictive behaviors relative to drug abuse consumption. Drugs of abuse share the property to enhance DA outflow in the nucleus accumbens (NAcc) and various 5-HT receptors such as 5-HT_{2A}, 5-HT_{2C} and 5-HT₃ receptors, are present in brain areas related to reward circuitry. However, their influence on drug abuse-stimulated DA outflow is unclear in that the influence of 5-HT receptors appears to be conditional and related to the mechanism of action of the drug of abuse considered. **Methods:** In vivo microdialysis in freely-moving or halothane anesthetized Sprague-Dawley rats was used to address the influence of 5-HT_{2A}, 5-HT_{2C} and 5-HT₃ receptors on NAcc DA outflow. Experiments were performed under various situations allowing us to study the specific DA cellular mechanisms altered by 5-HT receptors (synthesis, impulse, depolarization), the role of endogenous 5-HT tone or the region considered. DA outflow was stimulated by drugs of abuse known to affect DA outflow via distinct mechanisms, such as cocaine (COC, effect independent from increased DA neuronal firing), amphetamine (AMP, non-exocytotic DA outflow) and morphine (MOR, impulse-dependent DA outflow), as well as by the DA-D2 antagonist haloperidol (HAL, impulse-dependent DA outflow) or dorsal raphe nucleus electrical stimulation (DRNs). Selective 5-HT agents targeting 5-HT_{2A} (SR 46349), 5-HT_{2C} (SB206553, SB242084, Ro 60-0175) or 5-HT₃ receptors (ondansetron, MDL 72222) were administered 15 or 30 minutes before the treatments mentioned above. **Results:** Blockade of central 5-HT_{2A} receptors by SR46349, without altering either basal nor COC-, nor MOR-stimulated DA outflow, reduced the DA effect elicited by AMP. In contrast, blockade of 5-HT_{2C} receptor enhanced COC-, MOR-, HAL-, but not AMP-stimulated DA outflow whereas 5-HT_{2C} receptor stimulation inhibited HAL-stimulated DA outflow. Of note, 5-HT_{2C} receptor blockade and stimulation increased and decreased basal DA outflow, respectively. Blockade of central 5-HT₃ receptors by MDL 72222 or ondansetron, without affecting basal, AMP- or COC-stimulated DA outflow, reduced the DA effects of HAL, MOR or DRNs. Intra-NAcc application of ondansetron did not alter the effect elicited by HAL or by a low dose of MOR (1 mg/kg) but did reduce the DA effects elicited by a high dose of MOR (10 mg/kg) or by DRNs. Finally, lowering 5-HT tone by the subcutaneous administration of the 5-HT_{1A} agonist 8-OH-DPAT transiently reduced the effect elicited by the high, but not the low dose of MOR. **Conclusions:** 5-HT_{2A} receptors facilitate NAcc DA outflow originating from DA synthesis while 5-HT_{2C} receptors inhibit impulse-dependent DA release likely by inhibiting DA neuronal firing. Central 5-HT₃ receptors exert a state-dependent excitatory control on the impulse-dependent DA outflow and NAcc 5-HT₃ receptors are recruited when both 5-HT and DA tone are enhanced. The fact that 5-HT receptor subtypes control DA activity by acting at distinct levels of DA neuron function opens the possibility to selectively target the mechanisms involved in the DA-stimulating effects of the different drugs of abuse.

5-HT_{1B} Receptors Modulate Cocaine Sensitization

John F Neumaier*, Evelyn S Vincow and Blair J Hoplight

Psychiatry, University of Washington, Seattle, WA, USA

Background: Cocaine has well recognized effects mediated via the dopamine transporter, but cocaine also has high affinity for the serotonin transporter, and the role of serotonin receptors in the mechanisms of addiction is increasingly apparent. Among these receptors, the 5-HT_{1B} receptor has received a great deal of attention, partly due to contradictory results in different experimental models. We have focused primarily on the role of 5-HT_{1B} receptors expressed in nucleus accumbens shell (NAcc) neurons that project to ventral tegmental area (VTA), as these have been hypothesized to lead to disinhibition of dopaminergic projections from VTA back to NAcc. **Methods:** We injected an HSV viral vector expressing either epitope tagged 5-HT_{1B} receptor and GFP (HA1B/GFP) or GFP-only (as control) bilaterally into rat NAcc and performed in combination with

testing for cocaine induced locomotor hyperactivity or conditioned place preference. In the second experiment, rats were pretreated with a 5-HT_{1B} antagonist or vehicle, then cocaine or saline prior to locomotor testing. In the third experiment, a binge pattern drug administration strategy was used to investigate the regulation of 5-HT_{1B} mRNA by cocaine. **Results:** The HA1B/GFP vector induced intense 5-HT_{1B} expression by NAcc neurons and was also detected in axon projections to VTA. Increased 5-HT_{1B} expression in these projections increased the locomotor hyperactivity induced by acute cocaine injection without altering basal locomotor activity. In conditioned place preference, increased 5-HT_{1B} expression shifted the cocaine dose-response curve to the left, while GFP-only expressing animals had no change as compared to sham operated animals. These results suggest that 5-HT_{1B} receptors in NAcc projections to VTA sensitize animals to the behavioral effects of cocaine. In order to test this idea from another perspective, we determined whether SB 224289, a highly selective 5-HT_{1B} antagonist, would diminish the behavioral effects of cocaine. SB 224289 reduced cocaine-induced locomotor hyperactivity without affecting basal locomotor activity, although this drug also induced some behaviors suggesting increased anxiety. These results support the potential role of 5-HT_{1B} receptors in cocaine sensitization, and raise the possibility that 5-HT_{1B} antagonists may be potential therapeutic agents, although side effects may also be problematic. We next examined the effect of acute or chronic cocaine administration on 5-HT_{1B} mRNA expression in NAcc and striatum. Animals were treated with either saline or cocaine (15 mg/kg) three times daily for one day or 21 days; these animals were compared to unhandled cage controls. Both acute and chronic cocaine increased 5-HT_{1B} mRNA in striatum and NAcc by 60-80%. These data suggest that endogenous 5-HT_{1B} receptors are upregulated by binge type cocaine administration in a rapid and sustained manner, further supporting their role in cocaine sensitization. **Discussion:** These data suggest that 5-HT_{1B} receptors participate in cocaine sensitization. Cocaine exposure greatly increases 5-HT_{1B} expression and manipulation of 5-HT_{1B} receptors alters the behavioral effects of cocaine. It is now important to determine the role of 5-HT_{1B} receptors in brain reward circuits on other stages of psychostimulant addiction, such as withdrawal and reinstatement.

Serotonin Systems Modulate Cocaine-Seeking Behavior: Implications for Cocaine Craving and Relapse

Janet L Neisewander* and Jazmin I Acosta

Psychology, Arizona State University, Tempe, AZ, USA

Background: Exposure to cocaine-associated cues or sampling the drug itself can elicit incentive motivation for cocaine that may manifest as craving and relapse in cocaine abusers and cocaine-seeking behavior in animal models. We have conducted a series of experiments to investigate the role of serotonin systems in extinction and reinstatement of cocaine-seeking behavior. **Methods:** Animals first underwent self-administration training during which lever presses were reinforced by intravenous cocaine infusions paired with light and tone cues. Subsequently, animals underwent extinction training during which lever presses had no consequences and the responding observed in the absence of cocaine reinforcement was used as a measure of cocaine-seeking behavior. Across days of extinction training, cocaine-seeking behavior gradually declined and was then reinstated either by response-contingent presentations of the light/tone cocaine-paired cues or by priming injections of cocaine, reflecting incentive motivational effects elicited by these stimuli. **Results:** We found that enhancing serotonin release via d-fenfluramine administration decreased cue reinstatement of cocaine-seeking behavior, but did not alter cocaine-primed reinstatement reliably. The d-fenfluramine-induced decrease in cue reinstatement was reversed by co-administration of a 5-HT_{2C}-selective antagonist (SB 242,084) but not by a 5-HT_{1A}-selective (WAY 100,907) or a nonselective 5-HT_{2/α}-adrenergic (ketanserin) antagonist. The 5-HT_{2C}-selective agonist,

MK 212, decreased cue and cocaine-primed reinstatement at a dose that did not alter locomotion, and both effects were reversed by SB 242,084. The 5-HT1B/1A agonist, RU 24,969 also decreased both cue and cocaine-primed reinstatement at a dose that did not alter locomotion, and both effects were reversed by the 5-HT1B antagonist, GR 127,935. **Discussion:** The findings suggest that increased stimulation of either 5-HT2C or 5-HT1B receptors may decrease incentive motivation for cocaine elicited either by cocaine cues or priming injections. Understanding the role of 5-HT receptor subtypes in cocaine-seeking behavior may have important implications for developing treatments for cocaine dependence. (Supported by DA11064 and the Ford Foundation)

Panel Session

Accelerators and Regulators of G Protein Singaling: New Targets for Psychiatric Disorders

AGS3 in the Prefrontal Cortex Gates Cocaine Addiction

Peter Kalivas* and Scott Bowers

Neurosciences, MUSC, Charleston, SC, USA

Chronic cocaine administration reduces G-protein signaling efficacy. We recently examined the role of activator of G-protein signaling 3 (AGS3), which selectively binds to GiaGDP and inhibits GDP dissociation. AGS3 was upregulated in the prefrontal cortex (PFC) after withdrawal from repeated cocaine administration. Increased AGS3 was mimicked in the PFC of drug naive rats by microinjecting a peptide containing the Gia binding domain (GPR) of AGS3 fused to the cell permeability domain of HIV-Tat. Infusion of Tat-GPR mimicked the phenotype of rats pretreated with chronic cocaine by manifesting sensitized locomotor behavior and drug-seeking, as well as increased glutamate transmission in nucleus accumbens. By preventing cocaine withdrawal-induced AGS3 expression with antisense oligonucleotides, signaling through Gia was normalized and both cocaine-induced relapse to drug-seeking and locomotor sensitization were prevented. When antisense oligonucleotide infusion was discontinued, drug-seeking and sensitization were restored. Based on these findings it is proposed that AGS3 gates the expression of cocaine-induced sensitization and relapse by regulating G-protein signaling in the PFC.

The Striatal Enriched Protein RGS9 is a Major Modulator of Morphine Actions

Venetia Zachariou*, Dana Selley, Laura Sim-Selley, Rachael Neve, Stephen Gold and Eric Nestler

Pharmacology, University of Crete, Heraklion, Crete, Greece

Opioid alkaloids exert their analgesic and reinforcing effects by activating the mu opioid receptor. Upon activation this receptor couples via the Gi/o family of G proteins to various effectors, including the adenylyl cyclase signaling pathway, a system highly involved in the molecular adaptations following chronic drug use. Regulators of G-protein signaling (RGS) proteins are GTPase activating proteins that inhibit G protein function by reducing the duration of the activated GTP bound subunit state of the G protein. They may also have additional functions related to the scaffolding or trafficking of receptor signaling components. Most of the known RGS proteins are present in the brain and several subtypes exhibit striking regional specificity (Gold et al., 1997). An example is RGS9-2, which is very abundant in the striatum and also expressed at moderate levels in other areas mediating responses to opiates, such as the periaqueductal gray (PAG) and the superficial dorsal horn of the spinal cord. Each of these regions is involved in opioid actions, raising the possibility that RGS9-2 may modulate opioid receptor function. In this study, we are show-

ing that activation of the mu opioid receptor by acute or chronic morphine treatments regulates RGS9-2 protein expression in each of these three CNS regions. Acute morphine administration results in upregulation of RGS9-2 protein levels, an effect that can be perceived as a desensitization mechanism whereas chronic morphine causes a dramatic downregulation of RGS9-2, an effect that can be explained as an adaptive mechanism to enhance cell responsiveness. In cell culture models, we have also observed changes in RGS9 cellular localization upon acute opiate treatment, whereas mu opiate receptor internalization rate is also affected by this protein. To further investigate the influence of RGS9-2 on mu opioid receptor signaling, we used mice with a functional deletion of the RGS9 gene and monitored their responses to morphine in several behavioral paradigms. While deletion of the RGS9 gene does not affect pain thresholds, it potentiates morphine analgesia and slows the development of morphine tolerance. Morphine dependence is also affected by the absence of RGS9, since RGS9-/- mice experience exacerbated opiate withdrawal and neuronal activity in the locus coeruleus of dependent mice is twofold greater in the absence of RGS9. Deletion of the RGS9 gene is resulting in a tenfold increase in the sensitivity to the rewarding effects of morphine. To confirm that the reward phenotype is related to the actions of RGS9 in the nucleus accumbens we used viral mediated gene transfer, to infect the nucleus accumbens of RGS9 knockout mice with an HSV-RGS9-2 virus. While mice lacking RGS9 infected with control virus maintained their sensitivity to morphine, mutant mice infected with the HSV-RGS9-2 virus had similar responses to those of their wild type littermates. These data are suggesting that RGS9 in the nucleus accumbens plays a dramatic role in modulation of opiate rewarding effects. Our studies provide in vivo evidence for a physiological role of RGS9-2 as a negative regulator of mu opioid receptor function.

RGS4 As a Molecular Point of Convergence in Schizophrenia

Pat Levitt*, Philip J Ebert and Daniel B Campbell

Vanderbilt University, Nashville, TN, USA

Background: Transcript expression and genetic analyses indicate that disruption of Regulator of G-protein signaling 4 (RGS4) is associated with schizophrenia. Because of its potential role in modulating the extent of signaling by a variety of different G-protein coupled receptors, we have proposed that genetic susceptibility in a heterogeneous and complex disorder like schizophrenia might be related to disruption of RGS4 function. **Methods:** To begin to explore the biological role of RGS4, and how it might be involved in the pathogenesis of schizophrenia, we have generated a series of transgenic mice using bacterial artificial chromosome (BAC) methods to enhance the reliability of transgene expression in brain regions that normally express the transcript encoding RGS4 protein. **Results & Discussion:** Four transgenic lines express low, intermediate and high levels of the transgene. GFP reporter expression from the RGS4 locus allows us to map patterns of expression of RGS4 in detail. All lines exhibit identical patterns of GFP distribution, which strongly correlates with endogenous RGS4 mRNA expression. Our analysis reveals dynamic expression of RGS4 in the embryonic, juvenile and adult mouse, with expression noted in cortical, striatal, thalamic, hypothalamic, hippocampal, sensory and olfactory regions. GFP expression is evident as early as embryonic day (e) 9.5, peaks between the second and third postnatal week in the forebrain, and then decreases generally throughout the neuroaxis. GFP fiber staining is evident in the midbrain regions where monoamine neurons are located. This pattern is different compared to expression of RGS4 transcript in dopamine neurons in the primate midbrain. The transgenic mice also are engineered to over-express RGS4 in an endogenous pattern. We are analyzing the effect of RGS4 over-expression on G-protein coupled receptor expression and potential compensatory down-regulation of other RGS proteins in response to over-express-

sion of RGS4. Supported by: NARSAD Young Investigator award (PE), McKnight Neuroscience of Brain Disorders award (PL) and NIMH P50MH45156.

The Regulators of G Proteins Signaling (RGS) Family of Proteins: Novel Modulators of Mood and the Response to Stress

Cindy M Pudiak, Yan G Ni, Joseph M Arizpe, Eric J Nestler and Stephen J Gold*

Psychiatry, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA

The vast majority of antidepressant and anxiolytic drugs directly or indirectly modulate G protein coupled receptor (GPCR) signaling. Intracellular signals, initiated by activated GPCRs, are potentially regulated by the regulator of G protein signaling (RGS) family of proteins. We are evaluating whether RGS proteins modulate mood states and also whether RGS proteins influence the response to antidepressant and anxiolytic drugs. RGS proteins exert their negative regulatory function by acting as GTPase accelerating proteins for $G\alpha$ subunits. Thus, RGS proteins hasten the transition of the $G\alpha$ subunit from the active, GTP-bound state to the inactive, GDP-bound state. The majority of RGS proteins are abundant in brain with overlapping yet distinct patterns of expression that include the neural circuitry subserving mood and the response to stress. To evaluate the possibility that RGS proteins may be novel drug targets for the treatment of depression, ours and other laboratories have begun characterizing the role of these proteins in mood. Our animal studies on the regulation of RGS activity following stress and/or antidepressant treatments show that RGS proteins are regulated in a subtype- and region-specific manner. For example, following chronic unpredictable stress, RGS4 mRNA is down-regulated in two critical stress-response relays, the paraventricular hypothalamic nucleus and anterior pituitary, but is up-regulated in the locus coeruleus. A potential modulatory role for RGS proteins in antidepressant effects comes from a rat model of electroconvulsive therapy. Thus, chronic electroconvulsive seizures led to specific changes in RGS mRNAs in frontal cortex, hippocampus and hypothalamus. In addition to these studies on the regulation of RGS activity, our functional analyses suggest that RGS proteins modulate responses to antidepressant drugs. Specifically, increased RGS9 activity in rat nucleus accumbens via virally mediated RGS9 over-expression has an antidepressant effect in the Forced Swim Test. A role for RGS9 in regulating mood is further supported by studies of mice that are devoid of RGS9 activity. These mice are hypersensitive to the antidepressant effects of desipramine in the Forced Swim Test. Taken together these studies provide compelling evidence that RGS proteins are important modulators of affective state and the response to stress and may serve as valuable new drug targets for the treatment of depression. This work was supported by NIMH and NARSAD.

Panel Session Is Late Life Depression Alzheimer's Disease?

Epidemiology of Depression in Late Life: Relationship to Cognitive Disorders

Constantine Lyketsos*, Jeannie-Marie Sheppard, Joann Tschanz, Maria Norton, Annette Fitzpatrick and Johns C Breitner

Johns Hopkins University, Baltimore, MD, USA

Background: The Baltimore ECA study has reported two lifetime peaks of depression incidence, the second around age 55. The

same study has proposed a syndrome of "Depression without sadness" later in life, associated with functional decline. Data from many sources now report that depression is a risk factor for Alzheimer's disease. **Aims:** To examine the hypothesis of two depression syndromes in late life, and to study their association with cognitive disorders and conversion to dementia. **Methods:** Secondary analyses of combined data from the Cache County study and the CHS Cognition study, both population studies, in which the Neuropsychiatric Inventory (NPI) was assessed on individuals ≥ 65 years old ($n=1,643$). All were well characterized as to cognitive diagnosis. Six mood symptoms assessed by the NPI were extracted, and Latent Class Analysis (LCA) was applied to examine whether there were subgroups of individuals with different mood symptom profiles in this population. **Results:** 665 participants suffered from dementia, 399 from Cognitive Impairment No Dementia (CIND, aka Mild Cognitive Impairment or MCI), and 579 were cognitively normal. LCA analyses were supportive of three classes of participants based on profile of mood symptoms. Class 1 were individuals with very few to no mood symptoms. Class 2 were individuals with 2-3 mood symptoms on average, with sadness and self-depreciation being most prominent. Class 3 were individuals with 2-3 mood symptoms on average, with anhedonia and loss of interest being most prominent. In both Class 2 and Class 3, almost 30% reported delusions or hallucinations. The prevalence of Class 1 was highest in the cognitively normal, lower in CIND, and lowest in dementia (93%, 79%, 57% respectively). In contrast the prevalence of Class 2 increased substantially from the cognitively normal to CIND or dementia (4.2%, 9.4%, 14%); this increase was more dramatic with Class 3 (1.9%, 10.6%, 30%). The relationship between class membership and conversion to dementia could only be assessed in the Cache County sample. In the non-demented, rates of conversion to dementia after an average of about 3 years of follow-up were 18%, 22%, and 65% for the three Classes respectively. Compared to Class 1, membership in Class 3 ($p=0.02$) but not Class 2 (0.34) was associated with a significantly increased rate of conversion. **Conclusion:** These data support the existence of two mood syndromes in late life, one without sadness. Both were strongly associated with CIND and dementia, but only depression without sadness was associated with conversion to dementia.

The Course of Cognitive and Affective Symptoms of Late Life Depression

George S Alexopoulos*

Psychiatry, Weill-Cornell, White Plains, NY, USA

Case-control studies suggest an association between the syndromes of depression and dementia. In consecutively recruited elders with major depression ($N=236$), we observed that development of dementia was predicted by advanced age, lower education, impairment in instrumental activities of daily living, lower scores of middle insomnia, and higher scores of psychic anxiety, and paranoia. Further studies focused on the profile of cognitive impairment of depression and sought to identify cognitive dysfunctions influencing the course of depression. We observed that performance in tasks of inhibitory control and sustained effort are particularly impaired in late life depression, while tasks of selective attention are equally impaired in elderly and younger depressives. In two different samples, we noted that impairment in some executive functions is associated with poor, slow, and unstable response to antidepressant treatment. We followed these findings with a series of preliminary studies aiming to identify neural abnormalities influencing antidepressant response. Our early findings suggest that abnormal function of the conflict network, but not in the vigilance or the orienting networks, is associated with slow or poor response of late-life depression to citalopram. We also observed that compromised integrity (reduced fractional anisotropy) of white matter lateral to the anterior cingulate gyrus is associated both with executive dysfunction and poor antidepressant response to citalopram. Moreover, high amplitude of the left error

riety of sources at 7 sites in North America), and lack of randomized assignment to treatment conditions (a randomized clinical trial was conducted). Subjects were assigned to medication management (MedMgt), behavior modification (Beh), the combination of these two modalities (Comb), or a community comparison (CC). Intent-to-treat analyses at the end-of-treatment revealed initial growth suppression effects occurred (MedMgt vs Beh at the 14-month assessment documented stimulant-related suppression of height (-1.23 cm/yr) and weight (-2.48 kg/yr) gain, but the growth rates over the next 10-month follow-up phase did not differ for these 2 groups (MTA Group, 2004). However, in the MTA naturalistic follow-up, compliance with assigned treatment conditions decreases over time and progressively does not reflect actual treatment. The analyses of naturalistic subgroups based on actual treatment at the 24-month follow-up (MTA Group, 2004) suggested that growth suppression continue at the same rate from the 14- to the 24-month assessment (height suppression of about -1 cm/yr). At the 36-month assessment, naturalistic subgroups were based on medication use at entry into the MTA and actual treatment reported at the 14, 24 and 36 month assessments of the study, which resulted in 4 clinically meaningful subgroups: (1) Not Medicated (n = 65 stimulant-naive children), (2) Newly Medicated (n = 88 stimulant-naive children with stimulant medication initiated in the MTA), (3) Consistently Mediated (n = 70 children treated with stimulant medication before, during, and after the MTA treatment phase), and (4) Inconsistently Medicated (n = 213 children who started and stopped medication). We also measured height and weight of a local normative control group (LNCG) at the 24-month and 36-month assessments. The growth patterns of these subgroups will be presented and discussed. Similar growth measures were obtained in the Preschool ADHD Treatment Study (PATS), in which 4 and 5 year old children were treated with MPH using a prospective design. Growth patterns in this very young group of children with ADHD will also be discussed.

Growth in the Dopamine Transporter Knockout Animal Model of ADHD

Marc Caron*

Duke University Medical Center, Durham, NC, USA

Dopamine (DA) transporter knockout (DAT-KO) mice, generated through genetic deletion of the DAT by homologous recombination (Giros et al, 1996), display distinct behavioral phenotypes, including hyperactivity, impairments in cognitive tests, and paradoxical inhibitory responses to psychostimulants (Gainetdinov et al, 1999). Similarly, mice with reduced (<10%) expression of DAT demonstrate a modest hyperactivity, impaired response habituation, and paradoxical hypolocomotor reactions to amphetamine (Zhaung et al, 2001). In striking contrast, transgenic mice with modestly increased DAT expression (~25%) show hypoactivity particularly in a new environment (Donovan et al, 1999). Interestingly, in addition to the behavioral phenotypes, the absence of DAT inhibits the molecular and cellular events necessary to generate and maintain full complements of mature lactotrophs and somatotrophs during pituitary development (Bosse et al, 1997). Two striking properties of DAT-KO mice point to dysfunction of the hypothalamopituitary axis: (1) females show an impaired capability to nurse their young and (2) these animals are significantly growth retarded postnatally compared to wild-type littermates. The DAT-KO model has been used to demonstrate that DA reuptake by DAT plays a critical physiological role in the regulation of hypophysiotrophic DA function (Bosse et al, 1997). Hypothalamic DA is carried from the hypophysial portal blood to the anterior pituitary where it inhibits prolactin (PRL) synthesis and secretion through activation of DA-D2 receptors (Caron et al, 1978; Ben-Jonathan, 1985). These characteristics of DAT-KO mice suggest that efficacy and side effects of stimulants, i.e., blockade of DAT and increase in extrasynaptic DA, may exert similar long-term effects on the hypothalamopituitary axis. Human PET studies show that thera-

peutic doses of stimulants increase DA in the striatum (Volkow et al, 2001; Neto et al, 2003). Animal studies of DAT-knockout mice (the extreme of DAT blockade) show that high tonic levels of DA are produced in the hypothalamus, and the overflow is taken by the blood flow to the pituitary and results in growth inhibition (Bosse et al, 1997). Conversely, genetic inactivation of the D2 receptors in mice leads to the eventual development of pituitary hypertrophy suggesting that DA exerts a tonic inhibitory effect on pituitary development and function (Saiardi & Borrelli, 1997). These findings suggest a mechanism that could account for the abnormal growth patterns of the stimulant-untreated and the stimulant-treated children with ADHD: in the stimulant-untreated state, lower than normal DA levels may produce accelerated growth, while in the stimulant-treated state, higher than normal DA levels may produce growth suppression.

Panel Session

Schizophrenia Genes Implicate Convergent Signaling Pathways

NRG1 and Other Genes Affecting the Glutamatergic System Conferring Susceptibility to Schizophrenia

Hreinn Stefansson*, Sascha Haverfield-Gross, Valgerdur Steinthorsdottir, Thorkell Andresson, Maria Bjarnadottir, Mark Gurney, Jeffrey R Gulcher and Kari Stefansson
CNS, deCODE genetics, Reykjavik, Iceland

Several studies suggest that variation in the NRG1 gene confer susceptibility to schizophrenia although no causative mutation has been identified. Three of the nine follow up studies reported find the haplotype initially found at risk in the Icelandic population in significant excess, four of the reports show evidence for linkage disequilibrium with NRG1 and schizophrenia whereas two studies find no association with the disease. Functional studies also lend supportive evidence. Mutant mice heterozygous for either NRG1 or its receptor, ErbB4, show a behavioral phenotype that overlaps with mouse models for schizophrenia. NRG1 hypomorphs have fewer functional NMDA receptors than the wild-type mice and the behavioral phenotypes of the NRG1 hypomorphs are partially reversible with clozapine. Not only does NRG1 have impact on the number of functional NMDA receptors, it also affects the channel properties. We demonstrate that NRG1 signaling, through activation of Fyn and Pyk2 kinases stimulates NMDAR phosphorylation on NR2B Tyr1472, a key regulatory site. The non-receptor tyrosine kinases, Fyn and Pyk2, are associated with ErbB4, the prominent receptor for NRG1 on neurons and both have been implicated in regulation of NMDAR function through tyrosine phosphorylation. Furthermore, we show that NR2B Tyr1472 is hypophosphorylated in mutant mice heterozygous for NRG1 and this defect can be reversed by clozapine at a dose that reverses the behavioral abnormality. These data suggest that NRG1 induced susceptibility to schizophrenia is associated with hypofunction of NRG1 signaling through ErbB4, Fyn and other associated kinases like Pyk2 that phosphorylate regulatory sites on NMDAR subunits, resulting in abnormal modulation of excitatory glutamatergic neurotransmission.

Variation in DISC1 Affects Hippocampal Structure and Function and Increases Risk for Schizophrenia

Joseph Callicott*, Richard E Straub, Lukas Pezawas, Michael F Egan, Venkata S Mattay, Ahmad R Hariri, Beth A Verchinski, Andreas Meyer-Lindenberg, Rishi Balkissoon, Bhaskar Kolachana, Terry E Goldberg and Daniel R Weinberger
NIMH, IRP, NIH, Bethesda, MD, USA

Disrupted-in-schizophrenia-1 (DISC1 NCBI LocusLinkID 27185) is a promising schizophrenia candidate gene expressed predominantly within the hippocampus. We found that a three-marker

single nucleotide polymorphism (SNP) haplotype across 83 kb of the gene was associated with schizophrenia in a family-based sample. Furthermore, a common nonconservative SNP (ser704cys) within this haplotype was associated with schizophrenia. Based on this over-transmission of the ser allele to schizophrenic probands, we sought associations with other phenotypes in healthy individuals. We found that the ser allele was associated with subtle hippocampal deviations in structure and function, specifically reduced hippocampal gray matter volume and altered engagement of the hippocampus during several cognitive tasks assayed with functional magnetic resonance imaging (fMRI). These convergent data suggest that allelic variation within DISC1, directly either at the ser704cys SNP or a SNP or haplotype monitored by it, may increase risk for schizophrenia and that the mechanism of this effect involves structural and functional alterations in the hippocampal formation.

Postmortem Functional Genomics of Dysbindin

Steven Arnold*, D.J. Blake and K. Talbot

Center for Neurobiology & Behavior, University of Pennsylvania, Philadelphia, PA, USA

Twelve studies now report significant associations between schizophrenia and certain haplotypes of single nucleotide polymorphisms in the gene encoding dysbindin-1 at 6p22.3. Dysbindin-1 is best known as dystrobrevin binding protein 1 (i.e., DTNBP1) and is thus associated with the dystrophin glycoprotein complex found at certain postsynaptic sites in the brain. In this series of studies, we have mapped the distribution of dysbindin protein throughout the brain and demonstrate frequent and significant dysbindin-1 reductions at presynaptic sites in the hippocampal formation in two separate schizophrenia samples. The reductions occurred in intrinsic, glutamatergic pathways in strata oriens and radiatum of hippocampal field CA1-3 and especially in the inner molecular layer of the dentate gyrus (DGiml). An inversely correlated increase in vesicular glutamate transporter-1 (VGluT-1) occurred in DGiml of the same cases. Additional studies have assessed the expression of newly recognized dysbindin binding partners including snapin, pallidin, and BLOS-3 in DGiml. Our findings indicate that presynaptic dysbindin-1 reductions independent of the dystrophin glycoprotein complex are frequent in schizophrenia and are related to glutamatergic alterations in intrinsic hippocampal formation connections. Such changes may contribute to the cognitive deficits common in schizophrenia.

Variation in GRM3 Affects Cognition, Prefrontal Glutamate, and Risk for Schizophrenia

Michael Egan*, Richard Straub, Terry E Goldberg, Joseph Callicott, Ahmad Hariri, Venkata Mattay, Alessandro Bertolino, Thomas Hyde, Cynthia Shannon Weickert, Mayada Akil, Joel Kleinman and Daniel Weinberger

Clinical Brain Disorders Branch, NIMH, Bethesda, MD, USA

Background: The metabotropic glutamate receptor GRM3 modulates synaptic glutamate and dopamine concentrations and is therefore a promising candidate gene for schizophrenia. Several prior studies have found evidence for association between GRM3 and schizophrenia. **Methods:** Seven SNP markers within GRM3 were genotyped in a cohort including 217 probands, 311 siblings, 136 controls, all assessed with a battery of neurobiological phenotypes, and 362 parents. Effects of genotype on gene expression and protein levels were also assessed in human post mortem prefrontal cortex. **Results:** A common three SNP marker haplotype was strongly associated with schizophrenia in a family based analysis ($p=.0001$). Within this haplotype, the A allele of SNP 4 was significantly over transmitted to probands ($p=.02$). This allele was associated with poorer performance on several cognitive intermediate phenotypes involving prefrontal and hippocampal function. Physiologic responses to cog-

nitive challenges, assayed with fMRI, showed a relatively deleterious effect of the SNP4 A allele in both brain regions. This allele also predicted lower levels of prefrontal NAA, an in vivo MRI measure of tissue glutamate concentrations. In human prefrontal cortical brain tissue, SNP4 A allele was associated with lower mRNA expression of the glial glutamate transporter EAAT2, a protein regulated by GRM3 that critically modulates synaptic dopamine. **Discussion:** These convergent data suggest that variation in GRM3 increases risk for schizophrenia through its effects on glutamate neurotransmission, prefrontal and hippocampal function and cognition.

Panel Session

Synaptic Biology of Mood Disorders and their Treatment

Persistent Neurobiological Consequences of Early Life Trauma: Reversal by Antidepressant Treatment

Charles B Nemeroff* and Paul Plotsky

Psychiatry, Emory University, Atlanta, GA, USA

There is increasing evidence that severe stress early in life, e.g. sexual or physical abuse, markedly increases the risk of major depression in adulthood (see Maercker et al, *Brit J Psych* 184:482-487, 2004). The search for the neurobiological basis for this effect of child abuse and neglect on vulnerability to depression has been addressed in both preclinical and clinical studies. In rodents, maternal separation early in life is associated with the following alterations in adulthood: 1) increased CRF mRNA expression and CRF concentrations in the hypothalamus, amygdala LC/parabrachial region and the bed nucleus of the stria terminalis; 2) increased HPA axis activation in response to stress, 3) a blunted ACTH response to exogenous CRF and dexamethasone non-suppression of corticosterone; and 4) reduced hippocampal neurogenesis. Many of these alterations, as well as, the behavioral abnormalities of these animals (e.g. increased anxiety) are reversed by chronic treatment with antidepressant drugs (paroxetine, reboxetine and mirtazapine). These findings are concordant with data obtained in clinical studies in patients with mood and anxiety disorders. Supported by NIMH MH-52899 and MH-42088.

Listening to Neurotransmitter Transporters: What They Tell Us About Antidepressant Action

Susan G Amara*, S.L. Ingram, O.V. Mortensen, M.S. Sonders and S.D. Watts

Department of Neurobiology, Univ Pittsburgh Sch Med, Pittsburgh, PA, USA

The plasma membrane transporters for the biogenic amine neurotransmitters dopamine, norepinephrine, and serotonin are of significant therapeutic interest because they are the primary sites of action for many antidepressant drugs known to inhibit uptake, as well as for methylphenidate and psychostimulant drugs of abuse including cocaine, and amphetamine. Analyses of substrate transport, drug binding, ion conductances and carrier regulation have provided additional insights into distinct functional states of biogenic amine transporters and suggest different mechanisms through which drugs can affect carrier function. In some cases the transporters have been observed to have unanticipated functions. For example, using whole-cell and perforated patch clamp recordings, we have shown that substrates of the DAT, such as dopamine and amphetamine, increase the firing activity of dopamine neurons in culture independent of D2 autoreceptor activation. The change in firing rate appears to be regulated directly through the activation of a DAT-mediated anion conductance that can be blocked by cocaine and other DAT-inhibitors. This novel functional property suggests that, in addition to removing dopamine from the ex-

tracellular space, DAT has the capacity to directly modulate neuronal excitability and neurotransmitter release. Substrate selectivity and the modulation conformational states of the carriers can also be important determinants of which neurotransmitter systems are altered by transport inhibitors. Both in vivo and in vitro, serotonin transporters (SERTs) can readily accumulate dopamine, an aspect of SERT function that has generally been overlooked. The transport of dopamine by SERT occurs by a process that is mechanistically distinct from serotonin transport, has different ionic requirements, and is more potently inhibited by a variety of drugs including cocaine and selective serotonin reuptake inhibitors (SSRI's). Moreover, neurochemical and behavioral studies in rodents support the idea that SERT blockade can influence dopamine clearance, illustrating how even the selective blockade of SERT can impact removal of other biogenic amine transmitters. The talk will focus on work examining how the signaling properties, transport characteristics and regulation of biogenic amine transporters can influence and contribute to the actions of antidepressant and psychostimulant drugs in the central nervous system.

G Protein Trafficking and Synaptic Reorganization: Relationship to Depression and Antidepressant Therapy

Mark M Rasenick*, Robert Donati, Jiang-Zhou Yu, Brian T Layden and John Allen

Physiology&Biophysics, U. Illinois Chicago, Chicago, IL, USA;
Psychiatry, U. Illinois Chicago, Chicago, IL, USA

Both in vivo (rat) and in vitro (C6 rat glioma cells) studies demonstrated that Gsa migrates from a Triton X-100 (TTX-100) insoluble membrane domain (TIMD) to a TTX-100 soluble membrane domain in response to chronic treatment with tricyclic or SSRI antidepressants (Toki et al., 1999 J. Neurochem. 73(3): 1114-1120). Very closely related compounds, such as a chlorpromazine or an inactive fluoxetine analog (LY 368514) had no effect. Confocal microscopy revealed that Gsa, but not Goa, moves out of the long cellular processes and process tips of C6 glioma cells after antidepressant treatment (Donati et al., 2001 Mol. Pharm. 59(6):1426-1432). Gsa has been imaged in living cells using an internal-sequence GFP-Gsa fusion protein (Yu and Rasenick, Mol. Pharmacol. 61: 352-359, 2002). Studies in PC12 pheochromocytoma and rat hippocampal neurons reveal Gsa is the "pioneer" G protein in formation of neurite outgrowth. Current studies have focused on examining the possibility that the association between Gsa and tubulin at the membrane is altered in response to antidepressant treatment and that this is relevant to both Gsa redistribution and the increased coupling to adenylyl cyclase seen after chronic antidepressant treatment. We hypothesize that the region of Gsa that interacts with adenylyl cyclase and tubulin are the same. A structural analysis of the interactive domains is consistent with this hypothesis and selected mutations in Gsa can eliminate the influence of tubulin on Gsa. These data suggest that antidepressant-induced changes in the association of Gsa with the plasma membrane and with adenylyl cyclase is due to an alteration in its association with tubulin and that Gsa partitioning is a target of antidepressant action.

Regulation of Cellular Plasticity and Resilience by Mood Stabilizers: The Role of AMPA Receptor Synaptic Trafficking

Hussein Manji*, Neil Gray, Steven T Szabo and Jing Du

LMP, NIMH, Bethesda, MD, USA

Increasing data suggests that impairments of cellular plasticity underlie the pathophysiology of Bipolar Disorder. In this context, it is noteworthy that AMPA glutamate receptor trafficking regulates synaptic plasticity, effects mediated by signaling cascades which are targets for antimanic agents. The present studies were undertaken to

determine if two clinically effective, but structurally highly dissimilar, antimanic agents, lithium & valproate regulate synaptic expression of AMPA receptor subunit GluR1. Chronic (but not acute) treatment of rats with therapeutically relevant concentrations of lithium or valproate reduced hippocampal synaptosomal levels. The reduction in synaptic GluR1 by lithium and valproate was due to a reduction of surface GluR1 distribution onto the neuronal membrane as demonstrated by three independent assays in cultured hippocampal neurons. Furthermore, these agents induced a decrease in GluR1 phosphorylation at a specific PKA site (GluR1-p845), which is known to be critical for AMPA receptor insertion. Sp-cAMP treatment reversed the attenuation of phosphorylation by lithium and valproate and also brought GluR1 back to the surface, suggesting that phosphorylation of GluR1p845 is involved in the mechanism of GluR1 surface attenuation. In addition, GluR1p845 phosphorylation was also attenuated in hippocampus from lithium-or valproate-treated animals in vivo. By contrast, imipramine, an antidepressant which can trigger manic episodes, increased synaptic expression of GluR1 in hippocampus in vivo. It is now well established that glutamatergic neurotransmission plays a critical role in regulating various forms of plasticity; the regulation of synaptic AMPA receptors thus has the potential to contribute to the communication of critical circuits involved in affective functioning and buffering. The mechanisms by which glutamate receptors are actively recruited to synapses have long intrigued the neuroscience community; these novel results suggest that they may play also important roles in the pathophysiology and treatment of complex neuropsychiatric disorders. This progress holds much promise for the development of novel therapeutics for the long-term treatment of severe, refractory mood disorders.

Panel Session

Evolving Concepts in Chronic Pain

Activity-dependent Plasticity and Persistent Pain: Implications for Persistent Pain Management

Ronald Dubner*

Biomedical Sciences - Dental, University of Maryland, Baltimore, MD, USA

Background: Nerve signals arising from sites of tissue or nerve injury lead to long-term changes in the central nervous system and contribute to hyperalgesia and the amplification and persistence of pain. These changes are referred to as activity-dependent plasticity or central sensitization (CS). **Methods:** Molecular and pharmacological approaches are used to determine changes in gene expression and protein levels in the spinal dorsal horn and brain stem after peripheral inflammation and to correlate these changes with behavioral hyperalgesia and allodynia. **Results:** CS in ascending nociceptive pathways involves an increase in the excitability of spinal dorsal horn neurons brought about by a cascade of cellular events including neuronal depolarization, release of calcium from intracellular stores, phosphorylation of ionotropic and metabotropic glutamate receptors via activation of protein kinases, a change in the cell's excitability, and an increase in synaptic strength. Activity-dependent plasticity in descending pain modulating circuits complements the activity-dependent plasticity in ascending pain transmission pathways and leads to enhancement of descending mechanisms after injury. **Discussion:** These findings of activity-dependent neuronal plasticity in ascending and descending nociceptive pathways have important clinical implications in the development of new approaches to the management of deep tissue persistent pain.

Challenges in Translational Pain Research

Christine N Sang*

Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Boston, MA, USA

Background: Pain mechanisms in patients have been categorized by disease, by symptoms, by sensitivity to selective drugs, or by specific tests such as assessments of dynamic and thermal allodynia. Clinical trials show that, even within specific disease categories, there are subgroups of chronic pain patients with different responses to drug treatment. The heterogeneity of pain mechanisms within the study groups may account, in part, for the ceiling analgesic effects and limited response rates of each class of analgesics in these trials. The clinical utility of these compounds may also be limited by the development of dose-limiting side effects. **Methods:** Glutamate receptor antagonists suppress spinal sensitization following peripheral or central nervous system injury through mechanisms that are also thought to reduce pain, hyperalgesia and allodynia. This class of compounds has been extensively studied in animal models of neuropathic pain, but the results of analgesic trials in diagnostic subgroups of pain patients have been disappointing at best. We will present our data in human neuropathic pain models that illustrate potential strategies to widen the therapeutic ratio of glutamate antagonists, including the use of low affinity NMDA antagonists, combination therapy, subunit-selective (NR2B) NMDA antagonists, and selective kainate receptor antagonists. **Discussion:** Despite similar causative processes such as in diabetic neuropathy, mechanisms of pain are likely to be heterogeneous. Our challenge is to identify within individual patients which specific mechanisms are operating to produce which signs and symptoms, so that we may improve the diagnoses and initiate novel treatment strategies for pain.

Fibromyalgia: The Disease State and Treatment Strategies

Lesley M Arnold*

Psychiatry, University of Cincinnati Medical Center, Cincinnati, OH, USA

Background: Fibromyalgia (FM) is defined by the American College of Rheumatology as widespread pain of at least 3 months duration in combination with tenderness at 11 or more of 18 specific tender point sites on the body. Other characteristic symptoms include sleep disturbance, fatigue, and stiffness. Although FM affects an estimated 3.4% of women and 0.5% of men in the general United States population, and is associated with substantial morbidity and disability, the etiology of FM remains unknown. However, recent research has advanced our understanding of the pathophysiology of FM, and progress has been made in identifying potentially efficacious medications for treatment of FM. **Methods:** The following is a review of studies of FM that examine the possible etiologic roles of familial and genetic factors, dysfunction of monoaminergic neurotransmission, disturbances of the stress-response systems, and abnormalities in the central nervous system (CNS) processing of pain. Developments in the pharmacological management of FM from controlled trials will also be reviewed. **Results:** Both genetic and environmental factors probably contribute to the liability to FM. Controlled family studies of individuals with FM found that FM aggregates in families and coaggregates with major mood disorder in families. The coaggregation findings suggest that FM and mood disorders share important and possibly heritable-causal factors. Abnormalities in central monoaminergic neurotransmission might underlie both mood disorder and FM. Both serotonin and norepinephrine are implicated in the pathophysiology of mood disorders and the mediation of endogenous analgesic mechanisms through the descending pain pathways. Dysfunction of serotonin- and norepinephrine-mediated descending pain-inhibitory pathways is a potential mechanism for the pain experienced by patients with FM. Preliminary ge-

netic studies of FM have focused on the association of FM and genetic polymorphisms in monoamine-related genes. Environmental stressors have been associated with the development of FM, and chronic stress might promote disturbances in the stress response system that could lead to FM. Patients with FM have disturbances in the two major interacting stress-response systems, the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis. Although the interpretation of these disturbances is still debated, the available data on the neuroendocrine function in patients with FM suggest that there is mild to moderate reduction in the activities of the HPA axis. There is emerging evidence that FM is associated with aberrant CNS processing of pain. FM patients often develop an increased response to painful stimuli (hyperalgesia) and experience pain from normally nonnoxious stimuli (allodynia). Both hyperalgesia and allodynia reflect an enhanced CNS processing of painful stimuli that is characteristic of central sensitization. Various injuries, including trauma and infections, can also induce a CNS immune response that leads to subsequent production of pro-inflammatory cytokines, which have been implicated in the generation of chronic pain states. Neuroimaging studies of patients with FM have provided further evidence for central augmentation of pain sensitivity in FM. Emerging therapies for FM are based on an improved understanding of possible mechanisms involved in the pathophysiology of FM. Recent, large, randomized, controlled trials demonstrate that patients with FM experience reduction of pain and other symptoms of FM with dual serotonin and norepinephrine reuptake inhibitors, and an alpha2delta ligand. **Discussion:** Continued study of the pathophysiology of fibromyalgia will guide further development of promising treatments for this debilitating disorder.

Pain Disorders and Mood Disorder: Mechanisms and Management

Rollin M Gallagher*

Pain Management, Anesthesiology, Philadelphia VA Medical Center, Philadelphia, PA, USA; Psychiatry and Anesthesiology, University of Pennsylvania, Philadelphia, PA, USA

Background: Affective disorder (AD) is more closely associated with chronic pain conditions (CPC) than other chronic illnesses, suggesting a special relationship. Pain is common in patients with AD in psychiatric settings and in primary care where it is often the presenting symptom of AD. Treatment of AD lowers pain levels, implicating AD as a cause of pain, although AD more often follows the onset of CPC. Treatment studies demonstrate poorer outcomes in MDD cases with pain or in CPC cases with depression. Treatment resistance conferred by untreated co-morbidity may explain longitudinally persistent pain and depression found in prospective studies of older adults. Methodological inconsistencies, such as considering CPC as one illness and identifying AD by equating psychological distress with specific diagnoses (e.g., MDD), contribute to causal uncertainty. Recent epidemiologic studies examine two hypotheses about the nature of the pain-depression relationship. The first, that pain is an affective spectrum disorder, emerged from the theme of the psychogenic pathogenesis of CPC, such as the concept that CPC of unknown origin is masked depression. Studies showing high rates of depression in purported idiopathic pain occurred before imaging and diagnostic blocks that locate specific pain generators and before understanding pathophysiologic processes like sensitization and neuroplasticity that explain phenomena such as allodynia and hyperalgesia that were often labelled as ideopathic. The second hypothesis, AD as a consequence of the stress of living with pain, is suggested by studies showing pain preceding and increasing the risk of developing AD followed by methodologically more sophisticated studies comparing the rates of AD in first degree relatives of controls and persons with specific CPC. **Methods:** A study of patients with temporomandibular pain and dysfunction syndrome (TMPDS), ex-

amined rates of MDD in cases and controls and in first degree relatives of both groups. TMPDS cases with onset of MDD after TMPDS had rates of MDD in first degree relatives similar to rates in relatives of patients or controls without MDD, suggesting that the stress of living with chronic pain is causative of MDD, not familial risk. Cases with pre-morbid and/or family history of MDD developed MDD sooner after TMPDS onset, suggesting pre-pain vulnerability. Using similar methods a comparing community fibromyalgia (FM) sample with community controls showed that rates of MDD in relatives of FM cases without lifetime MDD were virtually identical to rates of MDD in relatives of FM cases with lifetime MDD, consistent with the affective spectrum disorder hypothesis in which FM and MDD share familiarly-mediated risk factors. **Discussion:** Speculation about the shared pathogenesis between FM and MDD includes abnormalities in serotonin and norepinephrine systems. That serotonin norepinephrine reuptake inhibitors (SNRIs) are effective for fibromyalgia and other CCD, particularly neuropathic pain, supports this mechanism. Shared genetic risk factors, such as genetic polymorphisms in serotonin related genes in FM, suggest that increased rates of MDD in families of FM, with or without depression, reflects vulnerability in neurotransmitter systems influencing both. Collectively, extant studies suggest that pain and depression are best treated together. Treating one without the other confers risk of poorer outcomes for either alone. General principles of support, psychotherapy and rehabilitation apply to all chronic illnesses; however, condition-specific, mechanism-based pharmacologic treatment is indicated for different co-morbidity combinations of psychiatric and pain disorders.

Panel Session

Physiological Functions and Therapeutic Potential of Metabotropic Glutamate 5 (mGlu5) Receptors

mGluR1/5 Regulation of NMDA Receptor Trafficking and Gating

R. Suzanne Zukin*

Neuroscience, Albert Einstein Coll. Med., Bronx, NY, USA

Dynamic regulation of synaptic efficacy is thought to play a critical role in formation of neuronal connections and for experience-dependent modification of neural circuitry. The molecular and cellular mechanisms by which synaptic changes are triggered and expressed are the focus of intense interest. Recent advances reveal that NMDA (N-methyl-D-aspartate) receptors (NMDARs) undergo dynamically regulated targeting and trafficking and that the physical transport of NMDARs in and out of the synaptic membrane is critical to several forms of long-lasting synaptic plasticity. Recent studies from our laboratory show that activation of mGluR1/5 increases NMDA receptor channel activity and receptor number at synaptic sites of hippocampal neurons. Recruitment of new channels to synaptic sites occurs via activation of PKC and SNARE-dependent exocytosis. Preliminary findings indicate that SNAP-25 is a downstream target of PKC critical to membrane insertion of channels. Synaptic NMDARs and mGluR1/5 are part of a large macromolecular complex linked to the cytoskeleton and downstream signaling molecules via scaffolding proteins. To identify novel mGluR1/5 binding partners, we developed a novel proteomics strategy based on Tandem Affinity Purification (TAP) and mass-spectrometry which enables isolation and identification of mGluR1/5 protein complexes in situ. mGluRs were N-terminally linked to an IgG-binding domain/TEV cleavage site/poly-histidine tag and expressed in polarized epithelial cells. Receptor-protein complexes were recovered and analyzed by mass-spectrometry. Our results validate the strategy and show its ability to detect proteins that bind mGluRs such as hetero-trimeric Gi and Gs proteins, and discriminate binding partners, such as tubulin, that recognize mGluR1a,

but not mGluR1b. The identification of novel mGluR1/5-interacting proteins is expected to aid in the discovery of novel treatment strategies for schizophrenia and other disorders associated with NMDA receptor dysfunction.

Synergistic Regulation of Prefrontal Cortex Neurons by NMDA and mGlu5 Receptors

Bitu Moghaddam*

Neuroscience, University of Pittsburgh, Pittsburgh, PA, USA

Tonic activation of metabotropic glutamate 5 (mGlu5) receptors is crucial for prefrontal cortex (PFC) dependent cognitive functions such as working memory and instrumental learning. Furthermore, mGlu5 receptors modulate the cognitive-impairing effects of NMDA receptor antagonists. To better understand the cellular mechanisms by which mGlu5 receptors influence cortical function, we recorded ensemble single unit activity in the medial PFC of awake rats in response to the selective mGlu5 receptor antagonist MPEP given alone or in combination with the NMDA receptor antagonist MK801. MPEP decreased the spontaneous bursting activity of the majority of mPFC neurons. This inhibition was activity dependent because greater decreases were observed in neurons with higher baseline firing rates. MPEP augmented the effects of MK801 on burst activity, variability of spike firing, random spike activity, and behavioral stereotypy. These studies suggest that in awake animals, mGlu5 receptors exert two related tonic influences on PFC neurons: (1) an activity-dependent excitatory influence on spontaneous burst activity and (2) potentiation of NMDA receptor mediated effects on firing rate and burst activity. These mechanisms may be critical for the adverse cognitive effects of mGlu5 receptor blockers and the therapeutic potential of mGlu5 receptor activators for treatment of cognitive dysfunction.

Influences of mGluR5 Receptors on Prepulse Inhibition in Wildtype and Mutant Mice

Mark A Geyer* and Suzanne A Brody

Psychiatry, University of California San Diego, La Jolla, CA, USA

Background: Schizophrenia-spectrum patients exhibit deficits in prepulse inhibition of the startle response (PPI), an operational measure of the gating abnormalities suggested to contribute to cognitive disorganization. The glutamate hypothesis of schizophrenia is derived from evidence that phencyclidine (PCP) and ketamine, non-competitive N-methyl-D-aspartate (NMDA) antagonists, produce schizophrenia-like symptoms in healthy humans. Similarly, PPI is disrupted in rats and mice by administration of NMDA antagonists such as the psychotomimetics PCP, dizocilpine, or ketamine. As with the psychotic symptoms induced by PCP or ketamine in humans, the PPI deficits produced by PCP-like drugs in rats are insensitive to dopamine antagonists and typical antipsychotics, but are sensitive to atypical antipsychotics such as clozapine, olanzapine, and quetiapine. Among the glutamate receptors that have been implicated in schizophrenia is the metabotropic glutamate receptor 5 (mGluR5). In rats, the mGluR5 antagonist MPEP, but not the mGluR2/3 agonist LY314582, potentiated the PPI-disruptive effects of PCP. Similarly, MPEP alone did not alter locomotor behavior, but augmented the complex, time-dependent locomotor-stimulating effects of PCP. The effects of MPEP on the response to PCP may reflect the cooperation and co-localization of NMDA and mGlu5 receptors. **Methods:** Startle in mice was assessed using stabilimeter chambers and acoustic or tactile startle stimuli. Both light and acoustic prepulse stimuli were presented at various intensities and prepulse-to-pulse intervals. Wildtype (WT), heterozygous, or null mutant (KO) mGluR5 mice on a C57BL/6 or

129SvPaslco background were derived from homozygous or heterozygous matings, with or without cross-fostering. Drug treatments were administered by subcutaneous injections, usually 10 minutes prior to testing. **Results:** A dramatic loss of PPI in mGluR5 knockout (KO) mice was observed on either the C57BL/6 or 129Sv-Paslco background, while a concomitant dramatic increase in startle reactivity is seen only in the former strain. This PPI deficit was not due to alterations in startle threshold, hearing, or modality of prepulse stimulus. Administration of raclopride, clozapine, or lamotrigine - at doses sufficient to reverse either an amphetamine or a ketamine-induced PPI deficit in mice - failed to reverse the PPI deficit of the mGluR5 KO mice. Thus, neither a typical nor an atypical antipsychotic was effective in rescuing this phenotype. C57BL/6-based mGluR5 KO mice exhibited deficient PPI when raised either by C57BL/6 WT or CD1 dams. Similarly, the PPI deficit in mGluR5 KO mice was seen in the offspring of either homozygous or heterozygous matings. However, the marked increases in startle seen in the mGluR5 KO offspring of homozygous matings were not reproduced in the offspring of heterozygous parents. **Discussion:** These and related findings indicate that mGluR5 receptors contribute to the glutamatergic modulation of PPI in rodents. The fact that neither raclopride nor clozapine attenuated the PPI deficit observed in the mGluR5 KO mice suggests that these mice do not represent a model for detecting antipsychotic effects. It is clear from the purely pharmacological studies that the administered doses of both raclopride and clozapine are sufficient to ameliorate drug-induced PPI deficits in mice. Further experimentation will be necessary to clarify the role of mGluR5 in sensorimotor gating and in the psychiatric populations characterized by deficits in this fundamental form of information processing.

Allosteric Potentiators of the mGlu5 Receptor as a Novel Approach to Treatment of Schizophrenia

Jeff Conn*

Department of Pharmacology, Vanderbilt Medical Center, Nashville, TN, USA

Background : Clinical and basic studies suggest that changes in signaling through the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor may play an important role in some of the pathological changes associated with schizophrenia. These studies have led to the hypothesis that novel compounds that potentiate the function of NMDARs may ameliorate the symptoms of schizophrenia. The mGlu5 subtype of metabotropic glutamate receptor, is a closely associated signaling partner with the NMDAR and may play an integral role in regulating NMDAR function. Activation of mGluR5 potentiates NMDA receptor function in forebrain circuits thought to be disrupted in schizophrenia. Furthermore, activation of NMDA receptors potentiates responses to activation of mGluR5 through a mechanism that is dependent on activation of calcineurin and dephosphorylation of mGluR5. These studies suggest that these receptors are closely associated signaling partners and raise the possibility that activators of mGluR5 could potentiate NMDA receptor mediated transmission. Based on this, it is possible that mGluR5 activators could provide a novel approach for treatment of schizophrenia. **Results:** To determine whether mGluR5 interacts with NMDA receptors in circuits responsible for the behavioral effects of NMDA receptor antagonists thought to reflect psychotomimetic effects, we examined the effect of the mGluR5 antagonist, MPEP, alone and in combination with PCP in a number of schizophrenia related animal models. MPEP alone did not disrupt pre-pulse inhibition (PPI) of the acoustic startle response or increase locomotor activity but potentiated the effect of PCP in both assays. Further, MPEP potentiated PCP-induced disruption of learning in the repeated acquisition procedure and of spatial working memory in a delayed non-matching to position (DNMTP) radial maze task. Finally, icv

injection of the mGluR5 agonist, CHPG reversed amphetamine-induced disruption of PPI. Collectively, these data suggest that mGluR5 regulates NMDA receptor function in circuits involved in behaviors that are used to predict efficacy of antipsychotic agents and in cognitive function. Unfortunately, it has been difficult to develop agonists that selectively activate mGluR5 and have properties that are desirable in drug-like molecules. However, we have now developed two compounds, DFB and CPPHA, that act as novel and selective allosteric potentiators of mGluR5. Neither compound alone has any agonist activity on mGluR5 but both potentiate activation of human and rat mGluR5 by glutamate and other agonists. Neither CPPHA nor DFB alter [3H]-quisqualate binding to the glutamate binding site, but DFB partially competes for [3H]-methoxyPEPy binding to a site for this allosteric antagonist, while CPPHA had no effect on the binding to this site. Furthermore, mutations that inhibit the response to DFB are without effect on the response to CPPHA, suggesting that these compounds act at distinct sites. Most recently, we have used structure similarity searches of a library of > 1 million compounds and high throughput screening of small molecule libraries to identify novel compounds with varying levels of allosteric potentiator activity at mGluR5. **Discussion:** 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 possibly other disorders that involve impaired cognitive function. Interestingly, behavioral studies with allosteric potentiators of multiple mGluR subtypes suggest that these compounds have behavioral effects similar to those of direct acting agonists. Supported by NIH, NIMH, NARSAD, The Stanley Foundation, and Merck & Co.

Panel Session

Medication Development for Addictions: New Approaches

New Treatments for Drug Addiction: Intracellular Targets for Drug Discovery

Eric J Nestler*

Psychiatry, UT Southwestern, Dallas, TX, USA

Most advances in addiction treatment to date have addressed the physical dependence and withdrawal that accompany addiction to some drugs of abuse. In contrast, it has proven more difficult to develop medications that effectively treat drug craving and relapse, the core features of addictive disorders. Current efforts focus on developing medications that prevent a drug from getting to its protein target (e.g., vaccines), that mimic drug action and thereby partially alleviate drug craving (e.g., methadone, buprenorphine, nicotine), or that affect the addiction process per se. The latter approach is most speculative, but also the most promising in terms of translating basic knowledge of addiction into clinical progress. My talk will focus on several examples of the latter approach which offer potential strategies for developing novel medications for treating drug addiction. I will discuss: 1) neurotrophic factors, such as BDNF and its signaling pathways, which modify the long-term actions of drugs of abuse on reward circuitry in the brain; 2) RGS proteins, which modify the actions of G protein-coupled receptors, and hence of drugs of abuse that act via these receptors; 3) the cAMP pathway and the transcription factor CREB, which mediate forms of drug tolerance and dependence; and 4) DeltaFosB, a long-lasting transcription factor, which mediates sensitized responses to drug of abuse. Analysis of target genes regulated by CREB and DeltaFosB reveal several additional potential strategies for medication development. There are many reasons why developing medications to post-receptor targets is challenging and difficult, and not the preferred path of the pharmaceutical in-

dustry. Nevertheless, the vast array of such targets available, and the compelling neurobiological evidence implicating them in the process of addiction, support the importance of this approach.

Genetic Modeling of Chemical Antagonists: Genome-scale Discovery of Neuropsychiatric Disease Drug Targets by In Vivo Functional Analysis

Arthur T Sands*, Ramiro Ramirez-Solis, Joel Edwards, Thomas Lanthorn and Brian P Zambrowicz

Lexicon Genetics, The Woodlands, TX, USA

While the sequencing of the human genome has provided a list of all potential host drug targets, there is a critical need to identify the small subset of these targets the modulation of which will lead directly to the discovery and development of new therapeutics. As a genetic model of a perfectly selective and potent chemical antagonist, the analysis of the phenotypes of knockout mice allows for the prediction of mechanism-based efficacy and side effect profile of target modulation within the context of mammalian physiology. The power of this approach has been demonstrated by studies examining the targets of the best-selling drugs and current pharmaceutical pipelines. These analyses have confirmed a strong (~85%) correlation between the target's knockout phenotype and the efficacy and/or side effects of drugs that modulate them. We have implemented a genome-scale target discovery and validation approach by developing systems and infrastructure to generate and comprehensively phenotype gene knockout mutant mice at a rate of more than 900 lines per year. Gene targeting by homologous recombination and gene trapping have been successfully scaled to mutate all genes in key families encoding proteins that could be modulated by antibodies or small molecule therapeutics. The battery of tests included in our phenotypic analysis program have been specifically selected to reveal those genes that encode key control points in mammalian behavior and physiology encompassing mechanisms with direct relevance significant areas of unmet medical need. With regard to neuropsychiatric disease, phenotypic screens have been implemented to discover genes that affect behaviors relating to surrogate measures of anxiety, depression, musculoskeletal strength, pain, schizophrenia, and learning and memory. Methods for accomplishing genome-scale knockout analysis and selected examples of phenotypes demonstrating new potential mechanisms for therapeutic intervention will be discussed.

Adenosine and Ethanol: From Cell Biology to Treatment

Ivan Diamond*

Ernest Gallo Clinic and Research Center at UCSF, Emeryville, CA, USA

The NAc/striatum is virtually unique because of the unusual co-expression of adenosine A2A and D2 receptors on the same medium spiny neurons. We have reported that ethanol activates PKA signaling primarily through the Golf coupled A2A receptor while D2 mimics ethanol activation of PKA even though it is coupled to Gi/o. Beta gamma subunits released from Gi/o by D2 activation stimulate cAMP/PKA signaling and CRE-mediated gene expression. Sub-threshold concentrations of a D2 agonist (NPA) and ethanol, which have no effect when added separately, act synergistically when added together to increase cAMP/PKA signaling in 10 minutes and CRE-mediated gene expression 5 hours later. Synergy is mediated by beta gamma dimers released from Gi/o and requires A2 and D2 receptors. Using adenovirus constructs to express antisense molecules in primary striatal neurons we will present data identifying the role of several specific signaling molecules required for beta gamma activation of PKA signaling and CRE-mediated gene expression. We have reported that beta gamma dimers in the NAc are required for voluntary alcohol consumption in rats. Because ethanol activates A2 receptors and because simultaneous activation of D2 receptors promotes beta gamma release, we asked whether inhibition of upstream postsynaptic A2A or D2 receptors in the NAc would reduce ethanol self-admin-

istration. We will show that adenosine and A2A receptors are required for operant ethanol self-administration in rats. After confirming that a D2 antagonist given systemically reduces operant self-administration, we will show that an A2A receptor antagonist (DMPX) reduces operant self-administration; an A1 antagonist (DPCPX) is without effect. These results provide evidence that adenosine A2 receptors appear to regulate ethanol preference and consumption. Our findings suggest that A2A antagonists appear to be novel and useful therapeutic agents in the management of human alcoholism. Supported by funds from State of California for Medical Research on Alcohol and Substance Abuse through UCSF and grants from NIAAA and the Department of the Army.

Therapeutic Targets from Brain Gene Expression Profiles

Robert A Harris*

Waggoner Center, University of Texas, Austin, TX, USA

Therapeutic Targets from Brain Gene Expression Profiles R. Adron Harris Waggoner Center for Alcohol and Addiction Research, University of Texas, Austin, TX 78712 Gene expression profiles are becoming available for animal models of addiction as well as some human samples from dependent individuals. This approach has been successful in leading to new therapeutic approaches for some other diseases (e.g., cancers) and emerging addiction databases provide opportunities for elucidation of new targets for addiction pharmacotherapy. We have taken three approaches. First, we used of mutant mouse models that differ in alcohol actions in conjunction with genomic databases (webqtl) delineate key genes and functional pathways that may regulate alcohol reward (Ponomarev et al., *Genes, Brain, Behavior*, in press). Second, expression profiles of brain tissue from human alcoholics delineates several functional pathways that are different from controls (*J Neurochem*. 2004;90:1050). Of interest for therapeutics are changes in genes important for neurodegeneration and neurogenesis. Third, therapeutics is advanced by disease biomarkers (theranostics) and our preliminary results suggest that blood gene expression profiles may provide markers for alcoholism and different therapeutic responses of alcoholics. Supported by NIH/NIAAA.

Panel Session

Differential Sensitivity & Intermediate Phenotype: The Role of Genes in Behavior

Genes for Estrogen Receptors in CNS, Controlling Behavior: Seven Lessons and One Theory

Elena Choleris and Donald Pfaff*

Laboratory of Neurobiology & Behavior, The Rockefeller University, New York City, NY, USA

The stereotypies of mouse social behaviors have encouraged their systematic analysis in this genetically tractable animal. Following experiments with genes for nuclear receptors and other neuroendocrine genes, we can state 7 'lessons' of gene/behavior causal relations bearing on sociosexual and aggressive behaviors. These are as follows: The effect of a given gene on a given behavior depends upon 1. Exactly when and where that gene is expressed in the brain; 2. The gender of the animal in which it is expressed; 3. The age of the animal; 4. The nature of the opponent; and 5. The form of aggression (e.g. testosterone-facilitated aggression vs. maternal aggression). 6. Better social recognition is correlated with lower levels of aggression. We have gathered evidence for a 4-gene micronet involving Estrogen Receptors alpha & beta, oxytocin and the oxytocin receptor as expressed in the hypothalamus and amygdala. 7. Some genetic influences on behavior derive from their effects on fundamental arousal of the mammalian brain, which underlies expression of any emotional behavior. Further, I'll present a theoretical summary of our data which

states: Gene products from ER-alpha are mainly concerned with a long chain of behaviors intimately associated with reproduction — courtship, mating behaviors, parental behaviors. Gene products from ER-beta are important for a range of cognitive and emotional behaviors, with an emphasis on social learning and mood regulation.

Rapid Effects of Estradiol in the Brain: Role in Sex Differences in Drug Abuse

Jill B Becker*

Psychology, University of Michigan, Ann Arbor, MI, USA

Background: Anyone who has gone through puberty will be aware that hormones can have powerful effects on both their body and their brain. We also know that the brains of males and females are different. These differences between males and females begin early during development due to a combination of genetic and hormonal events and continue throughout the lifespan of an individual. One potential impact of these sex differences is in drug abuse. It is estimated that approximately 30% of the 1.8 million Americans who use cocaine are now women. Women are more vulnerable than men to psychostimulant drugs, during all phases of the addiction process: initiation, maintenance, and relapse. In addition to genetic contributions to sexual differentiation of the brain, one mechanism through which sex differences in drug abuse may be caused is the effect of estradiol on ascending dopamine systems. Female rats show a greater behavioral response to striatal dopamine activation than do males, and during behavioral estrus, amphetamine-induced behaviors are potentiated relative to other days of the estrous cycle. Following ovariectomy, rotational behavior is attenuated and replacement with estradiol induces both rapid and long-term effects on dopaminergic activity in the striatum. Furthermore, the administration of physiological concentrations of estradiol to striatal slices directly enhances dopamine release in vitro. Results from studies using whole cell-clamp electrophysiology indicate that estradiol acts on striatal medium spiny neurons to inhibit L-type calcium current by acting at estradiol receptors on the extracellular membrane. Due to the rapid effects of estradiol on the striatum we have investigated how estradiol affects motivated behaviors. **Methods:** We used in vivo microdialysis to detect extracellular concentrations of dopamine and amino acids in striatum and nucleus accumbens, behavioral sensitization to assess long term changes associated with repeated exposure to cocaine and cocaine self-administration to look at how estradiol affects drug taking behavior. **Results:** Female rats exhibited greater behavioral sensitization to cocaine and acquired cocaine self-administration more rapidly than did males independent of gonadal hormones. Estradiol further enhanced this sex difference, while progesterone ameliorated the effects of estradiol when given concurrently. In a study looking at relapse of responding for cocaine, rats relapsed more severely if they were pre-treated with estradiol. Hormone treatment during training did not affect relapse, but estradiol when given with cocaine priming enhanced reinstatement of drug taking behavior. Finally, we find that estradiol attenuates stimulated GABA detected in dialysate from the striatum. So, we hypothesize that the estradiol-dependent enhancement of stimulated dopamine release is mediated through a release of the presynaptic GABAergic inhibition of dopamine terminals. **Discussion:** Estradiol has rapid effects on the striatum and nucleus accumbens that enhance stimulated dopamine release and dopamine-mediated behaviors. We propose that the mechanism mediating this effect is through inhibition of GABA release from medium spiny neurons that have recurrent collaterals that synapse on GABA-B receptors on pre-synaptic dopamine terminals. Thus, there is a release of inhibition in the presence of estradiol that enhances dopamine release. This effect results in greater behavioral activation when these dopamine systems are stimulated in the presence of estradiol. For the behavior of the animal, this means enhanced effects of drugs that act on dopaminergic systems. These results may be helpful for designing effective treatment programs by targeting times during the menstrual cycle when therapy may be more effective. Supported by a NIDA DA12677.

Gonadal Steroids and Mood: Phenotypic Antecedents to the Search for Genes

Peter J Schmidt*, Khursheed Khine, Paula Palladino-Negro and David R Rubinow

BEB, NIMH, Bethesda, MD, USA

Background: One of the targets of genetic research is the susceptibility to the development or expression of specific disorders. The discovery of the polymorphic genetic contributions to susceptibility in turn requires both refined phenotypes and greater understanding of the physiology underlying intermediate phenotypes. As both gender and gonadal steroids may influence the expression of mood and behavior, we performed hormone manipulation studies in several populations in an attempt to characterize both those at risk for hormone-dependent depression and the role of hormone levels (or changes in levels) in those depressions. **Methods:** Four groups of subjects have been studied: asymptomatic premenopausal women followed prospectively with behavioral and reproductive measures until their final menses (FMP), women with the onset of depression occurring during the natural perimenopause, and healthy men and women (with and without severe premenstrual dysphoria [PMD]) undergoing GnRH agonist-induced hypogonadism and sequential gonadal steroid replacement. **Results:** Twenty-nine premenopausal women were followed for an average of five years until their FMP. Nine of the 12 observed episodes of depression occurred in the 24 months surrounding the FMP, suggesting that the late perimenopause is a period of increased risk for depression. A past history of depression was not predictive of depression during the perimenopause. A double-blind, placebo-controlled trial of estradiol significantly improved mood in some (80%) but not all depressed perimenopausal women. Neither presenting symptoms nor baseline hormone levels predicted therapeutic response to estradiol. In women with PMD but not controls, short term exposure to ovarian steroids triggered the onset of mood symptoms; however, in contrast, prolonged exposure to ovarian steroids was associated with a maintenance of the remission of PMD symptoms observed during hypogonadism. These preliminary data suggest that symptoms of PMD are regulated by a physiologic change in rather than by the level of ovarian steroid secretion. In male and female asymptomatic volunteers, hypogonadism was associated with clinically significant mood symptoms in only 10% of men, whereas it more uniformly induced hot flushes and decreased libido. The magnitude of decline in libido varied across subjects, and it was baseline sexual functioning rather than hormone level achieved during replacement that predicted the sexual response to hormone replacement. **Conclusion:** While sex steroids regulate several CNS measures, the genetic polymorphisms to be pursued are those that permit a normal steroid signal to elicit an abnormal or distinct behavioral response.

From Genes to Behavior and Back

Daniel Weinberger

Abstract not available.

Panel Session

Neurobiological Consequences of Chronic Marijuana Exposure: A Translational View

A Longitudinal Evaluation of Cognitive Performance in the Offspring of Marijuana Users; Differential Effects to Those Exposed Prenatally to Cigarettes

Peter Fried*, Barbara Watkinson and Robert Gray

Psychology, Carleton University, Ottawa, ON, Canada

Background: The Ottawa Prenatal Prospective Study (OPPS) is an ongoing longitudinal project that was initiated in 1978. It has examined the neurobehavioral and developmental effects of prenatal exposure to marijuana and cigarettes in a low-risk cohort from birth

through adolescence. Since the early eighties, these children have been the subject of numerous reports examining the association between prenatal exposure to marijuana and a large number of cognitive and behavioral domains. The present report is an examination of cognitive performance in 13-16 year OPPS offspring. Several relatively consistent trends have been noted within the cognitive sphere when these children were younger. Prenatal marijuana exposure does not appear to impact on overall IQ but does have a negative association with tasks/problems requiring complex visual analysis. In contrast prenatal cigarette exposure appears to have a negative impact on IQ and tasks requiring auditory processing and verbal comprehension. **Method:** The outcomes presented in this report are derived from a large neuropsychological battery administered to the OPPS adolescents. The assessment instruments used to evaluate cognition included measures of achievement, general intelligence, memory and facets of executive functioning. Within each of these domains visual and auditory competence was examined. **Results & Discussion:** The major findings can be summarized as follows: prenatal marijuana exposure did not have an impact on IQ but was negatively associated with tasks requiring visual memory, analysis and integration. On the other hand, prenatal cigarette exposure was negatively associated with IQ and auditory memory. These observations remained statistically significant after controlling for a number of possibly confounding factors including the use of marijuana by the subjects. The findings are consistent with and extend the observations in the cognitive domain made in this sample when they were younger and serve to emphasize the differential and long-lasting consequences of in utero exposure to marijuana and cigarettes within this sphere of functioning. **Acknowledgements:** OPPS research over the years has been and continues to be supported by grants from NIDA to PAF

Neurobiological and Behavioral Consequences of Marijuana Exposure During Development

Xinyu Wang, Ellgren Maria, Sabrina Spano, Diana Dow-Edwards and Yasmin L Hurd*

Dept. Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

Drug exposure during the maturation and arrangement of neurotransmitter pathways and functions in the brain has long-term consequences that can alter an individual's vulnerability to substance abuse and psychiatric disorders. Marijuana (*Cannabis sativa*) is the illicit drug that is most used by pregnant women and adolescents, but information is still limited regarding the specific effects of cannabis on the development of neurotransmitter systems linked to the actions of cannabinoids. Our research investigations have focused during recent years on the effects of early marijuana exposure on cannabinoid-, dopamine-, and opioid-related systems in the human fetal brain. In situ hybridization histochemistry was used to measure mRNA expression levels of the cannabinoid receptor (CB1; predominant receptor subtype in the brain), dopamine D1 and D2 receptors, as well as opioid peptide precursors, prepro-dynorphin and preproenkephalin. Our findings have revealed significant gene- and region-specific alterations associated with maternal marijuana use. For example, marijuana-exposed fetuses have reduced D2 mRNA expression in the amygdala, a finding most predominant in males, which is directly correlated to the amount of maternal marijuana use during pregnancy. Studies of the human fetal brain also revealed significant alterations of, e.g., striatal preproenkephalin mRNA in association with in utero marijuana exposure. Animal models, used to examine the long-term effects of early cannabis exposure, also provide evidence of a significant disturbance of the endogenous opioid neuropeptide system when cannabis-exposed offsprings reach adulthood. Both neurobiological (e.g., mu opioid receptor functional coupling in mesolimbic brain structures) and behavioral (e.g., heroin self-administration) abnormalities are apparent in such animals. Altogether these human and animal studies emphasize that early marijuana exposure

has significant effects on mesolimbic and striatal neural systems that apparently lasts into adulthood with significant effects on behavior.

Effects of Marijuana on the Brain as Measured by BOLD fMRI and DSC MRI

Deborah A Yurgelun-Todd*

Psychiatry, McLean Hospital/Harvard Medical School, Belmont, MA, USA

Marijuana remains the most widely abused illicit drug in the United States. Nevertheless, surprisingly little data are available regarding the extent, duration, and potential mechanisms of brain changes associated with marijuana use. Recent studies, which have examined attention, learning, and memory processing in heavy marijuana users, found reduced abilities even after a one-day washout period. The duration of these neuropsychological deficits and the changes in the central nervous system (CNS) which may underlie them remain unclear. As marijuana has been shown to alter brain processes that are responsible for focused attention and memory, we have completed imaging studies to examine potential brain changes associated with these functions. To examine the association between changes in cortical brain regions and marijuana use, we acquired blood oxygen level-dependent (BOLD) fMRI data on current, long-term marijuana users during a supervised 28-day abstinence period. We measured frontal brain activation during tasks of working memory and selective attention. Since we were able to apply fMRI methods within the same individual on three occasions, (days 1, 7 and 28), we were able to characterize the relationship between functional brain activity and cannabis withdrawal. These findings are being used not only to examine the effects of long-term heavy marijuana use, but also to clarify whether or not CNS toxicity due to marijuana use is reversible or irreversible. A complementary set of studies was completed to examine changes in cerebral blood volume and cannabinoid concentration. In these studies, dynamic susceptibility contrast (DSC) MRI data was acquired on current, long-term marijuana users during the 28-day abstinence period and as above, imaging of heavy smokers was completed on days 1, 7 and 28 of the study. Study results suggest that brain response during cognitive challenges is most affected by marijuana in the acute phases of detoxification, whereas global circulatory effects in heavy marijuana smokers persist for a longer period of time than has been previously reported.

Cellular Substrates of Δ^9 -THC and Endogenous Cannabinoid Effects on Brain Drug Reward Circuitry

Carl Lupica*, Arthur Riegel and Alex Hoffman

Cellular Neurobiology Branch, NIH/NIDA-IRP, Baltimore, MD, USA

The addictive potential of marijuana likely reflect its actions on the brain reward circuitry, including the nucleus accumbens (NAc) and the ventral tegmental area (VTA). Also, recent evidence has implicated endogenous cannabinoids (endoCBs) as regulators of synaptic function in the NAc and the VTA, and has indicated that other abused drugs may act on reward circuitry through these molecules. EndoCBs are essential for the initiation of long-term depression (LTD) of glutamatergic cortical afferent input to medium spiny neurons in the NAc, and they also play an emerging role as modulators of synaptic input to the VTA where they are released on demand from dopamine (DA) neurons. A role for LTD and other forms of synaptic plasticity in compulsive drug use has been proposed because, single, or repeated exposure to abused

drugs, such as cocaine, alcohol, or Δ^9 -THC alters these phenomena. Because humans often use marijuana for extended periods, and because CB1 receptors appear to mediate the psychoactive effects of this drug (Huestis et al., Arch Gen Psychiatry 58: 322, 2001), we examined the effect of repeated 9THC exposure on synaptic transmission in the NAc. Electrophysiological recordings in rat brain slices containing the NAc were performed 24h following 7d of treatment with a single daily ip injection of vehicle, Δ^9 -THC (10 mg/kg), or the synthetic CB agonist WIN55,212-2 (WIN, 10 mg/kg). Electrically evoked synaptic glutamate potentials in the NAc were inhibited by WIN in a concentration-dependent fashion, in slices from vehicle-treated animals ($EC_{50} = 143$ nM). However, concentration-response curves were shifted to the right in slices from chronic WIN- or Δ^9 -THC-treated animals ($EC_{50}s = 1.2$ μ M). Also, the inhibition of GABAergic IPSCs by WIN was reduced in slices from chronic WIN- and Δ^9 -THC-treated rats. The consequences of this treatment on plasticity were also investigated. LTD was initiated via stimulation that mimicked a naturally occurring cortical glutamatergic input frequency (10 Hz, 5min), and was blocked by the CB1 antagonist SR141716A, indicating dependence on endoCBs and CB1 receptors. LTD was completely absent following chronic Δ^9 -THC or WIN in vivo, suggesting that adaptations occur in this brain reward circuit following prolonged CB exposure. Experiments were also performed to determine whether endoCBs regulate DA neuron activity in the VTA. IPSCs mediated by GABA^B receptor activation were elicited in DA neurons in the VTA. These currents were inhibited by WIN, and this was reversed by the CB1 antagonist AM251, localizing CB1 receptors on GABAergic terminals in the VTA. Additional experiments revealed that manipulations increasing DA neuron excitability (blockade of postsynaptic SK k⁺ channels, or antagonism of presynaptic metabotropic glutamate autoreceptors) could initiate endoCB release that then inhibited glutamate and GABA release onto the DA neurons. Chelation of Ca²⁺ with BAPTA in the DA neurons blocked endoCB production and implicated the DA neurons as the source of the endoCB. Collectively, these data indicate that endoCBs modulate the brain reward circuitry, and identify specific substrates upon which marijuana, and the endoCBs act within these circuits.

Panel Session

Face to Face: Face Processing in Health and in Schizophrenia

fMRI Investigations of the Fusiform Face Area (FFA)

Nancy Kanwisher*

McGovern Institute for Brain Research, MIT, Cambridge, MA, USA;
Brain & Cognitive Sciences, MIT, Cambridge, MA, USA

fMRI experiments from many labs over the last 8 years have demonstrated and explored a face-selective region of cortex called the fusiform face area (FFA), that can be easily identified in essentially any normal subject by its much higher response to faces than to a wide variety of other stimulus types. The response of the FFA is correlated on a trial-by-trial basis with success at both detection and identification of faces, but not with the detection or identification of a wide variety of nonface stimuli, including stimuli for which the subject has gained substantial expertise. Although it has been claimed that the FFA may play an important role in the processing of some classes of nonface stimuli, current evidence supports a strong degree of domain specificity of this region for the processing of faces per se. Ongoing research is now investigating these questions: i) what exactly does the FFA do with faces?, ii) what is the nature of the representations extracted in the FFA?, iii) how sharp is the profile of face selectivity across the cortex, and iv) how does the FFA arise in development?

Fusiform Gyrus Volume Reduction and Altered Face Processing in Schizophrenia

Robert W McCarley*, Martha E Shenton, Toshiaki Onitsuka,
Paul Nestor, Margaret A Niznikiewicz and Dean Salisbury

Psychiatry, Harvard/VAMC, Brockton, MA, USA

The fusiform gyrus (occipitotemporal gyrus) is thought to be critical for face recognition and may possibly be associated with impaired facial recognition and interpretation of facial expression in schizophrenia. Postmortem studies have suggested that fusiform gyrus volume is reduced in schizophrenia. In the initial in vivo structural study of fusiform gyrus in schizophrenia, we used high spatial resolution MRI to measure the gray matter volume of fusiform gyrus in 22 first-episode patients with schizophrenia (first hospitalization), 20 first-episode patients with affective psychosis (mainly manic), and 24 normal controls. First-episode patients with schizophrenia had overall smaller relative volumes (absolute volume/intracranial contents) of fusiform gyrus gray matter when compared with both controls (9%) and patients with affective psychosis (7%). For the left fusiform gyrus, patients with schizophrenia showed an 11% reduction compared with controls and patients with affective psychosis. Right fusiform gyrus volume differed in patients with schizophrenia only when compared with controls (8%). These findings suggest that schizophrenia is associated with bilateral reduction of fusiform gyrus gray matter volume that is evident at the time of first hospitalization, and is different from the presentation of affective psychosis. In a second study we used high spatial resolution MRI to investigate relationships between fusiform subregions and immediate and delayed memory for faces in patients with chronic schizophrenia. Compared with normal controls, patients with schizophrenia had overall smaller fusiform gray matter volumes, and their degree of poor performance on delayed memory for faces was significantly correlated with the degree of bilateral anterior FG reduction. This indicated that neuroanatomical FG abnormalities may underlie at least some of the deficits associated with facial recognition in schizophrenia. In a third study we looked at whether or not patients with schizophrenia show less activation in neural networks related to face processing than healthy subjects, and the relationship between functional abnormalities and MRI anatomical abnormalities in the fusiform gyrus (FG). The functional measure was the N170 evoked potential, which considerable evidence suggests indexes the processing of face information. EEG has the advantage of high temporal resolution compared to fMRI or positron emission tomography, and, when abnormalities in the N170 are linked to structural abnormalities, provides anatomical localization information. Twenty patients with chronic schizophrenia, in contrast to age and PSES-matched controls, showed bilateral N170 reduction to faces but not to other objects at posterior electrodes. Patients also showed bilateral anterior and posterior FG gray matter volume reduction (10%). In addition, the right posterior FG volume was significantly correlated with N170 to faces at the right occipitotemporal electrode ($r = -0.76$ at PO10) in patients with schizophrenia, but not in normal controls. These results provide clear evidence for deficits in the early stages of face processing in schizophrenia, whose association with FG volume reduction points to a defective anatomical substrate for face processing.

Amygdala, Ambiguity and Psychopathology: Functional Neuroimaging Studies of Facial Expressions of Emotion

Paul J Whalen* and Hackjin Kim

Psychiatry, University of Wisconsin, Madison, WI, USA

Background: Neuroimaging studies have shown that facial expressions of emotion, particularly fearful expressions, robustly activate the human amygdaloid complex. Work in our laboratory has sought to understand amygdala responsivity to fearful expressions by considering the information value of these stimuli, compared to other expressions. For example, we have suggested that the amygdala

is particularly sensitive to fearful faces because of what they do not predict for the viewer. That is, a fearful face is more context-dependent (e.g., something negative has occurred in your environment, and you would do well to figure out what that is) than say an angry face, which is itself the source of danger. Surprised facial expressions also provide an important comparison expression for fearful faces, and offer another opportunity to rethink the function of the amygdala. Like fearful faces, surprised faces signal the occurrence of an unknown event in the nearby context. But in the case of surprise, the predicted valence of the eliciting event is not clear (i.e., could be negative or positive), whereas fearful faces more clearly predict that the event is negative. **Methods:** Twenty-one subjects passively viewed repeating blocks of surprised and neutral faces during fMRI [Fifteen of these subjects were reported on in Kim et al (2003, *Neuroreport*, 14: 2317-2322) and all scanning parameters are described there]. Following scanning, subjects provided valence ratings of surprised and neutral face blocks based upon a scale from 1-9 where 1 was very positive, 5 was neither negative or positive and 9 was very negative. The additional subjects presented here allowed us sufficient numbers to separate subjects into categories based upon their valence ratings of surprised faces (i.e., positive, neither, negative). **Results:** Right ventral amygdala responsivity to surprised vs. neutral faces showed a significant interaction with medial prefrontal responsivity based upon valence rating group membership [negative (N=8) vs. positive (N=6)]. This effect was significant when the right amygdala was compared to either the right ventromedial prefrontal cortex (vmPFC; $F = 34.4$, $p = .00015$) or the left vmPFC ($F = 24.226$, $p = .00035$). Thus, subjects who interpreted surprised faces negatively showed high amygdala and low prefrontal activation, while subjects who interpreted the same stimuli positively showed low amygdala and high prefrontal activation. Subjects who rated surprised faces as neither neg or pos showed no appreciable signal change at these same amygdala and prefrontal loci. **Discussion:** If we assume that all subjects (regardless of valence rating) showed high initial amygdala responsivity (see Kim et al 2003), then these emergent individual differences related to valence interpretations may reflect individual differences in spontaneous prefrontal regulatory abilities. Further, this circuitry may be similar to that invoked in the extinction of conditioned responses. That is, surprised faces, like extinguished tones, offer two possible predicted outcomes: one positive, one negative. It is this ambiguity of valence that necessitates a regulatory input from the prefrontal cortex to gauge amygdala responsivity to surprised faces. A similar amygdala-prefrontal circuitry is hypothesized to play a role in the failure of regulation believed to contribute to a host of psychopathological disorders. The use of facial expressions of emotion as presented stimuli in human neuroimaging studies represents a simple and tolerable strategy for assessing potential dysfunction of this prefrontal-limbic circuit in psychopathology. Supported by NIMH and HHMI.

Face to Face: Face Processing in Health and in Schizophrenia

Nancy Kanwisher, Robert W McCarley, Paul J Whalen, Ruben C Gur* and Raquel E Gur

Psychiatry, Neurology, & Radiology, U Pennsylvania, Philadelphia, PA, USA

This symposium presents the cognitive neuroscience background and evidence for deficits in face and face emotion processing in schizophrenia. As first reported by Kanwisher, fMRI data indicated face recognition was mediated via a specialized processing area in the fusiform gyrus, the fusiform face area (FFA). Questions then arose if the FFA might also be engaged in within-category identification of non-faces and if face recognition might be a subclass of expert object recognition. Kanwisher (*Nat. Neurosci.* 2004 May;7:555) very recently showed that FFA activation was associated with both detecting the presence of faces and identifying specific faces. In contrast, for non-face objects (including cars seen by car experts), within-category identification was associated with activation outside the FFA. These

results support FFA domain specificity. McCarley's data indicate face processing is abnormal in schizophrenia. Both chronic and first episode schizophrenic patients (but not psychotic manic patients) have reduced MRI gray matter volume in the fusiform gyrus. The extent of the volume reduction predicted the extent of errors on delayed recall of faces. Moreover, new data have indicated schizophrenic patients (N=20) have a specific reduction in amplitude of the event-related potential elicited by faces, the N170, but not the N170 elicited by other objects. The extent of N170 amplitude reduction was highly correlated with the extent of right fusiform gyrus volume reduction ($r = -0.76$). Facial expression of emotion is a puissant feature in social interaction. fMRI data from Whalen indicate that separate brain and amygdala circuits likely process information about fear and about surprise. This resolves the debate as to whether the amygdala is primarily activated by fear or by surprise (novelty, uncertainty), since these are not mutually exclusive. Surprise evokes more medial prefrontal cortex activation and a more dorsal amygdaloid activation whereas fear (negative valence) evokes a stronger amygdaloid activation on the right & in a more ventral region. Interestingly, subjects with high state anxiety react to neutral faces with an activation pattern akin to the reaction to fearful faces. Gur will describe a novel methodology (based on shape transformation) that allows a quantitative description of changes in facial display as the intensity of an emotion evolves from neutral to peak and also allows a nearly complete control over the level of difficulty in emotion classification. In healthy subjects, 4T BOLD fMRI showed selective amygdala activation to judgment of emotional valence relative to judgment of age. Patients with schizophrenia showed an equal number of activated voxels throughout the brain, but diminished amygdala activation that was associated with deficits on emotion processing, greater severity of negative symptoms, and poorer outcomes. To our knowledge face processing has not been a topic of an ACNP symposium, although it is of obvious importance in social interaction. The data from the speakers have not heretofore been presented at ACNP, and are unique to their laboratories.

Panel Session

Functional Neuroimaging in Bipolar and Unipolar Disorders

Changes in Brain Structure in Depression

Yvette Sheline*, Mark A Mintun, Robert C McKinstry and Abrahm A Snyder

Psychiatry, Radiology, Neurology, Washington University School of Medicine, St. Louis, MO, USA

Both early onset and late onset depression have been associated with structural brain changes. Reports of brain changes within a specific neuroanatomical circuit, originally described by Nauta (1972), has been called the limbic-cortical-striatal-pallidal-thalamic (LCSP) tract (Swerdlow & Koob, 1987). The structures that comprise it are extensively interconnected (Price et al., 1987) and include the hippocampus, amygdala, caudate, putamen, and frontal cortex. Brain changes in this circuit associated with early-onset recurrent major depression (EORD) or primary depression will be reviewed, including both clinical and post-mortem studies. Data will be presented on the effects of antidepressant treatment on morphological changes. Studies will be presented of structural changes in late onset depression (LOD) both with and without known association with neurological disorders. A summary of studies to date will be discussed on effects of white matter hyperintensities (WMH) on treatment outcome and preliminary data on the association of quantitative measures of WMH and treatment outcome will also be presented. Finally, potential mechanisms for structural brain loss in depression will be explored and the question of whether depression is the cause or effect of abnormalities in brain structure will be addressed.

Functional Neuroimaging Abnormalities to Unipolar and Bipolar Depression: Relationship to Neuropathological Changes and Treatment Outcome

Wayne Drevets*

National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA

Neuroimaging studies of major depressive (MDD) and bipolar disorders (BD) demonstrate abnormalities of brain function and structure in areas of the prefrontal cortex, cingulate cortex, amygdala, hippocampus and anatomically-related areas of the striatum and thalamus. The structural abnormalities in these regions include differences in grey and white matter volume that persist between illness episodes, may become more prominent during recurrent illness, and may antedate the onset of mood episodes (as evidenced in never-depressed subjects at high familial risk for mood disorders). Post mortem studies of MDD and BD show reductions in grey matter, synaptic markers, and glial cells/markers and increases in neuronal density in regions where MRI studies show abnormal tissue volume. Treatment with mood stabilizing agents that exert neuroplastic effects in rodents appears capable of partly correcting the grey matter volumetric abnormalities in BD. The regions containing volumetric abnormalities in MDD and BD show elevated glucose metabolism in the depressed relative to the euthymic phase, and altered hemodynamic responses during exposure to emotionally valenced stimuli during depression. In the amygdala, hemodynamic responses to sad stimuli persist for abnormally long time periods and fail to show normal habituation during repeated stimulus exposure. During chronic antidepressant drug treatment regional metabolism returns to normative levels, and hemodynamic responses of the amygdala to emotionally valenced stimuli attenuate. Because the glucose metabolic signal is dominated by glutamatergic transmission, the metabolic data suggest depression is associated with elevated excitatory transmission in limbic-cortical-striatal-pallido-thalamic circuits. In rodents interactions between glutamatergic transmission and the elevated glucocorticoid secretion produced by repeated stress result in dendritic reshaping in these circuits. Because the regions implicated participate in modulating emotional behavior, the grey matter deficits in mood disorders may conceivably disinhibit emotional expression. If so, the neuroplastic effects of mood stabilizing and antidepressant drugs to restore or preserve neural tissue in such regions may partly underlie their therapeutic mechanisms.

Clarifying the Functional Neuroanatomy of Bipolar Disorder Using sMRI and fMRI

Stephen Strakowski*

University of Cincinnati, Cincinnati, OH, USA

Structural MRI studies of bipolar disorder suggest an anatomic substrate of abnormalities within anterior limbic (prefrontal-cortical-medial temporal) networks that are involved in the modulation of mood and cognition. Structural studies are limited by the uncertainty of whether structural abnormalities correspond with actual neural dysfunction. Integrating fMRI into the study of bipolar disorder addresses this limitation. Ideally, of course, we would want to study the spontaneous occurrence of mood episodes in the MRI scanner, but practically this is not possible. Instead, capitalizing on the well described reciprocal activation of mood and cognitive brain networks (Mayberg et al 1999), cognitive tasks can be applied in the scanner to activate brain networks of interest. Consequently, we have used attentional and working memory tasks as cognitive probes to study the functional neuroanatomy of bipolar disorder. We have focused this work on euthymic patients to eliminate the potential confound of mood state, and have been studying patients on medications as well as those who have taken themselves off of medications. In a study using a continuous performance task (the CPT-IP) as a probe, we observed inappropriate activation of brain regions that modulate mood, that appeared to be compensated for by posterior attentional regional activation

to permit normal task performance in euthymic, unmedicated patients (Strakowski et al 2004). In a slightly more difficult working memory (N-back) task, in which the patients exhibited mild impairments relative to healthy subjects, they also exhibited increased activation within cognitive brain areas (i.e., DLPFC; Adler et al, in press). Recently, we administered a more challenging counting Stroop task to euthymic bipolar patients, who exhibited moderate impairments relative to healthy subjects. While doing this task, patients exhibited decreased activation in brain areas that modulate error detection and response inhibition (e.g., cerebellar vermis, putamen, BA 10) consistent with an impulsive performance pattern observed in the patients. When comparing patients on and off of medications, however, those on medications exhibited increased activation in brain regions primarily involved in with Stroop tasks (DLPFC, anterior cingulate), suggesting that treatment helps to normalize brain activation and performance even in euthymic patients. Together these results suggest dysfunction within brain networks that maintain emotional and cognitive homeostasis, leading to the dynamic symptoms of bipolar disorder.

Regional Structure, Function and Connectivity Within an Amygdala-Ventral Prefrontal Neural System in Bipolar Disorder

Hilary Blumberg* and John H Krystal

Psychiatry, Yale University School of Medicine and VA CT Healthcare System, West Haven, CT, USA

Background: Converging evidence suggests that abnormalities in an amygdala-ventral prefrontal neural system in bipolar disorder contribute to disturbances in affective and cognitive regulation in the disorder. Structural and functional abnormalities within amygdala and ventral prefrontal cortex have been reported in bipolar disorder. The substantial reciprocal connections between these brain structures suggest that abnormalities originating within the individual structures, or within the connections between the structures or to other brain regions with significant connections to this system, could contribute to deficits in the optimal functioning of this neural system. **Methods:** Structural magnetic resonance imaging (MRI), functional magnetic resonance imaging (fMRI) and diffusion tensor MRI (DT-MRI) were performed in patients with bipolar disorder and healthy comparison research participants. **Results:** Volume and activation abnormalities in amygdala and ventral prefrontal cortex were observed in patients with bipolar disorder, as compared to healthy participants. DT-MRI and steady state fMRI findings provide preliminary evidence for abnormalities in the integrity of the structural connections within an amygdala-ventral prefrontal neural system, as well as abnormalities in the functional connectivity within this system. Early evidence also suggests influences of mood-stabilizing pharmacotherapies on the volume and functioning of structures within this circuitry, as well as on their connectivity. **Discussion:** These data point to amygdala and ventral prefrontal cortex, as well as connections between these structures, as targets for treatment interventions. Potential novel therapeutic approaches implicated by these data, considered in the context of emerging data regarding the involvement of specific cell types and particular neurotransmitter and growth factors in bipolar disorder, will be discussed.

Panel Session The Bed Nucleus of the Stria Terminalis: Where Stress and Anxiety Meet Drug Abuse

Role of Gonadal Steroids in Fear vs. Anxiety

Michael Davis*

Department of Psychiatry, Emory University, Atlanta, GA, USA

Emerging evidence indicates that the central nucleus of the amygdala (CeA) and lateral division of the bed nucleus of the stria

terminalis (BNST) are involved in stimulus specific fear vs. anxiety, respectively. We are now finding that gonadal steroids in both males and females affect BNST-dependent modulation of the acoustic startle reflex and not fear-potentiated startle, dependent on the CeA and not the BNST. Intact female rats and ovariectomized (OVX) rats with different ovarian steroid replacement regimes were tested for changes in CRF-enhanced startle (increased acoustic startle amplitude after intracerebroventricular (ICV) infusion of 1 µg CRF - dependent on the BNST and not the CeA). OVX rats injected with estradiol (E), followed by progesterone (P), showed a blunted CRF-enhanced startle effect compared to OVX and E-injected rats. CRF-enhanced startle also was reduced significantly in lactating females (high endogenous P levels) compared to cycling rats (low to moderate P levels), as well as in non-E primed rats when P was administered acutely (4 hrs prior to testing) or chronically (7 day P replacement). These effects were most probably mediated by P's metabolite allopregnanolone (tetrahydroprogesterone: THP) because THP itself had a similar effect and chronic administration of medroxy-progesterone (MPA), which is not metabolized to THP, did not blunt CRF-enhanced startle. Neither chronic P replacement nor acute THP affected fear-potentiated startle, suggesting that P's metabolites have an effect on the bed nucleus of the stria terminalis (BNST) and anxiety rather than on the amygdala and stimulus specific fear. Light-enhanced startle (increased acoustic startle amplitude when rats are exposed to bright light for about 20 min - dependent on the BNST and not the CeA) was greater in females compared to males. Castrated rats had light-enhanced startle comparable in magnitude to females. This could be reversed in males by chronic administration of testosterone (T) or a combination of its metabolites dihydrotestosterone (DHT) and estrogen but not by either one alone. T or E and DHT had no effect on light-enhanced startle in females nor did it alter fear-potentiated startle in either males or females.

Roles for the Extended Amygdala in the Reinforcing and Aversive Effects of Drugs of Abuse

George F Koob*

Neuropharmacology, CVN-7, The Scripps Research Institute, La Jolla, CA, USA

The extended amygdala is a forebrain neuroanatomical construct that involves elements of the basal forebrain with cytoarchitectural and connectivity similarities and includes the central nucleus of the amygdala, bed nucleus of the stria terminalis (BNST), and a transition zone in the shell of the nucleus accumbens. Recent evidence suggests that both the positive reinforcing effects of drugs of abuse and the aversive effects of withdrawal from drugs of abuse involve elements of the extended amygdala including the BNST and the central nucleus of the amygdala. The shell of the nucleus accumbens, BNST and central nucleus of the amygdala are particularly sensitive to the effects of dopamine antagonists in blocking the reinforcing effects of cocaine and γ -aminobutyric acid antagonists in blocking the reinforcing effects of alcohol. However, even more compelling is that the aversive stimulus effects of drug dependence which are hypothesized to contribute to the excessive drug intake associated with dependence recruit activity of brain stress neurotransmitters such as corticotropin-releasing factor (CRF) in the circuits of the extended amygdala. Extracellular CRF is increased in the extended amygdala during acute abstinence to alcohol, cocaine, Δ^9 -tetrahydrocannabinol and nicotine. CRF antagonists administered into the extended amygdala can block not only the anxiogenic-like effects of drug withdrawal, but also the excessive drug intake associated with drug dependence. These results suggest that the extended amygdala may be an important interface for the switch from positive reinforcement of initial drug taking to the negative reinforcement associated with dependence. [This work was supported by the National Institute on Alcohol Abuse and Alcoholism, National Institute on Drug Abuse, and the Pearson Center for Alcoholism and Addiction Research.]

The Bed Nucleus of the Stria Terminalis (BNST) and Circuitry Underlying Increased Drug Seeking During Protracted Withdrawal

Gary Aston-Jones*, Glenda C Harris and Jill Delfs

Psychiatry, University of Pennsylvania, Philadelphia, PA, USA

Elevated brain norepinephrine (NE) has long been implicated in opiate withdrawal, but which NE system(s) are involved is unknown. We found that an NE pathway from the A1/A2 neurons in the caudal medulla to beta NE receptors in the ventral bed nucleus of the stria terminalis (vBNST) is critical for the aversion of precipitated morphine withdrawal. However, this same pathway appeared to be minimally involved in somatic (physical) withdrawal responses, or in the aversiveness of somatic stimuli (footshock). Interestingly, projections from the locus coeruleus were not involved in either aversion or somatic signs of withdrawal. To determine if the NE innervation of the vBNST may also be involved in relapse after drug abstinence, we established a rat model of increased drug-seeking (morphine conditioned place preference) during protracted (> 5 weeks) morphine withdrawal. Staining for Fos revealed that areas of the extended amygdala were stimulated in proportion to morphine preference in non-withdrawn animals, including the cingulate cortex, basolateral and central amygdala and nucleus accumbens. However, the number of Fos neurons in the vBNST was particularly associated elevated preference during protracted withdrawal. We hypothesize that the increased drug-seeking observed during protracted withdrawal involves a conditioned release of NE in the vBNST (in response to the drug associated environment) which elevates anxiety, consistent with other studies. The anxiolytic effect of morphine during conditioning then acts as a negative reinforcer. We propose that this negative reinforcement summates with the positive reinforcement of morphine to produce elevated drug seeking during protracted withdrawal. This anxiety-related mechanism may summate with other responses to chronic morphine exposure (e.g., altered hedonic processing) to produce increased drug-seeking and relapse potential characteristic of protracted withdrawal. Supported by PHS award R37 06214.

Roles for CRF and Noradrenaline in BNST in Stress-induced Relapse

Jane Stewart*, Robert Sorge, Suzanne Erb and Francesco Leri

Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, QC, Canada

Background: In animals trained to self-administer cocaine and other drugs of abuse, stress is a powerful instigator of relapse after extinction. Extrahypothalamic CRF-containing cell groups in the central amygdala (CeA) and bed nucleus of the stria terminalis (BNST) are involved in the mediation of this effect. Furthermore, noradrenergic inputs to both of these regions most likely from the lateral tegmental nuclei of the brainstem play an important role in stress-induced relapse. **Methods:** Rats trained to self-administer cocaine intravenously for 7-10 days were given extinction sessions over several days during which no drug was available. Tests for stress-induced reinstatement of cocaine seeking followed during which rats were exposed to a period of intermittent footshock in the test chambers immediately before the lever became available. In another set of experiments, rats were given systemic injections of cocaine or saline for 7 days. After drug-free periods of up to 21 days, the effects of footshock stress on *c-fos* mRNA expression in the CeA and BNST and the effects of i.c.v. injections of CRF on locomotor activity were assessed. **Results:** Bilateral infusions of a CRF receptor antagonist given before exposure to footshock into the BNST, but not into the CeA, blocked stress-induced reinstatement. Also, bilateral infusions of

beta adrenergic receptor antagonists to the CeA or the BNST blocked stress-induced reinstatement. New evidence suggests that stress is a more potent instigator of relapse after a period of abstinence than it is immediately after termination of cocaine taking. In the second set of experiments, cocaine pre-exposed rats exhibited sensitized c-fos mRNA expression in the CeA in response to footshock and a potentiated locomotion to i.c.v. CRF. **Discussion:** These results suggest that

footshock stress activates the noradrenergic input to the amygdala and BNST and that in turn a CRF projection from the amygdala to the BNST is activated leading to reinstatement of drug seeking behavior. Furthermore, these and other results suggest that repeated exposure to cocaine and time since termination of drug alter the vulnerability to stress and to the activating effects of CRF. Supported by CIHR, NSERC Canada,