Neuropsychopharmacology (2005) 30, S1-S76

Monday, December 12, 2005

Panel Session
Dopamine Beta Hydroxylase Genetics and Substance Abuse: Of Mice and Men

Human Genetics of Plasma DBH Activity in Cocaine Dependence

Joseph F Cubells*, Richard S Schottenfeld, Alison Oliveto, Tony George, Marek C Chawarski, James Poling, Henry R Kranzler, Joel Gelernter and Thomas R Kosten

Human Genetics and Psychiatry & Beh. Sci, Emory Univ. Sch. Med., Atlanta, GA, USA

Background: Dopamine β-hydroxylase (DBH) catalyzes conversion of dopamine (DA) to norepinephrine (NE). It is secreted into the circulation during synaptic exocytosis by sympathetic neurons, and can be measured in the plasma. Studies dating to the 1970s have suggested that lower biochemical activity of plasma DBH associates with psychotic symptoms in a number of disorders, including major depression, schizophrenia and disulfiram-induced psychosis. Disulfiram and its metabolites potently inhibit DBH enzyme activity, through their ability to chelate copper, a necessary co-factor. Disulfiram appears to be effective for promoting abstinence from cocaine in some, but not all cocaine-dependent individuals. Plasma DBH levels are highly heritable. Prior linkage results suggested that *DBH*, the structural gene encoding DBH, accounts for much of that heritability. Our lab studies the relationship between sequence variation at *DBH* and variation in plasma DBH activity.
Methods: We performed a targeted re-sequencing study of *DBH*, targeting exons, flanking intronic regions, and several kb of upstream and downstream sequence, in subjects exhibiting very low, average, or very high levels of plasma DBH. That study identified a single nucleotide polymorphism (SNP) that appears to account for 35-50% of the variance in plasma DBH levels. The SNP, -1021C>T, is located in the upstream region of the gene, 1021 bp 5' of the translational start codon. We tested two hypotheses: (1) the low-activity allele of -1021C>T associates with cocaine-induced psychotic symptoms, as reported retrospectively by cocaine-depndent individuals. (2) that genotype at -1021C>T modulates response to disulfiram in cocaine-dependent patients.
Results: In contrast to our early findings, suggesting an association between a low-activity associated haplotype at *DBH* and cocaine-induced paranoia, more extensive analysis in a larger independent sample does not support an association between genotype at -1021C>T and cocaine-induced paranoia or other psychotic symptoms assessed by retrospective interview. However, preliminary results from a double-blind placebo-controlled trial of disulfiram in cocaine-dependent opiate users maintained on buprenorphine suggest that carriers of the low-activity-associated T allele at -1021C>T may be more responsive to the abstinence-promoting effects of disulfiram (250 mg/day) than CC homozygotes. A separate study, examining the relationship between plasma DBH activity and disulfiram response in cocaine-dependent opiate users maintained on methadone suggests that individuals with lower plasma DBH activity may also be more responsive to doses of 250 mg of disulfiram (but not to 125 mg). Those results are consistent with the hypothesis that the T allele at -1021C>T associates with better response to disulfiram.
Conclusions: (1) *DBH* is a major genetic determinant of plasma DBH activity. -1021C>T may be a functional variant that accounts for much of that association, but further research is necessary to test this hypothesis. (2) -1021C>T does not associate with cocaine-induced psychosis, as assessed by retrospective interviews of cocaine dependent individuals. (3) -1021 T allele carriers may be more responsive to the cocaine-abstinence-promoting effects of disulfiram. Support: NIDA grants R01 DA12422 (JFC), K02 DA 15766 (JFC), R01 DA 12849 (JG), R01 DA 13441 (AO), R01 DA 012979 (RSS), K24 DA 00445 (RSS), P50-DA12762 (TRK).

Dopamine β-Hydroxylase Knockout Mice Have Alterations in Dopamine Signaling and Are Hypersensitive to Cocaine Locomotion, Reward, and Aversion

Jesse R Schank, Rossella Ventura, Stefano Puglisi-Allegra, Antonio Alcara, L. C Liles, Philip Seeman and David Weinshenker*

Human Genetics, Emory University, Atlanta, GA, USA

Background: Multiple lines of evidence demonstrate that the noradrenergic system provides both direct and indirect excitatory drive onto midbrain dopamine (DA) neurons, and alterations in noradrenergic signaling can influence the degree and valence of psychostimulant responses.
Methods: We used dopamine β-hydroxylase knockout (Dbh -/-) mice that lack norepinephrine (NE) to determine the consequences of chronic NE deficiency on midbrain DA neuron function in vivo. DA release was assessed by in vivo microdialysis, DA receptor signaling was assessed by in vitro radioligand binding, the psychomotor effects of cocaine were assessed by automated locomotor activity assays, and cocaine reward and aversion were assessed by a place conditioning paradigm.
Results: Basal extracellular DA levels were significantly attenuated in the nucleus accumbens (NAc) and caudate putamen (CP), but not prefrontal cortex (PFC), of Dbh -/- mice, while amphetamine-induced DA release was absent in the NAc and attenuated in the CP and PFC. The decrease in dopaminergic tone was associated with a profound increase in the density of high-affinity state D1 and D2 DA receptors in the NAc and CP, while DA receptors in the PFC were relatively unaffected. As a behavioral consequence of these neurochemical changes, Dbh -/- mice were hypersensitive to the psychomotor, rewarding, and aversive effects of cocaine. Antagonists of DA, but not 5-HT receptors attenuated the locomotor hypersensitivity to cocaine in Dbh -/- mice.
Discussion: Because DBH activity in humans is genetically controlled and the DBH inhibitor disulfiram has shown promise as a pharmacotherapy for cocaine dependence, these results have implications for the influence of genetic and pharmacological DBH inhibition on DA system function and psychostimulant addiction.

DBH Genotype and Human Self-Administration of Cocaine

Robert Malison*

Psychiatry, Yale University, New Haven, CT, USA

Background: Previous studies suggest an association between 'low–activity' haplotypes at the gene for dopamine beta–hydroxylase (DBH) and retrospective self–reports of cocaine–induced paranoia (CIP) in clinically dependent populations. These data are supported by preclinical observations of increased conditioned place aversion to cocaine in dbh –/– knockout mice. The current study used human laboratory methods to more directly evaluate potential pharmacogenetic interactions between cocaine and a recently identified, functional, single nucleotide polymorphism (C—1021T) in DBH.
Methods: Thirty–one, non–treatment seeking, medically healthy, experienced cocaine users participated in a double blind, fully randomized laboratory study of intravenous cocaine (8, 16, and 32 mg/70 kg)

self–administration on three test days, during which time participants had 2 hours of 'ad lib' access to cocaine under a fixed ratio 1: timeout 5 minute schedule. Visual analog scale (VAS) self–ratings of paranoia were obtained at 5–min intervals throughout. Sessions were conducted blind to DBH C—1021T genotype.
Results: Procedures were well tolerated by participants, and no significant adverse events were noted. VAS self–ratings showed a significant main effect of cocaine dose ($F=19.3$, $p<0.001$), no effect of DBH genotype ($F=0.7$, $p=0.50$), but a significant dose x genotype interaction ($F=2.8$, $p<0.05$; rmANOVA, Huynh–Feldt), with 'very low–acitivity' TT homozygotes (N=5) reporting more paranoia than CT (N=11) or CC (N=15) individuals. Main effects of time ($F=11.4$, $p<0.001$), but not genotype ($F=1.64$, $p=0.20$), and a significant genotype x time interaction ($F=2.59$, $p<0.005$) were also observed at the highest (32 mg) dose. Cocaine consumption did not differ between genotypic subgroups.
Discussion: Human laboratory data suggest that homozygous carriers of 'very low–activity' (i.e., TT) DBH genotypes are more vulnerable to CIP.

Norepinephrine in Opiate Reward, Locomotion, and Withdrawal: Evidence for Distinct Contributions by the Locus Coeruleus and the Nucleus Tractus Solitarius

Valerie Olson*

Psychiatry and Behavioral Sciences, University of Washington/VA Puget Sound Health Care System, Seattle, WA, USA

Background: While norepinephrine (NE) is widely believed to play a critical role in opiate withdrawal (OWD), recent studies have brought this view into question. Further, relatively little is known about NE's role in the acute rewarding and locomotor-activating effects of opiates. Using mice lacking NE through targeted disruption of the dopamine β-hydroxylase (DBH) gene (DBH-KOs) we sought to ascertain the role of NE in opiate reward, locomotion, and withdrawal. To determine which noradrenergic nuclei contribute to these processes we used viral mediated gene transfer to selectively restore DBH expression into either the locus coeruleus (LC) or the nucleus tractus solitarius (NTS).
Methods: Behavior: To assess the ability of DBH-KO mice to experience opiate reward we used a conditioned place preference (CPP) paradigm. Conditioned place aversion (CPA) was used to assess the aversive properties of OWD. Locomotor activity was measured in activity chambers equipped with infrared beams for 2 hours following morphine injection. A blinded observer recorded OWD somatic signs for 20 minutes following induction of withdrawal by an injection of naloxone. Restoration of NE signaling in DBH-KO mice: We acutely restored systemic endogenous noradrenergic neurotransmission in DBH-KO mice by administering L-threo-3,4-dihydroxyphenylserine (DOPS), a compound converted to NE in the absence of DBH. To selectively restore DBH into noradrenergic nuclei, DBH-KO mice were intracranially injected with a DBH-containing adeno-associated viral vector into the LC or the NTS.
Results: DBH-KO mice were unable to form a CPP to morphine across a wide range of doses and showed a blunted locomotor response to morphine. This deficit in CPP was not due to a general inability to experience reward or to an inability to form a place association as DBH-KO mice formed a normal CPP for food. Systemic pharmacological restoration of NE rescued both morphine CPP and locomotion. Selective restoration of NE signaling in the NTS completely rescued morphine CPP, but in the LC failed to do so. Restoration of noradrenergic signaling in either the LC or the NTS partially restored morphine locomotion. DBH-KOs showed an attenuation of some OWD signs, but an exacerbation of others, and blunted CPA to OWD. Systemic pharmacological rescue of NE rescued CPA to OWD and normalized the expression of most OWD signs. Similar levels of cFOS expression in the LC and NTS of DBH-KO and control mice during OWD suggest that these behavioral changes are not due to a differential activation of noradrenergic nuclei. Restoration of DBH in the LC of DBH-KO mice normalized OWD signs and restored CPA to OWD. Restoration of DBH into the NTS did not restore OWD signs but did rescue CPA to OWD.
Discussion: These data suggest a novel role for NE in opiate reward and confirm a role for NE in opiate locomotion. Further, these data identify the NTS as a critical component in opiate reward neural circuitry. The LC and the NTS both seem to contribute to opiate locomotion. Previous findings support the idea that an increase in central NE neurotransmission underlies the somatic signs and possibly the aversive properties of OWD. Our data suggest a more complex role for NE in OWD, with NE attenuating some signs while intensifying others. Both the LC and NTS have been proposed to play a role in OWD, though their relative contributions remain in dispute. These findings support a role for the LC in OWD induced aversion and somatic signs and a role for the NTS in OWD induced CPA.

Panel Session
Specificity of Cortical GABAergic Systems: Development and Disturbances in Schizophrenia

Fate Determination of Cortical Interneurons. It Is Who You Are, Not How You Get There

Stewart A Anderson*

Psychiatry, Cornell Medical School, New York, NY, USA

Background: Alterations in cortical GABAergic interneurons are repeated findings of postmortem studies of schizophrenia. This presentation will discuss three general aspects of interneuron development and function.
Methods and Results: First, the origins of interneurons in rodents and humans will be discussed, with particular reference to the apparent primate-specific source of cortical interneurons in the human cortical subventricular zone. Second, evidence from in vivo transplantation studies will be presented that suggests that interneuron subgroup fate determination in rodents occurs in the subcortical telencephalon where the cells exit the cell cycle. This finding has allowed important progress towards identifying the molecular code for specifying interneuron subgroups. To this end, the roles of the transcription factors Gli3, Nkx2.1 and Lhx6 and signaling molecule Sonic Hedgehog for specifying the fates of interneuron subgroups will be discussed. Finally, advances in determining functions of distinct interneuron subgroups through genetic loss of function studies will be briefly presented.
Discussion: Implications of the above findings for directing future studies seeking to model the interneuron pathology of schizophrenia in the mouse, and the relevance of such a model for determining a molecular etiology of schizophrenia, will be discussed.

Regulation of Axon Growth and Inhibitory Synapses Formation by GAD67-Mediated GABA Signaling: Implications in Schizophrenia Pathogenesis

Z. Josh Huang*, Bidisha Chattopadhyaya, Graziella Di Cristo, Caizhi Wu and Richard Palmiter

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

Background: Reduced expression of the major GABA synthetic enzyme, GAD67, in dorsal lateral prefrontal cortex has been a replicated finding in Schizophrenia pathology, but it is not clear whether this is a cause or a consequence of the illness. Initially discovered as an inhibitory neurotransmitter, GABA has since been implicated in multiple processes of neural development, from cell migration to circuit formation. However, it is now known whether GABA signaling plays a role in the maturation of GABAergic synapses and innervation in neocortex.

Methods: We have generated a condition allele of the GAD67 gene in mice, which allows genetic manipulations in defined developmental stages and specific GABAergic cell types. We have also generated transgenic reporter mice, which label of specific classes of GABAergic interneurons and synapses with high-resolution. These experimental systems therefore allow us to examine whether and how GAD67-mediate GABA synthesis and signaling influence the construction of GABAergic synapses and circuits.
Results: The construction of GABAergic inhibitory synapses and circuits is crucial in regulating neuronal excitability and ensemble activities. A salient feature of GABAergic innervation is its local exuberance: a single parvalbumin-positive basket interneuron in neocortex innervates hundreds of target neurons in its vicinity, and forms multiple, clustered synapses onto the soma and proximal dendrites of each target. The mature pattern of target coverage and synapse density is achieved during adolescence, but the underlying mechanism is unknown. Here we show that GABA synthesis and signaling are crucial in regulating axon growth and synapse formation [during the maturation of GABAergic innervation field. Genetic manipulation of glutamic acid decarboxylase in single basket interneurons resulted in profound and dosage-dependent retardation of axonal branching, reduced target coverage and synapse density. GAD67, but not GAD65, appears to be required for the maturation but not the maintenance of perisomatic innervation. GABAa and GABAb receptor agonists partially rescue the effects of GABA deficiency on distinct aspects of perisomatic synapses. Since GAD67 transcription is strongly regulated by neuronal input, activity-dependent GABA synthesis and signaling may function to sculpt the architecture of synaptic innervation field.
Discussion: Allelic variation in regulatory region of GAD67 gene have recently been linked to childhood-onset schizophrenia and cortical gray matter loss. Our results suggest that GABA deficiency due to down regulation of GAD67 might lead to retarded development of GABAergic innervation, and aberrant neural circuit and information processing.

Differential Vulnerability of Specific Cortical GABA Systems in Schizophrenia

David A Lewis*, Takanori Hashimoto, Karoly Mirnics, Jaime G Maldonado-Aviles and Harvey M Morris

Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA

Background: A reduction in the mRNA expression for the 67 kD form of glutamic acid decarboxylase (GAD_{67}), a synthesizing enzyme for GABA, in the dorsolateral prefrontal cortex (DLPFC) is one of the most robust and widely replicated alterations in subjects with schizophrenia. We have previously reported that this deficit is present in the ~25% of cortical GABA neurons that express the calcium-binding protein parvalbumin (PV), but not in the ~50% of GABA neurons that express calretinin (CR). Furthermore, among the PV-positive GABA neurons, the chandelier subclass, which furnishes inhibitory synapses to the axon initial segment of pyramidal neurons, exhibits a pre-synaptic decrease in the GABA membrane transporter (GAT1) which is accompanied by a post-synaptic up-regulation of $GABA_A$ receptors containing α_2 subunits. However, abnormalities in PV neurons alone may not completely account for the decreased expression of GAD_{67} mRNA since such changes were also observed in cortical layers 1 and 2, where relatively few PV-containing GABA neurons are located, and where no changes in PV mRNA expression were found.
Methods: In order to determine whether other subpopulations of GABA neurons are affected in schizophrenia, and to place the alterations in PV-positive neurons in the broader context of the highly diverse cortical GABAergic systems, we used a customized DNA microarray platform containing ~100 GABA-related transcripts to simultaneously assess the tissue concentrations of these transcripts in the DLPFC of 14 subjects with schizophrenia and matched normal comparison subjects.
Results: Transcripts for both somatostatin and neuropeptide Y, which are co-localized in GABA neurons that contain neither PV nor CR, showed a substantial decrement in subjects with schizophrenia, and these changes were positively correlated with the changes in GAD_{67} mRNA. These findings were verified by both qPCR in the same subjects and by *in situ* hybridization in a second subject cohort. In addition, the mRNA expression levels of several subunits of the $GABA_A$ receptor were altered, suggesting that the normal ratio of phasic to tonic GABA neurotransmission is disrupted in schizophrenia. The potential pathogenetic mechanisms that may confer this differential vulnerability to selective components of cortical GABA neurotransmission are being evaluated in mouse genetic models.
Discussion: Together, these findings suggest that GABA neurotransmission is markedly disrupted in selective GABA circuits of the DLPFC in subjects with schizophrenia, and they reveal novel molecular targets for potentially improving prefrontal dysfunction in affected individuals.

New Clinical Evidence Implicating GABA Systems in Psychosis and Cognition: Implications for Schizophrenia

John H Krystal*, Deepak C D'Souza, Ismene L Petrakis, Jaakko Lappalainen and Joel E Gelernter

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

Abnormalities in GABAergic innervation of the cortex have been described in post-mortem tissue from schizophrenic patients. However, the functional implications of these abnormalities are not well understood. The purpose of this presentation is to present findings from two studies that suggest that deficits in GABA function increase the vulnerability to symptoms and cognitive deficits associated with schizophrenia that might arise in the context of monaminergic hyperactivity or deficits in NMDA glutamate receptor function. The first study explored whether a deficiency in GABA-A receptor function might convey a vulnerability to psychosis in the context of stimulation of serotonin receptors. This hypothesis emerged from studies where the serotonin partial agonist (predominately 5-HT2C), mCPP, worsened psychosis in some schizophrenic patients, but it did not produce psychosis in healthy subjects.
Methods: Twenty-three healthy subjects completed 4 test days involving the infusion of the benzodiazepine partial inverse agonist, iomazenil (3.7 microgm/kg, saline) prior to the infusion of mCPP (0.1 mg/kg).
Results: Neither iomazenil nor mCPP produced psychosis when administered by themselves. However, when subjects were pretreated with iomazenil, mCPP produced transient psychotic symptoms in healthy human subjects.
Implications: This study suggested that modest deficits in GABA-A receptor function might convey a vulnerability to psychotic states in the context of hyperstimulation of serotonergic, and perhaps other monoaminergic, receptors. This presentation will also present pilot data from an ongoing study of the role of GABA-related genes in modulating the effects of NMDA glutamate receptor antagonist, ketamine, in healthy human subjects. Building on the prior data and the findings from the Lewis laboratory, we hypothesized that deficits in GABA release or alpha-2 subunit-containing GABA-A receptors might exacerbate the psychotigenic and cognitive effects of ketamine in healthy human subjects.
Methods: To date, only 16 healthy subjects have completed testing, so reported findings must be considered extremely preliminary. Subjects who provided DNA completed two test days involving the infusion of saline or ketamine 0.5 mg/kg.
Results: Preliminary data suggest that polymorphisms in both the gene coding for the alpha-2 subunit of the GABA-A receptor (GABRA2) and the gene coding for GAD65, a key GABA synthetic enzyme, influence ketamine response. The GAD65 polymorphism may influence the magnitude of impaired verbal fluency, while GABRA2 seemed to influence psychosis, impaired working memory, and reduced grooved pegboard performance.
Implications: Heritable alterations in GABA function may influence the impact of deficits in NMDA receptor function upon outcomes

relevant to schizophrenia. Together these studies support the hypothesis that deficits in GABA function augment the impact of monoaminergic hyperactivity and deficits in NMDA receptor function that might be associated with schizophrenia.

Panel Session
Homo- and Hetero-Dimerization of G Protein-Coupled Receptors: Drug Mechanisms and Pharmacological Diversity

Functional and Pharmacological Consequences of Oligomeric Assemblies in G-protein-Coupled Receptor Signalling

Michel Bouvier*

Biochemistry, Universite de Montreal, Montreal, QC, Canada

Background: A growing body of evidence suggests that G protein-coupled receptors (GPCRs) form functionally relevant homo- and heterodimers that assemble into modular signalling complexes. In addition to their cognate G proteins, various scaffolding and signalling partners can be recruited to the receptors thus determining the selectivity and efficacy of the "signalosomes". Although the occurrence of these complexes has been well investigated *in vitro*, their ontogeny and their dynamic regulation in living cells are still poorly understood. Such understanding is crucial to apprehend the consequences that these newly appreciated complexes may have on drug discovery and screening.
Methods: To directly assess the real-time assembly of GPCR oligomers and of their signalling complexes in living cells, we used a combination of biochemical and biophysical approaches. In particular, multiplexing Bioluminescence and Fluorescence Resonance Energy Transfer (BRET and FRET) techniques allowed to monitoring the assembly of multiple partners simultaneously. These proximity-based assays rely on the non-radiative transfer of energy between energy donors and acceptors, which have different spectral properties, and permit the detection of both constitutive and dynamic interactions.
Results: We found that constitutive homo and hetero-oligomerization occurs early during the biosynthetic process for a number of GPCRs suggesting that oligomeric assemblies may be a general feature of these important pharmacological targets. Once at the cell surface, ligand binding to the receptor oligomers promote conformational changes within the oligomers and initiate the engagement of specific G protein complexes with a half time of ~250 msec followed by the recruitment of the scaffolding protein βarrestin to the receptor oligomers with a half-time of ~1 min. The βarrestin can then interact with the endocytic machinery adaptor protein AP2 to promote the internalization of the receptors and/or the assembly of mitogen-activated protein kinase (MAPK) signalling modules. In addition to play an important role in the ontogeny and trafficking of the signalling complexes, the occurrence of receptor oligomerization offers combinatorial possibilities to increase the pharmacological and functional potential of GPCRs. For instance, hetero-oligomerization between pharmacologically distinct GPCRs leads to signalling properties that differ from their corresponding homo-dimers. Specific examples of the influence of homo- and hetero-oligomerization on the formation of selective signalling modules will be discussed for the GABAb, the Sensory Neuron Specific, the delta-opioid and the adrenergic receptors.

Hetero-Dimerization of CNS Expressed G Protein-Coupled Receptors: Implications for Drug Function

Graeme Milligan*

Biochemistry and Molecular Biology, University of Glasgow, Glasgow, United Kingdom

Background: It is becoming increasingly accepted that G protein-coupled receptors have the capacity to homo-dimerize and that this may be key to cell surface delivery and function. It is also established that certain pairs of G protein-coupled receptors can hetero-dimerize but the importance of this is less clear in physiological settings. Some 865 genes in the human genome encode G protein-coupled receptors and a large percentage of the non-chemosensory G protein-coupled receptors are expressed in the central nervous system with array and immunohistochemical data indicating that many are co-expressed in small defined areas of the brain and indeed in individual neurones. We wished to assess how hetero-dimerization might impact pharmacology and function of specific pairs of CNS expressed G protein-coupled receptors.
Methods: Cell lines were established in the background of Flp-In T-REx-HEK293 cells to allow stable and constitutive expression of one G protein-coupled receptor and inducible expression of potential partner G protein-coupled receptors. Such cell lines were analysed for G protein-coupled receptor hetero-dimerization using co-immunoprecipitation and single cell fluorescence resonance energy transfer techniques. They were also used to study the ability of one G protein-coupled receptor to alter the cell trafficking characteristics of the other receptor as well as its maturation, cell surface delivery and function.
Results: Expression of the human orexin-1 receptor was induced in the presence or absence of the cannabinoid CB1 receptor. In the absence of the CB1 receptor the orexin-1 receptor was effectively delivered to the cell surface but in the presence of the CB1 receptor the orexin-1 receptor had a distribution pattern reflecting endocytic vesicle location. This reflected agonist-indepedent recycling of the CB1 receptor which, when hetero-dimerized with the orexin-1 receptor caused the orexin-1 receptor to adopt this phenotype. Treatment of cells co-expressing the two receptors with the CB1 receptor antagonist/inverse agonist rimonabant resulted in cell surface trapping of both receptors. Similar experiments were performed using pairs of mas-related gene (Mrg) receptors. Mrg D and Mrg E are co-expressed in dorsal root ganglia but although beta-alanine is an agonist for Mrg D it is not for Mrg E. These two receptors formed a hetero-dimeric pair and this restricted beta-alanine-mediated internalization of Mrg D and altered the sensitivity of beta-alanine-mediated ERK MAP kinase phosphorylation and the details of intracellular Ca2+ release.
Discussion: The use of cells stably expressing pairs of G protein-coupled receptors that are known to be co-expressed in the CNS has allowed demonstration of their hetero-dimerization and provided evidence that this can alter the pharmacology and function of these receptors. Such hetero-dimers may offer distinct therapeutic tragets for drug design.

Role of GPCR Oligomerization in Regulating Receptors by Endocytic Membrane Trafficking

Mark Von Zastrow*

UCSF, San Francisco, CA, USA

GPCR oligomer formation can have multiple effects, including effects on the regulation of receptors by endocytic membrane trafficking. Our laboratory and other groups have demonstrated that GPCR oligomerization can influence the ability of receptors to undergo regulated endocytosis following ligand activation. There is increasing evidence that oligomerization can also affect the 'sorting' of receptors, occurring after endocytosis, between divergent membrane pathways that produce essentially opposite net effects on cell signaling. Studies addressing the mechanistic basis of these endocytic sorting effects will be described, based largely on studies involving regulated endocytic trafficking of adrenergic catecholamine receptors and opioid neuropeptide receptors in heterologous cell models. Functional consequences of GPCR oligomerization, and potential implications for pharmacotherapy, will be discussed with an emphasis on receptor-receptor interactions influencing opioid regulation in physiologically relevant CNS neurons.

Crosstalk in G Protein-Coupled Receptors: Changes at the Transmembrane Homodimer Interface Determine Activation
Jonathan A Javitch*

Psychiatry and Pharmacology, Columbia University, New York, NY, USA

Functional crosstalk between G-protein-coupled receptors in a homo- or hetero-dimeric assembly likely involves conformational changes at the dimer interface, but the nature of this interface is not yet established, and the dynamic changes have not yet been identified. We have mapped the homodimer interface in the dopamine D2 receptor over the entire length of the fourth transmembrane segment (TM4) by crosslinking of substituted cysteines. Their susceptibilities to crosslinking are differentially altered by the presence of agonists and inverse agonists. The TM4 dimer interface in the inverse agonist-bound conformation is consistent with the dimer of the inactive form of rhodopsin modeled with constraints from atomic force microscopy. Crosslinking of a different set of cysteines in TM4 was slowed by inverse agonists and accelerated in the presence of agonists; crosslinking of these cysteines locks the receptor in an active state. Thus, a conformational change at the TM4 dimer interface is part of the receptor activation mechanism.

Panel Session
Successful Aging: A Neuropsychiatric Entity

A Psycho-Bio-Social Study of Successful Aging Among Community-Dwelling Seniors
Dilip V Jeste*, Robert D Langer, John W Daly, Colin A Depp, Jennifer L Reichstadt, Lori P Montross, Shahrokh Golshan and Barry D Lebowitz

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

Recent demographic trends as well as new research studies question the age-old concept of old age as being synonymous with dementia, degeneration, depression, disability, and other negative characteristics. Next 30 years will witness the largest-ever increase in the numbers of elderly people in the world, and especially, of those living highly functional lives. Yet, research on such "successful or healthy aging" has considerably lagged behind that on age-related diseases. Successful aging has been defined in different ways, with relatively few investigations having been done using seniors' self-perceptions of successful aging. The Stein Institute for Research on Aging at UCSD has been conducting a study of self-rated successful aging in more than 1,200 community-dwelling residents aged 60 to 99 years. These participants have completed a detailed survey questionnaire on their medical history, health behaviors, quality of life, resilience, cognitive performance, and self-ratings of successful aging. The questionnaire includes a number of published rating scales for evaluating various aspects of subjective functioning. The data suggest that nearly three-fourths of the respondents feel that they are aging well, often despite having physical illnesses and some disability. Significant associations of successful aging include overall level of activities, number of friends, cognitive functioning, resilience, and a positive attitude toward aging. On other hand, ethnicity, gender, education, and income have been found to have weak or no relationship with successful aging. Different biomarkers are also being examined in blood samples. We will discuss limitations of our results as well their implications for prevention and intervention trials.

Genome Survey for Loci that Influence Successful Aging
George S Zubenko*, Hugh B Hughes and Wendy N Zubenko

Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA

Objective: A systematic genome survey was initiated to identify loci that affect the likelihood of reaching age 90 with preserved cognition.
Methods: The genome survey was conducted at 10 cM resolution for simple sequence tandem repeat polymorphisms (SSTRPs) that identify genes for successful aging by virtue of linkage disequilibrium. Efficiency was enhanced by genotyping pools of DNA from 100 (50M/50F) cognitively-intact elders and 100 young (18-25 yrs.) adults matched for sex, race, ethnicity, and geographic location.
Results: Elders (94 nonagenarians, 6 centenarians) manifested preserved cognition as reflected by clinical and psychometric assessments, good average capacity to carry out their ADLs, and the majority were living independently despite multiple medical conditions. None had a history of mental disorders in early or middle adulthood, only one was a current smoker, and 80% consumed alcohol less than once each month. The genome survey method detected the expected elevation of the APOE E2 allele frequency, and reciprocal reduction in the E4 frequency, among the elders compared to the young adults. It also detected significant differences in the allelic distributions of DYS389 and DYS390, which are separated by only 2.6 Mb near the centromere of Yq. Evidence for additional genetic loci that selectively influence the development of successful aging in men or women was also observed.
Conclusions: These results suggest several behavioral and genetic factors that may contribute to the likelihood of achieving exceptional longevity with preserved cognition. These factors appear to differ for men and women.

Neurodegeneration and Regeneration in the Aging Nervous System
Laura L Dugan*

Medicine/Geriatrics, University of California San Diego, La Jolla, CA, USA

Degeneration of specific populations of neurons occurs in the mammalian brain during aging. This age-related neurodegeneration has been best characterized for dopaminergic neurons in the substantia nigra, and has been observed in humans as well as non-human primates, and several other mammalian species, including rat. We have focused on dopaminergic neurons as a model system to study neural cell death and regeneration during aging, and have extended the observation that there is loss of nigral neurons during aging to mice. We found a significant 25% decline in nigral dopaminergic neurons in healthy old (22 month) wild-type C57BL6 mice, as determined by unbiased stereological counts of tyrosine hydroxylase-positive (TH) neurons. This was associated with significantly higher levels of superoxide radical in nigral dopaminergic neuron. Superoxide levels were brought down to levels seen in young animals by treatment with a catalytic antioxidant compound, or by calorie restriction, the only universally-effective intervention which increases lifespan. Reactive oxygen species (ROS) such as superoxide and H2O2 are known to be triggers - and downstream effectors - of both apoptotic and necrotic neuronal death. We have established that ROS may induce neuronal apoptosis through several distinct pathways, including recruitment of the classic caspase cascade (Lotharius et al., 1999), activation of NFkB and p53 (de Erausquin et al., 2003), assembly of the mitochondrial membrane permeability transition pore (mPTP) (Reichert et al., 2001), activation of Erk1/Erk2 (Dugan et al., 1997), and stimulation of nitric oxide synthase (Reichert et al., 2001). In addition, the histone deacetylase, Sir2/SirT1, which is associated with enhanced longevity in C. elegans and Drosophila, and improved neuronal survival in mice, was decreased in old mice, and normalized by antioxidant treatment. We are currently in the process of determining 1) which apoptotic pathway(s) are involved in age-related death of dopaminergic neurons, 2) if similar apoptosis pathways are involved in loss of other neuronal populations with aging, 3) whether interventions which block ROS production or downstream pathways modified by ROS will rescue neurons, and 4) can effective antioxidant treatment promote proliferation and integration of neural stem cells into the aging brain. de Erausquin GA, Hyrc K, Dorsey DA, Mamah D, Dokucu M, Masco DH, Walton T, Dikranian K, Soriano M, Garcia Verdugo JM, Goldberg MP,

Dugan LL (2003) Nuclear translocation of nuclear transcription factor-kappa B by alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors leads to transcription of p53 and cell death in dopaminergic neurons. Mol Pharmacol 63:784-790. Dugan LL, Creedon DJ, Johnson EM, Holtzman DM (1997) Rapid suppression of free radical formation by NGF involves the MAPK pathway. Proceedings National Academy Science, USA 94:4086-4091. Lotharius J, Dugan LL, O'Malley KL (1999) Distinct mechanisms underlie neurotoxin-mediated cell death in cultured dopaminergic neurons. Journal Neuroscience 19:1284-1293. Reichert SA, Kim-Han JS, Dugan LL (2001) The mitochondrial permeability transition and nitric oxide synthase mediate early mitochondrial depolarization in astrocytes during oxygen-glucose deprivation. Journal Neuroscience 21:6608-6616.

Effects of a 14-Day Healthy Aging Lifestyle Program on Brain Function

Gary W Small*, Daniel H Silverman, Prabha Siddarth, Linda Ercoli, Karen Miller, Susan Bookheimer, Jorge R Barrio and Michael E Phelps

Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Background: Evidence suggests that several lifestyle choices may improve brain health and potentially reduce the risk for dementia. Objectives: To determine brain functional effects of a two-week lifestyle program combining healthy diet, physical and mental activity, and stress reduction techniques.
Methods: Seventeen non-demented subjects, aged 35 to 70 (mean age, 53) with normal baseline memory performance scores were randomly assigned to (1) the intervention group (N=8): a program combining a healthy diet plan (5 daily meals emphasizing omega-3 fats, low glycemic index carbohydrates, and anti-oxidant foods), relaxation exercises, aerobic conditioning (e.g., brisk daily walks), and mental activity (brain teasers, memory training techniques emphasizing verbal skills); or (2) the control group (N=9): usual lifestyle routine. Immediately before and after the two-week program, brain function was assessed using flurodeoxyglucose (FDG) positron emission tomorgraphy (PET) scanning during mental rest, which measures regional cortical glucose metabolic rates. Statistical parametric mapping (SPM) analysis was used to determine differences between groups in cerebral metabolic change.
Results: Subjects in the intervention group showed a 5% decline in left dorsolateral prefrontal glucose metabolism compared with controls ($Z=3.30$; $P<0.0005$), whereas the control group showed no change in brain metabolism. The region of change involved a stretch of cortex in Brodmann's areas 8,9,10.
Conclusion: A short-term healthy lifestyle intervention program combining mental activity, physical exercise, stress reduction, and healthy diet has significant immediate effects on brain metabolism. The reduced glucose metablic rates in the left dorsolateral prefrontal cortex suggest greater cognitive efficiency in a brain region that controls working memory. The significant change observed in the left hemisphere also is consistent with the verbal emphasis in the program's memory training exercises. Future studies will determine specific effects of individual components of the program, and whether a combination of healthy lifestyle strategies produces the optimum outcome.

Panel Session
Towards Building Translational Measures of Depression: Objective Characterizations of Depressive Phenotypes and Biomarkers in Humans and Animals

Objective Measures of Core Cognitive Symptoms of Depression

Barbara J Sahakian*

Psychiatry, University of Cambridge, Cambridge, United Kingdom

Objective measures of depression are needed to detect onset, monitor remission and relapse and assess the efficacy of pharmacological and psychological treatments. Dysfunctional attitudes, negative automatic thoughts or ruminations and anhedonia or the inability to experience pleasure are core cognitive symptoms in people suffering from depression. This talk focuses on objective measures of these cognitive symtoms in detecting depression in first-episode adolescents, in demonstrating sensitivity to mood state and in highlighting the importance of pharmacogenomics. Recent data demonstrate the importance of the ss allele of the serotonin transporter gene in risk for depression following the pharmacological stressor of chronic ecstasy use. In addition, new data emphasize the importance of the role of serotonin in the performance of a task of incentive motivation aimed at examining reward sensitivity, which may provide a novel measure of apathy in depression. Finally, a new study indicates that these objective measures may prove more sensitive in detecting differences between patients with unipolar or bipolar depression than clinical rating scales. In addition, certain of these measures have advantages over clinical rating scales in that they are more suitable for translational medicine models.

Laboratory Measures of Anhedonia in Humans: A Signal Detection Approach

Diego A Pizzagalli*

Department of Psychology, Harvard University, Cambridge, MA, USA

Background: The advancement of psychiatric genetics and clinical neuroscience is significantly impeded by the heterogeneity and lack of phenotypic definition of psychiatric disorders. Anhedonia - the loss of pleasure or lack of reactivity to pleasurable stimuli - is a core symptom of depression, and has been considered a potential trait marker related to depression vulnerability. Surprisingly, few studies have employed laboratory-based measures to objectively characterize this promising phenotype of depression. Further, although preclinical data have emphasized stress-mediated disturbances of mesocorticolimbic dopaminergic functions in the pathophysiology of depression, and particularly anhedonia, the mechanisms and substrates underlying these processes are largely unknown in humans.
Methods: As an initial step toward a better characterization of the anhedonic phenotype and underlying neural substrates, findings from four independent studies using a signal-detection approach will be presented. This approach uses a differential reinforcement schedule that permits the objective assessment of participants' propensity to modulate behavior as a function of reward-related cues.
Results: Findings from the first study indicate that, unlike control subjects, subjects with elevated depressive symptoms failed to show a systematic preference for the stimulus paired with the more frequent reward (i.e., they showed a decreased reward responsiveness). Interestingly, impaired reward responsiveness predicted higher anhedonic symptoms one month later after controlling for general negative affectivity. In a second study, subjects appraising their daily life as unpredictable, uncontrollable, and stressful showed impaired ability to modulate behavior as a function of reinforcements. In a third study using a within-subject design, two psychosocial stressors (threat-of-shock and negative feedback about task performance) negatively impacted reward responsiveness in a psychiatrically healthy sample. Finally, in a pharmacological challenge study, a single, 0.5 mg dose of the D2/D3 agonist pramipexole (likely acting on DA autoreceptors and thus leading to transiently suppressed dopaminergic transmission) was associated with decreased reward responsiveness, positive affect, and activation in orbitofrontal and cingulate regions which have been previously implicated in hedonic behavior.
Discussion: These findings suggest that (1) an impaired tendency to modulate behavior as a function of prior reinforcements may underlie diminished hedonic capacity in depression, particularly in the presence of stressors; (2) chronic stressors may exert their depressogenic effects by reducing individuals' propensity to modulate behavior as a function of reward-related cues. More generally, these findings are consistent with preclinical data highlighting stress-mediated

dysfunctions in mesocorticolimbic dopaminergic pathways subserving reward processing, and offer new insight into putative mechanisms linking stress to depression. This objective assessment of participants' propensity to modulate behavior as a function of reward may provide a powerful tool for improving the phenotypic definition of depression, and thus offer a reliable behavioral screening approach for neuroscience and genetic studies of depression.

Measuring Anhedonia in Animals: Reversal by Antidepressant Treatments

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

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

Anhedonia (i.e. diminished interest or pleasure in rewarding stimuli) is a core symptom of depression. Withdrawal from amphetamine or nicotine is also characterized by depressive symptoms and exhibits phenomenological similarity to depression in humans. Thus, we explored whether clinical antidepressant medications would reverse the anhedonia seen in rats undergoing spontaneous nicotine or amphetamine withdrawal. To measure anhedonia in rats, the intracranial self-stimulation procedure was used. This procedure involves preparing rats with self-stimulation electrodes in the lateral hypothalamus and allowing them to self-stimulate this brain site. Using a discrete-trial procedure that is based on the psychophysical method of limits, the lowest current-intensity for which the rat works to stimulate its own brain is measured. Cessation of chronic nicotine or amphetamine administration results in an anhedonic state defined operationally as elevated brain reward thresholds. The data indicated that the co-administration of the selective serotonin reuptake inhibitor fluoxetine and the serotonin-1A receptor antagonist p-MPPI reversed the threshold elevations seen in nicotine withdrawing rats. Similar results were seen in amphetamine-withdrawing rats when either of two selective serotonin reuptake inhibitors, fluoxetine or paroxetine, were co-administered with p-MPPI. Further, chronic, but not acute, treatment with the tricyclic antidepressant desipramine, that primarily inhibits the reuptake of norepinephrine, prevented the threshold elevations associated with nicotine withdrawal. Acute or chronic treatment with the atypical antidepressant bupropion, that acts primarily by inhibiting the dopamine transporter and is FDA-approved for smoking cessation, also reversed nicotine withdrawal. Thus, similarly to depression, pharmacological augmentation of decreased monoaminergic transmission ameliorates the depression-like anhedonic aspects of nicotine or amphetamine withdrawal. Moreover, chronic pretreatment with the atypical antipsychotic clozapine, that is the most effective antipsychotic medication against the negative depression-like aspects of schizophrenia, attenuated the severity of the nicotine withdrawal syndrome in rats. Finally, a series of studies in our laboratory led to the hypothesis that with the development of nicotine dependence, there is increased activity of metabotropic glutamate 2/3 receptors that are inhibitory receptors found primarily presynaptically where they control glutamate release. Hence, we predicted and subsequently showed that the metabotropic glutamate 2/3 receptor antagonist LY341495 reversed the threshold elevations associated with nicotine withdrawal in rats. Taken together, these data support the hypothesis of commonalities in the substrates mediating depressive symptoms of nicotine or amphetamine withdrawal and those seen in psychiatric patients. Thus, the anhedonic state associated with nicotine or amphetamine withdrawal and measured as elevations in brain reward thresholds can be used to study the neurobiology of depressive symptoms, and generate new hypotheses about novel pharmacological targets for antidepressants. For example, reversal of nicotine withdrawal by a metabotropic glutamate 2/3 receptor antagonist suggests that antagonists at these receptors may have antidepressant properties, and that decreased glutamate transmission may contribute to the pathophysiological dysregulation associated with anhedonia. Finally, these data indicate that the measurement of the anhedonic state associated with nicotine or amphetamine withdrawal and reflected in elevated brain reward thresholds can be a building block in the development of translational measures of depression.

Long-Term Neurobehavioural Effects of Early Life Adversity in Rats and Monkeys with Relevance to Depression

Joram Feldon*, M Leventopoulos and J Opacka-Juffry

Biology, Laboratory of behavioural Neurobiology, Swiss Federal Institute of Technology Zurich (ETH), Schwerzenbach, Switzerland

We have provided novel evidence, in rat and monkey studies, that repeated neglect-like social isolation of infants under specific conditions leads to long-term development of depression-like behavioural traits. The major effects obtained were: In adult Wistar rats, reduced motivation to obtain sucrose on a progressive ratio reinforcement schedule (reversed by fluoxetine) and reduced social interaction in an open field. In adult Fischer rats, increased escape failure in two-way active avoidance following pre-exposure to inescapable foot shock (reversed by fluoxetine). In juvenile-adolescent marmoset monkeys, reduced social behaviour, reduced motivation to obtain sweet reward on a progressive ratio reinforcement schedule, and impaired learning of reversal of stimulus-reward association. Recent in vivo and ex vivo studies have focussed on neurobiological phenotypes. In Fischer rats, serotonin content was increased and the 5-HIAA/5-HT ratio reduced in brain punchings from prefrontal cortex and hippocampus of socially-isolated adults. Also in Fischer rats, astroglial density (GFAP immunohistochemistry) was reduced in cingulate and prefrontal cortex, amygdala and hippocampus. In juvenile marmosets, using telemetric EEG, social isolation led to increased frequency of awakening. In infant marmosets, social isolation led to increased plasma basal cortisol titres and reduced hippocampal expression of the mineralocorticoid and glucocorticoid receptors (MR, GR immunohistochemistry). Therefore, building on our demonstration that neglect-like experience in infancy leads to depression-like behavioural phenotypes in rat and marmoset, we have obtained evidence for depression-like neurobiological correlates, which could also be relevant to elucidating the mechanisms via which infant experience can be fundamental to adult phenotype.

Panel Session
Effects of Immune Mediators on the Development of Brain Circuits Regulating Cognition and Emotion

The Effects of Prenatal Infection on Mechanisms Critical for Cortical Development

John H Gilmore*

Psychiatry, University of North Carolina, Chapel Hill, NC, USA

Prenatal exposure to maternal infections, viral and bacterial, increases the risk of neurodevelopmental disorders, including schizophrenia. We have hypothesized that inflammatory cytokines, generated by the maternal or placental immune system play a key role in the impact of maternal infection on the developing brain. We have utilized in vivo animal models of maternal infection and in vitro studies of cortical neuron development to understand mechanisms through which maternal infection acts on the developing brain. In animal models of bacterial and viral infections using e. coli lipopolysaccharide (LPS) and poly I:C respectively, we have shown that maternal infection significantly increases the inflammatory cytokines IL-1β, IL-6, and TNFα in the maternal-fetal unit. Maternal exposure to LPS increases brain-derived neurotrophic factor in the fetal brain, while ultimately reducing expression in the neonatal cortex. In cultures of embryonic cortical neurons, IL-1β, IL-6, and

TNFα, each significantly decrease the development of dendrite complexity, providing a link between the inflammatory cytokines generated during infection and the reduced cortical synapse density observed in postmortem studies patients with schizophrenia. In mature animals, inflammatory stimuli such as LPS causes activation of microglia, which in turn can generate inflammatory cytokines locally within the CNS. We will present recent data showing that prenatal exposure to maternal LPS causes persistent activation of microglia, as well as astrogliosis, in the neonatal cortex at 21 days after birth. Given emerging evidence that microglia and astrocytes play an important role in synapse development and maintenance, activation of glia in the cortex would likely have an adverse effect during this period of maximal synapse development. Finally, we will present data indicating that prenatal exposure to maternal infection can also inhibit fetal neurogenesis. Prenatal exposure to maternal infection, especially to the inflammatory cytokines that are generated acutely as well as by persistently activated glia, can have a significant impact on cortical neurogenesis, cortical neuron survival, and cortical dendrite and synapse development, processes thought to underlie the neuropathology of schizophrenia.

Sensorimotor Gating Deficits in Rodent Developmental Models Related to Psychotic Disorders

Mark Geyer* and Susan B Powell

Dept Psychiatry, Univ California Sch Med, La Jolla, CA, USA

Background: Circumstantial evidence has long supported the existence of developmental contributions to neuropsychiatric disorders, specifically schizophrenia. Novel models have been developed to address the effects of in utero or early post-natal perturbations, such as infection, on brain development and to identify mechanisms by which such challenges alter subsequent adult behaviors and susceptibility to psychiatric illnesses. Prepulse inhibition (PPI) of the startle reflex is an operational measure of sensorimotor gating that is amenable to cross-species comparisons and is deficient in patients with schizophrenia or other psychiatric disorders characterized by abnormalities in sensory, cognitive, or motor gating. Because of this link between some forms of schizophrenia and early developmental perturbations, many animal studies have examined the influences of various developmental manipulations on PPI in adulthood. For example, isolation rearing of rats from weaning into adulthood leads to a reorganization of brain circuitry including changes in monoamine systems that modulate PPI. Isolation rearing of rats leads to deficits in PPI that are not evident pre-puberty, endure through adulthood, and are developmentally specific, in that isolation of adult rats does not affect PPI. The PPI deficits in isolation-reared rats are reversed by antipsychotic treatments and not by several other psychoactive drugs. Thus, social isolation rearing of rats provides a developmentally specific, non-pharmacological manipulation that leads to deficits in sensorimotor gating that mimic those observed in schizophrenia patients and are responsive to antipsychotic medications. We have begun to compare the isolation rearing model with the effects of a prenatal immune challenge on PPI, based on the observation that schizophrenia is associated with increased rate of infection to the mother during gestation.
Methods: Following the methods described by Borrell et al. (Neuropsychopharmacology 26:204-15, 2002), we exposed pregnant Sprague-Dawley dams to 1.0 mg/kg of the bacterial endotoxin lipopolysaccharide (LPS; from Escherichia coli, Sigma L3755 Serotype 026:B6) on alternate days of pregnancy beginning on gestation day 1. Male and female offspring were then tested for PPI as adults.
Results: The results largely confirm Borrell's report. Male offspring of infected dams show significant deficits in overall percent PPI (mean +/-SEM): saline 49.8 +/-3.0; LPS 32.4 +/-4.7. In contrast, the small difference in the female offspring was not significant: saline 34.9 +/-6.5; 28.7 +/-4.0.
Discussion: Our findings further confirm that similar effects of LPS exposure on PPI are evident in Sprague-Dawley as well as Wistar rats. Borrell's work also demonstrated that such deficits in PPI are reversed by treatment with antipsychotic agents, paralleling the isolation rearing model. Further studies are warranted to assess the similarities and differences in the neuronal reorganization that is associated with these two developmental manipulations that produce similar deficits in sensorimotor gating measures that are reduced in patients with schizophrenia. Supported by MH52885.

The Impact of Infection Early in Life on the Formation of Long-Term Memories in Adulthood

Steven F Maier* and Staci D Bilbo

Psychology, Univ. of Colorado, Boulder, CO, USA

Background: Early exposure to infectious agents may have major consequences for the development of physiological systems throughout an individual's lifespan, a phenomenon that has been termed perinatal programming. For instance, neonatal exposure to bacterial products (e.g., lipopolysaccharide; LPS) influences reactivity to stress, immune regulation, and susceptibility to disease in adulthood, and there is increasing evidence to suggest that perinatal events involving the immune system may be involved in the etiology of a number of neuropsychiatric disorders. Few studies have examined, however, whether infection during the neonatal period alters cognitive functions in adulthood. Here we explore whether infection during the neonatal period alters the adult formation of long-term memories.
Method: Rat pups were infected with live, replicating E. coli on postnatal day 4 (PND4), or received vehicle. PND4 represents a time comparable to the third trimester in humans during which significant brain growth occurs. Subjects were tested for the ability to form long-term memory using a number of different paradigms in adulthood, some of which require the hippocampus for memory formation, and some which do not. In addition, subjects either received LPS or vehicle either before or after the learning experience. This was done to determine whether rats infected early in life might be more susceptible to disruptions produced by peripheral immune activation. In other experiments a variety of aspects of neural function were examined. Finally, rats were infected at times other than the early postnatal period to determine whether any effects obtained were truly developmental.
Results: Rats infected on PND4 but not challenged with LPS performed normally on all of the memory tasks. Thus, there were no gross impairments. LPS in adulthood had little effects on rats not infected early in life, but produced profound and long-lasting memory impairment In neonatally infected animals. This effect was specific to the formation of long-term memories. That is, neonatally infected animals that had received LPS in adulthood learned the tasks normally and remembered for a brief period of time, but were impaired in consolidating their memories to long-term memory. Moreover, this impairment was restricted to long-term memory formation for tasks that require the hippocampus. Importantly, these effects did not follow infection later in development. Immunohistochemistry and real-time PCR revealed that the early infection produced activation of microglia that persisted into adulthood, especially in the hippocampus. However, the expression of PICs by these microglia and other cell types was not elevated. The administration of LPS produced a much larger and more sustained increase in glial activation and PIC production in animals that had been infected on PND4. Finally, one of the PICs, interleukin-1beta, appeared to be critical to the disruption of memory. Inhibition of IL-1beta production during and shortly following the learning experience completely prevented the disruption of memory.
Discussion: Taken together, we have demonstrated that neonatal infection results in elevated glial cell markers and exaggerated PIC responses to LPS within the brain in adulthood, which appear to underlie memory impairment following an immune challenge. These

data suggest that neonatal infection creates a state of vulnerability that extends into adulthood. This vulnerability may involve glia that are primed, but not frankly pro-inflammatory. Here, stimuli that would normally produce an inflammatory response lead to an exaggerated response. Challenges other than immune activation may well lead to the same outcome.

Maternal Influenza Infection Leads to Neuropathology and Behavioral Abnormalities in Adult Offspring

Paul H Patterson*

Biology, California Institute of Technology, Pasadena, CA, USA

Background: Epidemiological evidence indicates that maternal viral infection increases the incidence of schizophrenia and autism in the offspring.
Methods: To explore this risk factor further, pregnant mice are given a respiratory infection with human influenza virus at mid-gestation. The pups are born normally and are analyzed behaviorally as adults and the brains taken for histology and molecular studies.
Results: The offspring of infected mothers display abnormalities consistent with those seen in schizophrenia and autism, including enhanced anxiety as well as deficits in social interaction and prepulse inhibition (PPI). The latter is corrected by anti-psychotic drug treatment. These adult offspring also display neuropathology in the hippocampus and cerebellum similar to that found in schizophrenia and autism, respectively. Furthermore, there are a number of changes in brain gene expression that are similar to those found in these disorders. The cause of these abnormalities is likely to be the maternal response to viral infection, as we find no evidence of virus in the fetus. Moreover, treatment of uninfected pregnant mice with dsRNA, which evokes an anti-viral-like immune response, also induces PPI deficits in the offspring. We are investigating whether cytokines mediate some of the effects of the maternal immune response on fetal brain development.
Conclusions: In a mouse model, the maternal response to influenza infection alters fetal brain development, leading to striking behavioral abnormalities, histopathology and altered gene expression consistent with such alterations seen in schizophrenia and/or autism.
Supported by the Cure Autism Now Foundation, Ginger and Ted Jenkins, the McKnight Foundation, the National Institute of Mental Health and the Stanley Medical Research Institute.

Panel Session
Genes and Brain Development in Health and Illness: The Trajectory is the Story

The Genetics of Cerebral Cortex Development in the Mouse

Susan K McConnell*

Department of Biological Sciences, Stanford University, Stanford, CA, USA

During development, the embryonic telencephalon is patterned into different areas that give rise to distinct adult brain structures. Several secreted signaling molecules are expressed at putative signaling centers in the early telencephalon, and other genes are expressed in layer-specific patterns in the cortex. We have used conditional and traditional knockout approaches in the mouse to assess the roles of secreted signaling molecules and transcription factors in the elaboration of neural circuitry during development.

Searching for the Cause of Dyslexia: To Gel and Bac

Albert M Galaburda*

Neurology, Beth Israel Deaconess Medical Center, Boston, MA, USA

Dyslexia is a disorder of mapping script to sound and quickly and efficiency linking up to language meaning. It is defined by a metaphonological problem characterized by difficulties with parsing words into phonemes and playing phonological games. Many dyslexic individuals show a problem with temporal processing of language and other sounds. We have developed a mouse model that exhibits equivalent auditory processing deficits. This mouse is produced by inducing neocortical neuronal migration anomalies, either surgically at birth or with the use of RNAi techniques. The cortical migration anomalies cause several plastic changes in cortical and subcortical structures and connectivity, which account for the auditory processing problem. There are attempts to compensate for the initial disorder, which work well in females but not in males. In this presentation the pathway beginning with gene mutation and continuing with gene function, neuronal migration anomaly, neural plasticity, and sound processing deficit will be outlined in surgical and genetic mouse and rat models of dyslexia.

Trajectories of Anatomic Brain Development in Children and Adolescents

Jay N Giedd*

NIMH, Bethesda, MD, USA

Background: As considerable strides have been made over the past decade in mapping the trajectories of anatomic brain development during childhood and adolescence, efforts have turned to examining the influences, for good or ill, on these developmental trajectories. Prominent among these investigations have been twin studies to address nature/nurture questions and genetic studies to examine the effects of specific genes.
Methods: Healthy MZ and DZ twins are recruited nationally for participation along with singletons in an ongoing longitudinal project at the Child Psychiatry Branch of National Institute of Mental Health which uses imaging, genetics, and psychological testing to explore the developmental neurobiology of cognition, behavior, and emotion. As of August 2005 the twin sample includes 654 scans from 122 MZ and 81 DZ pairs (with the longitudinal scans occurring at approximately 2 year intervals). For an independent sample of singletons 227 DNA samples with apoE status determination are available (82 E4 and 145 non E4).
Results: Structural Equation Modeling of brain morphometric twin data indicates a relatively low heritability for the cerebellum with age-by-heritability interactions for most structures examined. Gray matter heritability tends to decrease and white matter heritability tends to increase with age. Singletons with the E4 allele obtain peak cortical thickness earlier in polar temporal regions and later in frontal regions.
Discussion: As with sexual dimorphism, heritability and specific gene effects on brain morphometry during childhood and adolescent are age-dependent.

Genes, Brain Development and Clinical Outcome in Children with Attention Deficit/Hyperactivity Disorder (ADHD)

Philip Shaw*, W Sharp, M Gornick, A Addington, D Greenstein, X Castellanos and J Rapoport

Child Psychiatry Branch, NIMH, Bethesda, MD, USA

Context: ADHD is a highly heritable disorder in children, and while several risk genes have been identified it is unclear how these genes influence brain development.
Methods: Neuroanatomic images and clinical outcome data were acquired on 161 children with ADHD and age matched controls, with the majority of subjects having serial scans (627 scans in total) throughout adolescence. The overall and regional thickness of the cortex, volumes of the major lobes, cerebellum and subcortical structures were extracted automatically. We charted the effects of four risk genes for ADHD brain development in healthy children, and then examined deviation from this normal template among the subjects with ADHD. Additionally the relationship between risk alleles and

clinical outcome was examined and further linked to neuroanatomic measures.
Results: The risk alleles for DRD1 (rs2543), DRD4 (7 repeat VNTR), DAT-1 (10 repeat VNTR) were all associated with strikingly different and significant effects on the trajectory of cortical development in prefrontal regions. A similar interaction of diagnosis and genotype was found for caudate volume, with ADHD carriers of the DAT1 risk allele having a significantly smaller caudate. Additionally, ADHD subjects with the DAT1 risk allele who had a poor clinical outcome showed a marked, continuous loss in caudate volume throughout adolescence, unlike ADHD subjects without this risk allele.
Discussion: ADHD is associated with subtle abnormalities in cortical and basal ganglia development, particularly in the frontal lobes and caudate nucleus. In these regions risk alleles in healthy controls and subjects with ADHD are linked with significantly different developmental trajectories. We also observed different genotypic effects on brain development among ADHD subjects categorized according to clinical outcome. We speculate that risk genes may contribute to clinical outcome in ADHD through their effects on developing frontostriatal circuitry.

Panel Session
The Future of Phase IV Studies and Off-Label Prescribing: The Conundrum Facing Corporate Sponsors of Research, Regulators, Clinical Investigators and the Future of Psychiatric Practice

Dealing with the Challenging Legal Environment for Phase IV Clinical Trials and Off-Label Prescribing

Sandra H Johnson*

Center for Health Law Studies, Saint Louis University School of Law, St. Louis, MO, USA

This presentation will analyze the current legal environment and emerging legal standards influencing clinical research on pharmaceuticals, especially in Phase IV clinical trials. Legal claims levied against Phase IV sponsors and investigators also have serious implications for off-label prescribing generally. This presentation will indentify the impact of the current wave of legal actions related to off-label prescribing in routine medical practice as well as in a research context. The legal setting for clinical research generally has changed in the last several years, creating an environment in which judgments about research take place in the context of public scrutiny in the media and sometimes in the courts rather than within the scientific and medical professions alone. Phase IV trials raise particularly difficult legal issues for sponsor and researcher alike, and have triggered some prominent actions that illustrate some of the effects of opening the research endeavor to public review. Headlines in 2004 and 2005 have highlighted the legal risk of potential civil and criminal government enforcement actions on the part of the FDA, the Medicaid and Medicare programs, state attorneys general, and other agencies. The most well-known, but not the only one of these actions, against one firm for activities related to one drug led to a settlement with state and federal agencies of nearly $430 million in 2004. Other legal claims have asserted contractual liability to private health insurers for coverage of specific off-label uses of approved medication as well as liability to individual patients enrolled in trials. The clearest message from these headline cases is that "business as usual" and "professional custom" are not adequate defenses to charges of impermissible conduct. It is not enough to stay within the middle of the herd. Part of the challenge of the new legal environment for the study of and the expansion of off-label uses will be to develop standards that are clear enough to identify impermissible actions, for example in relation to clinical trials, industry-sponsored education, and marketing, without throwing the legitimacy of off-label uses into doubt in the practice of medicine. Key topics that will be discussed to illustrate these issues include litigation concerning federal and state criminal and civil enforcement actions against industry for marketing of off-label uses (e.g., actions relating to Neurontin); federal False Claims Act and anti-kickback litigation over claims for payment for off-label uses; products liability litigation concerning adverse events revealed in the course of clinical trials (e.g., those involving Vioxx and others); legal risks in specific financial arrangements or exchanges between physician investigators and sponsors of clinical trials; malpractice or other tort liability to individual patients for off-label prescribing; and other issues.

The Changing Legal Environment Around Phase IV Studies: The Industry Response

Roger Meyer, Dave Ceryak* and Doug Snyder

Eli Lilly & Company, Indianapolis, IN, USA

Phase IV studies have traditionally been supported by industry sponsors as a more efficient and flexible way (compared to registration-directed research) to work with external researchers and generate important efficacy and safety information about a product after it is on the market. The results of this scientifically-important research can, if positive, lead to initiation of registration trials for a new indication or might satisfy requirements for post-marketing safety data put in place by FDA as a condition of marketing approval. There are other benefits of Phase IV research that, in some cases, may have become the motive to support the research: 1) to encourage opinion leaders in academia to familiarize themselves with a new medication in investigator initiated studies; and 2) to utilize publications from these studies (which carry the names of these distinguished investigators) to highlight the product to the universe of clinicians in the relevant therapeutic area. Because this research is not meant to support registration and involves marketed products, industry's role in supporting the research might involve the sponsor's field Medical staff (Medical Liaisons) or its sales or marketing personnel. The changing legal environment around Phase IV studies and off-label prescribing has been well-described by the first speaker (Professor Johnson). In the context of the action against Pfizer in the Neurontin case, new theories of liability by government prosecutors and whistleblowers, and the volatile drug safety environment (as demonstrated by the litigation facing Merck — Vioxx), there has been significant re-thinking by industry sponsors about the solicitation, internal review process, and oversight of investigator initiated studies within each company. In many ways, these discussions have been shaped by the opinions of attorneys within the companies. For this panel, attorneys from Lilly (David Ceryak) and GSK (Douglas Snyder) will describe the changes that have, or will take place, in support of Phase IV studies in these companies. In addition, Mr. Snyder brings the perspective of having been at FDA as it initiated the Neurontin review that led to the action against Pfizer.

Reporting of Phase 4 Studies in Scholarly Journals

Richard M Glass*

JAMA, Chicago, IL, USA

Phase 4 clinical trials performed after a product has been approved and marketed can provide important information, such as systematic data on long-term effectiveness, optimal dosing, and the incidence and nature of adverse effects occurring with long-term use — information that is often not available from earlier phase studies. Phase 4 studies of off-label use for previously unapproved indications or patient populations (such as children or the elderly) can provide critically important systematic information on those uses that would otherwise be unavailable. Unfortunately, the use of phase 4 trials for marketing purposes has complicated their reputation and evaluation to the extent that careful, complete data collection and transparency in reporting of results have not occurred. This has resulted in such trials often not being published in scholarly medical journals, where

the results would have the most impact on clinical practice. The solution to these problems appears to be that phase 4 studies should be designed in consultation with academic researchers with the goal of obtaining the most useful scientific and clinical information and with a view toward reporting them following the same principles and procedures that can maximize the transparency and credibility of all clinical trials. These include registration in a publicly accessible clinical trial registry; appropriate use of an independent data and safety monitoring committee; independent investigator access and responsibility for study data, analysis, and reporting according to the CONSORT principles; full disclosure of financial support and conflicts of interest; and for commercially funded studies, analysis of the data by an independent biostatistician. Following these principles should maximize long-term outcomes for patients, investigators, commercial funders, and public confidence in biomedical research.

The Future of Phase IV Studies and Off-Label Prescribing: The Conundrum Facing Corporate Sponsors of Research, Regulators, Clinical Investigators and the Future of Psychiatric Practice
David Meltzer*

Medicine, University of Chicago, Chicago, IL, USA

Off label prescribing reflects a compromise position between regulatory and laissez faire approaches to pharamceutical access. As such, the consequences of alternative off-label prescribing policies can be considered as reflecting the relative strengths of the benefits and costs of the regulatory or laissez faire approaches to pharmacutical access for any given chemical entity. Key concerns include the scientific merit, speed, cost, and adpatability of the regulatory process, and the ability of firms, physicians, patients, and other actors in the health care system to produce desirable use of pharmaceuticals in the absence of regulation. Attributes of the chemical entity and its potential users may influence the relative magnitude of these forces and the desirable level of regulation of prescribing practice in any given setting.

Study Group Session

Developing a Consortium and Mechanism to Facilitate the Development of Radiotracers as Useful Biomarkers in Academia and Industry
Dean F Wong*, Linda Brady and Robert Innis

Radiology, Johns Hopkins University, Baltimore, MD, USA

This policy-related study group (SG) builds on last year's "Radiotracer Development: Barriers and Solutions to Study Pathophysiology and Enhance Therapeutic Drug Development." Both Pharma and academia have identified intellectual property (IP) as perhaps the single most significant barrier. Pharma is reluctant to release data related to a radiotracer because of the concern of loss of IP and competitive advantage. Academia has increased its surveillance and defense of IP, even where royalties are unlikely. Other policies may inhibit development in academia (e.g. non-exclusive licenses). These trends impede the development of imaging agents that could be used as tools in pathophysiological studies and drug development. This SG will address IP issues related to radiotracer development with the goal of reaching a consortium agreement to work towards their resolution. The ACNP Liaison Committee has identified at least 3 areas of potential cooperation. 1) Companies might work jointly to develop a radiotracer for a biomarker that would be useful to multiple experimental therapies with variable mechanisms of actions. 2) A radiotracer already developed in-house but not publicly disclosed could be made available for pathophysiological studies in academia. 3) A tracer already reported in the public domain but insufficiently validated could be released for critical quantitative analysis and then used for pathophysiological studies. One solution is a neutral clearinghouse, to which participants can submit blinded data on novel tracers and/or query if tracers are available for specific targets/mechanisms and the extent to which they have been validated. The use of these tracers could be brokered by the neutral party, if an organization does not wish to reveal publicly its interest in this therapeutic area. The clearinghouse could facilitate licensing agreements that would protect the developer of the tracer while still allowing limited use by other groups. Arrangements like these already occur informally but could be enhanced with a clearinghouse that would facilitate communication and mediate information exchange. This would enhance a more equitable sharing of IP while facilitating the development of radiotracers as research tools. Furthermore, this could offer developers (e.g., universities) a mechanism for more widespread use of any agent developed in-house instead of a restrictive IND and licensing policy. Participants will include individuals directly involved with drug development, and imaging biomarkers and have the support of a senior (VP level) decision maker to validate future commitments. The Liaison Committee can help coordinate future meetings and guide the development of the proposed clearinghouse.

Study Group Session

J'Accuse: COI in the News, but What of the Accused?
Daniel R Weinberger*, William Carpenter, Ezekiel Emanuel, Jack Gorman, Robert Muse, Jamie Talan and Alan Breier

NIMH/NIH, Bethesda, MD, USA

COI has become a critical issue for academic, government and industry scientists, and for organizations like the ACNP. Many of the recent high profile examples of conflicts of interest in industry and in academic and government research institutions have led to revised policies and procedures for preventing and managing potential conflicts. But COI vigilantism also is on the rise. Accusations may lead to trial in the press, secret investigations, lack of due process, and damages to the careers and personal lives of the accused even when conflict is not proven or real. This workshop will engage discussion about strategies for responding to such accusations, involving pariticpants from government, industry, University centers, the press and legal experts involved in these issues.

Tuesday, December 13, 2005

Panel Session
Animal Models of Schizophrenia: Making Progress or Just a Delusion?

Morphological and Molecular Changes in the Prefrontal Cortex of Rats Following Neonatal Ventral Hippocampus Damage
Lalit Srivastava*, Sanjeev Bhardwaj, Gonzalo Flores, Melissa Palardy, Clifford Cassidy and Remi Quirion

Psychiatry, McGill University, Montreal, QC, Canada

Background: Excitotoxic lesion of the ventral hippocampus (VH) in neonatal rats leads to a number of behavioral alterations in post-adolescent period, including disturbances in cognitive functions and sensorimotor gating. The neonatal VH (nVH) lesioned animals are often considered as developmental models of schizophrenia. The neural and molecular mechanisms of behavioral changes in nVH lesioned animals are poorly understood, though dysfunctional mesolimbic dopamine system is generally recognized to contribute to some behaviors. Recent evidence suggests that abnormalities in prefrontal

cortical neural circuits may be critical to behavioral alterations in nVH lesioned rats.
Methods: Sprague-Dawley rat pups, 7-day old, were microinjected with ibotenic acid or PBS (control) in the ventral hippocampus. At different postnatal periods, neuronal morphology, gene expression and behavior of nVH lesioned and control animals were assessed. In-situ hybridization and Golgi-Cox methods were employed to examine gene expression and neuronal morphology respectively. Affymetrix gene chips and real-time PCR were used to assess global gene expression changes. Locomotor activity of animals was measured in automated activity boxes.
Results: The results show that the length of basilar dendrites and branching and the density of dendritic spines on layer 3 pyramidal neurons were significantly decreased in post-pubertal rats with nVH lesions. Medium spiny neurons from the the nucleus accumbens (NAcc) showed a decrease in the density of dendritic spines without significant changes in dendritic length or arborization. In situ hybridization analyses showed that the basal level of an immediate early gene NGFI-B (Nurr77) mRNA in nVH lesioned rats was significantly reduced in the medial prefrontal cortex (mPFC) at post-pubertal age. No significant difference in NGFI-B mRNA levels was seen in the NAcc; however, a differential effect of amphetamine treatment on NGFI-B was observed between control and lesioned rats. Affymetrix DNA microarray analysis revealed altered expression (1.3-2.5 fold) of over 250 genes in the PFC of post-pubertal nVH lesioned rats; however, not all gene changes have yet been confirmed by real-time PCR. Interestingly, among confirmed genes are those for NGFI-B, activity-inducible protein Homer 2, and post-synaptic density protein Shank 1, which, along with Homer, is involved in dendritic spine formation.
Discussion: Taken together with other data, these results suggest significant structural and molecular realignment within the prefrontal cortex of nVH lesioned rats as they mature to adulthood. These changes may interact with neurochemical alterations in the PFC of nVH lesioned animals reported by us previously, e.g., in adrenergic and cholinergic systems, resulting in abnormal working memory functions and regulation of subcortical dopamine. Animal models are unlikely to reproduce the entire pathology of a disease. Nevertheless, data from nVH lesioned animals point to novel mechanisms of prefrontal cortical dysfunctions often described in schizophrenia. (Supported by the Canadian Institutes of Health Research).

Genetic Animal Models of Psychosis: The Dopamine/Glutamate Interaction
Marc G Caron*, Martin Beaulieu, Amy R Mohn, Tatyana Sotnikova and Raul R Gainetdinov

Cell Biology, Duke University Medical Center, Durham, NC, USA

Background: In the brain the dopaminergic and glutamatergic systems are presumably implicated in the control of locomotion, cognition and affect. Dysregulation in these systems has been implicated in the etiology of schizophrenia. In order to gain an understanding for a role of these neuronal systems to the elaboration of these symptoms, we have used a genetic approach in the mouse by creating an animal model in which the gene for the dopamine transporter (DAT) has been inactivated. DATKO mice mimic amphetamine-treated animals and recapitulate many of the endophenotypes attributed to positive symptoms of psychosis.
Methods: We have used in vivo pharmaco-genetic approaches in these mice to evaluate their responses to a variety of interventions in an attempt to understand the circuits as well as the signaling pathways implicated.
Results: Inactivation of the DAT gene results in a marked elevation of extracellular striatal dopamine (> 5 fold), which translate into a pronounced hyperactivity phenotype when exposed to a novel environment. DATKO mice also have marked deficits in PPI and various cognitive aspects of behaviors. We have used these mice to examine their responses to a variety of pharmacological interventions used in the treatment of schizophrenia. Antipsychotics and serotonin 2A receptor antagonists decrease hyperactivity and improve performance in PPI tests. Inhibition of glutamate transmission with NMDA receptor antagonists produces large further increases in locomotion whereas enhancing glutamate transmission with AMPAkines or inhibitors of the glycine transporter inhibit locomotion illustrating the link between the DA and glutamate systems. This link is also apparent in another animal model in which the NR1 subunit of the NMDA receptor has been genetically knocked down. These mice mimic MK-801 treated animal and display moderate hyperactivity, deficit in PPI and social interactions that can all be reversed by antipsychotics. Interestingly, in DATKO mice lithium can also inhibit locmotor activity and this is associated with an enhanced phosphorylation of Akt/PKB and its target substrate GSK3α&β. Thus in DATKO mice Akt and GSK3 are dephosphorylated whereas in wild type mice, apomorphine and amphetamine induce a dephosphorylation of Akt (inactivation) and GSK3 (activation). These responses are mediated through the D2 class of dopamine receptors. Inhibitors of GSK3 recapitulate the effects of Li in DATKO mice and mice lacking one allele of GSK3β show blunted responses to amphetamine. Recent experiments have shown that D2 class receptors mediate these effects through a novel signaling mechanism that involves the scaffolding of Akt and protein phosphatase 2A with βarrestin 2 a component of the desensitization machinery of G protein coupled receptors. Interestingly, mice lacking a functional βarrestin 2 gene show diminished response to amphetamine and inactivation of the βarrestin 2 gene on the DATKO background reduces their hyperactivity phenotype.
Discussion: These results suggest that the DATKO mice may recapitulate several endophenotypes of schizophrenia and provide a model to investigate the potential efficacy of known and novel antipsychotic drugs. Our results coupled with recent evidence linking dysregulation of the Akt signaling pathway with schizophrenia (Emamian et al, 2004; Ikeda et al, 2004) suggest that the regulation of Akt by the βarrestin 2 mediated signaling could contribute to the etiology and /or management of this condition.

New Genetic Mouse Models of Schizophrenia: Mimicking Cognitive Dysfunction by Altering Susceptibility Gene Expression
Jingshan Chen*, Barbara K Lipska and Daniel R Weinberger

Clinical Brain Disorders Branch, Genes, Cognition and Psychosis Program, NIMH, NIH, Bethesda, MD, USA

Translation of human genetic mutations into genetic mouse models is an important strategy to study the pathogenesis of schizophrenia, identify potential drug targets and test new drugs for new antipsychotic treatments. Recent discoveries of susceptibility genes for schizophrenia offer opportunities to develop a new generation of genetic mouse models of schizophrenia based on the genetic susceptibility. Most of the susceptibility genes have been linked or associated with cognitive dysfunction, a symptom relatively resistant to current antipsychotic treatments and viewed as a core symptom for schizophrenia. Unlike hallucinations and delusions, some domains of cognitive function, such as working memory, can be directly tested in mouse models. To mimic cognitive dysfunction, we over-expressed human catechol-o-methyl transferase (COMT)-val transgene, the high risk allele of the COMT gene for schizophrenia, in inducible tissue-specific transgenic mice. The COMT-val transgenic mice were analyzed in a radial arm-maze test. Our results demonstrated that the working memory was impaired in the COMT-val transgenic mice with high level of COMT over-expression in pyramidal cells of frontal cortex. The transgenic mice showed normal locomotor activity, but slower response to the baited food rewards in the maze. The working memory deficit and slower response are typical cognitive symptoms in schizophrenia. Therefore, the COMT transgenic mouse model is a valid model for cognitive dysfunction resulted from high activity of COMT in frontal cortex and the results suggest that a COMT inhibitor might be an effective drug for the treatment of cognitive dys-

function in schizophrenia. For other susceptibility genes, knocking out the gene by homologous recombination or knocking down the gene by siRNA silencing should be used if the lower activity allele is the risk allele. The new generation of the genetic mouse models could shed light on the etiology of schizophrenia and lead us to new hypotheses, novel diagnostic tools, and a more effective therapy.

Modelling Antipsychotic Action - Convergence from Studies of Mice and Men

Shitij Kapur* and Ming Li

CAMH - University of Toronto, Toronto, ON, Canada

Conditioned Avoidance Response (CAR), was one of the first (1952)″animal models″ used to study antipsychotics. In a series of experiments we have examined the mechanistic validity and the convergence between the action of antipsychotics in CAR and in humans. The data show that: a) typical or atypical, single or mulitreceptorial, antagonist or partial agonist, all antipsychotics that are known to be effective in humans are effective in CAR models; b) targets that have failed to be effective in humans (5-HT2, D4, A1) are ineffective in CAR; c) antipsychotics are generally effective in CAR at occupancies (~ 60-70% D2 occupancy) which are consistent with their doses/occupancies in patients; d) like in the clinic, clozapine is effective at lower than usual and aripiprazole requires saturating D2 occupancies to be effective in CAR; e) recent clinical data suggest that the anti″psychotic″ effect starts almost immediately after the first dose, grows with time and then asymptotes — repeated dosing in the CAR model replicates this trajectory; f) this progressive increase of the effect of antipsychotics in CAR not a function of dose accumulation or motor dysfunction, but, reflects a drug-induced, class-specific, practice-dependent, learning of new associations which allows the animal to overcome its spontaneous avoidance response. The implications of the model for the mechanism of antipsychotics will be discussed.

Panel Session
Noradrenergic and Dopaminergic Modulation of Prefrontal Cognition

Amygdala Modulation of Conditioning within the Prefrontal Cortex is Dependent on Cannabinoid and Dopamine D4 Receptor Activation

Anthony A Grace*, Steven R Laviolette and Witold J Lipski

Dept. of Neuroscience, University of Pittsburgh, Pittsburgh, PA, USA

Background: Previous studies from our lab have shown that conditioning can occur within the amygdala of anesthetized and non-anesthetized rats in a dopamine-dependent manner. In the present study, we found that the response of prefrontal cortical (PFC) neurons can be altered by pairing a noxious stimulus with an odor, and that this association is dependent on amygdala input and is modulated by dopamine (DA) D4 receptors and cannabinoids.
Methods: Rats were anesthetized with chloral hydrate, and single neurons in the medial PFC were recorded. Two different odors were presented to the nose of the rat; one that was paired with footshock (CS+) and one paired with absence of footshock (CS-). DA D4 antagonist L-741741, Cannabinoid-1 receptor (CB1) agonist AM 251 and antagonist WIN 55,212-2 were administered systemically. For behavioral experiments, rats were placed in a shock environment containing one odor (CS+) and a neutral environment containing the other odor (CS-). The same drugs or saline were microinjected into the PFC during the acquisition phase, and animals were tested for freezing and exploratory behavior to the CS+ and CS- in the drug-free condition.
Results: Only PFC neurons that exhibited direct excitation to amygdala stimulation exhibited an increased firing rate and burst firing in response to the CS+ but not the CS-. This no longer occurred if the amygdala was inactivated by muscimol injection during the pairing, showing that an intact amygdala is required for the PFC conditioning to occur. Following systemic administration of either the D4 antagonist or the CB1 antagonist, PFC neurons did not acquire alterations in firing rate or pattern to the CS+. In contrast, administration of the CB1 agonist the response to the CS+ was significantly greater. In the behavioral experiments, animals showed significant increases in freezing and decreases in locomotion to the CS+ but not the CS-. This was prevented if either the D4 antagonist or the CB1 antagonist was microinjected into the PFC during the pairing. Although microinjection of the CB1 agonist did not significantly alter the response to the CS+, it did enable conditioning to previously subthreshold levels of footshock.
Discussion: These data show that neurons in the PFC that receive a functional monosynaptic excitatory input from the amygdala will exhibit conditioned responses, provided that the baseline level of D4 and CB1 receptor stimulation is present. Moreover, this PFC system must be intact for the animal to acquire conditioned fear responses. Disruption of the ability of the PFC to acquire and modulate conditioned responses may underlie the pathophysiology of disorders related to learning and affect.

Relative Roles for Dopamine and Norepinephrine in Attentional Function and the Behavioral Effects of Methylphenidate and Other Therapeutic Agents for ADHD

Trevor W Robbins*

Experimental Psychology, University of Cambridge, Cambridge, United Kingdom

Background: Although it has commonly been assumed that the dopaminergic and noradrenergic innervations of the PFC have distinct modulatory functions, this has proven difficult to demonstrate in practice in terms of behavior. This issue is important for resolving how, for example, methylphenidate exerts its therapeutic effects in attention deficit/hyperactivity disorder, and to what extent selective noradrenergic agents will be effective in this condition. It is also relevant to understanding how cortical dopamine (DA) and norepinephine (NE) mechanisms may contribute to cognitive deficits in schizophrenia.
Methods: We employed the 5 choice serial reaction time task for rats to assess the effects of various pharmacological manipulations of the central noradrenergic system, including intra-prefrontal cortical saporin dopamine beta hydroxylase (DBH), the alpha-2 agonist guanfacine and methylphenidate on performance. We used a comprehensive battery of tests of attentional and cognitive function to assess the effects of guanfacine and the selective NA reuptake blocker atomoxetine on performance in helathy human volunteers. We also used several receptor antagonists to tests whether the behavioral effects of methylphenidate could be attributed to its actions on dopamine or noradrenergic mechanisms.
Results: Intra-cortical infusions of saporin DBH produced loss of NE neurons in the prefrontal cortex and other regions that led to selective deficits in attentional function under certain defined conditions. This treatment also enhanced the deleterious effects of systemic guanfacine on attentional performance, but had no effect on the effects of methylphenidate. However, systemic administration of the beta-blocker propanolol was effective in selectively antagonising the effects of methylphenidate on impulsive responding on the 5-choice serial reaction time task for rats. In human subjects, guanfacine at doses of both 1 and 2mg had few effects on performance of a battery including tests of working memory, attention set-shifting, planning and stop-signal reaction time (SSRT) although it tended to produce sedative effects on the latter. By contrast, the selective NE reuptake inhibitor atomoxetine produced potent improvements on the SSRT in human volunteers. Further studies have focused on the beneficial effects in humans of methylphenidate on working memory function, and their antagonism by the D2 receptor antagonist sulpiride.

Discussion: Our data in rats and humans suggest that manipulations of NE function have specific effects on attentional and inhibitory control functions. Moreover, some of the effects of methylphenidate may be mediated by central NE, as distinct from DA, mechanisms. Overall, it appears likely that both NE and DA mechanisms contribute to the behavioral effects of methylphenidate.

Noradrenergic and Dopaminergic Interventions in the Schizophrenia

Larry J Siever*, Philip Harvey, Marianne Goodman, Harold W Koenigsberg, Vivian Mitropoulou, Deanna Barch and Joseph Friedman

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

Background: Both dopamine and norepinephrine have been demonstrated to play critical roles in modulating prefrontal cortical processing, particularly working memory and executive function. In order to explore the role of these two neurotransmitter systems on cognitive performance as well as their possible pathophysiology in a schizophrenia spectrum, pharmacologic interventions with catecholaminergic agents as well as imaging studies of dopamine release and D_1 receptors were performed in subjects with schizotypal personality disorder (SPD) and a comparison group of patients with other personality disorders (OPD).
Methods: 24 SPD and 20 OPD patients entered a double-blind placebo controlled treatment trial and received guanfacine at a dose at 2 mg/day over four weeks. Of 16 SPD patients who entered a double blind placebo controlled trial of pergolide treatment, 10 received pergolide up to a maximum of .1 mg/wk over four weeks.
Results: Our results show that guanfacine had beneficial effects on several cognitive domains including substantial effects on maintenance working memory measured by the DOT test of visuospatial working memory. While SPD subjects made greater BX errors on a modified AX CPT which assesses context processing (reflecting poor context processing) and greater long delay AY errors (reflecting increased reliance on context) compared to controls ($p<0.05$), guanfacine treatment in SPD subjects reduced long delayed BX errors ($p<0.05$, covarying for baseline) and increased long delay AY errors. Performance on Trail Making Part A was not substantially affected but the more demanding Trail Making Part B was improved more substantially by guanfacine. Indeed, guanfacine led to improvement to the point of near normality on BX errors reflecting poor context processing. Pergolide resulted in a significant decrease in BX errors ($F(1, 16) = 6.62$, $p = .02$) reflecting an almost one third decrease in BX errors and a significant increase in AY errors was observed as well. For the pergolide, like the guanfacine, Trail Making Part B improved, as did the letter number sequence, but in contrast to the guanfacine trial, performance on an auditory working memory task, the PASAT, showed improvement with pergolide ($p = .0005$) while there was no significant improvement in the DOT test. These results may reflect differences in modality specific processing affected by the two types of agonists, or differences in efficacy of subcomponents of a working memory model of Baddely. Preliminary results suggest that D_1 receptors in the schizophrenia spectrum may be increased in subcortical regions in schizotypal subjects with relative reductions in binding associated with greater anhedonia ($p < .05$).
Discussion: Both guanfacine and pergolide improved working memory and reliance on context. Pergolide had a greater effect on an auditory working memory task involving maintenance/updating and guanfacine on a delay-dependent visuospatial working memory task. These results will be discussed in terms of potential differential roles of norepinephrine and dopamine in modulating cognitive function in the schizophrenia spectrum and relationships between responses to catecholaminergic agents and imaging variables.

COMT in Prefrontal Cortex and Beyond: Genetic Studies of Cortical DA Signaling

Daniel R Weinberger*, Andreas Meyer-Lindenberg, Venkatta Mattay, Callicott Joseph, Karen Faith Berman, Bhaskar Kolachana, Jingshan Chen, Terry Goldberg, Jose Apud and Michael Egan

Genes Cognition and Psychosis Program, NIMH/NIH, Bethesda, MD, USA

COMT has been shown to impact on cortical DA levels and have little impact on striatal DA and on NE (Tunbridge et al J. Neurosci 2004). Genetic variation (val158met) in COMT impacts on cortical processing of executive cognition, including working memory (Egan et al PNAS 2001) and attentional control (Blasi et al J. Neurosci 2005), and also hippocampal processing of memory and emotion (Drabant Arch Gen Psych under review). Augmentation of cortical DA levels in normal subjects, either by administration of amphetamine (Mattay et al PNAS 2003) or tolcapone (Apud et al Arch Gen Psych in press), enhances or impairs prefrontal cognition, depending on the load of val or met alleles, respectively. This genotype dependent variation in the effect of augmenting cortical DA is consistent with evidence of an inverted U shaped cortical DA dose response curve. Recent data in human subjects correlating cortical rCBF with F18-DOPA uptake in the brainstem based on COMT genotype shows a relationship between prefrontal function and brainstem DA activity consistent with the inverted U model and with models of tonic cortical inhibition of brainstem DA neuronal activity (Meyer-Lindenberg et al Nat Neurosci 2005). A similar result had earlier been reported in postmortem normal human brain tissue showing that COMT genotype predicted TH mRNA expression in brainstem DA neurons, and the predictions were consistent with models of tonic cortical inhibition of brainstem DA activity (Akil et al J. Neurosci 2003). Finally, recent genetic data indicates that at least three loci within COMT are functionally variant, including a promoter variant, the val/met coding variant (Chen et al AJHG 2004), and a variant in the region of the 3'UTR affecting mRNA abundance (Bray AJHG2003). Functional analysis of the effects of these variants on prefrontal cortical physiologic activity measured with fMRI indicates that varying combinations of alleles at these loci (haplotypes) can interact to produce common functional states of the COMT gene, likely accounting for inconsistent clinical associations based solely on specific alleles or haplotypes (Meyer-Lindenberg et al under review). These various findings illustrate the complex biologic associations of genetic variation in COMT, which reflect local and downstream ramifications of varied cortical DA signaling, and the complex interactions of varying functional loci within the gene on these biologic associations.

Panel Session
Steroid Hormones and Addiction: From Genes to Behavior

Effects of Estrogen in the Brain: Roles of ER Alpha and Beta in Neurons and Glia

Daniel M Dorsa*, Damani Bryant, Laird Sheldahl, Andrew Mhyre, Lisa Marriott and Robert Shapiro

Physiology and Pharmacology, Oregon Health and Science University, Portland, OR, USA

In addition to its well known effects on sex behavior, estrogen has been reported to, such as ameliorate menopausal symptoms, enhance of motor function and cognition, promote neuroprotection, and modulate the reinforcing effects of drugs. Rapid, membrane–based effects of the hormone have been implicated in many of these actions. While recent findings of the Women's Health Initiative Study (WHI) are still being evaluated, it is clear that more selective, mechanism specific, estrogenic agents will be required to separate positive from adverse effects, many of which are the result of nuclear effects of the

hormone. Several laboratories, including our own, have shown that the same estrogen receptors (ER's) that mediate nuclear effects, ER αand β, also transduce rapid, membrane-initiated effects of the hormone. Transfection of these receptors into either immortalized murine hippocampal (HT22) neurons, or C6 astrocytic gliocytoma cells, promotes rapid responses to estrogen exposure not seen in untransfected cells. In HT22 cells, MAP kinase and CREB phosphorylation and CRE–mediated gene transcription is noted within minutes after treatment with estrogen. While ER α appears resident at the membrane, ERβ exhibits movement from the cytoplasmic compartment to the neuronal membrane within 5 minutes of estrogen exposure, and can be detected by ICC, in cell fractionation experiments, or using GFP–tagged receptor. In C6 glioma cells, transfection of ERα confers sensitivity to estrogen's rapid effects as well, but in this case appears to involve coupling to PI3 Kinase, promoting a Ca++–dependent reduction in cAMP accumulation and CRE–activation. Thus, the rapid effects of estrogen may differ in neurons and glia. In order to determine whether these rapid, membrane–initiated effects observed in in vitro cell systems also occur in the brain in vivo, ovariectomized rats were given a single injection of 15ug/kg of 17β estradiol (or its 17α isomer). 17β (but notα) estradiol promotes MAP kinase phosphorylation in several regions of the brain within 20 min as measured by Western blot of extracts from microdissected brain tissue. These effects occur in many regions implicated in addiction, and which express either ER. These include the nucleus accumbens, paraventricular nucleus, arcuate nucleus, diagonal band of broca, and the cerebral cortex. These effects can also be seen when brain tissue slices are treated with estrogen or its membrane–delimited BSA conjugate. Given the importance of CRE–depenent gene transcription in drug addiction, these effects are likely to be important mediators of the effects of estrogen. (Supported by NIH NINDS NS20311-23 and the Alzheimer's Disease Research Center of the Univ. of Washington.)

Sex Differences and Influences of Steroid Hormones in Drug Abuse
Jill B Becker*

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

Cocaine abuse by women has increased rapidly in the last decade. Approximately 30% of the 1.8 million Americans who use cocaine are women, and sex differences in patterns of cocaine use and addiction are well documented. Women begin using cocaine at an earlier age than men, after first use they take less time to become addicted, they enter treatment at a younger age than men, and when they present for treatment they have a more severe habit than men. Sex differences in cocaine self-administration behavior are also found in rodents. Female rats are more sensitive to the psychomotor activating effects of psychostimulants than are males and with repeated drug treatment they show greater psychomotor sensitization. We have found that independent of the presence of gonadal hormones in the adult, female rats exhibit greater behavioral sensitization to cocaine and acquire cocaine self-administration more rapidly than males. Thus, some of the sex differences in the response to drugs of abuse are due to sex differences in the adult brain. In addition, estradiol enhances stimulated dopamine detected in dialysate as well the acute behavioral response to cocaine, and behavioral sensitization to cocaine. Female rats exhibit greater behavioral sensitization to cocaine, acquire cocaine self-administration more rapidly than males, and estradiol enhances this sex difference. Recent studies from this laboratory indicate 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. These effects of estradiol are mediated, at least in part, by the action of estradiol at membrane estradiol receptors. To develop a good understanding of the all of causes of drug abuse it will be important to delineate the neurological bases for sex differences in drug abuse as well as the mechanisms mediating the effects of estradiol on the responses to cocaine and other drugs of abuse.

Steroid Hormones, DARPP-32 and Signal Integration Mechanisms in Brain and Behavior
Shaila Mani*

Molecular & Cellular Biology, Baylor College of Medicine, Houston, TX, USA

Ovarian steroid hormones, estradiol and progesterone, modulate neuroendocrine functions in the central nervous system resulting in alterations in reproductive physiology and behavior in female mammals. Classical model of steroid hormone action assumes that these neural effects are predominantly mediated via their intracellular receptors functioning as "ligand-dependent" transcription factors in the steroid-sensitive neurons regulating genes and genomic networks with profound behavioral consequences. Steroid receptors are phosphoproteins and are regulated by phosphorylation. Thus, steroid hormone-dependent, receptor-mediated transcription is dependent on the state of phosphorylation of the cognate receptors and/or their co-regulator proteins. Studies from our laboratory demonstrate that in addition to the steroid hormones, intracellular steroid receptors can be activated in a "ligand-independent" manner by neurotransmitter, dopamine, which can alter the dynamic equilibrium between neuronal phosphatases and kinases via DARPP-32. Using biochemical and molecular approaches we have elucidated that the signaling cascade initiated by neurotransmitter, dopamine, converges with steroid hormone-initiated pathways to regulate neuroendocrine pathways associated with reproductive behavior. Signal transduction via protein phosphorylation is common to the molecular mechanisms and pathways through which steroid hormones and neurotransmitters mediate their physiological effects in the central nervous system. Current studies suggest a high degree of cross-talk and reinforcement among non-classical, membrane-initiated pathways at the G-protein level and the classical intracellular signaling pathways at the transcriptional level in the mediation of steroid hormone-dependent behavior in mammals. The molecular mechanisms, by which a multitude of signals converge with steroid receptors to delineate a genomic level of cross talk, provide new avenues for understanding the role of steroid hormones in brain and behavior. Supported by NIH grants MH057442 and MH063954.

Anabolic Steroid Addiction? Insights from Animal Studies
Ruth I Wood*

Cell & Neurobiology, Keck School of Med of USC, Los Angeles, CA, USA

Androgenic-anabolic steroids (AAS) are drugs of abuse. They are taken in large quantities by athletes and others to increase performance, with negative long-term health consequences. Although AAS were banned from Olympic competition in 1975, and classified as controlled substances in 1991, steroid abuse continues. However, the potential for reinforcement and addiction to AAS has received relatively little attention. In particular, it is difficult in humans to separate the direct psychoactive effects of AAS from reinforcement due to their systemic anabolic effects. Recent studies in rats and hamsters have demonstrated that testosterone is reinforcing using conditioned place preference and self-administration. In particular, our laboratory has provided evidence of voluntary testosterone intake, including oral, intravenous, and intracerebroventricular (icv) self-administration. Male Syrian hamsters self-administer testosterone icv across a range of concentrations (0.1 -2.0 ug/ul). Icv testosterone induces Fos in the medial amygdaloid nucleus, bed nucleus of the stria terminalis, and ventral tegmental area. Although hamsters will also self-administer estradiol, the reinforcing effects of AAS do not require aromatization to estrogen because hamsters will self-administer the non-aromatizable androgens dihydrotestosterone or drostanolone. Moreover, reinforcement is related to androgenic potency: male hamsters will

self-administer the potent injectable androgens drostanolone or nandrolone decanoate, but do not develop a preference for the active nose-poke with self-administration of the weak oral androgens, oxymetholone or stanozolol. Likewise, male hamsters will acquire self-administration of recently-controlled androgen precursors such as androstenedione. Circulating androgens appear to enhance sensitivity to self-administration of exogenous androgens because operant responding for testosterone at 0.1 ug/ul is reduced in females and castrated males. When taken in large doses (>40 ug in 4h), testosterone icv produces torpor and, occasionally, death. Symptoms of androgen overdose resemble those of opioid intoxication, and androgen withdrawal can be induced by the opioid antagonists naloxone and naltrexone. However, with continued exposure, hamsters develop tolerance to the depressive effects of testosterone. Tolerance and withdrawal are key criteria for addiction. Thus, it appears that AAS may be addictive, even in an experimental context where athletic performance is irrelevant. (Supported by NIH DA-12843).

Panel Session
Drug Development: Biomarkers in Alzheimer's Disease: Early Diagnostic and Treatment Implications

D10S1423 Identifies Susceptibility Locus AD7 for Alzheimer's Disease in a Prospective, Longitudinal, Double-Blind Study of Asymptomatic Individuals

George S Zubenko*, Hugh B Hughes and Wendy N Zubenko

Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA

Typical, later-onset forms of Alzheimer's disease (AD) appear to be influenced by multiple susceptibility loci, combinations of which contribute to the development of this disorder. We previously reported the results of a systematic survey of the human genome for the identification of highly informative DNA polymorphisms (SSTRPs) that target new AD risk genes. In addition to the APOE locus, our survey detected five new candidate susceptibility loci for AD, including D10S1423. An association of the D10S1423 234-bp allele with AD has been reported in three independent samples of AD cases and controls (Boston, Pittsburgh, Bonn). Data from our case-control studies suggest a strong synergistic interaction between the D10S1423 234-bp and APOE E4 risk alleles. This report describes the prospective, longitudinal, double-blind assessment of the age-specific risk of AD encountered by 325 asymptomatic first-degree relatives of AD probands who carried the D10S1423 234-bp allele, the APOE E4 allele, or both, after 13.9 years of systematic follow-up. A total of 30 incident cases of AD were detected during the first 3752 subject-years of this longitudinal study. The effects of carrying either or both of the D10S1423 234-bp and APOE E4 alleles on the age-specific risk of developing AD were determined using Kaplan-Meier survival analysis. The age-specific risk of developing AD was the greatest for individuals who carried both alleles (Mantel-Cox statistic = 16.42, df = 3, P= 0.0009; Breslow statistic = 13.36, df = 3, P= 0.004). A Cox proportional hazards model was developed to estimate the risk ratios for each genotype, controlling for the potential effects of age at recruitment, sex, and years of education. In the resulting model, only individuals who carried both risk alleles exhibited a risk ratio that differed significantly from 1 (risk ratio = 4.7, P= 0.002, 95% CI = 1.8-12.6). After controlling for these genotypes, neither age at recruitment, sex, nor years of education made significant contributions to the model. D10S1423 is located within chromosome 10 open reading frame 112 (C10orf112), whose predicted product encodes multiple LDL receptor domains and other domains commonly associated with membrane receptors (NCBI, 2005). These results suggest that the D10S1423 234-bp allele affects the risk of AD among APOE E4 carriers by identifying a functional variant of a novel LDL receptor gene whose product binds to apoE.

Cerebrospinal Fluid Peptides as Biomarkers for Presymptomatic AD in "At Risk" Controls

Trey Sunderland*, Nadeem Mirza, Karen Putnam, Edward D Huey, Deepa Bhupali, Judith L Rapoport, Holly Soares, Gyorgy Csako and Cohen M Robert

NIMH, Bethesda, MD, USA

Background: Lower levels of cerebrospinal fluid (CSF) β-amyloid$_{1-42}$ and higher levels of total tau and hyperphosphorylated tau (p-tau 231) have been linked with the known neuropathology of AD. Furthermore, it is known that individuals with at least one APOE ε4 allele on chromosome 19 are at increased risk of developing AD. It is possible, therefore, that measures of CSF β-amyloid$_{1-42}$, total tau and p-tau 231 might serve as biomarkers of incipient pathology. We hypothesized that using the absence or presence of the APOE ε4 allele as a predictive variable in the controls, one or several of these CSF measures might help identify subjects at risk for subsequently developing clinical AD.
Methods: We assessed the levels of β-amyloid$_{1-42}$, total tau and p-tau 231 in the CSF of 230 subjects (188 young and older controls and 42 mild-to-moderate AD subjects) who were research subjects at the National Institutes of Mental Health in the BIOCARD (Biomarkers in Older Controls At Risk for Dementia) study. The group of older controls was enriched with a high percentage of subjects with a positive family history of AD in a first-degree relative. The younger controls consisted of a group of college graduates and a group of young schizophrenics. CSF β-amyloid$_{1-42}$ and p-tau 231 were measured with modified enzyme-linked immoabsorbent assays. CSF total tau was measured with a commercial enzyme immunoassay (Innotest, Ghent, Belgium).
Results: As expected, the AD patients had lower levels of CSF β-amyloid$_{1-42}$ and higher CSF total tau and p-tau 231 levels than the normal control group ($p<0.01$). When the control group was divided by the absence or presence of the APOE ε4 allele, the older controls with at least one ε4 allele also had a significantly lower CSF β-amyloid$_{1-42}$ than controls without an APOE ε4 allele ($p<0.01$). CSF total tau and p-tau 231 levels did not differ significantly by APOE allele status in the controls. Furthermore, there were no significant differences in any of these biomarkers associated with the absence or presence of the APOE ε4 allele in the younger subjects.
Conclusions: The association of APOE ε4 alleles and lower, more AD-like levels of CSF β-amyloid$_{1-42}$ in older controls is consistent with previous studies showing possible neuroimaging and cognitive abnormalities with ε4 carriers and suggests that CSF β-amyloid$_{1-42}$ may represent an early biomarker of AD. This finding appeared to be age-associated, as there was no suggestion of an APOE allele effect on any of the biomarkers in the younger control subjects. Additionally, there was no significant APOE allele effect on either total tau or p-tau 231 levels in the older controls. Longitudinal follow-up is of course required to verify if this CSF β-amyloid$_{1-42}$ finding or any other biomarker is indeed predictive of clinical conversion to AD, and this work is ongoing in the BIOCARD study.

Antecedent Biomarkers for Alzheimer's Disease

John C Morris*

Neurology, Washington University, St Louis, MO, USA

Background: Alzheimer's disease (AD) likely has a long preclinical period in which brain changes gradually accumulate without symptoms. When sufficient synaptic and neuronal damage occurs to overcome brain reserve, mild cognitive impairment (MCI) is apparent and represents the earliest expression of dementia of the Alzheimer type (DAT). At this early symptomatic stage, however, substantial neuronal damage and cell loss already is present in vulnerable brain regions, such as the entorhinal cortex. This suggests that potential disease-modifying therapies for AD may need to be introduced prior to the occurrence of irreversible brain injury (ie, prior to MCI or early stage

DAT) for optimal benefit. Imaging techniques and biomarkers relevant to the disease process that are antecedent to the appearance of symptoms thus must be developed to detect preclinical AD.
Methods: The Adult Children Study (ACS) of the Washington University Alzheimer's Disease Research Center compares middle age and older adults at different genetic risk for AD on an array of biomarkers and imaging modalities. We will enroll 120 individuals, age 45y to 74y, with a parent with AD (age at onset prior to age 80y) and 120 similarly aged individuals for whom neither parent had AD to test the hypothesis that the ACS indivduals with an AD parent will demonstrate changes on one or more of the study measures that hearld eventual deveopment of AD. All ACS participants have the following assessments at entry and every three years thereafter: 1) comprehensive clinical, cognitive, and behavioral (including personality) batteries; 2) blood draw for genetic analysis (including apoE genotyping) and assays of amyloid-beta levels; 3) cerebrospinal fluid (CSF) assays for relevant analytes, including amyloid-beta, tau, phospho-tau, and sulfatide, and for proteomic studies; 4) magnetic resonance imaging (MRI) for volumetric studies of targeted brain regions and whole brain volume; and molecular neuroimaging for brain amyloid deposits using the benzothiazole derivative developed by University of Pittsburgh investigators, Pittsburgh Compound-B (PIB).
Results: To date, 67 children of parents with AD have been enrolled; these 44 women and 23 men have a mean educational level of 16.6y and a mean MiniMental State (MMS) score of 29.5. Of these, all but 5 have completed lumbar puncture for CSF studies and all but 6 have completed the MRI protocols; 51% have at least one apoE4 allele. Seventeen children of parents without AD have been enrolled also; 13 women and 4 men with a mean educational level of 16.3y and mean MMS score of 29.5. All have completed the MRI protocols and 16 have completed lumbar puncture; only 8% have at least one apoE4 allele.
Conclusion: The willingness of adult children to participate in this study is confimed by the high rate of completion of all procedures in those with and without an AD parent. Cross-sectional comparison of group differences in the imaging and biochemical assays can identify candidate antecedent biomarkers for AD and longitudinal analyses then can determine their predictive value for the disorder. Supported by National Institute on Aging grants P01 AG026276, P01 AG03991, and P50 AG05681.

CSF Biomarkers Add to Delayed Recall and Hippocampal Volume in Diagnosing MCI

Mony J De Leon*, S. DeSanti, R. Zinkowski, P.D. Mehta, D. Pratico, H. Rusinek, J. Li, W. Tsui, B. Reisberg, J. Zhan, K. Rich and P. Davies

New York University School of Medicine, New York, NY, USA

Background: It is well known that MRI and PET neuroimaging is informative in the mild cognitive impairment (MCI) stage of impairment for predicting future Alzheimers disease (AD). We recently extended this predictive capacity by reporting three separate neuroimaging techniques for detecting preclinical AD in normal elderly. These advanced techniques rely on FDG-PET and MRI to precisely evaluate the hippocampal formation for evidence of glucose metabolic or structural atrophic changes. While these new techniques are very sensitive to the effects of AD on tissue and quite informative of disease progression, the general application of such AD diagnostic tools to normal subjects and to patients with mild cognitive impairments (MCI) will be limited because the imaging measurements are based on non-specific findings. We contend that direct evidence for the involvement of the major pathological pathways of AD: amyloid beta pathology, tau pathology, and cellular oxidative damage, is necessary to make a meaningful early and specific in vivo diagnosis.
Method: In a 2-year two-point longitudinal study of MCI and normal elderly subjects, we examined the hypothesis that several pathologically validated CSF markers for AD pathology would improve the diagnostic accuracy over independently acquired neuropsychological and MRI hippocampal volume assessments. Two complete clinical observations with cognitive testing, MRI, and LP were performed on 7 MCI patients and 9 normal controls. All results stated are significant at $p<.05$.
Results: At both observations, the MCI patients (defined by history and clinical criteria but not neuropsychological performance) and the controls were correctly classified by decreased delayed recall memory (~70%), by reduced hippocampal volume (~90%), and with elevated CSF levels of both hyperphosphorylated P-tau231 (>80%), and 8,12-isoiPF2?-VI isoprostane (~90%). Amyloid beta 1 to 40 levels were elevated in MCI only at the follow-up (~75% accuracy) and no cross sectional effects were found for the amyloid beta 1 to 42 levels. At both observations, both the P-tau231 and isoprostane measures individually incremented the diagnostic classification accuracy of the memory measures raising the total accuracies to ~90%. Only the isoprostane increased the follow-up diagnostic accuracy of the hippocampal volume from 88% to 94% ($p<.05$). Significant univariate longitudinal effects were restricted to isoprostane which based on the change scores, demonstrated an accuracy of (80%). In analyses restricted to the MCI patients, the longitudinal reduction in the hippocampal volume was closely associated with progressive increases in hyperphosphorylated tau levels ($r= -.79$) and decreases in amyloid beta-42 levels ($r= .82$). Prior studies suggest that failure to observe longitudinal changes in CSF biomarkers is complexly explained by a host of clearance mechanism and degradation and pathway factors. We will show preliminary evidence demonstrating the utility of an MRI correction for the longitudinally increasing ventricular CSF on the estimated P-tau231 level.
Discussion: The results from these studies demonstrate that CSF biological markers for AD increment conventional diagnostic measures in the separation of normal controls and MCI patients. Overall, these data suggest the combination of advanced neuroimaging with CSF biomarkers will enable an earlier and more specific clinical AD diagnosis.

Panel Session
The Prefrontal Cortex and Cognitive Flexibility

Reversal Learning in Primates: A Neuroanatomical, Neurochemical and Behavioral Analysis

Angela Roberts*

Anatomy, University of Cambridge, Cambridge, United Kingdom

Background: Behavioral inflexibility, i.e. the continued expression of previously appropriate, but currently inappropriate, behaviour, is associated with a variety of neurodegenerative and neuropsychiatric disorders including Parkinsons disease, Alzheimers disease, Obsessive Compulsive disorder, Attention Deficit Hyperactivity Disorder and Schizophrenia. Dysfunction within prefrontal circuitry, including neuromodulatory dysregulation by the monoamines and acetylcholine, is common to many of these disorders and is known to contribute to behavioural inflexibility but the precise nature of this contribution is poorly understood. Previously we demonstrated that different forms of behavioural flexibility were associated with distinct regions of prefrontal cortex (PFC), lateral regions were important for shifting attentional sets and orbitofrontal regions were important for reversing stimulus-reward associations (Dias et al, 1996, Nature 380;69). Attentional selection, but not reversal learning, was subsequently shown to be modulated by prefrontal dopamine (Crofts et al, 2001, Cerebral Cortex, 11;1015). This paper reviews our more recent studies of the role of prefrontal serotonin in behavioural flexibility (Clarke et al, 2004, Science, 7:878; Clarke et al, 2005, J. Neurosci. 25:532) and describes unpublished findings that demonstrate the neurochemical and behavioural specificity of serotonins effects.
Methods: Two studies examined the ability of New world monkeys, common marmosets, to perform a series of pattern discriminations

presented on a touch sensitive computer screen. Study 1 (Clarke et al, unpublished findings) compared the effects of 5,7 dihydroxytrptamine-induced prefrontal serotonin depletions with that of 6-hydroxydopamine-induced prefrontal dopamine depletions on a marmosets ability to perform a series of visual discrimination reversals. Thus, post surgery, animals had to acquire a novel pattern discrimination that involved responding to one of two patterns in order to gain access to banana juice reward. Subsequently, they received a series of reversals whereby for each reversal they had to learn to inhibit responding to the previously rewarded, but now currently unrewarded stimulus and respond instead to the previously unrewarded, but now currently rewarded stimulus. Study 2 (Clarke et al, unpublished findings) characterized the behavioural nature of the reversal deficit induced by prefrontal serotonin depletion, by comparing performance on a modified reversal task. To determine whether the deficit was due to enhanced learned avoidance of the previously unrewarded stimulus or perseverative responding to the previously rewarded stimulus, a novel stimulus replaced either the previously rewarded stimulus (learned avoidance test) or the previously unrewarded stimulus (perseveration test) on the second reversal.
Results: Prefrontal serotonin depletion disrupted performance across a series of discrimination reversals while prefrontal dopamine depletion was without effect (Study 1). The reversal deficit induced by prefrontal serotonin depletion was due to a failure to inhibit responding to the previously rewarded stimulus and not due to enhanced avoidance of the previously unrewarded stimulus (study 2).
Significance: These findings highlight the neurochemical and behavioural specificity of the effects of prefrontal serotonin on reversal learning. They also provide insight into the differential contribution of dopamine and serotonin to prefrontal functioning.

The Primate Prefrontal Cortex and the Executive Control of Attention
Leslie G Ungerleider*, Andrew Rossi and Luiz Pessoa

NIMH, Bethesda, MD, USA

Background: The prefrontal cortex is thought to play a prominent role in the cognitive control of sensory information. To examine the contribution of the prefrontal cortex to the allocation of visual attention, we removed the prefrontal cortex unilaterally in combination with transection of the forebrain commisures in two adult rhesus monkeys. As a result, visual processing in only one hemisphere could be modulated by feedback inputs from the prefrontal cortex.
Methods: Monkeys were trained to fixate a central spot and discriminate the orientation of a colored target grating presented among colored distracter gratings in either the control visual hemifield or the hemifield affected by the prefrontal lesion. The color of the central fixation spot cued the identity of the target on each trial, and we manipulated the frequency at which the fixation spot changed colors, that is, indicated that the identity of the target had switched.
Results: A comparison of the monkeys' performance in the two hemifields indicated no deficit when the frequency of the target switching seldom occurred (for example, every 50 trials). However, a significant deficit in the hemifield affected by the prefrontal lesion was found when target switching was frequent, and this deficit was a function of the frequency of switching; the most severe deficit was found when the target switching occurred on every trial. Control experiments showed, however, that performance in the lesion hemifield was not differentially affected by the frequency of target change when the target was defined by color pop-out. In addition, the prefrontal lesions did not impair the monkeys' ability to attend to a target when its spatial location was uncertain or when it was surrounded by potent distracters but its identity remained constant from trial to trial.
Discussion: The results indicate that the deficit observed in the color cueing task can be attributed to a disruption of the mechanism of top-down switching. When the target's identity was constant or defined by bottom-up pop-out, target selection was presumably accomplished by intact cortical mechanisms. Thus, the prefrontal cortex appears to play an important role in the selection of a behaviorally relevant target for processing resources and in the updating of this information from moment to moment. Parallel brain imaging studies in humans reveal the focus within the human prefrontal cortex for these attentional effects.

The Prefrontal Cortex and Cognitive Flexibility
Cameron S Carter*

Imaging Research Center, University of California Davis, Sacramento, CA, USA

Mature cognitive functions depend upon a distributed neural network to which late developing frontal cortical elements play a key role. Disturbances in the development of these systems are thought to contribute to many forms of adult psychopathology, including schizophrenia. A wealth of new information from cognitive neuroscience suggests that the frontal cortex is organized in a relatively modular manner such that medial and lateral elements contribute unique component processes to cognitive control functions, and that these systems are maturing during the window of risk for a number of adult disorders. In this session the organization and development of these circuits, as revealed through recent non invasive neuroimaging studies of healthy typically developing subjects, will be reviewed. New data will also be presented on the function of these circuits early in the course of schizophrenia. The potential for this applied cognitive neuroscience approach to enhance early diagnosis and risk prediction in schizophrenia, and hence to support early intervention and disability prenvention in this illness, will also be discussed.

Response Flexibility in Bipolar Disorder: A Developmental Perspective
Ellen Leibenluft*, Daniel Dickstein, Eric Nelson, Monique Ernst and Daniel Pine

NIMH, Bethesda, MD, USA

Background: Mania and depression are characterized by extreme deficits in response flexibility. Depressed patients do not respond to positive stimuli (anhedonia) and manic patients respond maladaptively to negative stimuli by denying their existence (euphoria) or responding angrily (irritability). These deficits are developmental: data from longitudinal and family studies relate abnormal responses to emotionally salient stimuli in childhood to risk for mood disorders in adults. However, the neural architecture and behavioral perturbations accounting for these developmental relationships are poorly understood. We hypothesize that the inability to update and correct one's behavior in response to emotional stimuli is a trait deficit in bipolar disorder (BPD); that the core symptoms of mania and depression occur when the underlying neural dysfunction causing this deficit is exacerbated; and that prefrontal dysfunction mediates this response inflexibility. Moreover, we argue that this deficit emerges in childhood, before complete manifestation of the clinical syndrome. Historically, the literature on prefrontal dysfunction in BPD has focused on ventral regions. However, some data (i.e., indicating deficits in sustained attention) implicate dorsolateral prefrontal (DLPFC) areas in the illness. Here, we review a series of studies testing the hypothesis that response inflexibility is a trait deficit in pediatric BPD associated with dysfunction in prefrontal regions.
Methods: Response flexibility was assessed in children with BPD (N=25-35) and matched controls using tasks that engage the PFC i.e., 1) probabilistic response reversal (PRR) task; 2) motor flexibility task (the change task); and 3) antisaccade inhibitory task. fMRI data have been obtained on the change task and are being obtained on the PRR task; data in nonhuman primates indicate that the latter is mediated by ventral PFC. Structural MRI data were also obtained.
Results: Data demonstrate a response flexibility deficit in pediatric BPD, compared to controls. PRR data indicate that children with

BPD learn initial reward contingencies as well as euthymic children, but perseverate when reward contingencies change. Change task data indicate that euthymic children with BPD are deficient in flexibly adapting their motor responses to changing cues on a trial-by-trial basis. fMRI data suggest that deficits on the change task may be mediated by dysfunction in both the dorsolateral prefrontal and motor cortex. Saccade task data indicate that children with BPD have inhibitory deficits, manifest as a greater rate of errant pro-saccades on anti-saccade trials, but normal performance on a delayed-response pro-saccade task. This deficit emerges under conditions of low, as well as high, motivation. Structural MRI data indicate decreased DLPFC volume in children with BPD compared to controls.

Discussion: Across several tasks, children with BPD show deficits in response flexibility. Direct evidence from imaging studies and indirect evidence from other work suggest that these deficits might arise from underlying dysfunction in both dorsal and ventral PFC. Ventral dysfunction may cause behavioral deficits when response flexibility is required in response to the changing emotional salience of stimuli. DLPFC dysfunction may cause response inflexibility on tasks that include significant attentional demands associated with maintenance of a stable task-related attention focus. While these deficits appear independent of mood state in patients, studies in individuals at risk for BPD are required to determine whether response inflexibility is an endophenotype for BPD.

Panel Session
Stress, Pain and Emotions

Brain Systems Regulating the Organism's Response to Stress: Preclinical and Clinical Studies

Charles B Nemeroff*, Christine Heim, Dominique L Musselman and Andrew H Miller

PsychBehSci, Emory Sch of Med, Atlanta, GA, USA

There is considerable evidence that stress is an important etiological factor in the pathophysiology of depression — both early life stress such as child abuse and more recent life stressors. In addition, there is increasing evidence of an over lap between mood disorders and somatic syndromes (e.g. chronic fatigue syndrome and fibromyalgia) and somatic symptoms (e.g. pain, fatigue). This presentation will focus on the CNS, endocrine and immune factors that likely underlie these comorbidities. Although overlap between pain syndromes and depression have been reported in relatively small clinical studies, Ohayon and Schatzberg (2004) using the Sleep-Eval Expert System sampled almost 19,000 individuals and reported that 38% of patients with major depression without any medical disorder had painful physical symptoms. There is burgeoning evidence that neurotransmitter systems that are involved in the pathophysiology of stress and depression, e.g. CRF, NE, 5HT, are also involved in the pathophysiology of chronic pain and other somatic symptoms. In this presentation, data from our clinical studies of women with a history of child abuse will be described with a focus on the comorbidity of depression, PTSD and somatic symptoms. One major class of mediators that have received considerable attention are inflammatory cytokines. In women with a history of child abuse and current major depression, ACTH and cortisol responses to a standardized laboratory stressor are markedly increased compared to depressed women without a history of child abuse and normal volunteers. When administered a standardized CRF stimulation test, women with a history of child abuse exhibited an increased IL-6 response compared to controls. The women with early life stress also exhibited increased rates of acyclic chronic pelvic pain, loss of energy/fatigue and a marked increase in somatic symptoms referable to a variety of organ systems. Remarkably, several studies have reported elevated IL-6 levels in drug-free depressed patients. Cytokines in the periphery enter the brain and there is also a CNS cytokine network. There is much evidence that cytokines effect neurotransmitter turnover, neuroendocrine function and ickness behavior. Our group has studied the endocrine and behavioral consequences of treatment with interferon-alpha, used for managing cancer and viral diseases including malignant melanoma and hepatitis-C infection. Interferon-alpha is a potent inducer of IL-6, IL-1, TNF-alpha and CRF, and produces depression, fatigue, cognitive dysfunction. The HPA axis response to initial interferon-alpha therapy predicts which patients will develop subsequent depression. The depression may in part be due to reduced serotonin availability. Paroxetine pretreatment prevents the mood and cognitive symptoms but not the neurovegetative syndrome (fatigue, anorexia, motor slowing and sleep disturbance) induced by interferon-alpha. Recently, we demonstrated using [18]fluorodeoxyglucose and PET imaging, an increase in basal ganglia activity during interferon-alpha therapy for malignant melanoma. These data, taken together, shed light on the biological substrates underlying the comorbidity of mood disorders and several so-called "psychosomatic" syndromes. Supported by NIMH MH-58299 and 42088.

Chronic Pain and Depression

Alan Schatzberg* and Maurice Ohayan

Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA, USA

Data from clinic-based samples point to high rates of comorbidity of chronic pain and major depression. There has been a relative lack of data on the relationship between the two in epidemiological based samples. We present data from 2 epidemiologic studies - one conducted in 5 European countries; the other in California. A computer generated, interactive system (Sleep-EVAL) was used to assess the prevalence of sleep disorders, medical illness, and psychiatric disorders. In the European study approximately 19,000 subjects in 5 European countries (United Kingdom, Germany, Italy, Portugal and Spain) were interviewed. To receive a diagnosis of chronic pain, the condition had to be present for the past 6 months and had to result in consulting a physician; the taking of medication for the pain or reports that pain interfered with functioning. Chronic pain was observed in 16% of subjects without major depression and 43% of those with. In patients with comorbid pain and depression, an organic cause for the pain was observed in about a third of subjects. The most common forms of comorbid pain in depressed subjects were headache/neck pain (24.5%) followed by limb pain (16.3%) and backache (12.8%). All 3 were more significantly commonly seen than in subjects without major depression (headache/neckache = 6.9%; limb pain = 5.4%; and backache = 2.7%). Data on over 2,000 subjects from California reveal similar rates and patterns of chronic pain and depression with pain comorbidity seen in 53% of subjects who met criteria for major depression. Implications of these data for understanding the common biology and pharmacological treatment of the 2 disorders are discussed.

Irritable Bowel Syndrome: Brain Mechanisms Involved in the Generation of Chronic Visceral Pain and How Emotions and Cognitions Can Alter Normal Processing of Visceral Stimuli

Emeran A Mayer*

Center for Neurovisceral Sciences & Women's Health, UCLA, Los Angeles, CA, USA

Background: Irritable bowel syndrome (IBS) is a common syndrome which shows high comorbidity with depression, anxiety disorders and symptom-related fears. A positive family history as well as a history of aversive early life events is common. Gastrointestinal symptoms are stress sensitive and key pathophysiologic mechanisms to explain IBS symptoms of abdominal pain, discomfort and altered bowel habits are visceral hypersensitivity and autonomic dysregulation. Cognitive factors play an important role both in the severity of symptoms (ineffective coping styles), as well as in the effectiveness of various therapeutic approaches (cognitive behavioral therapy, hypnosis).

Studies in rodents have shown a relationship between aversive early life events, psychological stress, central stress mediators (CRF/CRF1R and SP/NK1R signaling systems), stress induced visceral hyperalgesia and stress induced intestinal autonomic dysregulation.
Results: IBS patients show enhanced perception of naturally occurring and experimentally induced visceral stimuli. This enhanced perception involves different pathophysiological mechanisms including preattentive hypervigilance, altered attentional mechanisms, and altered endogenous pain modulation mechanisms to noxious stimuli. CSF levels of substance P and CRF are elevated in a subset of patients. Early functional brain imaging studies have shown enhanced responses of dorsal ACC to visceral stimuli in IBS. More recent studies suggest a dysregulation of corticolimbic pontine circuits in response to delivered or anticipated visceral stimuli, with sex-related differences seen in this dysregulation. Preliminary results using voxel based morphometry techniques suggest structural abnormalities in pontine regions in IBS patients. Neuroimaging studies are revealing the brain correlates of different therapeutic approaches: while treatment with a 5-HT3 receptor antagonist, and amitryptiline is associated with decreased limbic and paralimbic activity, placebo responders show increased prefrontal activity associated with decreased limbic responses.
Conclusions: A wealth of epidemiological and experimental studies suggest a close pathophysiological relationship between affective disorders and IBS. Preclinical and clinical studies are consistent with alterations in the emotional motor system regulating emotional, perceptual, and autonomic responses to stimuli related to the digestive system. Even in those patients without a psychiatric diagnosis of anxiety disorder, symptom related fears and anxiety play a prominent role in symptom severity. Supported by NIH grants RO1 DK 48351, P50 DK 64539, and R24 AT 002681.

Fibromyalgia: Brain Mechanisms Involved in the Generation of Chronic Somatic Pain and How Somatic Pain Processing is Altered in the Setting of Altered Mood and Cognitions

Daniel Clauw*, David Williams and Richard Gracely

University of Michigan, Ann Arbor, MI, USA

Fibromyalgia is a common condition defined on the basis of chronic widespread pain and tenderness. Although only these features are necessary to meet established criteria for fibromyalgia, most individuals with this condition also suffer from a number of non-defining symptoms and syndromes such as fatigue, sleep disturbances, memory difficulties, irritable bowel syndrome, etc. Although the "tenderness" required for the diagnosis of fibromyalgia is measured on the basis of the pain threshold in eighteen distinct regions of the body in fibromyalgia (tender points), these individuals have been found to be tender throughout the entire body. This widespread hyperalgesia/allodynia to multiple types of stimuli (e.g. pressure, heat, electrical) as well as a decreased noxious threshold for multiple types of other stimuli (noise, bright light, etc.) suggests a central disturbance in sensory processing as a fundamental mechanism in causing symptom expression in this and related conditions. Functional imaging studies have corroborated the augmented central processing of pain in fibromyalgia. When pressure or heat is applied to a patient with fibromyalgia, they will rate this as being more painful than a control group (i.e. hyperalgesis/allodynia), and fMRI identifies increased cortical activation in areas of the brain that code for the sensory-discriminative aspect of pain (e.g. primary and secondary somatosensory cortices). The presence of co-morbid depression (or the degree of co-morbid depressive symptomatology) does not influence either the degree of pain report or the strength of the neuronal activation in these brain regions, but is associated with increased activation in regions that code for affective and cognitive elements of pain, such as the amygdalae and contralateral anterior insula. Studies of pharmacological therapies that have variable degrees of anti-depressant and analgesic properties support the notion that the affective and analgesic effects of these drugs are independent. However, maladaptive "cognitions" regarding pain such as an external locus of control, or catastrophizing, are associated with increases in activation in both sensory and affective/cognitive regions. Ongoing studies will further delineate how pain and emotion are processed, and whether cognitive interventions aimed specifically at modifying cognitions can reduce clinical and experimental pain by modifying pain processing in sensory regions of the brain.

Panel Session
New Neuroimaging Findings in the Pathophysiology and Treatment of Panic Disorder

Functional Magnetic Resonance Imaging Studies of Amygdalo-Cortical Function in Panic Disorder

Scott L Rauch*, Paul J Whalen, Lisa M Shin and Mark H Pollack

Psychiatry, Massachusetts General Hospital, Charlestown, MA, USA

Background: Neuroimaging research has already substantially influenced neurocircuitry models of anxiety disorders. However, whereas large convergent bodies of data support cohesive neurobiological models of posttraumatic stress disorder (PTSD) and obsessive compulsive disorder, the neural substrates of panic disorder (PD) remain less well established. Moreover, the extent to which various anxiety disorders share common versus distinct pathophysiological underpinnings remains unclear. We previously developed functional magnetic resonance imaging (fMRI) probes of amygdala responses to general threat (masked fearful faces) and rostral anterior cingulate cortex (rACC) engagement during suppression of attention and response to higher order threatening stimuli (words, in the context of an emotional Stroop task). Subsequently, we demonstrated that subjects with PTSD, in comparison with trauma-exposed non-PTSD controls, exhibited exaggerated amygdala responses to masked fearful faces and attenuated rACC responses during performance of the emotional Stroop. Here we present findings from a parallel series of experiments in subjects with PD vs. healthy controls.
Methods: Subjects meeting DSM-IV criteria for PD were studied and healthy controls were used as the comparison group. Anxiety sensitivity was also quantified using the Anxiety Sensitivity Index. Previously described masked faces and emotional counting Stroop paradigms were employed in conjunction with fMRI at 1.5T. Data were analyzed using voxelwise and region of interest based methods.
Results: As in PTSD, we found that PD subjects exhibited both exaggerated amygdala responses and deficient rACC responses in comparison with controls. In addition, as the magnitude of activation within the amygdala has been correlated with PTSD symptom severity, here we found that the magnitude of amygdala response was correlated with scores on the Anxiety Sensitivity Index.
Discussion: The current findings suggest commonalities in the pathophysiology of PD and PTSD. The implications of these results will be discussed in terms of the phenomenological similarities (as well as differences) between PTSD and PD, and the functions mediated by amygdalo-cortical circuitry. In this context, conceptualizations regarding the role of rACC in anxiety disorders will be considered against the backdrop of recent findings suggesting that extinction retention is mediated by more ventral territories of medial prefrontal cortex.

Functional Magnetic Resonance Imaging of Attention and Emotion Recognition in Panic Disorder

Srinivasan S Pillay*, Staci A Gruber, Jadwiga Rogowska, Norah Simpson and Deborah A Yurgelun-Todd

Harvard Medical School, Cognitive Neuroimaging Laboratory/ McLean Hospital, Belmont, MA, USA

Background: Impaired cognitive control over threat-related information may play a key role in the behavioral responses of anxious in-

dividuals. Understanding the biological substrates that underlie the interaction between attentional demands and response to fear or safety from threat is crucial. We have begun probing these interactions in a series of fMRI studies.
Hypotheses: Relative to controls: (1) patients with panic disorder (PD) would demonstrate an increased error-monitoring rate that would result in increased activation in the prefrontal cortex and anterior cingulate cortex (ACC), as well as decreased posterior brain activation; (2) in response to fearful faces, patients with panic disorder (PD) would demonstrate hypoactivity of the ACC but increased amygdala activation; (3) in response to happy faces, patients with PD would demonstrate hyperactivity of the ACC and amygdala.
Methods: For all components, eight patients with PD and eight age- and sex matched controls were recruited for the study. Scanning was performed on a GE Signa 1.5 Tesla scanner retrofitted with a whole body echo planar coil. Using a quadrature head coil, echo planar images and high-resolution MR images were acquired. To probe attention, a standard color-word Stroop was administered, and to probe responses to fear and happiness, standard Ekman faces were presented to all subjects.
Results: Relative to controls: (1) PD patients demonstrated statistically significantly greater ACC and prefrontal activation and less activation in the precuneus, lingual gyrus and cerebellum. In PD patients, activation of the ACC correlated with HAM-A and HAM-D, but activation in the prefrontal cortex correlated with HAM-D only. Cerebellar activation correlated with STAI-Y1. PD patients exhibited a greater number of errors that did not reach statistical significance when compared to controls; (2) PD patients demonstrated statistically significantly less ACC and amygdala activation in response to fearful faces. Negative correlations with HAM-A were noted in the left cingulate gyrus but these correlations were not sustained for the ACC or the amygdala. There were no other statistically significant correlations for HAM-D or STAI-Y1 or Y-2; (3) PD patients demonstrated greater ACC activation bilaterally in response to happy faces compared to controls and compared to the neutral condition also. However, there were no differences in amygdala activation between both groups. There were no statistically significant correlations between anxiety indices and regional brain activation.
Discussion: In these preliminary studies of attention and emotion recognition, patients with PD appear to activate the ACC and amygdala differently from controls in different conditions. The ACC is activated more on attentional tasks and on seeing happy faces in PD patients compared to controls. However, when exposed to fearful faces, patients with panic disorder activate the ACC less, and in concert with this, also activate the amygdala less than controls. Thus, emotional dysregulation may alter attentional mechanisms in patients with PD. We speculate that this disturbed attention is part of a feedback loop that further exacerbates panic attacks until a threshold for panic attacks is reached. This sudden fear of loss of contact with conscious attentional mechanisms may describe why panic attacks occur and the time it takes for recovery of attention may partly explain the time-limited nature of panic attacks.

fMRI Studies of Fronto-Limbic Fear Circuitry in Panic Disorder
David A Silbersweig*, Hong Pan, Oliver Tuescher, Daniel Zimmerman, Xenia Protopopescu, Martin Goldstein, Almut Engelien, Jane Epstein, Margaret Altemus, Marylene Cloitre, Jack Gorman, Elizabeth Phelps, Joseph LeDoux, Yihong Yang and Emily Stern

Psychiatry, Weill Medical College of Cornell University, New York, NY, USA

Background: The symptoms of Panic Disorder, including panic attacks, anticipatory anxiety and agorophobia, suggest abnormal functioning of fronto-limbic fear circuitry.
Methods: A set of translational fMRI studies were performed, using animal-derived fear circuitry models and complementary neuropsychological tasks to probe fronto-limbic function in patients with panic disorder (PD). Profiles of fear circuit activity in PD patients during instructed fear conditioning and during the processing of disorder-specific linguistic stimuli were examined, in comparison to normal control subjects, with BOLD echoplanar fMRI imaging and hypothesis-driven SPM analyses. Targeted analyses were also performed correlating fMRI measures with specific clinical, behavioral, structural imaging, physiological and neuroendocrine data from these subjects.
Results: The diagnosis of PD and the presence of core PD anxiety symptoms were associated with particular condition-specific and temporal profiles of activity in amygdalar, ventral hippocampal, ventromedial prefrontal and related subcortical regions. In PD patients, amygdalar activation did not follow the normal stimulus pattern, and was present in the setting of decreased ventromedial prefrontal activity.
Discussion: The findings suggest that specific clinical features of PD are associated with dysfunction within and among fronto-limbic-subcortical subregions, in a manner that builds upon translational fear circuitry and autonomic dysfunction models. Patients demonstrate inappropriate generation of, and diminished control over, physiological and psychological anxiety responses. These profiles of abnormal activity can be contrasted with those that we detected with the same neurocognitive probes in another anxiety disorder, PTSD, and can seen in the context of emerging models of fronto-limbic dysfunction in anxiety and affective disorders.

Regional Metabolic Brain Activation in Patients with Panic Disorder During Doxapram Infusion
Jack M Gorman*, Amir Garakani, Jose Martinez, Cindy Aaronson and Monte Buchsbaum

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

Vigorous attempts have been made to relate the neurobiology of Pavlovian fear conditioning to anxiety disorders. So far, it has been shown that patients with social phobia, specific phobia, and posttraumatic stress disorder have in common increased activation of the amygdala and decreased activation of the medial prefrontal cortex (mPFC) compared to controls. For panic disorder, however, only the latter has been shown whereas documenting increased amygdala activation during the actual panic attack has been difficult. This is probably because hyperventilation during the panic attack causes widespread cerebral vasodilatation, compromising the ability of blood flow based measures of brain activity, like 15O-PET and fMRI, to detect increases in amygdala activity. In an attempt to circumvent this problem, we used FDG-PET to capture regional metabolic activity during panic attacks. First, in two separate studies, we replicated the findings of the Abelson group that doxapram, a respiratory stimulant, reliably causes panic attacks at a higher rate in panic disorder patients than normal comparison subjects. Then, we administered placebo (saline) and doxapram on two separate days, to six patients with panic disorder and seven normal comparison subjects during FDG-PET imaging. Patients showed great amygdala and orbitofrontal cortical (OFC) activation than comparison subjects during doxapram compared to placebo conditions. We further showed that OFC activation during doxapram infusion was normalized in patients following a 12-week course of cognitive behavioral psychotherapy. We believe that this is one of the first demonstrations of greater amygdala activity in patients with panic disorder compared to controls and of the effect of psychosocial therapy on brain activation in panic disorder.

Panel Session
ECNP Panel: Bipolar Disorders: European Contributions

Exploring the Endophenotype in Bipolar Disorder
Guy M Goodwin*

Psychiatry, Oxford University, Oxford, United Kingdom

Background: Bipolar disorder is an increasingly broad and complex diagnosis. It is now characterised by critical boundaries - with schizophrenia on the one hand and unipolar depression on the other - and

extensive co-morbidity with anxiety disorders and substance misuse. The clinical criteria on which this classification has evolved appear increasingly to support dimensional rather than categorical perspectives on bipolarity. However, symptomatic descriptions are atheoretical and have limited heuristic potential. Cognitive neuroscience offers, in contrast, alternative formulations of how brain function is organized in health and, speculatively, in disease. Components of cognition appear to contribute to the endophenotype in bipolar disorder and may provide alternative targets for drug development and measures of clinical outcome.
Methods: The clinical background has been established by appropriate community surveys which confirm high rates of bipolarity. The experimental approach to the endophenotype has employed parallel group designs and investigation of neuropsychological function, brain structure and function with MR.
Results: The rates of bipolarity in college students surveyed by email/web based methods were comparable with earlier surveys of the general population: rates of medication in a UK sample were low. Meta-analysis of published investigation of cognitive function in bipolar samples confirms the consistency of memory and executive deficits. In our hands these effects and deficits of sustained attention are especially marked but may have different heritability (1). Attention deficits in particular may be acquired in bipolar samples and/or reflect illness course. Executive deficits may be a genetically determined abnormality. The use of tasks that explicitly tap reward mechanisms are of growing interest given the prominence of elation and addiction in the lives of bipolar patients. One 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 (2). Tryptophan depletion reduced the sensitivity to reward in the gaming task: hence serotonin may mediate decision-making in healthy volunteers within the orbitofrontal cortex (3).
Discussion: The dimensions of the endophenotype in bipolar disorder appear to be multiple and their importance is likely to be established pragmatically on the basis of their demonstrated capacity to illuminate heredity, prognosis and response to treatment. References 1. Clark et al. (2005) Biol. Psychiatry 57, 183-187. 2. Rogers et al. (2004) Biol. Psychiatry 55, 594-602 3. Rogers et al. (2003) Neuropsychopharmacology 28, 153-62.

Diagnosis of Bipolar Disorder in Children and Adults (A European Perspective)
Jan Buitelaar*

Psychiatry 333, Radboud University Medical Center, Nijmegen, Netherlands

Background: Bipolar disorder according to DSM-IV is a spectrum or group of overlapping clinical entities that include bipolar I, bipolar II, cyclothymia, and bipolar disorder not-otherwise-specified. The international classification of ICD-10 puts less emphasis on the spectrum idea and does not recognize bipolar II as a separate entity. Retrospective data indicate that about 60% of adults with bipolar disorder had symptom onset prior to the age of 20 year. Increasingly, bipolar disorder is diagnosed in children and adolescents, particularly in the US. Despite continuing or even increased clinical and research interest, a number of controversies surround the diagnosis of bipolar disorder in children and adults. Some of these controversies may be linked to different perspectives from the European continent and from the US into bipolar disorder.
Aim: The aim of this presentation is to review and discuss different perspectives on bipolar disorder in children and adults, and to outline strategies to resolve these controversies.
Methods: This review is based on key papers identified from the literature by Medline and on summary chapters by experts in handbooks and textbooks.
Results: The European perspective as noted in ICD-10 is more focused on the classical type of bipolar disorder, bipolar I which is characterized by the lifetime presence of both full manic and major depressive episodes. Further, ICD-10 pays greater attention to the description of the clinical phenomenology. The more heated discussions concern the diagnosis of early-onset mania and the status of bipolar disorder in children. There is disagreement whether the diagnosis of mania should require the presence of clearly defined episodes; if so, what the minimum duration of these episodes should be; and whether there are specific hallmark symptoms of mania that should be required for the diagnosis (Leibenluft et al., 2003). On the basis of this disagreement and the various diagnostic possibilities a narrow phenotype of juvenile mania can be described in which the child exhibits clear episodes that meet the full DSM-IV criteria, including duration, and at least one of the hallmark criteria elevated or expansive mood, or grandiosity. At the other side of the spectrum is a broad phenotype that is marked by childrens increased reactivity to negative emotional stimuli as well as by chronic signs of hyperarousal as distractibility, hyperactivity, etc. Though this broad phenotype tends to be recognized by many European clinicians, often they will consider alternative primary diagnostic formulations, such as a subtype of pervasive-developmental disorder (PDD-NOS, or multiple complex developmental disorder), or disruptive behaviour disorder, or major depressive disorder, or ADHD, or, even more likely, as a comorbid combination of two or more of these conditions. A consensus conference of experts organized by the British Institute for Clinical Excellence (NICE) in 2004 carefully reviewed existing evidence and came to the conclusion that the diagnosis of bipolar disorder in children should be limited to the narrow phenotype (i.e. bipolar I) and that this narrow phenotype is quite rare in schoolage children. Although manifestations of bipolar II, bipolar disorder NOS (broad phenotypes) in children were granted, the validity and reliability of the diagnoses were considered to be insufficiently supported by empirical evidence sofar.
Discussion: Strategies to resolve these controversies are systematic longitudinal studies, treatment studies, family-genetic studies and studies into the underlying neurobiology of both narrowly, intermediately and broadly defined phenotypes of early-onset bipolar disorder.

A Critical Analysis of the Value of Psychoeducation in Bipolar Disorder
Eduard Vieta*

Institute of Neuroscience, University of Barcelona Hospital, Barcelona, Catalonia, Spain

Background: On the basis of significant European contributions to current knowledge in the filed of manic-depressive illness, we aimed to examine the historical and current relevance of psychosocial approaches to bipolar illness by conducting a systematic review of prospective studies assessing the effectiveness of psychological interventions for bipolar disorder, with particular emphasis on group psychoeducation, as developed by the Barcelona Bipolar Disorders Program.
Method: A systematic literature search was conducted using EMBASE, MedLine and PsychLIT and reference sections of papers were scrutinized for further relevant reports. Only five trials met the criteria of a prospective study and achieved the necessary methodological standards. Further emphasis was made on group psychoeducation, as the only intervention that has been tested against a sort of "placebo" intervention (a non-structured group psychosocial intervention).
Results: The studies showed benefits for patients in terms of relapse prevention and the reduction of hospitalization rates. Psychoeducation (delivered in groups or as part of a family intervention) and cognitive behavioural therapy were also found to be effective prophylac-

tic treatments for bipolar disorder in medicated patients. There is some recent evidence on acute benefits by means of interpersonal and social rythm therapy in particular subpopulations. Other interventions do not appear to be supported by sufficient evidence.
Discussion: Psychological approaches, and particularly psychoeducation and cognitive-behavioural therapies, are evidence-based prophylactic therapies for bipolar patients receiving pharmacotherapy. They should be used as adjuncts to medication where possible in the prevention of bipolar disorder.Psychoeducation is aimed at providing the bipolar patients with a theoretical and practical approach towards understanding and coping with the consequences of illness -within the context of a medical model-, and allows them to actively collaborate with the physician in some aspects of the treatment. Our group has shown the efficacy of group psychoeducation in preventing all sort of bipolar episodes and increasing time to relapse at the two-year follow-up. The number of hospitalizations per patient was lower for the psychoeducated group. This study had a reasonably large sample size (N=120) and a random allocation of subjects to either a treatment condition (psychoeducation plus standard pharmacological treatment) or non-intervention (non-structured meetings plus standard pharmacological treatment). In a subsequent trial, we obtained very similar results when the study was repeated including only highly compliant bipolar I patients, suggesting that, though psychoeducation is surely working by means of adherence enhancement, there may be other supplementary mechanisms of action, such as early detection of prodromal signs and habits regularity. Thus, the mechanisms of action of psychoeducation may be the enhancement of adherence, the improvement of illness management skills such as early recognition of warning signs of relapse, the development of strategies for effective coping with symptoms and stressful events, and the regularity of sleep and other habits/rythms that may influence outcome. The biological correlates of these mechanism are currently being actively researched.

The Treatment of Depression in Bipolar Disorder (An European Perspective)

Heinz Grunze* and Hans-Juergen Moeller

Department of Psychiatry, LMU Munich, Munich, Germany

Despite the fact that bipolar depression is the predominant mood state for the vast majority of patients with bipolar disorder, the focus of research and randomised clinical trials in the management of bipolar disorder has mainly been on mania. Although clinical treatment algorithms are now available taking into account the particular characteristics of bipolar depression, data from randomised clinical trials fulfilling the requirements for regulatory approval of the indication bipolar depression are limited. The antidepressive efficacy of traditional mood stabilizers like lithium or several anticonvulsants is not well proven, at least not following the methodological standards that are commonly used to establish the efficacy of antidepressants. Although there are some hints for an antidepressive efficacy of mood stabilizers, especially for lithium, and to a lesser degree for lamotrigine with contradictory results, the question remains open whether their antidepressive efficacy is comparable to that of antidepressants. At least some data show a lower efficacy of lithium compared to antidepressants or to co-medication of lithium with antidepressants. Particularly this question needs further evaluation before a final conclusion can be drawn whether antidepressants should be replaced by lithium or other mood stabilizers generally, or under certain conditions, in the treatment of acute bipolar depressions. Of course, the tolerability of traditional and modern antidepressants compared to the recommended mood stabilizers also has to be taken into consideration. Some of the mood stabilizers have an unfavourable side effect profile, at least compared to modern antidepressants. Thus in the current situation, antidepressants still appear generally indicated to obtain a good antidepressive response, at least in moderate and severe acute bipolar depression. In acute bipolar depression the main goal should be to obtain an optimal antidepressive response as it is the first aim in the treatment of unipolar depression. In particular the view, which is increasingly respected in the field of treatment of unipolar depression, that not only response but also remission of the depressive symptoms should be achieved (Hirschfeld et al. 2002; Keller 2003), has to be transferred to the field of treatment of acute bipolar depression. Of course, the risk of switch has to be critically considered but with the use of modern antidepressants like the SSRIs and with the protection of mood stabilizers this risk can be reasonably well controlled (Bottlender et al. 2001; Gijsman et al. 2004). Thus patients suffering from acute bipolar depression should not be left without this powerful treatment of their depressive symptoms (Gijsman et al. 2004; Moeller and Grunze 2000).The bipolar depression guidelines of the World Federation of Societies of Biological Psychiatry (Grunze et al. 2002) and those of the British Association of Psychopharmacology (Goodwin 2003), as well as the North American Expert Consensus on Medication Treatment of Bipolar Disorder (Sachs et al. 2000), are open to accepting the necessity of this medication, especially of second generation antidepressants, not only in severe but also in moderate depression.

Panel Session
Lipid Rafts, Caveolin-Related Proteins and Neuronal Signaling

Caveolin: A Novel Regulator of GPCR Signaling

Bryan L Roth*

Biochemistry, Case Western Reserve University Medical School, Cleveland, OH, USA

5-HT2A serotonin receptors are important for a variety of functions including vascular smooth muscle contraction, platelet aggregation, and the modulation of perception, cognition, and emotion. Virtually all atypical antipsychotic drugs function as 5-HT2A inverse agonists while many hallucinogens are 5-HT2A agonists. In a search for 5-HT2A receptor-interacting proteins, we discovered that caveolin-1 (Cav-1), a scaffolding protein enriched in caveolae, complexes with 5-HT2A receptors in a number of cell types including C6 glioma cells, transfected HEK-293 cells, and rat brain synaptic membrane preparations. To address the functional significance of this interaction, we performed RNA interference-mediated knockdown of Cav-1 in C6 glioma cells, a cell type that endogenously expresses both 5-HT2A receptors and Cav-1. We discovered that the in vitro knockdown of Cav-1 in C6 glioma cells nearly abolished 5-HT2A receptor-mediated signal transduction as measured by calcium flux assays. RNA interference-mediated knockdown of Cav-1 also greatly attenuated endogenous Gq-coupled P2Y purinergic- and bradykinin-receptor-mediated signaling without altering the signaling of PAR-1 thrombin receptors. Cav-1 appeared to modulate 5-HT2A signaling by facilitating the interaction of 5-HT2A receptors with Gq. Similar results were obtained upon cholesterol depletion using methy-β-cyclodextran. These studies provide compelling evidence for a prominent role of Cav-1 in regulating the functional activity of not only 5-HT2A serotonin receptors but also many other Gq-coupled GPCRs.

Caveolin-1 Interacts with Metabotropic Glutamate Receptors and Participates in Receptor Trafficking

Anna Francesconi* and R S Zukin

Neuroscience, Albert Einstein College of Medicine, Bronx, NY, USA

Background: Group I metabotropic glutamate receptors (mGluR1/5) are G protein-coupled receptors that regulate glutamatergic transmission and are enriched at excitatory synapses throughout the brain. Signaling by group I mGluRs is implicated in cortical development,

activity-dependent synaptic plasticity and neuropsychiatric disorders such as schizophrenia, Parkinson's disease and addiction. Regulated trafficking of glutamate receptors is critical to the establishment and maintenance of synaptic circuitry and to activity-dependent synaptic plasticity. Although a great deal is known about activity-dependent trafficking of ionotropic glutamate receptors, relatively little is known about activity-dependent trafficking of mGluRs.
Results: The overall objective of this study was to elucidate mechanisms of mGluR trafficking and to identify novel mGluR1 binding partners involved in receptor trafficking. Toward this end we used a novel proteomic strategy termed Tandem Affinity Purification. Thus far, we have identified five novel mGluR1 binding proteins including caveolin-1, a lipid raft-associated adaptor protein involved in cell signaling and trafficking. Caveolin-1 is abundantly expressed in hippocampal neurons, where it colocalizes with mGluR5 at excitatory synapses. Here we show that constitutive mGluR1 internalization occurs via both clathrin- and caveolar/lipid raft-dependent pathways. Moreover, over-expression of caveolin-1 in heterologous cells and neurons inhibits mGluR1 internalization. We further show that endocytosis via the caveolar/lipid raft pathway promotes sorting of mGluR1 to the endosomal recycling compartment, thereby increasing the pool of intracellular receptors available for re-insertion at the cell surface in a protein synthesis-independent manner.
Discussion: Together, these findings suggest that caveolin-1 and lipid rafts regulate group I mGluR trafficking in neurons. An important prediction of our study is that the caveolar/lipid raft pathway may play a critical role in the remodeling of the postsynaptic membrane and thereby impact on synaptic strength and neuronal function. Dysregulation of the interaction between caveolin-1 and group I mGluRs and/or of caveolar/lipid raft-mediated endocytosis could profoundly affect mGluR trafficking and signaling leading to dysfunctions in neuronal activity. Understanding the molecular mechanisms underlying mGluR trafficking could help in the design of novel therapeutic strategies for amelioration of cognitive deficits underlying schizophrenia.

Agonist-Induced Lipid Raft Trafficking and Subsequent Internalization of G alpha s Regulates cAMP Signaling
John Allen, Anushree Bhatnagar, Bryan Roth and Mark M Rasenick*

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

Many G protein-coupled receptors are internalized by endocytosis and recent studies have indicated that some G proteins also undergo agonist induced internalization. Previous data have demonstrated that stimulation of beta adrenergic receptors (bAR) results in lipid raft-mediated internalization of G alpha s (Gs). The objective of this current study is to determine whether agonist induced internalization of Gs from lipid rafts regulates cAMP synthesis. C6 glioma cells or C6 cells in which caveolin-1 was stably knocked down by RNAi (C6 Cav-1) were transfected with Gs-GFP and trafficking was assessed using fluorescence microscopy. Upon stimulation of C6 cells with the bAR agonist isoproterenol, Gs-GFP was rapidly removed from the plasma membrane and internalized within vesicles. However, Gs-GFP internalization was blocked by disrupting lipid rafts/caveolae with cyclodextrin and other cholesterol disrupting drugs. Subcellular fractionation studies revealed that agonist treatment significantly increased Gs localization in Triton X-100 insoluble lipid raft membrane fractions, while bARs were removed from lipid rafts, completing endocytocis through a different pathway. In addition, cyclodextrin disruption of rafts significantly increased isoproterenol and forskolin stimulated adenylyl cyclase activity. In experiments with C6 cells in which caveolin1 expression was supressed throgh siRNA, Gs-GFP did not internalize during agonist treatment, suggesting that rafts/caveolae are necessary for Gs internalization. In addition, isoproterenol and forskolin stimulated adenylyl cyclase activity was significantly increased in the C6 Cav-1 cells vs. wild type C6 cells. These results suggest that during receptor activation, Gs is internalized through lipid raft/caveolae microdomains of the plasma membrane where it is less available to adenylyl cyclase, diminishing cAMP synthesis. We suggest that lipid rafts/caveolae act as negative regulators of bAR/Gs/adenylyl cyclase signaling.

Lipid Rafts and Neural Cell Adhesion Molecules in Axon Growth
Vance Lemmon*, Suni Lee and Yoshimasa Kamei

Neurological Surgery, Univ. of Miami, Miami, FL, USA

Axon growth and branching are critical aspects of development and neural plasticity. Detergent-insoluble membrane microdomains, or lipid rafts, have been implicated in many cellular processes, such as polarized sorting and signal transduction, and are enriched in GPI-anchored molecules and Src family protein tyrosine kinases (PTKs). GPI-anchored molecules include Cell Adhesion Molecules (CAMs) essential for axon growth. Src family kinases are also important in regulating axon growth. We have examined the distributions of different CAMs, such as L1 and Contactin subfamily members in the central nervous system. In mouse and chick brains, Contactin subfamily CAMs were found predominantly in the rafts but their distributions were not identical. L1 was mostly outside the rafts but some L1 was detected in the rafts. A variety of experiments show that src family kinases are critical in CAM mediated axon growth and branching. Disrupting rafts also disrupts L1 mediated axon growth. These results suggest that L1 interacts with signaling molecules, like src family kinases in lipid rafts. The studies on CAMs will be compared to similar studies on growth factor stimulated axon growth.

Panel Session
JSNP Panel: Molecular Mechanisms of Drug Dependence Induced by Amphetamine and Related Drugs

Candidate Gene Analysis of Methamphetamine-Related Disorders
Hiroshi Ujike*, Toshiya Inada, Mutsuo Harano, Tokutaro Komiyama, Mitsuhiko Yamada, Yoshimoto Sekine, Nakao Iwata, Masaomi Iyo, Ichiro Sora and Norio Ozaki

Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

Background: Methamphetamine has been a most popular drug in Japan after the World War II, and estimated methamphetamine abusers are more than 200,000. Repeated consumption of methamphetamine can easily induce intense psychogenic dependence and psychosis resembling to schizophrenia. Many lines of evidence from twin and family studies have clearly showed that genetic factors play major roles in susceptibility of substance-related disorders, including methamphetamine dependence and psychosis. To assess individual genetic risk factors for methamphetamine dependence and psychosis, JGIDA (Japanese Genetics Initiative for Drug Abuse), a multi-center collaboration group, has been established in 2001. Candidate genes were selected based on pharmacological actions of methamphetamine in the CNS such as genes encoding dopamine, serotonin, glutamate, GABA and opioid receptors and those metabolize enzyme and transporters. Genes associated with susceptibility to schizophrenia, such as FZD-3, AKT-1 and XBP-1, those from findings of animal models of dependence, such as TNF alpha, BDNF, CART, t-PA and Mrt-1, and those related to neurotoxixity, such as glutathion-S-transferese and SOD were also candidate.
Methods: Patients with methaphetamine-related disorders (N=197, 156 males and 41 females) and controls (N=219) were analyzed by case-control association. Among patients, 183 subjects were diag-

nosed as methamphetamine dependence according to ICD-10, and 167 of them are also suffered from methamphetamine-induced psychosis. Fourteen patients were methamphetamine abuser without dependence.
Results: We detected several significant associations in genes encoding D1 dopamine receptor, mu opioid receptor, prodynorphin, the GABAA receptor gamma2 subunit, AKT1 and FZD-3 with susceptibility to methamphetamine-related disorders. In addition, several physiologically functional polymorphisms were associated with prognosis and clinical phenotypes of methamphetamine psychosis. Nine- or fewer repeat alleles of the VNTR of dopamine transporter gene, which produce less density of the transporter, were associated with prolonged psychosis even after therapy in comparison with 10- or more repeat alleles (Odds ratio was 4.2). An allele of -141C del in the promoter of the DRD2 gene encoding dopamine D2 receptor, which may produce enhanced transcription of the DRD2 gene, 4-repeat allele of the MAO-A gene, a hyperfunction allele, and 158Met allele of COMT gene, a hypofunction allele, were found to be genetic risk factors for shorter latency of psychosis, worse prognosis, and spontaneous relapse of psychotic state. In contrast, A1/A1 genotype of TaqIA polymorphism of the DRD2 gene, which produces a decrease in D2 receptor density, was negative risk or protective factor for them.
Conclusion: JGIDA study have revealed that certain genetic variants were potent genetic risk or negative risk factors which should potentially influence individual susceptibility to substance dependence, and individual variation of prognosis or several clinical phenotypes of substance-induced psychoses.

Brain Imaging Reveals Corticolimbic Dysregulation in Methamphetamine Abusers

Edythe D London*, Steven M Berman, John R Monterosso, Thomas Newton, Timothy Fong, Sara L Simon, Paul M Thompson, Kiralee M Hayashi, Arthur L Brody, Mark A Mandelkern, Walter Ling and Richard Rawson

Psychiatry & Biobehavioral Sciences, UCLA, Los Angeles, CA, USA

Methamphetamine (MA) abuse is the fastest growing drug abuse problem in the world, and amphetamines are used by more people worldwide than any illicit drug besides cannabis (SAMHSA, 2004; UN, 2004). Nonetheless, adequately effective treatments for MA dependence are lacking, and knowledge of the brain's condition when the addict enters treatment is needed. As current treatment for MA dependence is almost exclusively by outpatient, behavioral therapy in the US, we tested MA abusers during early MA abstinence, when engagement in treatment is particularly important. We used subjective self-reports and cognitive testing, as well as FDG PET and structural MRI imaging (London et al., 2004; Thompson et al., 2004). In tests of 17 abstaining (4-7 days) MA abusers and 18 control subjects, using measures of mood and relative estimates (PET FDG) of regional cerebral glucose metabolism (rCMRglc) during performance of a vigilance task, MA abusers gave higher self-ratings of depression and anxiety. They also differed significantly from the controls in rCMRglc: lower in the anterior cingulate and insula and higher in the lateral orbitofrontal cortex (OFC), middle and posterior cingulate, amygdala, and ventral striatum. In MA abusers, self-reports of depressive symptoms covaried positively with relative glucose metabolism in limbic regions (e.g., perigenual anterior cingulate gyrus and amygdala) and ratings of state and trait anxiety covaried negatively with relative activity in the anterior cingulate cortex and left insula. Trait anxiety also covaried negatively with relative activity in the OFC and positively with amygdala activity. Using high-resolution MRI, we studied 22 MA abusers and 21 age-matched healthy control subjects (Thompson et al., 2004). These two groups included the samples studied with PET. We used surface-based computational image analyses to map regional abnormalities in the cortex, hippocampus, white matter, and ventricles. Cortical maps revealed severe deficits in the gray matter volumes of cingulate, limbic, and paralimbic cortices of MA abusers (averaging 11.3% below control; $p < 0.05$). MA may selectively damage the medial temporal lobe and, consistent with metabolic studies, the cingulate-limbic cortex. Along with the observed metabolic abnormalities, these structural findings are consistent with a loss of cortical control of subcortical limbic responsivity to environmental stimuli that can trigger craving and drug use. Preliminary evidence for additional gray matter deficits (i.e., in right inferior frontal gyrus) (Thompson et al., 2004) may provide an anatomical basis for deficits among MA abusers in a test of response inhibition (Stop Signal Task) (Monterosso et al., 2005). ED London, SL Simon, SM Berman, MA Mandelkern, AM Lichtman, J Bramen, NK Shinn, K Miotto, J Learn, Y Dong, JA Matochik, V Kurian, T Newton, R Woods, R Rawson, W Ling (2004): Arch Gen Psychiatry 61:73. JR Monterosso, AR Aron, X Cordova, J Xu, ED London (2005): Drug Alcohol Depend 79:273. SAMHSA (2004): Drug and Alcohol Services Information System (DASIS) Report. Office of Applied Studies, SAMHSA. PM Thompson, KM Hayashi, SL Simon, JA Geaga, MS Hong, Y Sui, JY Lee, AW Toga, W Ling, ED London (2004): J Neurosci 24:6028. United Nations (2004): World Drug Report 2004, Vol 1UN Office on Drugs and Crime. Accessed February 25, 2005 at http://www.unodc.ord/pdf/WDR_2004/volume 1.pdf.

Mesolimbic Dopamine D2/D3 Receptor-Mediated Signaling in Methamphetamine-Sensitized Rats

Jin-Chung Chen*, Pei-Chun Chen and Yao-Chang Chiang

Pharmacology, Chang-Gung University, Tao-Yuan, Taiwan

Dopamine D3 receptors belong to dopamine D2-like receptor family, localized in both presynaptic nerve terminals and postsynaptic neurons. Functionally, dopamine D3 receptors participate in various limbic functions such as mood, emotion and cognition. Due to lack of selective D3 receptor ligand, the physiological functions among D2-like receptors could not be well differentiated. The application of D3 receptor expressed cell lines or genetically engineered D3 knock-out mice would provide valuable information to probe the functional significance of D3 receptor. Previously, we found that chronic amphetamine treatment resulted in dopamine D3 receptors down-regulation in D3-enriched limbic forebrain (olfactory tubercle, island of Calleja and nucleus accumbens). In addition, intracranial administration with D3 antagonist significantly blocked the development of amphetamine sensitization. To correlate the possible cellular signaling(s) with D3 receptor down regulation in behavioral sensitized rats, we first investigated in chronic methamphetamine (METH) treated rats, the significance of Cdk5/DARPP-32 pathway during the development of behavioral sensitization. The results shown that acute and chronic METH treatment enhanced Cdk5 activity, p35 translocation, a Cdk5 activator, from cytosol to membrane and DARPP-32 phosphorylation at Thr75. Intra-accumbal microinjection with Cdk5 inhibitor, roscovitine not only inhibited acute METH-induced behavioral activation, but suppressed the development of METH sensitization. To further characterize the role of D3 receptors in METH-mediated behavioral sensitization, we applied D3 KO mice and treated chronically with METH. The results shown that the rate of development of METH sensitization in D3 KO mice was faster than wild-type. In addition, signaling of PI3K/Akt, ERK1/2 and Cdk5/DARPP-32 not only altered in na?ve D3 KO mice, but significantly different from wild-type after METH sensitization. Further analyses revealed that difference in the development of METH sensitization could be caused by super-sensitivity of dopamine D1 receptor, since repetitive D1 agonist treatment accelerated the development of locomotor sensitization in D3 KO mice as compared to wild-type. The finding that increase in pERK1/2 in the limbic forebrain of METH-sensitized D3 KO mice prompted us to test if SL327, a MEK inhibitor that can penetrate into the brain, would affect the development of METH sensitization in wild-type mice. The results showed that MEK blockade suppressed the behavioral sensitization. In order to delineate the role of D3 on dopamine activity, we tested in PC-12/hD3 cells if D3 receptor

could functionally associated with dopamine release. Our results indicate D3 receptor activation could initiate the Cdk5/DARPP-32 as well as PI3K/Akt signalings, in addition, dose-dependently inhibited [3H]DA release. Pretreatment with Cdk5 inhibitor, roscovitine or olomocine, but not PI3K inhibitor, wortmannin effectively relieve the D3 receptor-mediated autoinhibition on [3H]DA release. Over-expression of Cdk5 in PC-12/hD3 cells, on the other hand, potentiated the inhibitory effect of D3 agonist, PD128907. The overall results suggest that dopamine D3 receptors play an essential role in determining the forebrain dopamine activity and drug-induced neural plasticity.

The Role of Tissue Plasminogen Activator in Methamphetamine-Related Reward and Sensitization

Kiyofumi Yamada*, Taku Nagai, Mina Ito, Kazuhiro Takuma and Toshitaka Nabeshima

Neuropsychopharmacology, Kanazawa University Graduate School of Natural Science, Kanazawa, Japan

Background: Accumulating evidence suggests that tissue plasminogen activator (tPA) plays a role in synaptic plasticity in the brain. We have recently demonstrated that tPA contributes to the rewarding effects of morphine by regulating dopamine release via the formation of plasmin from plasminogen. In the present study we investigated the role of tPA in methamphetamine (METH)-related reward and sensitization.
Methods: Male Wistar rats and wild-type (C57BL/6J) and tPA-/- mice were used in the present study. The levels of tPA mRNA were determined by real-time RT-PCR using TaqMan probe. The enzymatic activity of tPA was assayed by gel zymography. The rewarding effect of METH was assessed by using a conditioned place-preference test. The extracellular dopamine levels in the brain were measured by in vivo brain dialysis. Laminin degradation induced by tPA and plasmin was assayed by Western blotting.
Results: Repeated METH treatment dose-dependently induced tPA mRNA expression in the frontal cortex, nucleus accumbens (NAc), striatum and hippocampus whereas single METH treatment did not affect the expression in these brain areas. The METH-induced increase in tPA mRNA expression in the NAc was completely inhibited by pretreatment with R(+)-SCH23390 and raclopride, dopamine D1 and D2 receptor antagonists, respectively. In addition, repeated METH treatment increased the enzymatic activity of tPA in the NAc. METH-induced conditioned place preference and behavioral sensitization after repeated METH treatment were significantly reduced in tPA-/- mice compared with wild-type mice although there was no difference in basal locomotor activity or acute METH-induced hyperlocomotion between wild-type and tPA-/- mice. The defect of behavioral sensitization in tPA-/- mice was reversed by bilateral microinjections of recombinant tPA into the NAc. Behavioral sensitization induced by repeated METH treatment in wild-type mice was associated with a marked potentiation of dopamine release-stimulating effect of METH in the NAc. On the other hand, tPA-/- mice failed to show the potentiation of METH-induced dopamine release after repeated METH treatment. Treatment of brain homogenate in vitro with tPA in the presence of plasminogen caused a marked degradation of laminin. A similar degradation of laminin was also observed by plasmin treatment.
Discussion: In the present study we have demonstrated that repeated METH treatment results in an increase in tPA mRNA expression and the enzymatic activity in the brain through the activation of dopamine D1 and D2 receptors and that tPA is involved in the rewarding effects of METH. Furthermore, it is suggested that tPA plays an important role in the development of behavioral sensitization induced by repeated METH treatment, which is associated with the potentiation of dopamine release-stimulating effect of METH. Degradation by the tPA-plasmin system of extracellular matrix proteins such as laminin may be related to long-lasting changes in synaptic structure and function in METH dependence.

Serotonin Modulation of the Effects of Amphetamine and Phencyclidine: Locomotor Hyperactivity and Prepulse Inhibition Studies in Rats

Maarten Van den Buuse* and Snezana Kusljic

Behavioural Neuroscience Laboratory, Mental Health Research Institute, Parkville (Melbourne), VIC, Australia

Background: The mechanism of the effects of psychotomimetic drugs in the brain, such as amphetamine and phencyclidine, is not completely understood. While we conducted a series of experiments to elucidate the role of brain serotonin projections in animal models of schizophrenia, we also obtained new information about the role of brain serotonin in the action of amphetamine and phencyclidine.
Methods: We stereotaxically micro-injected the serotonin neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) or vehicle into the dorsal raphe nucleus (DRN) or median raphe nucleus (MRN) of anesthetized male Sprague-Dawley rats. In follow-up studies, lesions were targeted at major projection regions of the MRN and DRN, the dorsal hippocampus, ventral hippocampus, central amygdala, basolateral amygdala and prefrontal cortex. After behavioural experiments, serotonin levels were measured with HPLC.
Results: Two weeks after surgery, MRN-lesioned, but not DRN-lesioned rats showed a marked and significant enhancement of the locomotor hyperactivity induced by phencyclidine (2.5 mg/kg). In contrast, there was no change in the locomotor hyperactivity response to treatment with amphetamine (0.5 mg/kg). Rats with selective MRN lesions, but not DRN lesions, also showed significant disruption of prepulse inhibition (average PPI 38% vs. 56% in sham-operated controls). Serotonin depletion in DRN-lesioned rats was most marked in frontal cortex, striatum and ventral hippocampus (70-80% depletion). MRN lesions caused serotonin depletion particularly in dorsal hippocampus (73%). Specific 5,7-DHT induced lesions of the dorsal hippocampus caused marked and significant enhancement (+100%) of the effect of phencyclidine. In contrast, the effect of amphetamine was slightly, although significantly reduced. Total distance travelled after phencyclidine treatment was 7271 ± 984 cm in controls vs. 14552 ± 1077 cm in lesioned rats. Total distance travelled after amphetamine treatment was 17884 ± 1603 cm in controls vs. 13628 ± 1109 cm in lesioned rats. Rats with dorsal hippocampus lesions also showed significant disruption of PPI. In contrast, 5,7-DHT lesions of the ventral hippocampus, central or basolateral amygdala, or the frontal cortex, had no significant effect on locomotor hyperactivity. PPI was also not disrupted by these lesions except by central amygdala lesions. Measurement of serotonin levels in these brain areas by HPLC confirmed the effectiveness and selectivity of the lesion approach.
Discussion: Locomotor hyperactivity induced by treatment with amphetamine or phencyclidine in rats is widely used as an animal model of psychosis. We used this behaviour to explore the role of different serotonin projections in the brain in schizophrenia. Our studies show that the effect of amphetamine is generally not affected by differential serotonin depletion in the brain. Only in dorsal hippocampus-lesioned rats was there a small, but significant reduction of the effect of amphetamine. In contrast, the action of phencyclidine was markedly enhanced in rats with dorsal hippocampus serotonin depletion, either by local lesions or MRN-lesions. The mechanism of action of phencyclidine might include activation of a serotonergic inhibitory pathway, which is eliminated by the lesions in our study. PPI was also differentially affected by regional serotonin depletion in the brain. Our studies thus provide new insight into a possible role of serotonin projections in the brain in aspect of schizophrenia, particularly those modelled by the effect of phencyclidine and by PPI. The effect of amphetamine, at least at the acute dose used in these rat studies, seems to be modulated only to a minor extent by serotonin projections.

Panel Session
What Does Dopamine Say: Clues from Computational Modeling

The Role of Dopamine in the Temporal Difference Model of Reinforcement Learning

Read Montague*

Baylor College of Medicine, Houston, TX, USA

Background: Reinforcement learning models now play a central role in modern attempts to understand how the brain categorizes and values events traditionally framed by psychology as rewards and punishments. These models provide a way to design and interpret of reward expectancy experiments in humans across a wide range of rewarding dimensions. They also provide a connection to computational models of optimizing control, and hence connect the neurobiology of reward processing to simple forms of decision-making, even decision-making about social exchanges. A central signal in these computational accounts is the reward prediction error signal encoded by burst and pause responses in midbrain dopamine neurons. Numerous experiments have now provided strong evidence for the existence of such reward prediction error signals. Despite these successes, there is a missing piece to this story. The missing piece is a learning signal known as regret. By regret, we mean the difference between what 'could have been obtained' and what 'actually was obtained'.
Methods: We used several event related fMRI and hyperscan-fMRI experiments to probe both reward prediction error signals and regret signals in humans subjects. We studied the reward prediction error signals using a simple conditioning paradigm where a light predicted the temporally consistent arrival of a juice squirt in the mouth of 25 human subjects. We also probed the existence of reward prediction error signals in another domain, social exchange, using a two-person economic exchange game (a trust game) and hyperscan-fMRI (n=96 subjects). A third experiment was carried out on to probe neural correlates of regret single human subjects carrying out an investment task (basically a gambling game).
Results: All three experiments revealed strong correlates of these computational learning signals: reward prediction error and the regret signal. In both cases strong responses were observed in the ventral striatum, and in the case that choices were actually made by the subjects the prediction error signal activated ventral portions of the caudate nucleus consistent with previous reports using different tasks. The regret experiment showed exceptionally strong responses in the ventral putamen and also responses in Lateral Interparietal Sulcus area (LIP) that correlated with the value of the market fluctuation. In the trust experiment, we observe a signal in the ventral caudate that displays features of a reward prediction error signal.
Discussion: These results address three major issues. (1) They show that reward prediction error signals possess detectable correlates in human brains using functional magnetic resonance imaging. (2) They show that reward prediction error signals show up in the ventral putamen when no action is required by the subject to obtain reward and the ventral caudate and putamen when an action is required. (3) Regret signals are treated by the brain as real losses and drive changes in behavior (behavioral results) and furthermore that this signals represent another form of learning signal, a counterfactual reward error signal, that has detectable neural correlates in the striatum, thus suggesting one physical substrate for the experience of regret. (4) Collectively, these results show the utility of using computational models to search for neural correlates of signals involved in reward learning and perturbed by disease. This approach provides a new direction to more traditional methods of searching for neural correlates of reasonable psychological categories.

Dopamine Encodes a Quantiative Reward Prediction Error for For Reinforcement Learning

Paul W Glimcher*, O'Dhaniel A Mullette-Gillman, Hannah M Bayer, Brian Lau and Robb Rutledge

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

Background: There is much evidence that the activity of midbrain dopamine neurons is correlated with the reward prediction error signal postulated by all reinforcement learning models. There has, however, been little effort devoted to testing the hypothesis that the activity of these neurons specifically encodes the reward prediction error term of any particular model or that the activity of these neurons can account for behaviors related to reinforcement learning processes. Our laboratory has attempted to address this with a three pronged approach. First, we have developed behavioral tools for quanifying reinforcement learning in humans and primates. Second, we have linked the trial-by-trial activity of dopamine neurons, measured in awake behaving primates, to the history of recent rewards which serve as the input data for reinforcement learning. Third, we have examined how changes in dopamine unit activity influence the ways in which the history of recent rewards influence behavior.
Methods: Reinforcement learning combines information about previous rewards and punishments in order to place values on actions. This is, however, not the only class of information that can influence the desirability of an action. Biases and the history of ones own choices (irrespective of the rewards that they have yielded) can also influence choice. We therefore developed a mathematical technique for analyzing the choices made by monkeys that allows us to determine the specific contribution previous rewards and punishments make to decision-making; a quantitative estimate of the reinforcement learning process. We performed this analysis on monkeys and humans performing a Matching-Law task of the type pioneered by Herrnstein. Our single unit approach is broadly similar. Once again we ask, here by linear regression, how the firing rates of single dopamine neurons are related to the previous history of rewards. If the behavioral and neuronal processes are identical then these two sets of measurements should also be identical.
Results: Our behavioral studies indicate that the segment of choice behavior which is driven by the history of recent rewards is strongly influenced by recent rewards and weakly influenced by rewards that are more distant in time. This weakening with time occurs with an exponential decay having a time course of about 7 trials, exactly as predicted by reinforcement learning theories like the TD model of Sutton and Barto. We find that the weighting function which relates the firing rates of dopamine neurons to the magnitudes and times of previous rewards precisely matches both the theoretical weighting function predicted by Sutton and Bartos model and the behaviorally derived estimates of the reinforcement learning process described above. Most recently we have begun to explore how changes in the activity of dopamine neurons influence our sophisticated behavioral measures of the reinforcement learning process. To this end we have examined the behavior of monkeys who receive electrical stimulation in the substantia nigra and of human Parkinsons patients, both on and off medication, during reinforcement learning tasks.
Discussion: Our results support the conclusion that midbrain dopamine neurons carry a reward prediction error of precisely the type required by reinforcement learning models. This activity appears sufficient to account for behavioral measurements of reinforcement learning and the contribution that these processes make to behavior.

Implications of the Temporal Difference Reinforcement Learning Model for Addiction and Relapse

A David Redish*

Neuroscience, University of Minnesota, Minneapolis, MN, USA

Temporal difference reinforcement learning (TDRL) algorithms have gained popularity to explain both behavior and the firing paterns of

dopaminergic cells. These models learn to predict value (expected predicted reward). If the agent (the animal or simulation) knows the value of the consequences of its actions, it can act to maximize that value. Estimated value is updated through a value-error term δ, defined as the difference between expected and observed changes in value. Addictive drugs have been hypothesized to access the same neurophysiological mechanisms as natural learning systems. A non-compensable drug-induced dopamine increase will drive a TDRL model to over-select actions leading to drug receipt. In this model, the agent incorrectly assigns ever-increasing value to drugs due to the noncompensable dopamine signal. Because willingness to pay is proportional to estimated value, as the estimated value approaches infinity, the willingness to pay increases proportionally. This willingness to pay provides an explanation for addicts continued attempt to find drugs, even at the expense of great and terrible costs. Because responses are so easily renewed after extinction, extinction cannot entail unlearning of the original association (Pavlov 1927, Bouton LearnMem 2004). Because standard TDRL models are generalizations of standard associative models, they do not differentiate learning from unlearning: a missing reward produces $\delta<0$, which produces a decrease in value (expectation of reward), which produces a decrease in action-selection. We propose instead that acquisition and extinction are driven by separate processes: Acquisition entails the development of an association, is based on phasic increases in dopamine, and is learned through increases in the value-estimate. Once this association has been learned, it is permanently stored and cannot be unlearned. Extinction entails the development of a new state space, which has no associated value-estimate. Tonically low δ (signaled by repeated pauses in dopamine neuronal firing) produces a splitting of the state space, such that a new state s' is created which can be differentiated from s. Evidence for dopamine antagonists producing representational instability has been found in frontal cortex (Zahrt et al. JNsci 1997), auditory cortex (Bao et al. Nature 2001), and hippocampus (Kentros et al. Neuron 2004). Relapse, then, occurs when the neural representation returns to the original representation which leads to the addictive path to drug-use. As with extinction processes, this implies that relapse will be particularly sensitive to context and other cues which can drive the representation back to the original representation. This learning-theory explanation of relapse is independent of whether the association produces positive desire for drugs or negative symptoms which need to be relieved. In either case, relapse occurs when the representation returns to the state s and makes the pathway to drug use available again. Reward/aversion can be categorized into four separate processes: reward (positive value larger than expected), disappointment (lack of expected positive value), aversion (negative value larger than expect), and relief (lack of expected negative value). We suggest that they arise from different neurological mechanisms and have different neurological consequences. Whether aversion and relief work in similar ways to reward and disappointment is unknown at this time, but the similarity of extinction processes on negative value consequences (e.g. cue leads to shock) to positive value consequences (e.g. cue leads to food) suggest that they might.

Dopamine-Norepinephrine Interactions: Exploitation versus Exploration

Jonathan D Cohen*, Samuel M McClure, Mark S Gilzenrat and Gary Aston-Jones

Psychology, Center for the Study of Brain, Mind and Behavior, Princeton University, Princeton, NJ, USA

Background: Adaptive behavior involves a trade-off between exploiting known sources of reward and exploring the environment for new, potentially more valuable ones. Research over the past decade suggests that DA mediates a learning signal that reinforces responses predictive of reward. Reinforcement learning (RL) models have successfully described DA activity in stable environments, but have not addressed more realistic conditions in which contingencies between responses and rewards may change — requiring that previously learned associations be ignored and new ones discovered. For such adaptations, RL models require additional apparatus ("annealing mechanisms") that detect when the environment has changed and promote exploration of new behaviors. Models of DA currently lack such mechanisms. However, recent studies suggest that the locus coeruleus-norepinephrine (LC-NE) system may serve this role. These studies have revealed two modes of LC function: a phasic mode, selectively favoring responses to task-relevant events, and a tonic mode producing a more generalized enhancement of responding. These findings suggest that the LC-NE system may implement an annealing mechanism for DA-mediated RL. This theory presupposes that the LC has access to evaluations of current task utility necessary to adjudicate between exploitation (high utility) and exploration (low utility). Recent anatomic findings support this, indicating that the two primary cortical projections to LC are from orbitofrontal and anterior cingulate cortex — areas consistently implicated in the evaluation of rewards and costs, respectively.
Methods: We implemented a model of interactions between DA-mediated RL (using the method of temporal differences), cortical mechanisms for decision making and evaluation of utility (reward rate and conflict), and an LC-NE annealing mechanism (simulating the dynamics of LC-NE activity). All of the mechanisms were drawn from previous models that accurately simulate relevant behavioral and physiological findings concerning these systems. We tested the model in a reversal conditioning experiment using a target detection task, in which the target identity was periodically reversed. The model's performance was examined with and without the LC-NE system, and was compared with behavioral and LC recordings from a non-human primate performing the same task.
Results: Without the LC-NE system, the model rapidly learned the initial target but took a protracted amount of time (several hundred trials) to learn to respond accurately following reversals. Introducing the LC-NE system dramatically improved learning following reversals (within 25-50 trials). Reversals were associated with transient decreases in LC phasic responding and increases in baseline firing (shift to tonic mode), followed by a return to the LC phasic mode as the new contingency was acquired. Both the performance of, and dynamics of LC activity in the model closely matched empirical observations in the same task performed by a monkey.
Discussion: The model demonstrates how DA-NE interactions may support the self-regulation of exploitation vs. exploration, a function critical to adaptive learning and decision making. More generally, it highlights the importance of interactions between neuromodulatory systems, above and beyond their individual functions. This is likely to have direct relevance to psychiatric disorders, which almost certainly involve disturbances of interactions between neuromodulatory systems that go beyond the simple excesses or deficits of individual systems commonly postulated by many existing theories.

Panel Session
The Role of Feeding Neuropeptides in Alcohol and Drug Dependence

Galanin and Opioid Peptides in Relation to Alcohol Intake and Dietary Fat: Possible Positive Feedback Mechanisms

Sarah Leibowitz*, Olga Karatayev, Valerie Gaysinskaya, Pedro Rada, Michael Lewis, Nicole Avena, Carmen Carrillo and Bartley Hoebel

Rockefeller University, New York, NY, USA

Recent experiments in our lab have demonstrated a close link between hypothalamic feeding-stimulatory peptides and both fat consumption and an associated rise in circulating triglycerides (TG). When injected into the paraventricular nucleus (PVN), which is involved in controlling food intake, the peptide galanin (GAL) and the opioids, enkephalin (ENK) and dynorphin (DYN), stimulate feeding

behavior in Sprague-Dawley rats, and this response is stronger on a high-fat diet than a high-carbohydrate diet and in rats showing a preference for dietary fat. Also, measurements of the endogenous peptides demonstrate that chronic or acute consumption of a high-fat diet stimulates the expression and production of these feeding-stimulatory peptides specifically in the PVN, revealing strong, positive correlations with circulating TG. The importance of these elevated lipids and their metabolism in stimulating the peptides is suggested by the findings that their expression in the PVN is stimulated by injection of Intralipid, which raises TG, and reduced by polyunsaturated fat diets, which lower TG, or by compounds that inhibit fat metabolism. These findings support the existence of non-homeostatic, positive feedback circuits, or "vicious cycles", that relate dietary fat and circulating lipids to PVN peptides that stimulate feeding. They suggest that these peptide systems contribute to the overeating and large meal size generally associated with fat-rich foods. Further studies of GAL, ENK and DYN in Sprague-Dawley rats demonstrate a similar relationship between these peptides and the consumption of alcohol. Evidence demonstrates that the drinking of alcohol is stimulated by PVN injection of GAL and ENK, while reduced by peptide receptor antagonists. Further, the consumption or injection of alcohol stimulates the expression and production of GAL, ENK and DYN, specifically in the PVN. Thus, there exists a positive feedback loop between these PVN peptides and alcohol intake, which is similar to that seen with dietary fat and may be involved in promoting the over-consumption of alcohol. These feed-forward peptide systems related to dietary fat and alcohol intake are linked by other peripheral and central mechanisms. In particular, alcohol consumption like fat intake increases circulating TG, which are positively correlated with blood alcohol levels as well as peptide expression. Also, dietary fat and alcohol have similar effects on central mechanisms, e.g., the dopamine (DA) system in the nucleus accumbens, which mediate the rewarding properties of consummatory behavior. Microdialysis experiments demonstrate that extracellular DA in the accumbens is increased by consumption of alcohol. A similar effect is produced by PVN injection of GAL, systemic injection of opiate drugs, and ingestion of fat-rich diets. Thus, PVN peptides are likely to act, together with accumbens DA as well as circulating TG, in promoting excess consumption of alcohol as well as a high-fat diet. Further evidence suggests that dietary fat itself can stimulate alcohol intake. In a series of experiments, we found that the drinking of 7-9% alcohol is significantly greater in rats previously shown to be hyperphagic (vs normophagic) on a chronic high-fat diet or given a single high-fat (vs low-fat) meal. It is also increased in rats given a single injection of Intralipid, which raises TG levels. Thus, the PVN feeding-stimulatory peptides, GAL, ENK and DYN, may have an important role in promoting alcohol consumption, specifically on diets rich in fat. Supported by: USPHS grants MH 43422 and AA 12882.

Neuropeptide Y and Melanocortin Neuropeptides Regulate Alcohol Intake and Feeding: Are Overlapping Pathways Involved?

Todd E Thiele*, Montserrat Navarro, Inmaculada Cubero, Darin J Knapp and George R Breese

Psychology, University of North Carolina, Chapel Hill, NC, USA

Background: Ethanol is a caloric compound, and ethanol drinking and feeding involve both appetitive and consummatory behaviors. It is therefore possible that overlapping central pathways are involved with uncontrolled eating and excessive ethanol consumption. Neuropeptide Y (NPY) and the melanocortin (MC) peptides produce orexigenic and anorectic effects, respectively. Recent investigations have implicated NPY and MC neuropeptides in the regulation of ethanol consumption. The purpose of the present research was to determine if compounds targeting central NPY or MC receptors (MCR) would produce similar effects on behaviors associated with the ingestion of ethanol or food.
Methods: Subjects were non-dependent C57BL/6J mice. For studies involving central infusions, mice were implanted with cannulae aimed at the lateral ventricle (ICV). Mice were individually housed, had ad libitum access to food, and were presented with 24-h access to a water bottle and an ethanol bottle. To determine the contributions of the NPY Y1 receptor, mice were given intraperitoneal (IP) or ICV administration of the Y1 receptor antagonist (Y1RA), [(-)-2-[1-(3-chloro-5-isopropyloxycarbonylaminophenyl)ethylamino]-6-[2-(5-ethyl-4-methyl-1,3-tiazol-2-yl)ethyl]-4-morpholinopyridine]. To study the contributions of MCR signaling, mice were given ICV infusion of the non-selective MCR agonist melanotan II (MT-II), the non-selective MCR antagonist agouti-related protein (AgRP)-83-132, or the selective MC-4 receptor agonist (MC4RA), cyclo(NH-CH_2-CH_2-CO-His-D-Phe-Arg-Trp-Glu)-NH_2. Additionally, we studied MC-3 receptor (MC3R) knockout mice to determine the role of this receptor.
Results: In C57BL/6J mice, IP (25-75 mg/kg) and ICV (100 μg) administration of the NPY Y1RA significantly reduced ethanol drinking and food intake over a similar time-frame. Similarly, ICV infusion of MTII (1.0 μg) reduced, while AgRP-(83-132) (0.05 or 5.0 μg) increased, feeding and ethanol consumption. MC3R knockout mice showed normal ingestive behavior and showed normal MTII-induced reduction of feeding and ethanol intake. ICV infusion of the selective MC4RA (1.0 and 3.0 μg) reduced food and ethanol intake, implicating the MC4R. Importantly, none of the drugs examined influenced ethanol metabolism.
Discussion: In non-dependent C57BL/6J mice, administration of compounds targeting NPY Y1 or MC receptor produced similar and concurrent effects on ethanol and food ingestion. These results are consistent with the hypothesis that there is overlapping neuropeptide control of ethanol consumption and food intake. Several important questions remain. It will be important to determine if the effects of these compounds are similar in dependent versus non-dependent mice. In fact, recent data suggest that NPY produces very different effects in ethanol-dependent animals, implying that different NPY circuits are recruited over the course of ethanol dependence. A second important issue requiring resolution is whether compounds targeting NPY or MC receptor control ethanol drinking through a mechanism involving the regulation of calories or by a mechanism that modulates the pharmacodynamic effects of ethanol (e.g., the reinforcing properties of ethanol). Finally, it will be important to determine if ethanol alters NPY and/or MC signaling during the development of ethanol dependence, and if such alterations have subsequent effects of feeding behaviors. (Supported by NIH grants AA13573, AA011605, and AA14949).

Not Your Mother's CRF: Type 2 Urocortins, Selective CRF2 Receptor Agonists, Reduce Excessive Ethanol Drinking and Overeating

George F Koob* and Eric P Zorrilla

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

Background: Members of the corticotropin-releasing factor (CRF) family, including the urocortins, have been hypothesized to be major mediators of the stress response through two receptors (CRF1 and CRF2) and their isoforms. Genes encoding two selective CRF2 agonists, type 2 urocortins (urocortin 2 and urocortin 3), have been cloned from both mouse and human genomic libraries. Type 2 urocortins are >1000 times more selective than urocortin 1 or CRF in their relative affinities for CRF2 over CRF1 receptors. The present series of studies tested the hypothesis that type 2 urocortins, via CRF2 receptors, influence behavioral responses to stressors, palatable food intake or excessive ethanol drinking in the rat.
Methods: Adult male Wistar rats were chronically exposed to intermittent ethanol vapor to produce dependence. Thereafter, rats were pretreated (i.c.v.) with urocortin 3 during acute (2-hr) ethanol withdrawal and tested for operant ethanol self-administration in a Latin square design. The effects of urocortin 3 (i.c.v.) on withdrawal-induced anxiogenic-like behavior in the elevated plus maze were studied in separate rats. For feeding studies, the effects of urocortin 2 and urocortin 3 pretreatment on palatable food intake was studied in

non-deprived rats consuming precision pellets in a nosepoke microstructure procedure. The ability of urocortin 2 to reduce palatable cafeteria diet-induced hyperphagia also was examined. Potential non-specific aversive properties of type 2 urocortin infusion were examined in tests of malaise (conditioned taste aversion, kaolin intake) and anxiety-like behavior, using LiCl and stressin-1 (a selective CRF1 agonist), respectively, as positive controls.
Results: Urocortin 3 (i.c.v.) blocked the anxiogenic-like effects of early alcohol withdrawal and the excessive drinking associated with alcohol dependence. Urocortin 2 decreased 6-hr intake of a highly palatable cafeteria diet that promoted hyperphagia, and both urocortin 2 and urocortin 3 reduced the quantity of food intake and local eating rate of free-feeding rats consuming palatable precision pellets. Doses that reduced feeding or ethanol drinking did not induce visceral illness or produce anxiogenic-like responses in the elevated plus-maze, defensive burying or social interaction tests.
Discussion: These results suggest that the type 2 urocortin system when activated can reduce excessive drinking of dependent rats and excessive intake of palatable food without producing malaise-like effects and suggest that the brain CRF2 system may be important as a common neuroadaptative response to or therapeutic target for drug taking and excessive food intake.

Sugar Bingeing Produces Effects on Opioid and Dopamine Systems: Possible Roles in Drug Abuse

Nicole M Avena, Pedro Rada, Miriam Bocarsly and Bartley G Hoebel*

Psychology, Princeton Univ, Princeton, NJ, USA

Background: Opioids and dopamine (DA) play a role in addiction to a variety of drugs, including heroin, morphine and alcohol. Food can activate parts of the same neurochemical systems. The question is whether this is an addiction system that may have evolved in part for survival by food seeking. If so, this system might contribute to signs of addiction that are seen with foods that release opioids and DA.
Methods: Rats with ad libitum water were given plain chow plus a bottle of sugar solution 12 h per day beginning 4 h into the dark phase, thereby causing 12 h food deprivation and delaying the first meal of the night. Microdialysis was used to measure DA and acetylcholine (ACh) in the nucleus accumbens (NAc). Autoradiogrphy was used for receptor binding. Behavioral tests were used as noted, with details found via the reference below.
Results: The animals escalated their sugar intake, until by the end of 10 days they were taking a very large first meal of sugar. After 3 weeks, these animals were sustaining excessive DA release. The taste of a sugar binge released excessive DA even if the sugar drained out through a gastric fistula. In the NAc, D1 and mu-opioid receptors were up-regulated. Sugar abstinence, even with ad libitum chow, then caused behavioral signs of anxiety in the elevated plus maze and defensive burrowing in the cage bedding. Corticosterone was suppressed, and blood glucose levels were normal, suggesting that the observed behaviors during spontaneous withdrawal were not due to hypoglycemia. With total deprivation from food and sugar, after 24 h forepaw tremor and shaking were observed in experimental, but not control, animals. One week of sugar abstinence caused locomotor sensitization (hyperactivity) to a low dose of amphetamine, suggesting that the DA released by sugar had sensitized the mesolimbic DA system. Sugar abstinence also caused avidity for ethanol. This was shown in rats that were first trained to binge on sugar, then given forced abstinence, and then trained to drink ethanol. The group sensitized with sugar consumed significantly more 9% ethanol than the control groups. Rats that binged daily on sugar showed CNS withdrawal signs, both behavioral and neurochemical, within minutes of receiving naloxone (3 mg/kg, s.c.), suggesting that sugar acts, in part, via the endogenous opioid systems. Neurochemical manifestations of this opioid withdrawal were a significant decrease in extracellular DA in the NAc, coupled with an increase in ACh, similar to that seen during naloxone-induced withdrawal from morphine or ethanol. The cause in this case, however, was intermittent intake of sugar, not a drug of abuse. Reference: Rada, P, Avena, NM, Hoebel, BG. (2005) Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. Neuroscience (in press). Supported by USPHS grants DA-10608 and MH-65024.

Panel Session
Drug Development: Development of Novel Metabotropic Glutamate Receptor Agents for the Treatment of Schizophrenia and Anxiety-Related Disorders

Positive Allosteric Modulators of Metabotropic Glutamate Receptor 5: A New Generation of Antipsychotics?

Vincent Mutel*, Anne-Sophie Bessis, Mark Epping-Jordan, Emmanuel Le Poul, Bernard Ludwig, Sonia-Maria Poli and Jean-Philippe Rocher

Addex Pharmaceuticals SA, Plan Les Ouates, Switzerland

mGluR5 positive allosteric modulators (PAM) recently appeared as very attractive new pharmacological principles with a potential application in the treatment of broad CNS disorders. Novel mGluR5 positive allosteric modulators discovered at Addex Pharmaceuticals have been extensively characterized in vitro and in vivo. Several of these molecules demonstrate high potency and selectivity versus the other members of the GPCR family III. They have excellent general tractability properties (solubility, metabolic stability, cytochrome inhibition, permeability, hERG, etc.) and have been used to initiate a lead optimization program at Addex. In parallel with this development effort, the effect of one specific molecule; ADX47273 was investigated in animal models of psychosis and cognition. ADX47273 is a moderately potent mGluR5 PAM which is highly selective for mGluR5 versus the other members of the mGluR family and many other receptors, ion channel and transporters. This molecule has an intermediate to high plasma clearance, a half life of 2 hours, a bioavailability around 40% and has a brain to plasma ratio > 2, in rats. ADX47273 in vivo reduces the [18F]fluoro-2-deoxy-D-glucose metabolism in hippocampus, nucleus accumbens and cortex and dose-dependently inhibits the locomotor stimulation induced by amphetamine and PCP. In addition it also reverses the natural or scopolamine-induced forgetting in the novel object recognition model in mice. This compound was devoid of sedative, pro-cataleptic, pro-convulsant and pro-nociceptive potential. In addition it did not affect prolactin level and rotarod performance. Taken together these results suggest that mGluR5 PAM have the potential to be effective against symptoms observed in schizophrenia and might alleviate the cognitive impairment observed in this pathology. Finally, these molecules also could be active on the cognitive deficit associated with other pathologies and may represent an attractive novel alternative to the existing therapies for memory impairment.

Description of a Clinically Validated Anxiolytic with mGlu5 Antagonist Properties

Richard Porter*, Georg Jaeschke, Will Spooren, Theresa Ballard - Yardy, Eric Prinssen, Andreas Muehlemann, Juergen Wichmann, Sabine Kolczewski, Bernd Buettelmann, Eric Viera, Vincent Mutel and Pari Malherbe

Pharma Research Basel Discovery CNS, F.Hoffmann-La Roche Ltd., Basel, Switzerland

Here we report on an atypical anxiolytic agent that has been demonstrated to be effective in a double blind placebo controlled Phase II trial of anxious patients. The compound appears to be a selective and potent mGlu5 receptor antagonist acting at an allosteric modulatory site shared with 2-methyl-6-phenylethynyl-pyridine (MPEP), the protypical selective mGlu5 receptor antagonist. The compound inhibited quisqualate-evoked [Ca2+]i response mediated by human mGlu5 receptor with an IC50 = 70 nM. It acted in a non-competitive

mechanism, similar to MPEP and demonstrated inverse agonist properties. It blocked the mGlu5 constitutive activity with IC50 = 87 nM. It bound to rat and human recombinant receptors with Kd values of 54 nM and 31 nM respectively, and exhibits anxiolytic activity in the stress induced hyperthermia model, Vogel conflict test, rat Geller-Seifter procedure, and conditioned emotional response with an MED of 10 to 30 mg/kg. Furthermore, it is devoid of GABA-ergic activity supporting the assertion that it acts by a mechanism distinct from benzodiazepines. The non-GABA-ergic activity, coupled with its robust anxiolytic activity and rapid onset in man supports the potential of developing mGlu5 receptor antagonists with an improved therapeutic window over benzodiazepines.

Anxiolytic and Cognitive Properties of the mGlu1 Receptor Negative Allosteric Modulator JNJ16259685

Anne S Lesage*, Hilde Lavreysen, Arjan Buist, Ana F Oliveira, Ana A Mateus, Sandrina Nobrega Pereira, Xavier Langlois, Christoph Van Dyck, Hansfried Van Craenendonck, Jos Prickaerts, Anton Megens and Thomas Steckler

Psychiatry, Johnson&Johnson Pharmaceutical Research and Development, Beerse, Belgium

Background: The precise assessment of the therapeutic potential of mGlu1 antagonists has been impeded by the lack of potent, systemically active and selective mGlu1 receptor antagonists. We here describe a novel mGlu1 selective negative allosteric modulator, a structurally related radioligand and a PET radiotracer. We document in vitro potency, ex vivo occupancy and in vivo activity in animal models of anxiety and cognition.
Results: JNJ16259685 is a novel negative allosteric modulator of the mGlu1 receptor. JNJ16259685 has high affinity for a site different from the glutamate binding pocket, and inhibits binding of [3H]R214127 to rat and human mGlu1 receptors (Ki 0.61 and 2.15 nM). It potently blocks signaling of recombinant rat and human mGlu1 receptors expressed in CHO and HEK293 cells respectively (IC50 3.24 and 1.21 nM) and blocks endogenous mGlu1 receptor signaling in rat cerebellar neuron culture (IC50 1.73 nM). The compound readily penetrates the brain, as illustrated by the ED50 for occupancy of thalamic and cerebellar mGlu1 receptors in rat (0.014 and 0.04 mg/kg s.c.). Anxiolytic potential of JNJ16259685 was explored in the lick suppression test. Acute administration of JNJ16259685 increased the number of licks (lowest active dose: 2.5 mg/kg i.p). Co-administration of the mGlu1 antagonist JNJ16259685 with the mGlu5 receptor antagonist MPEP had additive effects. Chronic administration of JNJ16259685 over 14 days (5 mg/kg bid) increased the number of licks to a level comparable to that seen after acute administration, suggesting that no behavioural tolerance or sensitisation developed after chronic administration. No anxiolytic-like properties of JNJ16259685 were observed in the elevated zero maze, suggesting that the anxiolytic-like effects observed are task-dependent. The effects of JNJ16259685 on learning and memory were investigated in mice by means of a water maze task. JNJ16259685 impaired acquisition and re-acquisition, already at the lowest dose tested (0.63 mg/kg s.c.). In contrast, effects on spatial retention performance were relatively mild in mice that had learned the task prior to treatment.
Discussion: We conclude that the selective mGlu1 antagonist JNJ16259685 is a valuable tool compound to investigate the role of mGlu1 receptors in vivo.

mGluR7: A Novel Therapeutic Target for Stress-Related Disorders

John F. Cryan*, Kayo Mitsukawa, Deepak Thakker, Cedric Mombereau, Erika Loetscher, Doncho P Uzunov, Francois Natt, Dieter Huesken, Rainer Maier, Kevin McAllister, Daniel Hoyer, Herman Van der Putten and Peter J Flor

Novartis Institutes for Biomedical Science, Basel, Switzerland

Glutamatergic neurotransmission has been strongly implicated in the pathophysiology of affective disorders such as major depression and anxiety. Metabotropic glutamate receptors (mGluR) act as important pre- and postsynaptic regulators of neurotransmission in the central nervous system and comprise at least eight subtypes (mGluR1 to -8). Of all mGluRs the role of presynaptic group III mGlu receptors (mGluR4; -6; -7; -8), and in particular mGluR7, in brain function is the least investigated because of the lack of specific pharmacological tools. To this end we examined the behavioural profiles of mice with a targeted deletion of the gene for mGluR7 (mGluR7-/-) in animal models of depression and anxiety. Mice with a targeted deletion of the gene for mGluR7 (mGluR7-/-) showed antidepressant and anxiolytic-like effects in a variety of stress-related paradigms, including the forced swim stress and the stress-induced hyperthermia tests. Deletion of mGluR7 reduces also amygdala- and hippocampus-dependent conditioned fear and aversion responses. Recently, we have focused on the role of mGlur7 in modifying the stress response. Upon examining parameters of the hypothalamic-pituitary-adrenal (HPA) axis, mGluR7-/- mice showed only moderately lower serum levels of corticosterone and ACTH compared with mGluR7+/+ mice. More strikingly however, we found strong evidence for up-regulated glucocorticoid receptor (GR)-dependent feedback suppression of the HPA axis in mice with mGluR7 deficiency: (i) mRNA transcripts of GR were significantly up-regulated in the hippocampus of mGluR7-/- animals, (ii) similar increases were seen with 5HT1A receptor transcripts, which are thought to be directly controlled by the transcription factor GR and finally (iii) mGluR7-/- mice showed elevated sensitivity to dexamethasone-induced suppression of serum corticosterone when compared with mGluR7+/+ animals. These results indicate that mGluR7 deficiency causes dysregulation of HPA axis parameters, which may account, at least in part, for the phenotype of mGluR7-/- mice in animal models for anxiety and depression. In addition, we present evidence that protein levels of brain-derived neurotrophic factor (BDNF) are also elevated in the hippocampus of mGluR7-/- mice, which we discuss in the context of the antidepressant-like phenotype found in those animals. We conclude that genetic ablation of mGluR7 in mice interferes at multiple sites in the neuronal circuitry and molecular pathways implicated in affective disorders. However, as is the case with all knockout animals it is not clear if the phenotypic changes are due to developmental compensations. Most recently, we have developed a method for the use of short interfering RNA in the brain, in adult animals (Thakker et al., Proc. Natl. Acad. Sci. U. S. A. 101, 17270-17275, 2004). This work has demonstrated that it is possible to generate widespread gene knockdown in the adult brain, translating to a behavioural phenotype similar to that observed after pharmacological blockade of the target gene product. Consequent to the knockdown of mGluR7 expression in the brain, mice displayed a very marked reduction in anxiety in both the light-dark box and the stress-induced hyperthermia tests compared with animals treated with vehicle or negative controls (a scrambled or a luciferase-targeting siRNA). Taken together these data support the role of mGluR7 in the modulation of stress-related disorders and that the development of selective pharmacological tools to target mGluR7 is warranted.

Panel Session
Drug Development: Practical Clinical Trials of Antipsychotic Drugs in Schizophrenia

First Generation versus Second Generation (non-clozapine) Antipsychotic Drugs versus Clozapine in Schizophrenia: The CUtLASS Trials

Shon Lewis*, Thomas Barnes, Robin Murray, Linda Davies, Robert Kerwin, David Taylor, Graham Dunn, Karen Hayhurst and Peter Jones

Division of Psychiatry, University of Manchester, Manchester, United Kingdom

Background: Two non-commercially-funded, pragmatic, open, multisite, randomised, controlled trials (CUtLASS 1 and 2) were conducted in the UK NHS in order (i) to compare SGAs (other

than clozapine) with FGAs, in people with schizophrenia requiring a change of treatment because of inadequate response or adverse effects and (ii) to compare clozapine with the class of other SGAs in people who had responded poorly to two or more prior antipsychotic drugs. The hypotheses were that SGA drugs would outperform FGA drugs; and clozapine would outperform other SGA drugs.
Methods: Participants were aged 18-65 with DSM-4 schizophrenia and related disorders. In CUtLASS 1, participants were randomly allocated to either FGA or SGA classes (amisulpride, olanzapine, quetiapine, risperidone). In CUtLASS 2, participants were randomly allocated to clozapine, or to one of the other SGA drugs. The choice of individual drug within the allocated class was made in advance by the managing clinician. Randomised samples were 227 and 136 respectively. Outcomes were assessed blind to treatment allocation at 12, 26 and 52 weeks. Complete follow up assessments at one year were obtained in 81% and 87% of the samples respectively.
Results: In CUtLASS 1, there were no advantages of SGA over FGA drugs. Participants in the FGA arm showed a trend toward greater improvements in QLS and PANSS scores. Participants reported no clear preference for either class of drug; costs were similar. In CUtLASS 2, an intent to treat comparison showed an advantage for commencing clozapine in QLS score at trend level (3.6 points; CI 0.5-7.7; p=0.08), and in PANSS total score that was statistically significant (4.9 points; CI 1.1-8.8; p=0.01), at one year. At 12 weeks, participants receiving clozapine reported that their mental health was significantly better compared with those receiving SGA drugs.
Discussion: In people with schizophrenia whose medication is changed for clinical reasons, there is no clinical or cost advantage over one year in using non-clozapine FGA drugs rather than clozapine SGA drugs. Neither inadequate power nor patterns of drug discontinuation could account for this result. There is an advantage to commencing clozapine rather than other SGA drugs in terms of symptoms and patient preference over one year. Keywords Schizophrenia First generation antipsychotic Second generation antipsychotic Quality of life Randomised controlled trial Clozapine

Effectiveness of Antipsychotic Drugs in Patients with Chronic Schizophrenia: Primary Efficacy and Safety Outcomes of the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) Schizophrenia Trial
Jeffrey A Lieberman*, T. Scott Stroup, Joseph P McEvoy, Marvin S Swartz, Robert A Rosenheck, Diana O Perkins, Richard S Keefe, Sonia M Davis, Clarence E Davis, Barry Lebowitz, John Hsiao and Joanne Severe

Psychiatry, Columbia University, New York, NY, USA

A second generation of (atypical) antipsychotic drugs was introduced in the 1990s that now accounts for the vast majority of prescriptions for treating schizophrenia in the United States. The relative effectiveness of these new drugs is not known, and they have not been proven more effective than the first generation of antipsychotic drugs. To address this question, a comparison of an older drug, perphenazine, and the newer drugs olanzapine, quetiapine, risperidone, and ziprasidone was conducted supported by an NIMH contract. 1493 subjects with schizophrenia recruited at 57 U.S. sites were randomly assigned in phase I of the study to flexibly-dosed treatment with olanzapine 7.5-30 mg/day, perphenazine 8-32 mg/day, quetiapine 200-800 mg/day, or risperidone 1.5-6 mg/day under double-blind conditions and followed for up to 18 months. Ziprasidone 40-160 mg/day was included after its approval by the FDA when 40% of the sample had already been randomized. The primary aim was to determine if there were differences between the treatments in overall effectiveness, efficacy and tolerability. Subsequent phases of the study allowed for rerandomization to other treatments not received in phase I. Cost and service utilization was assessed throughout all phases of the study. The results and conclusions of phase I in will be presented and discussed

Effectiveness of Antipsychotic Drugs in Unresponsive and Intolerant Patients
Joseph P McEvoy*, T S Stroup and Jeffrey A Lieberman

Psychiatry, Duke University Medical Center, Butner, NC, USA

Background: The CATIE Schizophrenia Trials were designed to determine the comparative effectiveness and tolerabiltiy (as measured by all-cause treatment discontinuation) of specific antipsychotic drugs in representative samples of patients with schizophrenia.
Methods: Patients who discontinued their assigned blinded treatment with a newer atypical antipsychotic in Phase 1, 1A, or 1B because of inadequate therapeutic effect were expected to preferentially enter the Phase 2 Clozapine Trial. The Phase 2 Clozapine Trial offered a 50:50 randomization to clozapine versus a newer atypical antipsychotic (olanzapine, quetiapine, or risperidone) different from that which the patient previously received. For ecological validity, and because of the logistical complexities that blinding would entail, patients assigned to clozapine received this treatment open-label. Patients assigned to a newer atypical antipsychotic received blinded treatment.
Results: 90 patients were randomized and had at least one follow-up assessment in this trial; 17 received olanzapine, 14 quetiapine, and 14 risperidone, and 45 received clozapine. The mean modal daily doses were 23.4mg for olanzapine, 643mg for quetiapine, 4.8mg for risperidone, and 332mg for clozapine. Eighty percent were male and 64% were white. Their mean Positive and Negative Syndromes Scale total score was 80.6 at baseline. We will present the percentages of patients in each treatment group that completed the trial, the median duration of treatment before discontinuation and the reasons for discontinuation for each treatment group, and associated measures of effectiveness and tolerability.
Discussion: These data will address the question, if a patient does not get adequate therapeutic benefit from one of the newer atypical antipsychotics, is it better to try another or to proceed directly to clozapine?

Cost-Effectiveness of Atypical Antipsychotics in the CATIE Schizophrenia Trial
Robert A Rosenheck*

Psychiatry, Yale Medical School, New Haven, CT, USA

This presentation will review data comparing the cost and aggregate effectiveness of drugs used during Phase 1 of the CATIE trial. Data were obtained monthly from all participants on use of inpatient, residential and outpatient, medical and psychiatric care. Cost were based on administrative data and research publications from diverse providers at the State level. Data on costs of study and concomitant medication were based on average wholesale prices. Effectiveness is assessed by a measure of utility based on the PANSS, and a second aggregate measure weighted for consumer preferences. Mixed effects model analyses will be used to compare mean monthly costs of patients treated on olanzapine, risperidone, quetiapine, and perphenazine. Data on ziprasidone will be presented for the subsample in which ziprasidone was used.

Panel Session
Corticolimbic Changes in Bipolar Disorder: A Developmental Perspective

Prefrontal-Amygdalar Abnormalities in Pediatric Bipolar Disorder
Kiki Chang*, Amy Garrett, Asya Karchemskiy, Kim Gallelli, Christopher Wagner, Meghan Howe and Allan Reiss

Psychiatry, Stanford University, Stanford , CA, USA

Background: Prefrontal amygdalar circuits that regulate mood have been implicated in the pathophysiology of bipolar disorder (BD). Re-

cently, several research groups have reported decreased amygdalar volume and prefornal/limbic overactivation in children with BD. We report here converging evidence for prefrontal and amygdalar abnormalities that may represent both trait and state markers of BD.
Methods: We performed neuroimaging on two groups of children and adolescents. First, using a high resolution T1 weighted SPGR 3D MRI sequence at 3T, we studied 42 children and adolescents with a parent with bipolar I or II disorder, and 22 healthy controls (13.3 +/- 2.5 years, 15M/7). Bipolar offspring had BD I (BD Group; n = 22, 14.6 +/- 2.8 years, 16M/4F) or ADHD and significant mood symptoms but no history of full mania (Prodromal Group; n = 22, 12.3 +/- 2.5 years, 15M/7F). Amygdalae were manually traced and segmented into gray/white matter using BrainImage. We also used 1H-MRS and fMRI to study 11 adolescents (the LTG group; 16.1 +/- 1.6 yrs) with BD I, II, or NOS who were in a depressive episode, before and after 8 weeks of lamotrigine adjunct or monotherapy. For MRS, an 8cm3 voxel was placed in bilateral DLPFC and for fMRI subjects performed a task involving evaluating affectively valenced pictures.
Results: After covarying for total brain volume and age, the BD Group and Prodromal group had decreased amygdalar gray matter (10.4%, p = .003; 9.7%, p = .06, respectively) compared to controls. In the LTG group left DLPFC N-acetyl aspartate (NAA)/Creatine-phosphocreatine (Cr) (1.59 +/- .13 to 1.66 +/- .10, p=.037) and myoinositol (mI)/Cr ratios (.49 +/- .06 to .56 +/- .11, p=.038) increased after lamotrigine treatment. Amount of decrease in amygdalar activation after lamotrigine treatment was positively correlated with amount of decrease in depressive symptoms by the CDRS-R (r = .74, p = .037), particularly for right amygdala (r = .88, p = .004).
Conclusions: Prefrontal-amygdalar abnormalities may represent both trait (present before the onset of BD) and state (varying with mood state) markers in pediatric BD. Decreased amygdalar gray matter may represent a trait finding in early-onset BD, or may be due to abnormalities in pruning or neurotoxic processes shortly before the onset of BD. Prefrontal NAA and mI levels may normalize after remission of depressive episodes. Amygdalar activation may be normal during euthymic states, but abnormally elevated during mood episodes. Implications for early identification and treatment of BD with neurotrophic agents will be discussed.

Progression of Anterior Limbic Dysfunction in Adolescent Bipolar Disorder
Melissa P DelBello*, Cal M Adler, James Eliassen, Neil Mills and Stephen M Strakowski

Division of Bipolar Disorders Research, Department of Psychiatry, University of Cincinnati, Cincinnati, OH, USA

Background: Structural and functional magnetic resonance imaging (MRI) studies suggest that adolescents with bipolar disorder exhibit abnormalities in anterior limbic brain regions, including the ventral prefrontal cortex, amygdala and striatum. However, whether these abnormalities are present at illness onset, and therefore, are potentially etiologic, or instead develop over time, secondary to illness progression and associated factors such as medication exposure or substance use, remains unclear. We will review recent data from structual and functional neuroimaging studies of adolescents with bipolar disorder and report the results of a study examining anterior limbic function among unmedicated BP adolescents at their first hospitalization for mania, unmedicated BP adolescents who have had multiple previous mood episodes and healthy controls, in effort to identify the progression of anterior limbic functional abnormalities in adolescent bipolar disorder.
Method: Unmedicated adolescents hospitalized for their first manic or mixed episode of bipolar disorder [FE, N=14, mean (SD) age, 15 (2), 57% female, and 43% ADHD], unmedicated adolescents hospitalized for a manic or mixed episode of bipolar disorder and with at least two prior manic or mixed episodes [ME, N=12, mean (SD) age, 16 (2), 42% female, and 50% ADHD) and demographically matched healthy control adolescents free of any psychiatric disorder [HC, N=19 mean (SD) age, 14 (2), and 53% female] underwent functional magnetic resonance imaging (fMRI) scans using a 3 Tesla MR scanner. Areas of activation during administration of the continuous performance test-identical pairs version contrasted with a control condition of viewing flashing numbers were measured. Functional images were analyzed using Analysis of Functional Images (AFNI), t-test were used to create individual maps, and t-coefficient were used for between group multiple linear regression models, controlling for gender and performance. Additionally, the effects of age on brain function within each group were examined.
Results: FMRI data revealed that compared with HC, FE adolescents exhibit increased activation in ventral (Brodmann area 10) and anterior cingulate prefrontal cortical regions, striatum, and thalamus. Compared with HC and FE adolescents, ME adolescents exhibit decreased activation in Brodmann area 10 and increased activation in anterior insular cortex. There was an age related increase in prefrontal activation in HC, but not in FE or ME adolescents. ME adolescents exhibited an age related increase in posterior parietal cortical activation.
Conclusions: Our results suggest increased activation in anterior limbic brain regions between FE and HC adolescents, indicating that anterior limbic abnormalities are present at illness onset. However, other differences, such as increased activation in anterior insular cortex, are present only in ME adolescents, and therefore, are not etiologic, but instead may be associated with illness progression or associated factors. Additionally, our results indicate that there in a progression of ventral prefrontal dysfunction with potentially compensatory activity in insular and posterior parietal cortex.

Multimodality Magnetic Resonance Imaging Evidence for Abnormalities in Cortico-limbic Stuctural and Functional Connectivity in Bipolar Disorder
Fei Wang, Jessica H Kalmar, Monique M Jones, Linda Spencer, Carolyn Fredericks and Hilary Blumberg*

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

Converging evidence suggests the presence of abnormalities in the development of cortico-limbic neural circuits that subserve adaptive regulation of emotions and impulses in bipolar disorder (BD). We used multimodal neuroimaging techniques to examine morphology and function within major nodes of cortico-limbic circuitry, as well as the integrity of their structural and functional connections. Morphometric analyses showed reduced amygdala volumes in adolescents and adults with BD and the emergence of ventral prefrontal cortex (VPFC) volume deficits in late adolescence/early adulthood, relative to healthy comparison participants (HC). These regional structural abnormalities are associated with functional abnormalities that emerge with a similar developmental trajectory, i.e. with elevated amygdala activation in adolescents and adults with BD and deficits in VPFC function in adults with BD, compared to HC. Moreover, during both resting state and emotional processing, BD participants showed relative uncoupling of function between cortico-limbic structures in BD. Diffusion tensor magnetic resonance imaging (DT-MRI) data suggest that abnormalities in the integrity of white matter connections contribute to this uncoupling. A model for the development of cortico-limbic neural circuitry abnormalities in BD will be presented.

Complementary Morphometric and Functional Corticolimbic Changes in Adult Bipolar Patients
Caleb M Adler*, Melissa P DelBello, Ari Levine, Kelly Jarvis, John Adams, Scott K Holland, James Eliassen and Stephen M Strakowski

Center for Imaging Research, University of Cincinnati College of Medicine, Cincinnati, OH, USA

Background: Bipolar disorder remains a major source of psychiatric morbidity and mortality that affects approximately 1.5% of the pop-

ulation. The clinical course of the disorder is marked by periodic affective episodes, as well as evidence of significant cognitive decline. Although the neuropathology linked to these behavioral changes remains poorly understood, morphological and functional neuroimaging studies appear to implicate dysfunction in the anterior limbic network (ALN), a group of limbic and paralimbic structures hypothesized to be involved in emotional regulation and modulation. Several lines of evidence suggest that bipolar neuropathology involves a combination of developmental abnormalities, present in even first and early-episode patients, and progressive changes linked to illness duration and symptoms, and resulting in increasing affective and cognitive symptomatology over the course of bipolar illness. We have utilized a combination of methodological approaches, including voxel-based morphometry (VBM), functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI) to study anterior limbic structure and function in early episode patients.
Methods: We utilized structural and functional magnetic resonance imaging to examine groups of single and multi-episode patients with bipolar disorder using VBM, fMRI and DTI. Comparisons were made with matched groups of healthy controls.
Results: FMRI findings in adult bipolar patients suggest the involvement of portions of the prefrontal cortex, as well as other limbic and paralimbic structures. A follow-up VBM study in first episode bipolar patients found increased gray matter density and volume in some of these limbic and paralimbic regions but did not observe changes in the prefrontal cortex. In contrast, a group of early episode, bipolar patients demonstrated increased prefrontal gray matter volume. DTI findings in both multi- and first-episode patients found increase FA in prefrontal white matter tracts linking these prefrontal and subcortical structures.
Discussion: These findings suggest that bipolar disorder is associated with a cluster of structural and functional abnormalities involving limbic and paralimbic structures, as well as linking white matter tracts. Our VBM studies found that while elements of prefrontal neuropathology are present at the start of the illness, some changes in prefrontal volume and density appear to be related to illness progression. Our DTI findings further suggest that while some white matter abnormalities may similarly be related to illness progression, at least some elements of the axonal pathology observed is present at the start of bipolar symptomatology. Together, these studies indicate that bipolar neuropathology may be related to both developmental abnormalities, present at the start of bipolar disorder, and neuropathic changes over the course of the illness, that may be related to the neuropathological effects of affective symptomatology.

Panel Session
Novel Pharmacological Approaches to the Treatment of Drug Addiction: Animal and Human Studies

The Differential Neuroplasticity and Differential Inhibition Hypotheses of Drug Addiction: Empirical Evidence and Implications for Treatment

Laura L Peoples*, Alexxai V Kravitz and Kevin G Lynch

Psychology, University of Pennsylvania, Philadelphia, PA, USA

Various lines of evidence indicate that a drug-induced decrease in the activity of accumbal neurons may contribute to drug addiction. How these decreases in activity might contribute to drug addiction is unknown. It has been proposed that certain neuroadaptations critical to addiction may occur differentially between neurons that are activated in relation to drug seeking and taking (Task-Activated neurons) and neurons that do not contribute to that behavior (Differential Neuroplasticity Hypothesis). In line with this proposal, it has also been hypothesized that drug-induced hypoactivity may be activity-dependent and affect Task-Activated neurons to a lesser degree than Task-Non-Activated neurons (Differential Inhibition Hypothesis) (Peoples and Cavanaugh 2003; Peoples et al., 2004). Such differential plasticity would be associated with a relative increase in the transmission of signals that contribute to drug seeking as well as an absolute decline in the transmission of non-drug-related signals. Such a narrowing of information flow could contribute to a selective increase in drug seeking and a simultaneous decline in other behaviors, essentially locking an individual into an uncontrollable, involuntary, pattern of drug seeking and taking. Given the global involvement of accumbal circuits, in appetitive and aversely-motivated behaviors, the differential changes in neural activity could also potentially contribute to other, negative, symptoms of addiction such as anhedonia, and the loss of inhibitory control by adverse behavioral outcomes. In the first test of this hypothesis, chronic extracellular recording techniques were used to record the activity of accumbal neurons in rats self-administering cocaine. Recordings were made during Session 1-3 and Session 30 of a 30-day regimen of daily long-access (6 hrs) self-administration sessions. Between-session comparisons showed that the Task-Non-Activated neurons exhibited a significant decrease in firing during baseline periods, as well as during periods of drug seeking and taking; whereas, Task-Activated neurons showed either no change, or an increase. More detailed characterization of sub-types of Task-Activated and Task-Non-Activated neurons showed that the direction and magnitude of the between-session change exhibited by those neurons was positively correlated with the change in firing rate that the neuron groups exhibited during individual self-administration sessions. The differential between-session change in firing increased the difference between the firing of the Task-Activated and Task-Non-Activated neurons. The magnitude of this difference was related to the propensity of animals to seek and take drug. The differential inhibition in firing of Task-Activated and Task-Non-Activated neurons was not observed when similar recordings were conducted in drug-naive animals trained on sucrose reinforcement. These findings are consistent with the Differential Inhibition Hypothesis. Additional tests of the hypothesis are required. However, if correct, the hypothesis has implications for the development of therapies. A commonly considered treatment strategy is to suppress the activity of neurons that contribute to drug seeking and taking. An additional strategy might be to reverse, or otherwise compensate for, the decreased activity of neurons that contribute to behaviors other than drug seeking and taking. This latter strategy may be particularly interesting in that it might treat negative as well as positive symptoms of addiction. Supported by DA06686, DA05186, and DA13401.

Novel Pharmacological Targets for Relapse Prevention: Animal Studies

Friedbert Weiss*

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

Background: Vulnerability to relapse is a defining feature of drug addiction. The neurobiological basis of relapse and its prevention has therefore attracted major attention in addiction research. Key factors implicated in relapse are craving induced by drug-related environmental stimuli and stress. Our studies on the neurobiological basis of relapse linked to these risk factors have revealed novel potential treatment targets, including the nociceptin/orphanin FQ (N/OFQ) opioid peptide system, metabotropic glutamate receptors (mGluR), and the sigma1 receptor.
Methods and Results: In reinstatement models of relapse, central administration of N/OFQ and activation of Group II mGluRs by LY379268 (a potent mGlu2/3 agonist) dose-dependently reversed reinstatement induced by either drug cues or footshock stress. Antagonism of Group I mGluR receptors by MTEP (a potent mGlu5 antagonist) and by BD1045 (a selective antagonist of the intracellular sigma1 receptor) dose-dependently reversed the response-reinstating effects of drug cues. All agents attenuated drug-seeking behavior without producing non-specific inhibitory effects as determined by

the absence of interference with behavior motivated by conventional reinforcers including food pellets, sweetened condensed milk, or sucrose. Additionally, with exception of N/OFQ, all drug targets proved to be more sensitive for the prevention of relapse compared to behavior maintained by the primary reinforcing effects of cocaine or ethanol. In functional follow-up studies, the attenuation of stress-induced reinstatement was traced to a functional CRF antagonist action of N/OFQ within the bed nucleus of the stria terminalis (BNST), and the peptide's inhibitory effects on cue-induced reinstatement to a functional opiate antagonist action in the nucleus accumbens (Nacc). In the case of Group II mGluR agonist LY379268, these studies implicate increased inhibitory GABAergic output from the central nucleus of the amygdala in the anti-stress actions of this agent, and interference with hippocampal contextual information processing in the reversal of cue-induced reinstatement. Experiments to elucidate the mechanisms and neurocircitry through which Group I mGluR and sigma1 antagonists exert their inhibitory actions on drug-seking are in progress.
Discussion: The results implicate Group I and II mGluRs, the N/OFQ system and sigma1 receptors as promising targets for further scrutiny with respect to treatment drug potential for relapse prevention. (Supported by NIH/NIAAA AA10531, AA014351 and NIH/NIDA DA07348, DA017097).

Regulating Glutamate Homeostasis in Treating Cocaine Addiction
Peter Kalivas*, Megan Moran, Roberto Melendez, Steve LaRowe, Hugh Myrick, Roberto Malcolm, Jeremy Seamans and Antonieta Lavin

MUSC, Charleston, SC, USA

A variety of electrophysiology, neurochemical and behavioral data suggest that glutamate transmission in the nucleus accumbens is altered after withdrawal from chronic cocaine administration. Glutamate transmission is regulated by many factors, including neuronal firing frequency, presynaptic inhibition and postsynaptic signaling. Data will be shown demonstrating that withdrawal from repeated cocaine affects the membrane potential of prefrontal cortical neurons projecting to the accumbens, indicating that the ability to stimulate this glutamatergic projection is altered. Specifically, after withdrawal from self-administered cocaine, in vivo intracellular recordings of prefrontal pyramidal cells in anesthetized rats reveals a loss of membrane bistability. Presynaptic regulation of glutamate release in the accumbens is accomplished largely by glutamatergic tone on mGluR2/3 presynaptic receptors. Data will be presented showing that these receptors are relatively uncoupled and desensitized after chronic cocaine. Moreover, glutamatergic tone on presynaptic receptors arises only in part from synaptic glutamate release, and relies more on glutamate released in the process of cystine/glutamate exchange (rate limiting step in glutathione synthesis). Whole cell patch data from accumbens slices will be presented showing that glutamate derived from cystine-glutamate exchange does indeed regulate synaptic glutamate release in the accumbens, and it will be shown that regulation of the exchanger by N-acetylcysteine administration prevents cocaine-primed reinstatement in animals extinguished from cocaine self-administration. In order to determine if the cocaine-induced adaptations described above are important in cocaine addicts, a double-blind clinical trial was run to determine if N-acetylcysteine treatment could inhibit craving for cocaine in a cue-induced craving paradigm. Hospitalized cocaine addicts were administered N-acetylcysteine (4 X 600 mg oral over 12 hrs) or placebo and were exposed to visual stimuli (slides) containing cocaine related pictures or neutral pictures. Self-reported craving induced by cocaine-associated slides was significantly reduced in the N-acetylcysteine group. In some patients (n=13) fMRI was conducted during cue exposure. The only brain area showing a reduction by N-acetylcysteine compared with placebo was the anterior cingulate, a brain region shown in many studies to be activated during cocaine craving. These data demonstrate that regulating glutamate homeostasis in the accumbens by controlling extracellular, extrasynaptic glutamate levels may be a pharmacotherapeutic target for controlling cocaine craving and relapse.

Translating Data from Animal Models to New Treatments for Addiction
Charles O'Brien*

Psychiatry, Univ. of Pennsylvania, Philadelphia, PA, USA

Animal models of addictive disorders have had some remarkable successes in discovering medications that were subsequently found to work in the clinic. In the 1970s the endogenous opioid system was not known to be involved in alcoholism. Subsequently, studies in non-human primates and rodents demonstrated that alcohol activated endogenous opioids and that blocking opiate receptors reduced alcohol drinking in a variety of animal models. Based on these results, randomized clinical trials in alcoholic patients were conducted with positive results and FDA approval. Studies in mouse knockouts and rat relapse models have further delineated mechanisms by which blocking endogenous opioids can have a therapeutic benefit. The alcoholism/endogenous opioid story has been consistent across human laboratory studies, epidemiological studies, and pharmacogenetic studies. Similarly, acamprosate for the treatment of alcoholism was initially discovered in rodent models of alcohol drinking. Cocaine addiction is also yielding to medications that have worked first in animal models. GABAergic drugs suppress cocaine self-administration behavior and recent evidence shows that they also prevent relapse among human subjects. A major problem in translating data from animal models to the human population is that animals are usually considered to produce a consistent response and variability is not often pursued. Humans, in contrast, have great genetic heterogeneity and consequently a variable response to drugs. For example, some alcoholics have a large endorphinergic response to alcohol accompanied by reports of euphoria while others have minimal or no endorphinergic response and do not report euphoria. Recent genetic studies are shedding light on these differences and may enable us to select patients for specific medications according to genetic criteria. Mu opiate receptors coded by the Asp40 polymorphism have been found to have three times the binding affinity for beta-endorphin as measured by in vitro binding compared to receptors from the Asn 40, another common variant. Human subjects with the Asp40 variant have reported more euphoria from alcohol and population studies have found an increased risk of both alcoholism and opiate addiction. In a retrospective analysis of alcoholism treatment trials, patients with the Asp 40 allele responded poorly to placebo, but when randomized to an opiate receptor antagonist, the non-relapse rate was over 80%. Thus knowledge of the alcoholism-endogenous opioid interaction has developed through a bi-directional interplay beginning with animal models and continuing through human laboratory, clinical trials and more complex animal models of relapse.

Wednesday, December 14, 2005

Panel Session
The Warrior/Worrier Paradox: Varying Allelic Effects and Counter Balancing Selection of Phenotypes

The 5HTTLPR Low Versus High Activity Alleles are Associated with High Versus Low Aggressive Behaviour, But Not Suicidality
James L Kennedy*, Paul D Arnold, Vincenzo DeLuca, Peggy A Richter, Sahar Ehtesham and Joe H Beitchman

Neuroscience Dept, Centre for Addiction and Mental Health, Univ of Toronto, Toronto, ON, Canada

The current state of psychiatric genetics is characterized by many small gene effects that have varying degrees of replication. It may be useful to look at a given functional gene variant across a range of phenotypes in order to understand better the gene effect on behavior. The serotonin transporter gene is relevant to the spectrum of worrier to warrior in view of SSRI efficacy in disorders lying in this spectrum,

and the apparent effect of the 5HTTLPR in determining 25% of the variance in amygdala activation (Hariri et al, 2004). In OCD, we have shown that the LA allele is associated with the disorder ($p = 0.0018$) when the G>A variant of the repeat is considered. The LG allele becomes a functional short allele (Goldman et al, 2004). Our recent data shows the LA allele to be associated in particular with the need for symmetry / ordering subtype of OCD ($p = 0.02$). We also have a unique sample of children (N=90) with pervasive and persistent high aggression, as measured by CBCL scores and both parent and teacher rating scales. Genotyping for the 5HTTLPR revealed a significant positive association between aggressive behavior and the low expressing promoter genotypes (S/S, LG/S, LG/LG) (n=77, p=0.049, OR=2.37, CI=1.10-5.08), as well as with the S/S genotype alone (p=0.047, OR=2.65, CI=1.12-6.28). We have also found an association with the MAOA gene in this aggression sample, but cannot as yet assess 5HTTLPR x MAOA interactions due to small sample size. Another phenotype of interest is suicide attempt, that could be speculatively described as the worrier who becomes a warrior unto himself. We have suicide attempt measures in both schizophrenia (N=150) and bipolar samples (N=240), and we do not find an association with the 5HTTLPR polymorphism. Overall, our OCD sample (worrier) shows association with the high expressing LA allele of the 5HTTLPR, and our aggression (warrior) sample is associated with the low expressing S or LG allele.

CNS Serotonergic Functioning, Alcohol, Genotype X by Rearing Interactions and Violence Using a Nonhuman Primate Model

James D Higley*, Christina S Barr and Stephen J Suomi

Section of Primate Models of Psychopathology, LCTS, NIH, NIAAA Intramural, Poolesville, MD, USA

A relationship between alcohol intake and aggressive behavior has been demonstrated in a variety of studies. Primary among the factors that contribute to aggression during intoxication are a previous history of violence and impaired CNS serotonin functioning. In addition, aggressive behavior, a low level of response to alcohol (LOR), and alcoholism have been attributed to impaired CNS serotonergic function. When adolescent rhesus macaques received an intravenous dose of ethanol (2.2g/kg) and were provoked by an investigator making eye contact, sensitivity to alcohol, alcohol-induced increases in CSF MHPG, and pre-alcohol CSF levels of 5HIAA were associated independently with aggression during intoxication. Studies have also demonstrated gene-environment interactions between 5HTTLPR variation and early environmental deficits on violent behavior. As in humans, there is a serotonin transporter gene promoter biallelic length (short s and long L) polymorphism in rhesus macaques that produces similar decreases in transcriptional efficiency. Macaques with histories of parental deprivation (peer reared PR subjects) have been shown to exhibit impulsive aggression and incompetent social behavior. Not all PR subjects show such deficits. Studies show that subjects with the less efficient 5HTTLPR s allele are particularly sensitive to the effects of parental deprivation, showing high rates of violence as adolescents and adults, whereas the mother reared controls (MR) are undifferentiated by genotype. New studies show that these differences are even more exaggerated when the adolescents are exposed to alcohol, with the PR subjects possessing the s allele showing high rates of aggression. MR subjects were again not differentiated by genotype. More recently we have investigated the developmental history of this phenomenon. Rough and tumble play is widely held to be a socializing influence on aggression in monkeys. We found that play in PR infants with the short allele is infrequent, but in PR subjects with the L allele high rates of play are the norm. As juveniles, low rates of play in the PR subjects and the s allele were predictive of frequent violent behavior. As in the earlier studies, there was gene by environment interaction with the MR subjects not differentiated by genotype in rates of play or violence. Such studies illustrate the emerging evidence that the phenotype is not simply an additive effect of genes and environment, but that genotypic effect on the phenotypic outcome is instead dependent on the environment and that risk for violence and aggression are a complex interplay between impoverished experiences and deleterious genotypes.

Variation in Allelic Expression of Genes Associated with Aggression

Larry J Siever*, Janine D Flory, Ke Xu, Michelle Romero, Antonia S New, Francesca Ducci and David Goldman

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

Background: Candidate genes involving the serotonin and dopamine systems, trophic factors such as BDNF, and steroids have been implicated in the regulation of aggression in clinical and/or animal model studies. Aggression is a phenotype not necessarily associated with a single disorder but is integral to the diagnosis of intermittent explosive disorder revised (IED-R) and is common in the Cluster B personality disorders. Specific alleles related to aggression may vary in their phenotypic effects depending on their context, e.g., the disorder involved, interaction with other relative genotypes, and environmental interactions. For example, the Val^{158}Met polymorphism of Catecholamine-O-Methyltransferase (COMT) may express itself in externalizing, aggressive or "warrior behaviors" as well as anxiety-based, internalizing "worrier" behaviors depending on both allele and psychiatric disorder (e.g., schizophrenia vs. substance abuse). In another non-coding SNP of COMT, RS165599 (located near the three prime UTR), the "G" allele is associated with lower COMT expression.
Methods: 412 subjects were characterized clinically and genotyped with respect to the Val^{158}Met and RS165999 alleles of COMT as well as polymorphisms of the $5HT_{2a}$, $5HT_{1b}$, $5HT_{1a}$, $5HT_{2c}$ receptors. Subsets of these patients received the Buss Durkee Hostility Inventory (BDHI) and performed the Point Subtraction Aggression Paradigm (PSAP) a laboratory test of aggression, the Continuous Performance Test (CPT) and as well as other laboratory tasks measuring impulsivity, imaging studies of the serotonin transporter and 5HT2a receptor and responses to serotonergic probes.
Results: In this sample of personality disorder patients, the Val allele tended to associated with higher aggression in 112 non-Hispanic Caucasians. The "G" allele of the RS165999 polymorphism of COMT in this sample was significantly associated with higher scores on BDHI irritability/assault subscales ($\beta = .20$, $t = 2.1$, $p = 0.04$) as well as higher assault subscale scores ($\beta = .2$, $t = 2.1$, $p = .04$). However, more people with AA genotype had attempted suicide relative to the GG genotype (X^2 score = 5.4, $p = .07$) so that the relationship between externally oriented and internally oriented aggression appeared to differ with regard to their association to this allele. While there were no specific associations in 267 subjects with regard to the $5HT_{2a}$, $5HT_{1a}$ and $5HT_{1b}$ polymorphisms and aggression, subjects meeting criteria for IED-R tended to be more likely to have the low activity $5HT_{2c}$ serine allele ($r = .78$, $p = < .1$), consistent with reduced physiologic responses to 5HT2c agonists. The "A" allele of the $5HT_{2a}$ polymorphism was found to be significantly associated with aggressive responses on the PSAP ($r = -.39$, $p < .05$).
Discussion: These findings will be discussed in terms of the relationship of these genotypes to imaging studies of serotonergic receptors and transporter, responses to probes, and prefrontal activity to explore how alterations in circuitry may interact with specific genotypes to yield varying phenotypic outcomes with respect to internally and externally directed aggression.

Novel Functional Variation in the Serotonin Transporter and Tryptophan Hydroxylase Genes and Linkage to Complex Behavioral Phenotypes

David Goldman*, Zhifeng Zhou, James Kennedy, Matti Virkkunen, Robert Robin, Alec Roy, Dennis Murphy and Xianzhang Hu

Lab of Neurogenetics, NIH/NIAAA, Rockville, MD, USA

Background: Synthesis and reuptake are regulated components of serotonin function. Effects of genetic variation in these processes are

likely to be pleiotropic because a multitude of behaviors are modulated by serotonin, including externalizing behaviors, internalizing behaviors and obsessive compulsive disorder. The genotype effects are presaged by behavioral effects of inhibition of serotonin synthesis and reuptake as well as behavior of mice lacking the serotonin transporter. Identification of a functional promoter polymorphism (HTTLPR) led to replicated linkage of the low expression S allele to anxiety, but the effect size was small. The effect of HTTLPR on dysphoria is enhanced by stress. Effects on brain intermediate phenotypes such as amygdala activity and structure are also larger. Recently, we reported that a rare gain-of-function allele, Val425, was linked to severe treatment resistant OCD. As detailed here, a third common functional allele exists in HTTLPR itself. In line with the role of Val425 in OCD, gain of function HTTLPR genotypes were also linked to OCD, in two datasets. Further, identification of TPH2 as rate-limiting for brain serotonin synthesis enabled us to link TPH2 haplotype to dysphoric behaviors in multiple datasets.
Methods: HTT mRNA levels were correlated with the triallelically genotyped HTTLPR in 62 lymphoblastoid cell lines representing six genotypes. Altered AP2 binding was shown by EMSA and supershift assay. Linkage of HTTLPR to OCD was performed in 169 cases and 253 controls from NIMH, and in 175 cases, including 86 informative parent-child trios from the Clarke Institute. Fifteen TPH2 SNPs spanning the 106 kb region were genotyped (not including His441, a functional allele not observed in 779 individuals, including 403 with major depression), haplotype linkage was performed to anxiety/dysphoria phenotypes in the following case/control datasets: U.S. Caucasians (85 cases, 61 controls), Finnish Caucasians (317 cases, 196 controls), African Americans (342 cases, 315 controls) and Southwest Indians (420 cases, 62 controls). Linkage to CSF 5HIAA was performed in 94 Finns.
Results: The HTTLPR locus is functionally triallelic, with common alleles LA and LG produced by an A>G SNP within the first of two extra 22 bp repeats that differentiate the 16-repeat L allele from the S allele. The S and LG alleles are equivalently low in expression. All alleles are functionally codominant. Linkage studies in NIMH case/controls and Clarke Institute parent-child trios (using TDT) revealed an excess of LA alleles and LALA homozygous genotypes in OCD. Two common, opposite configuration TPH2 haplotypes of apparently ancient origin are present, and are indeed found across populations worldwide. One TPH2 haplotype was associated with low CSF 5HIAA levels and linked to increased rates of suicidality, anxiety and depression.
Discussion: Identification of functional alleles has increased power to resolve effects of genes in behavior as shown by linkage of gain-of-function serotonin transporter HTTLPR LA and rare Val425 missense variants to OCD. Previously, the loss-of-function S allele was linked to dysphoria and intermediate phenotypes relevant to internalizing disorders. As illustrated by TPH2, a haplotype predictive of an intermediate phenotype for serotonin function (low CSF 5HIAA) may also predict anxiety/dysphoria. However, functional TPH2 loci remain unknown.

Panel Session
Subregions in the Medial Temporal Lobe: Implications for Normal and Disordered Cognition

The Hippocampus and Episodic Memory: Cognitive and Neural Mechanisms
Howard Eichenbaum*

Center for Memory and Brain, Boston University, Boston, MA, USA

Background: In humans, the hippocampus plays a critical role in episodic memory. Episodic memory is characterized by threshold of retrieval for events in the context of experience, by memory for the flow of events in unique experiences, and by our ability to network related experiences and use these networks to make inferences in novel situations. Animal models that emphasize these features of memory can be used to explore the neurobiological bases of normal and disordered episodic memory.
Methods: We use a combination of behavioral and neurophysiological techniques. Exploiting rats' superb ability to use olfactory cues in their natural foraging behavior, we have measured the dynamics of memory retrieval, memory for the order of events, and the ability to link memories and make novel inferences from memory. By recording extracellular spike patterns of hippocampal neurons in animals performing spatial and olfactory memory tasks, we have also examined whether hippocampal neuronal networks encode items in the context in which they are experienced, represent the order of events in unique experiences, and encode common events in multiple experiences that could serve to link memories.
Results: Signal detection analyses have distinguished the retrieval dynamics of episodic recollection and familiarity in humans. Using the same analytic techniques, we found that olfactory recognition in rats also reflects a combination of recollection and familiarity. Selective lesions of the hippocampus abolished recollection while sparing familiarity. We also tested whether rats could remember the order of a once-presented sequence of odors, as reflected in the ability to select the earlier of an arbitrarily selected pair from the sequence. Normal rats demonstrated memory for the order of events in a unique experience. Rats with selective hippocampal damage were severely impaired even though they could distinguish presented odors from other odors that were not presented. In addition, we trained rats on paired associate and discrimination problems where the pairs of elements overlapped (e.g. A-B, and B-C), then tested whether these associations were linked to support inferences between indirectly related elements (A-C). Normal rats performed well in making inferences, indicating the development of the memory networks. Rats with hippocampal damage learned the separately trained associations but could not make the inferences, indicating the absence of linkage. In recording studies, we observed a high proportion of hippocampal neurons that encode odors in the context that they were experienced. Furthermore, these neuronal networks distinguish experiences that are defined by unique sequences of events. At the same time, some elements of these networks also encode the common events that link related experiences.
Discussion: These observations suggest that the hippocampus is essential to cognitive functions in animals that characterize the fundamental features of episodic memory in humans. Our findings suggest that episodic memory is based on three fundamental properties of hippocampal information processing: (1) representation of events in the context in which they are experienced, (2) representation of event sequences that compose unique experiences, and (3) linking of related memories by common events, supporting ability to make novel inferences from memory. Further studies on these properties of hippocampal function and the neural coding mechanisms that support them may help elucidate the role of medial temporal areas in psychiatric disorders.

Binding Processes and Structures in Working Memory and Long-Term Memory: Interaction of Hippocampal and Neocortical Areas
Jaap Murre*

Department of Psychology, University of Amsterdam, Amsterdam, Netherlands

Models of long-term and working memory assume various forms of binding processes. Long-term memory consolidation involves a process whereby memory representations are first bound by the hippocampus and certain surrounding areas. Then, a consolidation process is assumed whereby the binding role is transferred from the hippocampus to neocortical sites, a movement from hippocampal-cortical to cortico-cortical connectivity. Many models of working

memory assume that high levels of neural synchrony or simultaneous firing rate represent its memory contents. Short-term binding is thus accomplished through firing rates and temporal correlations of firing that emerge from the complex interplay of existing connections and from the effects of the recent activation history (from 'thinking', planning, perception, setting of motor movements, etc.). In a few seconds this content can in principle be transferred to long-term memory, specifically to the hippocampus, via Hebbian plasticity. I will present a new binding perspective building, on two connectionist models: a model of binding in working memory and a model of trace binding in long-term memory consolidation. The latter model was recently extended and applied to persistent episodic memory impairments in schizophrenia, focussing on the effects of reduced parahippocampal connectivity (from perirhinal and parahippocampal cortex to entorhinal cortex and from entorhinal cortex to hippocampus). In the model, parahippocampal processing subserves integration of different cortical inputs to the hippocampus and feature extraction during recall. Reduced connectivity in this area resulted in a pattern of deficits that closely mimicked the impairments in schizophrenia, including a mild recognition impairment and a more severe impairment in free recall. The results can be interpreted as a neuroanatomically induced binding deficit in schizophrenia.

Hippocampal Contribution to the Novel Use of Relational Information in Declarative Memory

John Gabrieli* and Alison R Preston

Brain and Cognitive Sciences, MIT, Cambridge, MA, USA

Background: The medial temporal lobes (MTL) are essential for declarative memory, and are comprised of multiple structures, including the hippocampus, entorhinal, perirhinal, and parahippocampal cortices. Animal research from Eichenbaum and colleagues has indicated that the hippocampus specifically underlies the flexible expression of declarative memory. The goal of this study was to examine, via functional magnetic resonance imaging (fMRI), whether the hippocampus in the human brain has a specialized role in the flexible expression of declarative memory.
Methods: Ten healthy young adults participated. Prior to scanning, participants learned to associate specific faces (stimuli A) with specific houses (stimuli B). Then, participants learned to associate another set of faces (stimuli C) with the same specific houses (stimuli B). Thus, each house was associated with two different faces in the two learning phases, and specific pairs of A and C faces could be flexibly related to one another through their overlapping associations with the same house (B). Finally, participants learned to associate pairs of novel faces (stimuli D and E) as a baseline. During the scanned retrieval phase, participants made two-alternative forced-choice judgments on learned face-house pairs (AB and BC), learned face-face pairs (DE), and, critically, A and C faces.
Results: Memory judgments for recognition of related face-face pairs (AC) yielded greater activation bilaterally in the hippocampus than any other condition (AB, BC, or DE). A more posterior hippocampal region exhibited activation for all four retrieval conditions. Bilateral anterior parahippocampal activations were greater for the AB, BC, and AC conditions than the DE condition, and posterior parahippocampal activations were greater for the learned face-house pairs (AB, BC) than the face-face pairs (AC, DE).
Discussion: Multiple MTL regions were activated during recognition, but only the hippocampus yielded activation that was specific to the flexible use of elements of previous experience, i.e., for the recognition of information not explicitly learned. These findings are consistent with animal studies about the specific role of the hippocampus in the flexible expression of declarative memories, and also go beyond prior animal and patient studies by showing that activation associated with the flexible expression of declarative memory may be unique to the hippocampus because no other MTL region demonstrated similar activation. Thus, the human hippocampus may contribute to the encoding and retrieval of associations that allow for the flexible use of experience in novel situations

Hippocampal Dysfunction in Psychiatric Disorders

Stephan Heckers*

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

Background: Hippocampal dysfunction has been implicated in the pathogenesis of several psychiatric disorders, including schizophrenia, depression, PTSD, and anxiety disorders. The presentation will review converging evidence from neuroimaging and postmortem studies that support a model of hippocampal dysfunction in psychiatric disorders.
Methods: Findings from postmortem and neuroimaging studies are reviewed for evidence of regionally specific deficits of hippocampal function and structure in psychiatric disorders.
Results: Postmortem studies have demonstrated that the well-established finding of decreased hippocampal volume in schizophrenia and depression is not due to a generalized loss of pyramidal cells. Studies of protein and gene expression have revealed subtle and regionally specific deficits of subsets of hippocampal neurons. Structural imaging studies have demonstrated a temporal profile of hippocampal volume change throughout the course of schizophrenia and depression. In addition, several studies have reported regionally specific hippocampal volume deficits in schizophrenia. Functional imaging studies have linked impaired hippocampal recruitment in schizophrenia, depression, and PTSD to impaired memory encoding and retrieval.
Discussion: While neuroimaging and postmortem studies have provided compelling evidence for abnormalities of hippocampal structure and function in schizophrenia, depression, PTSD, and anxiety disorders, the details remain unclear. This is in contrast to disorders such as dementia and epilepsy, for which comprehensive models of hippocampal and parahippocampal cortex dysfunction have been developed. Further studies are needed to properly constrain hippocampal-based neural network models of psychosis, mood disorders, and anxiety disorders.

Panel Session
Drug Development: GSK-3: A Novel Target for Improved Therapeutics for Severe Neuropsychiatric Diseases

Involvement of an Akt/β-arrrestin2/PP2A Signaling Complex in the Actions of Dopamine

Marc G Caron* and Martin Beaulieu

Cell Biology, Duke University Medical Center, Durham, NC, USA

Background: Neurotransmitters interact with ligand-gated ion channel receptors to mediate fast synaptic transmission and metabotropic receptors of the G protein-coupled receptor (GPCR) class for slow synaptic transmission. These seven transmembrane domain GPCRs engage different signal transduction pathways by activating heterotrimeric G-proteins. In the brain dopamine (DA) through prototypic GPCRs regulates locomotion, reward and affect and most of these actions have been associated with cAMP or Ca^{2}-dependent signaling events. Dysregulation of dopaminergic neurotransmission is associated with multiple neurological and psychiatric conditions such as Parkinson's and Huntington disease, attention deficit hyperactivity disorder, mood disorders and schizophrenia. Recent studies in genetically engineered mice have suggested that not all physiological effects of DA are mediated through canonical GPCR signaling pathways (Waddington et al 2003). Moreover, evidence from the GPCR field has indicated that at least in cellular systems, GPCRs can signal not only through second messenger pathways but also through

kinase cascades such as MAPkinases. Interestingly, activation of these cascades can occur in a G protein-independent manner that involves components of the GPCR desensitization machinery (i.e. receptor kinases and arrestins) (Luttrell et al 2001; Lefkowitz and Shenoy 2005). Previously, we have demonstrated that D2-class DA receptors activation leads to dephosphorylation/inactivation of the serine/threonine kinase Akt and that mice lacking the β-arrestin 2 gene have a diminished response to DA stimulation (Beaulieu et al 2004; Gainetdinov et al 2004).
Methods: We have used in vivo pharmaco-genetic approaches in mice to examine the molecular mechanisms underlying the DA-mediated modulation of the Akt signaling pathway.
Results: Inhibition of Akt phosphorylation by DA was observed in the striatum of mice that display persistently elevated levels of extracellular DA due to a lack of the DA transporter (DATKO) mice or in normal mice (WT) treated with direct or indirect DA agonists. Dephosphorylation of Akt in response to DA leads to a reduction in kinase activity and a concomitant dephosphorylation/activation of its substrates GSK3α and GSK3β. Pharmacological activation of Akt or inhibition of GSK3α/β results in reduction of DA-associated locomotor activity in both DAT-KO mice and WT mice treated with amphetamine. Moreover, mice lacking one allele of the GSK3β gene show markedly reduced locomotor responses to amphetamine thus supporting a role for the Akt/GSK3 signaling pathway in the expression of DA-associated behaviors. Interestingly, the time course of inactivation of Akt following administration of DA agonists was more sustained than responses mediated through canonical G-protein/cAMP/PKA pathways and these effects were independent of increases in cAMP or Ca^{2}. Thus using biochemical approaches we showed that D2-class receptor-mediated Akt regulation involves the formation of signaling complexes containing β-arrestin 2, Akt and phosphatase 2A (PP2A). β-arrestin 2 deficiency in mice also results in loss of Akt regulation by dopamine and disruption of the Akt/PP2A/β-arrestin2 interaction.
Discussion: These results demonstrate that apart from its classical function in receptor desensitization, β-arrestin 2 also acts as a signaling intermediate through a kinase/phosphatase scaffold and may be important in sustained dopaminergic synaptic transmission. Thus, Akt, GSK3 and β-arrestin 2 may represent legitimate pharmacological targets for dopamine related psychiatric disorders.

Targeting Glycogen Synthase Kinase-3 as a Treatment for Bipolar Disorder

Todd D Gould*, Haim Einat, Alyssa M Picchini, Kelley C ODonnell, Robert J Schloesser and Husseini K Manji

Lab Mol Pathophysiology, NIMH, Bethesda, MD, USA

Initial interest in glycogen synthase kinase-3 (GSK-3) as a target for the treatment of mood disorders arose from the finding that lithium directly inhibited the enzyme. GSK-3 is implicated in either the direct or downstream mechanism of action of many other mood stabilizing and antidepressant medications currently in use. The transcription factor β-catenin is a primary protein target of GSK-3. Normally active GSK-3 phosphorylates β-catenin leading to its degradation. Inhibition of GSK-3, for example by lithium, increases β-catenin levels. We utilized validated rodent behavioral models responsive to lithium (the forced swim test (FST) and decreased activity following administration of amphetamine) and pharmacological and genetic approaches to study the relevance of GSK-3 and β-catenin to the behavioral effects of lithium. AR-A014418 (AR) is a selective ATP competitive GSK-3 inhibitor that readily crosses the BBB and increases β-catenin. Administration of AR reduced hyperactivity following amphetamine administration in both rats and mice. Subacute intraperitoneal injections of AR reduced immobility time (increased activity) in the FST compared to vehicle treated rats. We additionally utilized transgenic mice that over express β-catenin in the brain. Similar to the effects with AR and lithium, the transgenic mice demonstrated decreased immobility time in the FST, as well as significantly decreased activity following both acute and repeated exposure to amphetamine. The specificity of these results is supported by the absence of changes in learning and memory paradigms, open field activity, and anxiety measures among many behavioral tasks performed. These data support the hypothesis that lithium may exert its behavioral (and perhaps therapeutic) effects through inhibition of GSK-3 and subsequent modulation of β-catenin mediated signaling events.

GSK-3 as a Target of Lithium Action in Bipolar Disorder and Alzheimer's Disease

W. Timothy O'Brien, Michael O'Donnell, Christopher J Phiel, Christina A Wilson, Virginia M Lee, James R Woodgett, Silvia Maretto, Stefano Piccolo and Peter S Klein*

Medicine (Heme-Onc), University of Pennsylvania School of Medicine, Philadelphia, PA, USA

Background: Lithium is widely used to treat bipolar disorder, but its mechanism of action in this disorder is unknown. Lithium directly inhibits glycogen synthase kinase-3 (GSK-3), a critical regulator of multiple signal transduction pathways. However, other targets of lithium have also been identified, and it is not known which of these putative targets is responsible for the behavioral effects of lithium in vivo. A robust animal model for the effects of lithium would greatly facilitate the characterization of lithium action. The observation that lithium is a GSK-3 inhibitor has led to recent findings from this laboratory and from others that suggest a novel potential role for lithium in treating Alzheimer's disease (AD). AD is associated with increased production and aggregation of amyloid-beta (A-beta) peptides, which are generated from the amyloid precursor protein (APP), and with increased phosphorylation of tau protein, a primary constituent of neurofibrillary tangles (NFTs). GSK-3 phosphorylates tau protein at sites associated with NFT formation and also associates with presenilin, an essential component of the gamma-secretase complex that cleaves APP.
Methods/Results: We have identified a set of simple behaviors in mice that are robustly affected by chronic lithium and show that these behaviors are similarly affected in mice lacking one copy of the Gsk-3beta gene. In addition, lithium inhibition of GSK-3 induces Wnt-dependent transcription within the amygdala, hippocampus, and hypothalamus, and this effect is also observed with valproic acid, another widely prescribed mood-stabilizing drug. Lithium salts, as well as other GSK-3 inhibitors, markedly reduce production of A-beta peptides in cultured neurons and in vivo in a mouse model of AD. RNA interference studies indicate that GSK-3alpha is a critical target of lithium in this setting.
Conclusion: These observations, together with recent work from other laboratories showing that alternative GSK-3 inhibitors mimic the behavioral effects of lithium and Gsk-3beta loss of function, support a central role for GSK-3beta in mediating behavioral responses to lithium. These observations support the hypothesis that GSK-3 is a direct target of lithium action in the treatment of bipolar disorder, although further work is necessary to confirm this hypothesis. As GSK-3 is required for generation of A-beta peptides and also phosphorylates tau protein, inhibition of GSK-3 with lithium or other inhibitors offers a novel potential approach to reduce formation of both amyloid plaques and neurofibrillary tangles, two pathological hallmarks of Alzheimer's disease.

Genetic Analysis of GSK-3α and β in Conditional Knockout Mice and Stem Cells

Jim Woodgett*, Bradley Doble, Satish Patel and Lisa Kockeritz

Ontario Cancer Institute, Toronto, ON, Canada

Glycogen synthase kinase-3 (GSK-3) has been directly implicated in several human conditions including Alzheimer's disease, bipolar disorder and type-2 diabetes. Lithium is a physiologic inhibitor of GSK-3

both in vitro and in vivo and at least part of the behavioral response to lithium is mediated through this enzyme. To understand the contribution of this protein kinase to these human disorders, we have derived conventional and conditional knockout mice for both GSK-3α and β allowing the selective deletion of these protein kinases in whole animals and specific tissues. Using embryonic stem cells that have been engineered to lack both alleles of both genes, we have investigated the roles of the kinase in differentiation and responses to specific agonists including the Wnt and mitogenic pathways. ES cells lacking both alleles exhibit a profound block to differentiation, maintaining their pluripotency in the absence of any external signals or conditions. Conditional mice lacking either or both alleles of GSK-3 in specific tissues are being exploited to determine the effect of selective loss of GSK-3 activity in particular organs as a model for predicting therapeutic consequences of small molecule inhibitors. The role of this protein kinase in modulating neuronal cell differentiation and functions will be discussed as well as its potential as a therapeutic target.

Panel Session
Validation of Screening Models for Medications Development to Alcoholism

High Throughput Pharmacological Screening for Lead Compounds at Molecular Targets of Relevance to Alcohol Dependence

John M Littleton*

Kentucky Tobacco Research & Development Center, University of Kentucky, Lexington, KY, USA

Effective medications for drug and alcohol dependence are greatly needed, but progress has been slow. One problem is that, although several molecular targets can be identified, many of these present specific difficulties. For example it is quite clear that nicotinic receptors for acetylcholine (nAChRs) are targets for smoking cessation medications, and the involvement of nAChRs in "reinforcing" pathways means that they are important for other types of dependence also. The difficulty here is the complexity of the subtypes of nAChRs, and their many roles in physiological function. Drugs that targeted different nAChR subtypes specifically might be valuable for different types of dependence, including alcoholism, and might have fewer of the side effects and abuse potential that reduce the value of agents like nicotine. Similarly, there is little doubt that the glutamatergic transmitter system in brain also plays an important role in reinforcement, and that glutamate receptors are therefore important molecular targets. In addition, the role of glutamate/NMDA receptors (NMDARs) in conditioning is well known, suggesting that this receptor is a target for medications aimed at reducing relapse caused by conditioned stimuli ('cues') previously associated with drug-taking (for example acamprosate in alcohol dependence). Once again, abuse potential and side effects related to the physiological role of this receptor (e.g. in learning and memory) make the NMDAR a difficult molecular therapeutic target. In this case the problem is less one of sub-type selectivity and more of targeting mechanisms that prevent NMDAR "overactivation" but which preserve normal function. The first stage of drug discovery is to develop high throughput pharmacological screens (HTPS) for the molecular activity required. While it is easy to devise screens that measure "activity" at a receptor, it is more difficult to develop screens that identify compounds with more subtle selectivity, as is required here. We have attempted to do this using two or more HTPS sequentially, so that differences in activity at closely related binding sites are compared. Compounds with the selectivity we are seeking give a specific "signature" of activity in these screens. This differential/ sequential screening (DSS) has been applied to the identification of synthetic compounds and natural products that bind to nAChRs or to NMDARs with the kind of selectivity we require in potential medications for drug dependence. For NMDARs we have used radioligand binding of [3H]MK801 in the presence and absence of spermidine and have identified synthetic iminoguanidines that appear to inhibit the receptor only when it is co-activated by polyamines. For nAChRs we have used [3H]epibatidine, [3H]cytisine and [3H]methyl-lycaconitine sequentially and have identified several native plant extracts that contain compounds with receptor subtype selectivity that differs from that of known compounds. These may have been evolved as protection against insect predation, because some of them bind with very high affinity to insect nAChRs. If so, their affinity for different subtypes of mammalian nAChRs is "accidental" but potentially valuable. These synthetic compounds and plant extracts are now being tested in more complex screens and models of drug dependence to establish whether they are of any potential value as medications, lead compounds, or research tools. Supported by NIAAA (AA12600).

Development of Animal Models of Excessive Drinking, Protracted Abstinence, and Cue-Induced Relapse for Medications Development

George F Koob*

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

Background: Animal models for various stages of the alcohol addiction cycle have been developed and are providing a rational basis for medication development for treatment of alcoholism. Animal models of excessive drinking include selective breeding of strains for alcohol preference, drinking during withdrawal, and drinking during protracted abstinence. Animal models of relapse include reinstatement of alcohol taking behavior following stress, conditioned cues, and drug administration.
Methods: Animal models that comprise core elements of three major components of the human alcoholism syndrome—baseline drinking, excessive drinking during dependence and during protracted abstinence, alcohol cue-induced reinstatement of drinking, and stress-induced reinstatement—will be presented. The face and construct validity of these models will be explored in the context of both established and potential medications for the treatment of alcoholism.
Results: Results with two proven medications, naltrexone and acamprosate, show efficacy for different animal models. Naltrexone blocks baseline drinking, the increased drinking associated with dependence and protracted abstinence, and cue-induced reinstatement. Acamprosate does not affect baseline drinking but blocks the increased drinking associated with dependence, protracted abstinence, and cue-induced reinstatement. For example, corticotropin-releasing factor antagonists do not affect baseline drinking but block the increased drinking associated with dependence, protracted abstinence, and stress-induced (but not cue-induced) reinstatement. Novel medications that target γ-aminobutyric acid and glutamate systems will be presented in these same models.
Discussion: These results suggest that different medications have unique profiles of effects on animal models with face validity for different aspects of the alcohol addiction cycle. Such profiles will provide a 'Rosetta stone' for interpretation of the clinical treatment potential of compounds directed at novel targets and at the same time provide construct validity for the animal models. Such an approach provides a heuristic framework for medication development as it relates to a 'Go/No Go' decision of lead compounds.

Application of Functional Genomics to Animal Models of Alcoholism for Target Discovery and Validation

Markus Heilig*, Anita Hansson, Wolfgang Sommer, Anton Terasmaa, Karl Bjork and Roberto Rimondini

LCTS, NIAAA/NIH, Bethesda, MD, USA

Background: Currently available medications provide proof of principle for pharmacological treatment of alcoholism, but leave ample

room for improvement, prompting a need to identify novel treatment targets. Animal models have demonstrated their utility for the development of clinically effective pharmacological treatments in alcoholism, but presently available compounds have been developed based on a priori knowledge of the role of opioid, amino acid and serotonergic transmission, respectively. To allow for discovery of novel targets, we have applied unbiased microarray analysis to animal models.
Methods: Two animal models have been used for Affymetrix-based gene expression analysis to identify novel candidate treatment targets. To model neuroadaptive processes underlying progression to dependence, we established and subjected to microarray analysis a model of dependence induced drinking, based on prolonged exposure of the brain to repeated cycles of intoxication and withdrawal using inhalation of EtOH vapor. This leads to a marked and long-lasting increase in voluntary ethanol intake. Dependence-induced intake is antagonized by acamprosate, a compound clinically effective in human alcoholism. To model genetic susceptibility, we have analyzed expression profiles in genetically selected alcohol-preferring AA rats with the alcohol-avoiding counterpart ANA line. Hits from the gene expression screens have been confirmed for differential expression using in situ hybridization, and validated for functional role in vivo.
Results: 1. Support for a validity of the Affymetrix approach has been obtain through analysis of regional gene expression, wherein this method correctly identified several genes known to be preferentially expressed within the Nc. Accumbens, e.g. dopamine D1 and D2, adenosine A2a receptors, and substance P. Among pathways indicated by differential expression in relation to alcohol preference, two that are of particular interest have been followed up in depth and validated functionally: 2. The cannabinoid system was early flagged in the dependence induced drinking model by differential expression of the CB1 receptor. Following up on this observation, we found decreased expression and activity of the endocannabinoid degrading enzyme, FAAH, in the alcohol preferring AA rats. This leads to increased endocannabinoid drive. A functional role for this is supported by the finding that both systemic and prefrontocortical injections of the CB1 antagonist rimonabant markedly suppressed EtOH self-administration in AA rats, while local administration of an FAAH inhibitor into the PFC in normal rats led to an increase of self-administration. 3. A differential expression of beta-arrestin 2 (BARR2) was found in several brain regions of the AA rat, providing a possible mechanism underlying the increased responsiveness in dopamine and opiate systems observed in this line. Expression differences between the lines have been linked to haplotype segregation at this locus. A functional role of the BARR2 gene has been obtained through data showing markedly lowered EtOH intake in BARR2 null-mutants, and altered signal transduction in response to EtOH challenge.
Discussion: Application of GeneChip technology to animal models of alcoholism is able to generate novel candidate targets. Extensive confirmatory expression analysis using targeted methods, and in vivo functional validation studies are required to move from gene lists to targets for development.

Development and Validation of Human Laboratory Models for the Prediction of Medication Efficacy in Clinical Trials

Barbara J Mason*

The Scripps Research Institute, La Jolla, CA, USA

Background: Alcoholism and addiction have been conceptualized as consisting of 3 repeating phases: 1.) binge/intoxification, 2.) acute withdrawal and 3.) protracted abstinence. Opioid antagonists have shown efficacy for reducing severity in the binge/intoxification phase in clinical trials, as well as in animal and human lab studies involving alcohol administration in both dependent and nondependent subjects. The protracted abstinence phase involves a state of heightened vulnerability to relapse following acute withdrawal that has been linked to prolonged activation of the brain stress arousal systems, and a heightened responsivity to internal cues, e.g. negative affect, and external cues, e.g. the sight and smell of alcohol, that are precipitants of relapse in dependent but not nondependent subjects. We developed a human laboratory model of risk factors for relapse in protracted abstinence to provide an early phase screen for potential medications for protracted abstinence, as an alternative to older human laboratory methods involving alcohol administration.
Methods: The human lab model of protracted abstinence involves randomly assigning non treatment-seeking paid volunteers with alcohol dependence to one week of double-blind, placebo-controlled study medication. Standardized assessments of drinking and key aspects of protracted abstinence, i.e. mood, sleep, and urge to drink, are collected at the end of the medication phase. Subjects' subjective and physiological responsivity to pairs of affective (negative, neutral, positive) and beverage (alcohol, water) cues are measured to determine if study drug modifies responsivity to these laboratory analogues of risk factors for relapse. Additionally, standardized measures of safety, compliance and abuse potential are collected. It is hypothesized that medications from different neuropharmacological domains will be active in different components of this human model of risks for relapse in protracted abstinence.
Results: Data from our human lab study of gabapentin offer an illustrative "case study" of this early phase evaluation of a potential medication for protracted abstinence. Gabapentin was selected for study because it alters neurochemical systems (e.g., GABAB) associated with the development of alcohol dependence, in a manner similar to that of acamprosate, a newly-approved medication for alcohol dependence. Subjects were 30 non treatment-seeking paid volunteers with alcohol dependence. Gabapentin showed positive effects on key symptoms of protracted abstinence (sleep and mood), which may be predictive of relapse prevention effectiveness in motivated subjects receiving concomitant alcoholism counseling.
Discussion: This highly standardized laboratory assessment of responsivity to alcohol and affective cues, in combination with naturalistic measures of drinking, mood and sleep, offers a screen for potential anti-drinking relapse medications that can be reliably replicated across multiple laboratories, and which offers an alternative to older human laboratory methods involving alcohol administration, that violate the condition of protracted abstinence.

Panel Session
Is it Worth the Risks? Treating the Elderly with Antipsychotics

What Are the Risks?

Lon S Schneider*

Psychiatry, USC Keck School of Medicine, Los Angeles, CA, USA

Background: Antipsychotics are widely used to treat delusions, aggression and agitation in people with Alzheimer's disease and other dementia. During the last decade, the newer atypical antipsychotics (e.g., risperidone, olanzapine, quetiapine, and aripiprazole) have substantially replaced conventional antipsychotics and have been considered preferred treatments. Reasons include perceived relative safety advantages compared to other medications, the opinions of experts, and expectations of efficacy. The perceived safety advantages include lesser cardiovascular adverse effects, sedation, postural instability, falls, and movement disorders although no direct comparison trials adequately address this. Recently, concern has been expressed about risk for cerebrovascular adverse events (CVAEs), metabolic syndrome, death and other adverse events (AEs) specifically caused by atypicals. The FDA added warnings of increased CVAEs to prescribing information for risperidone, olanzapine, and aripiprazole between April 2003 and February 2005. In April, 2005 the FDA issued a health advisory for increased risk for death with atypicals in people

with dementia but did not provide data. There is limited access to the data because most trials have not been published and key adverse events were not reported in some.
Methods: Fifteen trials (9 unpublished), generally 10 to 12 weeks in duration, including16 contrasts of atypical antipsychotics with placebo met criteria: aripiprazole (k=3), olanzapine (k=5), quetiapine (k = 3), risperidone (k=5). AEs were assessed using standard methods to calculate odds ratios [OR] and risk differences [RD] by meta-analysis. In addition adverse events were assessed from 36-week CATIE AD trial of AD outpatients initially randomized to placebo, risperidone, olanzapine, or quetiapine.
Results: From the combined trials, there were no differences in dropouts. Adverse events were mainly somnolence (OR = 2.84 [95% CI 2.25, 3.58]), urinary tract infection or incontinence (OR = 1.28 (1.02, 1.61)) across all drugs, and extrapyramidal signs and symptoms or abnormal gait due mainly to risperidone or olanzapine. There were no increased risks for falls, syncope or injuries (ORs = 0.93 to 1.00). There was a non-significant risk across the trials overall for CVAEs, RR = 2.01 [0.88, 4.60], with significance for risperidone, RR = 3.06 [1.43, 6.57] but not for olanzapine with an equally high RR = 3.34 [0.76, 14.61]. Death occurred more often among subjects randomized to drugs (118 (3.5%) vs. 41 (2.3%) with a RR from meta-analysis = 1.65 [1.19 - 2.29]. Sensitivity analyses did not show evidence for differential risks for individual drugs, severity, sample selection or diagnosis. From CATIE-AD, 421 subjects were randomized to one of 3 atypicals or placebo, could be double-blindly switched to another drug if they were not responding, and were observed up to 36 weeks. There were increases in sedation with all the drugs approximately three to five times that of placebo, confusion and movement disorders with olanzapine and risperidone. There were too few deaths or CVAEs to evaluate.
Discussion: Atypical antipsychotics may be associated with small risks for death and CVAEs compared with placebo over a 10 to 12 week period of the trials. In addition, they are associated with increased risks for sedation, urinary tract infection (probably from sedation or being bed bound). In limited outpatient trials with relatively younger patients, there is similar risk for somnolence and sedation, as well as confusion. Efficacy evidence and risk for adverse events should be considered within the context of medical need, effectiveness, medical co-morbidity, and the efficacy and safety of alternatives.

Long-Term Adverse Effects: Old and New?
Dilip V Jeste*, Hua Jin and David P Folsom

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

Considerations of safety are paramount in any age group, but particularly in the elderly. This presentation will focus on data from published trials as well as new data from ongoing studies at UCSD on side effects of antipsychotics in older people. It is worth noting that these medications have been approved by the FDA only for schizophrenia and bipolar disorder; however, they are used commonly for various off-label indications in elderly persons. We have reported that the annual incidence of tardive dyskinesia with typical neuroleptics is very high in older patients (about 30%), and that the newer atypical antipsychotics have a several folds lower risk of inducing tardive dyskinesia than do the typical agents. At the same time, a number of studies have found an increased risk of adverse metabolic effects including diabetes mellitus and other components of the metabolic syndrome, and also of cerebrovascular adverse events and mortality in individuals with dementia. This presentation will address possible mediators and moderators of these risks including endocrine susceptibility factors and cognitive impairment. A careful consideration of risk:benefit ratio of atypical antipsychotics as well as that of available alternative treatments is required in each patient, especially in one with dementia. There are currently few suitable alternative treatments that have been shown in large-scale controlled trials to be both effective and safe in elderly psychotic patients. We will discuss a proactive approach to regular monitoring of the patients on antipsychotics.

What About Efficacy?
Pierre N Tariot*, Lon S Schneider, Karen Dagerman and Philip S Insel

Psychiatry, University of Rochester, Rochester, NY, USA

An unpublished meta-analysis was performed by Schneider and colleagues to assess the evidence for efficacy of atypical antipsychotics in dementia. 15 trials met criteria (9 unpublished), generally 10-12 weeks in duration, including 16 contrasts of atypical antipsychotics with placebo: aripiprazole (k=3), olanzapine (k=5), quetiapine (k = 3), risperidone (k=5). A total of 3353 subjects were randomized to drug and 1757 placebo. Standard meta-analysis methods were used to calculate OR's and risk differences for dichotomous outcomes and adverse events, and weighted mean differences were calculated for continuous variables. There were no overall differences from placebo in all-cause dropouts. Overall efficacy by metaanalysis was found for aripiprazole (3 trials, BPRS NPI) and risperidone (4 or 5 trials BEHAVE-AD), but not for olanzapine (3 trials, BPRS, NPA). The 3 quetiapine trials could not be statistically combined; 2 had non-significant results and 1 using agitation scores as outcome was significant. Subgroup analyses did not suggest evidence for differential efficacy by individual drugs. Adverse events were mainly somnolence and urinary tract infection or incontinence across all drugs, and extrapyramidal signs and symptoms or abnormal gait due mainly to risperidone or olanzapine. In comparison, the CATIE-AD trial enrolled 421 outpatients with AD and agitation and psychosis who were randomized in the first phase to olanzapine (mean last dose 5.5 mg/d), quetiapine (mean last dose 56.5 mg/d), risperidone (mean last dose 1 mg/d), or placebo for up to 36 weeks. There was no overall difference in all-cause discontinuation, whereas there was an overall drug-placebo difference in discontinuation due to lack of efficacy, with supplemental analyses favoring olanzapine and risperidone. Conversely, there was a lower rate of discontinuation from placebo than from active medication due to safety or tolerability problems. Examination of Clinical Global Impression of Change data showed trend or significant differences in favor of active treatments, results supported by supplemental ratings of behavior, but with varying effects of different treatments on functional capacity and caregiver burden. Atypical antipsychotics overall are associated with small statistical effect sizes, and evidence for efficacy is not seen consistently for all drugs and methods of assessment. Efficacy evidence and risk for adverse events in these frail patients should be considered within the context of medical need, effectiveness, medical co-morbidity, and the efficacy and safety of alternatives.

Cost-Effectiveness of Atypical Antipsychotics in the CATIE Alzheimers Disease Trial
Robert A Rosenheck*

Psychiatry, Yale Medical School, New Haven, CT, USA

This presentation will review data comparing the cost and aggregate effectiveness of drugs used during the CATIE Alzheimers disease trial. Data were obtained monthly from all participants on use of inpatient, nursing home and outpatient, medical, psychiatric and eldercare. Costs were based on administrative data and research publications from diverse providers at the State level. Data on costs of study and concomitant medication were based on average wholesale prices. Effectiveness is assessed by a measure of utility based on the Health Utilities Index, Mark III. Mixed effects model analyses will be used to compare mean monthly costs of patients treated on olanzapine, risperidone, quetiapine, and placebo. There was no difference between treatment groups in either Quality Adjusted Life years or costs. While experimental drug costs were significantly lower for the placebo group, ancillary drug costs were higher, so that total drugs costs for placebo were only $40 (15%) less than for patients assigned to atypicals. Study

medications were relatively inexpensive due to low dosages used.There were no significant differences between groups in total health care costs which averaged $1,268 per month. There was essentially no difference in cost-effectiveness of the agents evaluated in this trial.

Panel Session
Neuregulin and Schizophrenia: From Genetic Sequence to Pathogenic Mechanisms

The Relevance of NRG1-erbB Signaling in Oligodendrocytes to Schizophrenia

Gabriel Corfas*

Neurology, Harvard Medical School/Children's Hospital Boston, Boston, MA, USA

Recently, the Neuregulin-1 (NRG1) gene has been identified as a susceptibility gene for schizophrenia. NRG1 is a pleiotropic factor that, signaling through its erbB2, erbB3 and erbB4 tyrosine kinase receptors, regulates diverse processes of brain development. In vitro studies indicate that NRG1-erbB signaling may be important for the regulation of oligodendrocyte development. Interestingly, a number of histological and imaging studies of schizophrenic patients have revealed white matter defects while others have uncovered alterations in the expression of specific oligodendrocyte and myelin-related genes. I will discuss the data showing that NRG1-erbB signaling is a key factor in oligodendrocyte development and myelination in the central nervous system and the evidence provided by human studies and animal models that oligodendrocyte abnormities may be a contributing factor to schizophrenia.

Neuregulin, Nicotine and Schizophrenia

David A Talmage*, Chuang Du, Melissa Hancock, Chongbo Zhang, Ying-Jiun J Chen and Lorna W Role

Institute of Human Nutrition, Columbia University, New York, NY, USA

The Neuregulin 1 (Nrg 1) gene and the alpha 7 nicotinic acetylcholine receptor subunit (α7 nAChR) gene both have been identified as possible susceptibility loci for developing schizophrenia. Our studies test the hypothesis that perturbations in the expression of neuregulin 1 alter the maturation and maintenance of key synaptic connections that, in the normal adult, are modulated by cholinergic input. I will present results of studies with mice carrying an isoform-specific targeted disruption of one allele of Type III Nrg1 (Nrg1tm1.1Lwr) demonstrating functional interactions between the products of these two genes. Specifically, I will report that (a) Nrg1tm1.1Lwr mice have reduced expression of α7 nAChR in the ventral hippocampus; (b) chimeric synapses between Nrg1tm1.1Lwr ventral hippocampal slices and wild-type nucleus accumbens neurons lack sustained, α7 nAChR mediated nicotine-enhanced glutamatergic transmission; (c) back-signaling by the Type III Nrg1 intracellular domain regulates the levels of functional α7 nAChRs in axons. This increase in functional α7 nAChRs results from local activation of a phosphatidylinositol 3-kinase/Akt signaling pathway. Based on these results, we propose (a) Type III Nrg1 mutant mice are useful for studying biochemical and molecular interactions between the products of different schizophrenia susceptibility loci; (b) that Type III Nrg 1 back-signaling is necessary for establishing functional pre-synaptic α7 nAChRs which play a critical role modulating synaptic transmission from ventral hippocampal projections to the nucleus accumbens; and (c) that convergent effects of genetic modifications of Nrg1 and altered nicotinic responses might contribute to the high incidence of smoking seen in schizophrenics.

Neuregulin mRNA and Protein in Patients with Schizophrenia

Cynthia S Weickert*, Amanda J Law, Senda Beltaifa, Maree J Webster, Paul J Harrison, Joel E Kleinman and Daniel R Weinberger

MiNDS, CBDB, NIMH, Bethesda, MD, USA

Background: NRG1 is a potent developmental protein capable of directing cell migration, differentiation and viability by acting through ErbB receptors. Inheritance of certain genetic polymorphisms of NRG1 is associated with increased risk to developing schizophrenia and we have shown that NRG1 type I mRNA is increased in frontal cortex and hippocampus of patients with schizophrenia. However, the anatomical distribution of the various NRG1 isoforms, and thus, the cell type demonstrating an increase in type I NRG1 mRNA has not been determined. Furthermore, it is not known how changes in NRG1 mRNA may relate to changes in NRG1 protein. Although alterations in NRG1 function in patients with schizophrenia have not been described, a down-regulation of mRNA encoding one NRG1 receptor, ErbB3, has been found in the frontal cortex of patients with schizophrenia. This observation has been used to putatively link NRG1 to cellular and molecular pathology in the disease. We sought to replicate the finding of reduced ErbB3 mRNA in the brain of patients with schizophrenia.
Methods: We performed in situ hybridization with NRG1 isoform specific (types I-VI) and ErbB2, 3 and 4 riboprobes. First, we mapped the expression of the six NRG-1 splice variants and the three ErbB receptors in normal rhesus monkey brain. Then, we compared expression in the brains of normals (N=14) and schizophrenics (N=14) also using in situ hybridization. Next, we measured NRG1 protein in the brains of patients with schizophrenia (n=15) compared to controls (N=15) using Western blotting methods with nuclear versus cytoplasmic extracts from middle frontal gyrus.
Results: We found that NRG1 types I, II, III and IV are detectable in widespread regions of the primate brain, while NRG1 types V and VI mRNA were not detectable. By in situ hybridization, we found moderate-high expression of ErbB2 and ErbB4 mRNAs, with low expression of ErbB3 mRNA throughout the monkey telencephalon. We have quantitatively examined ErbB mRNAs in the frontal cortex of patients with schizophrenia compared to controls and we have been unable to find a robust change in the levels of ErbB2, 3 or 4 mRNAs in the brains of patients with schizophrenia from film-based analysis. In our preliminary studies, we did not detect a significant difference in NRG1 protein levels in patients with schizophrenia compared to controls by Western blotting.
Discussion: Our data suggest that alterations in NRG1 protein or ErbB mRNAs may not be readily detectable in all groups of patients with schizophrenia. This suggests the need for development of more specific assays and the need to study a larger group of patients and controls where the effect of NRG1 genotype can be explored.

SNPing Away at NRG1 and ErbB4 Gene Expression in Schizophrenia

Amanda J Law*

Dept Psychiatry, University of Oxford, Oxford, United Kingdom

Background: There is strong evidence that genetic variation in NRG1 is associated with schizophrenia and data suggests that genetic interaction between disease-associated SNPs in NRG1 and variants in its primary receptor, ErbB4 may increase susceptibility to the disease. The disease-associated SNPs in both genes are non synonymous and we propose that the mechanisms behind the clinical association involves altered gene transcription.
Methods: Utilizing quantitative real-time RT-PCR we have measured mRNA for NRG1 splice variants-types I-IV in the hippocampus of 84 controls and 48 patients with schizophrenia. In each individual we examined 4 SNPS (SNP8NRG221132; SNP8NRG221533; SNP8NRG 241930; SNP8NRG243177) previously reported to constitute the original schizophrenia "at-risk" haplotype. Each SNP and the haplo-

type were tested for association with NRG1 mRNA levels. In addition, we examined expression levels for the ErbB4 splice isoforms, JMA, JMB, CYT1 and CYT2 in the hippocampus and prefrontal cortex of the same individuals.
Results: With regards to NRG1, we report isoform specific alterations of NRG1 Type I in schizophrenia and interaction of genotype at a single disease associated SNP. We also show that a single disease associated SNP and the classic risk haplotype predicts increased expression of a newly discovered Type IV isoform. Bioinformatic promoter analyses indicate that both SNPs lead to a gain/loss of putative binding sites for specific transcription factors. Data on Erbb4 isoform expression in schizophrenia will be discussed in the presentation.
Discussion: I will present postmortem and genetic evidence suggesting that the molecular mechanism behind the clinical association of NRG1 with schizophrenia involves aberrant transcriptional regulation of the gene. Interactions between ErbB4 splice isoform gene expression, NRG1 and schizophrenia will be presented. The functional consequences of altered NRG1-ErbB4 signaling on cortical neural development, plasticity and neurotransmission will be discussed. Since schizophrenia is likely the result of a combination of mutations/variations in several genes, with each locus contributing modestly to disease etiology, understanding the interactions between genes (and their biological pathways) becomes critical to understanding the genetic architecture and pathophysiology of the disease.

Panel Session
Animal Models of Deficient Sensorimotor Gating: A New Role for the Corticotropin-Releasing Factor System?

CRF1 and CRF2 Receptor Modulation of Information Processing

Victoria B Risbrough*

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

Corticotropin Releasing Factor (CRF) has recently been shown to modulate information processing and response inhibition of acoustic startle in animals. Across species, presentation of a neutral, non-startling "prepulse" 30-300 ms before a startling stimulus reduces startle magnitude, termed prepulse inhibition (PPI), theoretically by requiring the organism to allocate attentional resources to process the prepulse and hence filter or "gate" the subsequent startling stimulus. PPI is used clinically as an operational measure of sensorimotor gating that is deficient in a number of neuropsychiatric disorders. Certain anxiety disorders, post-traumatic stress disorder (PTSD) and panic disorder (PD), exhibit deficits in PPI, as well as abnormalities in other measures of startle plasticity (increased startle magnitude or reduced startle habituation). These disorders also appear to exhibit pathology in the CRF system, either CRF hypersecretion or increased receptor signaling. CRF is a neuropeptide that coordinates many behavioral and neuroendocrine responses to stress via activation of 2 known receptor subtypes, CRF-R1 and CRF-R2. We have used the murine model of acoustic startle to examine the respective roles of the two known CRF receptors, CRF1 and CRF2, in information processing as measured by startle plasticity. Previously we have shown that acute CRF administration significantly increases startle magnitude and reduces PPI of startle in mice. CRF-induced deficits in PPI and increases in startle were reversed by CRF1 selective antagonism or in mice homozygous for CRF1 receptor gene deletion (CRF1 KO mice), indicating that activation of CRF1 reduces PPI while increasing startle. CRF-induced deficits in PPI are potentiated by CRF2 selective antagonism and selective CRF2 agonists increase PPI, indicating that activation of CRF2 enhances PPI. The CRF2 antagonist antisauvagine-30 attenuates CRF-induced increases in startle, indicating that CRF2 activation may act in concert with CRF1 to increase startle. We have extended these studies by (1) determining the effects of CRF on startle plasticity and subsequent response recovery in CRF2 KO mice (2) testing if forebrain CRF1 receptors are necessary for CRF effects on startle and PPI, and (3) determining the effects of chronic CRF release on startle plasticity measures. Based on our previous data and these new studies we have developed a model of the respective roles of CRF receptors in startle plasticity during both acute and chronic CRF release. We suggest that CRF1 activation increases defensive startle via increasing the magnitude of the response as well as by decreasing inhibition of the response (e.g. PPI) while CRF2 activation can both maintain (block habituation) and initiate recovery of CRF1-initiated increases in startle, via increasing startle inhibition (PPI). However we suggest that in situations of chronic CRF release, CRF1 receptor mediated behaviors may predominate over CRF2 mediated behavioral recovery.

Is Dopamine to Blame for CRF-Induced Sensorimotor Gating Deficits in Rats?

Ronald P Hammer* and Isabel C Arrillaga-Romany

Psychiatry and Neuroscience, Tufts University, Boston, MA, USA

In rats, sensorimotor gating deficits are induced by selective dopaminergic, noradrenergic and serotonergic agonists, as well as by non-competitive NMDA receptor antagonists. In general, only atypical antipsychotics or specific non-dopaminergic antagonists prevent sensorimotor gating deficits produced by non-dopaminergic compounds, while typical antipsychotics effectively block only dopaminergic deficits. We examined the influence of corticotropin releasing factor (CRF) on prepulse inhibition of acoustic startle response (PPI), as well as the ability of clozapine and raclopride to alter this response. Adult male Harlan Sprague-Dawley rats were assessed for baseline PPI, which was used to normalize groups. Rats then received intracerebroventricular guide cannulas and recovered for one week. Various dose or drug pretreatment trials were conducted at weekly intervals using a counter-balanced design. We found that human/rat CRF (0, 1.0 or 3.0 μg) produced a dose-dependent reduction of PPI assessed 40-60 min after central administration. Although only the highest dose significantly disrupted PPI, both doses increased grooming and locomotor behavior suggesting that both were centrally active. These effects were blocked by co-infusion of a non-selective CRF receptor antagonist. Clozapine (5 and 10 mg/kg, i.p.), an atypical antipsychotic with broad receptor-binding properties, and raclopride (0.05 and 0.1 mg/kg, s.c.), a selective D_2-like receptor antagonist, were then administered prior to CRF. Clozapine pretreatment dose-dependently prevented CRF-induced PPI disruption, with complete reversal at the highest dose. In contrast, the highest dose of raclopride only partially reversed CRF-induced PPI disruption. Neither drug altered PPI nor locomotor activity in the absence of CRF. These doses of raclopride are known to block PPI disruption in response to apomorphine (0.5 mg/kg), but CRF-induced PPI disruption appears to be less sensitive to raclopride. Thus, clozapine blocks CRF-induced PPI disruption more effectively than does raclopride in rats. These data suggest that dopamine plays a role in CRF-induced PPI disruption, but that other neurotransmitters whose actions are influenced by clozapine are important modulators of this CRF effect.

Effects of Antipsychotics and Manipulations of the Serotonin System on the Corticotropin-Releasing Factor-Induced Decrease in PPI in Rats

Lisa H Conti* and Jane E Tayler

Psychiatry, University of Connecticut Health Center, Farmington, CT, USA

Background: Sensorimotor gating, assessed by prepulse inhibition (PPI) of the startle response, is deficient in a number of psychiatric disorders in which stress has a role, including schizophrenia. Corticotropin-releasing factor (CRF) is released during stress, not only to activate the pituitary-adrenal axis, but from extrahypothalamic brain

areas to result in behavioral changes, and alter brain extracellular concentrations of the monoamines.
Methods: Male Wistar-Kyoto (WKY) rats, which show high levels of PPI, and Brown Norway (BN) rats, which show low levels of PPI, had guide cannula (22 gauge) implanted in the lateral ventricle for intracerebroventricular (ICV) infusions. To test PPI, rats were placed into a cylindrical chamber enclosed in a sound- and vibration-attenuating cabinet (San Diego Instruments). The startling stimulus (120 dB, 40 msec) was presented on all trials. Prepulse stimuli (ranging from 3-18 dB above background; 20 msec) were presented 100 msec prior to the startling stimulus on some trials. Average startle amplitude during the 100 msec following the onset of each startling stimulus was recorded and stored on a computer (San Diego Instruments, SR Lab). In most experiments, either saline or CRF (0.1 -3.0 μg) was infused ICV (6.0 μl) 30 min prior to testing. In other experiments, rats were treated as follows: 1) Saline or CRF was administered by a subcutaneous (SC) route; 2) Either the non-selective CRF antagonist, D-Phe12-41, or astressin was infused (ICV); 3) The typical antipsychotic, haloperidol (0.5 or 1.0 mg/kg), or the atypical antipsychotic, clozapine (5.0 or 10.0 mg/kg) was administered (SC) 10 min prior to CRF (3.0 mg, ICV); 4) To assess whether manipulations of the serotonin (5-HT) system alter the effect of CRF on PPI, rats were either treated with the 5-HT synthesis inhibitor para-chlorophenyalanine (PCPA) prior to CRF infusion, or with the 5-HT2A/2C receptor antagonist (2.0 mg/kg, SC), or the 5-HT1A receptor antagonist, WAY 100635 (1.0 mg/kg, SC) 10 min prior to receiving CRF (ICV).
Results: CRF (ICV) diminishes PPI in both WKY and BN rats, although only the high dose (3.0 μg) has this effect in WKY rats, and a relatively low dose (0.3 μg) is more effective in BN rats. This effect of CRF is independent of changes in baseline startle amplitude, and can be achieved more than once in the same rats. Systemic administration of CRF has no effect on PPI in either rat strain. Neither of the non-selective CRF antagonists tested enhanced PPI in the BN strain. Additionally, neither the typical, not the atypical antipsychotic enhanced baseline PPI in the BN rats. However, the high dose of haloperidol reversed the CRF-induced decrease in PPI in WKY rats, and clozapine increased PPI in CRF-treated rats of both strains. The CRF-induced decrease in PPI was not reversed by 5-HT depletion, although 5-HT depletion potentiated the effect of CRF on baseline startle amplitude. Similarly, administration of the postsynaptic 5-HT receptor antagonist, ketanserin, did not alter the effect of CRF on PPI. However, ketanserin did attenuate the CRF-induced increase in baseline startle amplitude seen in WKY rats. Administration of the 5-HT1A receptor antagonist, WAY 100635, itself caused a slight reduction in PPI in BN rats, and this effect was additive with the effect of low-dose CRF.
Conclusions: Central, but not peripheral administration of CRF diminishes PPI in rats, although tonic levels of CRF do not appear to increase PPI in BN rats, which show low levels of PPI. The effects of CRF on PPI can be reversed by antipsychotic drugs, but are not altered by manipulations of the 5-HT system. CRF may have a role in the diminished PPI seen in psychiatric disorders such as schizophrenia.

Acute and Long-Term Effects of Stress and CRF on Prepulse Inhibition

Vaishali P Bakshi*, Karen M Alsene and Elenora E Connors

Psychiatry, UW-Madison School of Medicine, Madison, WI, USA

Prepulse inhibition (PPI), an operational measure of sensorimotor gating, refers to the phenomenon in which a weak stimulus presented immediately before an intense startling stimulus inhibits the magnitude of the subsequent startle response. PPI is deficient in a number of psychiatric illnesses including schizophrenia that involve a putative breakdown in sensorimotor gating. Stressful events can exacerbate schizophrenic symptomatology, yet the effects of stress on PPI have been relatively understudied. These studies compared the sequelae of a purely psychological stressor (predator exposure; a rat within a protective cage is placed inside of a ferret's homecage) to those of a nociceptive physical stressor (footshock) and to those of the stress hormone corticotropin-releasing factor (CRF) on PPI and baseline startle responses. Separate groups of male Sprague-Dawley rats either received one of three CRF doses (0, 0.5, or 3.0 μg/5μl, into the lateral ventricles) or were exposed to one of three stress conditions (no stress, predator stress, or footshock) and then tested in startle chambers; all rats were tested for PPI again 24 hours, 48 hours, and 9 days later. Blood samples (via chronic indwelling jugular catheters) were also collected for the acute and 24-h timepoints to measure plasma levels of corticosterone in response to these manipulations. None of the treatments affected PPI acutely, even though they all significantly increased plasma corticosterone levels, and in the case of CRF, potently elicited grooming and locomotion for 60 min after infusion. Twenty-four h later, however, PPI was significantly lower in the predator stress and CRF groups (both doses) compared to their respective controls; corticosterone levels in no group differed significantly from the vehicle/no stress values at this timepoint. By 9 days after stress/CRF administration, PPI in rats that had undergone predator stress or those that had received the lower CRF dose returned to vehicle/no stress levels, but PPI in rats that had received the high dose of CRF remained disrupted. In contrast to predator stress or CRF, footshock failed to affect PPI at any timepoint. Changes in PPI were dissociable from alterations in baseline startle magnitude for all treatments. Taken together, these results indicate that: 1) a purely psychological stressor produces a delayed but reversible disruption of PPI; 2) low levels of CRF receptor stimulation recapitulate this effect, whereas high levels cause a long-lasting PPI deficit; 3) different types of stressors have distinct effects on startle plasticity; and 4) stress or CRF effects on PPI are independent of alterations in circulating glucocorticoid levels or baseline startle reactivity. These findings may have important implications for identifying the mechanisms through which stress may influence sensorimotor gating in various psychiatric illnesses.

Panel Session
Molecular Mechanisms of Synaptic Alterations Associated with Neuropsychiatric Disorders and Addiction

Synapse Assembly in the Developing Brain: The Active Role of the Adhesion Molecule SynCAM

Thomas Biederer*

Molec. Biophysics & Biochemistry, Yale University Medical School, New Haven, CT, USA

Background: Synapse formation is required to establish neuronal networks and ultimately to organize the human brain. Intense synaptogenesis is initiated in the central nervous system (CNS) after birth and synapses continue to form throughout adulthood. Huge progress has been made in the unraveling of principles of synapse formation at the neuromuscular junction. Yet, for the CNS, only two protein interactions are known to directly induce new synapses, mediated by SynCAM and the neuroligin/neurexins proteins. Both constitute adhesion systems that connect pre- and postsynaptic sites. It remains unknown how these proteins demarcate synaptic membranes and how synapse formation proceeds. Our laboratory focuses on the roles of SynCAM 1 in synaptic differentiation. Understanding these processes is important for human health. Alterations in synapse formation affect synaptic plasticity, which is associated with changes in human behavior, learning and memory, and addiction.
Methods: We employ multiple approaches. We biochemically purify synaptic proteins and their binding partners, and characterize their interactions. For in vitro studies of these proteins in synaptogenesis, we use dissociated hippocampal and cortical neurons and analyze

them by immunocytochemistry, electrophysiology, and live cell imaging. These experiments involve manipulations that increase formation of synaptic complexes or interfere with synaptic protein interactions. Our in vivo studies are based on mouse genetic approaches.
Results: We identified SynCAM 1 (Synaptic Cell Adhesion Molecule) in a search for adhesion proteins that bind intracellular adaptor molecules containing PDZ protein interaction domains. PDZ-domain containing proteins function as molecular scaffolds at synaptic sites, providing the rationale for our approach. SynCAM 1 contains three extracellular immunoglobulin (Ig)-like domains that mediate homophilic adhesion, a single transmembrane region, and a short cytosolic tail. It is expressed throughout the developing brain during the peak of synaptogenesis. SynCAM 1 drives formation of fully functional presynaptic terminals de novo, as shown by three approaches. First, SynCAM 1 induces the clustering of synaptic vesicle markers when presented to cultured neurons. Second, these SynCAM-induced specializations contain actively recycling synaptic vesicles. Third, we used this activity of SynCAM 1 to reconstitute synaptic transmission for the first time. This activity of SynCAM 1 is only shared by the postsynaptic neuroligin adhesion proteins. Notably, SynCAM 1 promotes synaptic transmission in dependence on its intracellular sequence. Our key goal is to characterize the extra- and intracellular interactions of SynCAM 1 and their role in synaptic differentiation and physiology. SynCAM 1 is member of a small, vertebrate-specific family of four Ig-domain containing proteins expressed in the developing brain. Our work also includes studies of these additional family members, comparing them to SynCAM 1.
Discussion: Our studies helped to establish that synaptic adhesion molecules like SynCAM 1 can be sufficient to drive the formation of synaptic specializations. Our in vivo studies will complement these approaches to examine the activity of synapse-inducing proteins in the complex environment of the developing brain. Potentially, the outcome of our work will advance our understanding from the description of synaptic adhesion molecules to the identification of the molecular principles of CNS synapse formation, and of the roles synapse-inducing proteins play in synaptic plasticity.

Identification and Analysis of a Rac1 Regulator That Mediates Dendritic Pruning

Phil Buttery, Asim Beg, Ben Chih, Arkady Broder, Carol A Mason and Peter Scheiffele*

Dept. of Physiology & Cell Biophys, Columbia University, New York City, NY, USA

Dendritogenesis is a complex process which remains inadequately understood. A number of extracellular factors have been implicated in the regulation of dendrite extension, branching and spine formation, including the Trk, Notch, Slit-Robo, and semaphorin pathways. Evidence from a variety of systems suggests that regulated retraction of individual dendritic branches is also an integral part of normal dendritic growth. Such selective pruning of minor dendritic branches is likely to be fundamental to activity-dependent sculpting of cell-specific dendritic arbors. By gene expression profiling of postnatal cerebellar tissues, we have identified a group of genes that are up-regulated during the later stages of synaptic maturation. One EST identified in this screen encodes a GTPase activating protein (GAP) for Rac1. Using the Purkinje cell dendritic arbor as a model system, we show that a major function of this molecule is to prune dendritic arbor growth. Thus over-expression results in pruning of dendrites and inhibition of spine formation. That this activity is dependent upon an intact GAP domain suggests that inhibition of Rac1 activity may be central to pruning events in growing dendrites. By further mutational analysis, we demonstrate that the pruning effect is also dependent on an intact phorbol ester-binding C1 domain. Through its ability to bind diacylglycerol, this domain potentially links Rac GAP activation (and hence Rac inactivation), to signaling from the cell surface via receptor-activated phospholipases. We therefore investigated the activation of the molecule by a combination of GTP pull-down assays and live confocal imaging in primary neurons; this has allowed us to demonstrate that activation of the molecule involves rapid regulation of its subcellular localization, and is linked directly to signaling through muscarinic acetylcholine and metabotropic glutamate receptors. We thus show that a Rac-GAP may be a key component in the regulation of pruning events during dendritogenesis, and hence crucial to the normal patterning of dendritic arbors. As the minor branches that are subject to potential pruning also bear nascent synapses, such synapto-dendritic 'sculpting' may be also be fundamental to experience-dependent refinement of neuronal interconnectivity.

Developmental Synapse Elimination is Mediated by the Complement Cascade

B Stevens, AD Huberman and Ben Barres*

Department of Neurobiology, Stanford University, Stanford, CA, USA

Background: During development, competition between axons causes permanent removal of synaptic connections within the brain. The synapses to be eliminated become progressively weaker, are eliminated, and then the competing axon extends axonal processes to occupy those sites. These findings have lead to a simple model in which synaptic transmission produces two postsynaptic signals: a short range protective signal and a longer range elimination signal. Functionally weak synapses are not protected from the elimination signal of neighboring stronger synapses, resulting in the disappearance of postsynaptic receptors and withdrawal of the axon. This withdrawal then provides the opportunity for the stronger axon to expand into the vacated territory. The identities of the punishment and protection signals are unknown. In this presentation, we will describe our recent studies that provide evidence that the complement cascade mediates developmental synapse elimination.
Methods: Described below.
Results: To understand the effects of astrocytes on synaptogenesis, we examined the effects of astrocyte-conditioned medium on gene expression by highly purified retinal ganglion cells in culture. We found that expression of most of the 8,000 neuronal genes that we examined was not significantly altered with the exception of mRNAs encoding the complement protein C1q A, B, and C chains, which were each upregulated by 30-fold. We confirmed the presence of these 3 C1q subunit mRNAs and proteins within the neurons by using RT-PCR, Western blotting, and immunostaining. To find out if C1q was localized to synapses in the developing brain, we performed immunostaining experiments in cryosections of P8 mouse and rat brain. We found bright punctate C1q immunoreactivity throughout the developing, but not adult, brain that colocalized with synaptic markers. C1q-immunoreactivity was not detected when the same antibodies were used to stain cryosections prepared from C1q-deficient P8 mouse brains. Thus C1q is highly localized to synapses in the developing but not adult CNS. To investigate whether complement proteins are required for developmental synapse elimination, we next examined refinement of synapses in the developing visual system. Retinogeniculate segregation provides an excellent model system for studying the activity-dependent mechanisms that refine synapses during development into their mature pattern of connections. It has previously been found that retinal activity is required for normal retinogeniculate segregation during the first week of postnatal development. To test the possible roles of the complement proteins C1q and C3 in developmental synapse refinement, we performed similar experiments by anterograde tracing of retinal afferents in P30 wild type and mutant mice. We found that mice deficient in C1q and C3 have significantly expanded and diffuse ipsilateral projections patterns compared to littermate controls.
Discussion: These findings implicate the complement cascade in developmental synapse elimination, lead to predictions about the iden-

tities of the synapse protection and elimination signals, and have implications for understanding the cause of synapse loss in Alzheimers Disease.

Synapse to Nucleus Signaling During Long-term Synaptic Plasticity: A Role for the Classical Active Nuclear Import
Kelsey C Martin*

Psychiatry, Biological Chemistry, UCLA, Los Angeles, CA, USA

Background: Long lasting forms of synaptic plasticity, such as those that underlie learning and memory or drug addiction, have been shown to depend on new RNA synthesis. This requirement for transcription indicates that signals are transported from the synapse, where they are generated, to the nucleus, where they are converted into changes in gene expression. We have focused on the role of the classical nuclear import pathway in carrying signals from distal synapses to the nucleus during long-term plasticity. In this pathway, proteins bearing nuclear localization signals are recognized by a family of transport factors called importins, which dock the karyophilic proteins at the nuclear pore and mediate their translocation from the cytoplasm into the nucleus.
Methods: We have studied importin-mediated plasticity in two model systems of learning-related plasticity: long-term facilitation (LTF) of Aplysia sensory-motor synapses and long-term potentiation (LTP) of mouse hippocampal neurons.
Results: We find that importins are present at distal synaptic sites and that they associate with the post-synaptic density. As such, they are appropriately localized to mediate signaling from synapse to nucleus. We further find that stimuli known to produce long-lasting, transcription-dependent changes in synaptic strength trigger translocation of the importins into the nucleus. Inhibition of the active nuclear import pathway by microinjection of anti-nuclear pore antibodies (which block active transport but not passive diffusion through the pore) blocks LTF of Aplysia sensory-motor synapses without affecting basal transmission.
Discussion: These results indicate that synaptic stimulation can recruit importin-mediated transport of signals from the synapse to the nucleus. We are currently 1) examining the cell biological pathways whereby the importins carry their cargoes through the neuronal process and 2) using the importins to identify synaptically localized cargoes that function as signals to alter gene expression in response to synaptic activity.

Panel Session
Tourette Syndrome-A Window into Neurochemistry and Neuropharmacology of Many Comorbid Neuropsychiatric Disorders

Striatal Biology and Tourette Syndrome
Roger Albin*

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

Tourette syndrome is a polygenic neurodevelopmental disorder characterized by involuntary stereotyped movements and phonations - tics - and comorbid behavioral abnormalities, particularly obsessive-compulsive behaviors (OCBs). Indeed, tics and OCBs probably represent a spectrum ranging from simple tics to pure obsessions. In this presentation, I review emerging data indicating that TS arises from a primary disorder of the basal ganglia. Recent clinical, neuroimaging, and anatomic data suggesting basal ganglia abnormalities will be discussed. Basic research into striatal function over the past 2 decades has indicated core roles of the basal ganglia, specifically the striatum and the dopaminergic nigrostriatal projection, in the execution of stereotyped, repetitive behaviors with social significance, incremental learning of specific stimulus response associations (habits), and rewarded behaviors. These basic science discoveries have plausible analogies in the clinical phenomenology of tics and OCBs. Improved understanding of striatal function and evidence indicating basal ganglia abnormalities in TS converges to help explain the pathophysiology of tics and OCBs, and suggests hypotheses for research in TS.

Tics and Tourette Syndrome in Children: Genetics and Public Health Impact
Lawrence D Scahill*

Child Study Center, Yale University, New Haven, CT, USA

Background: Transient tics are common in school-age children. Tourette syndrome (TS), which is defined by the enduring presence of both motor and phonic tics is less common and is associated with significant impairment. An important question is whether TS represents a vertically transmitted genetic vulnerability with expression across a wide range has been examined using various research strategies.
Methods: Literature review of all recent community surveys on the prevalence of tics, tic disorders and TS were examined to identify the best estimate of prevalence and the public health impact of tics and TS in children. These findings were placed in the context of recent results of family genetic and affected sib-pair studies.
Results: Transient tics are common in childhood affecting as many as 20% of school-age children. Tic disorders, in which motor or vocal tics persist over time, are far less frequent affecting an estimated 2 to 5% in the pediatric population. Current estimates for Tourette syndrome (TS), defined by the enduring presence of both motor and phonic tics range between 1 and 10 per 1000 (0.1% to 1.0%). TS clearly has a genetic basis with TS, chronic tic disorders and obsessive-compulsive disorder as variable expressions of the same genetic vulnerability. Linkage analyses suggest an association between TS and a region of chromosome 17 and a region of chromosome 11. The association of other genes and TS is currently being evaluated through the TS International Genetics Consortium.
Discussion: Tics may be a marker for abnormal brain development with social and educational implications. Based on data from both community and clinical samples, the impairment associated with TS is greater when accompanied by attention deficit hyperactivity disorder.

Abnormalities of the Dopamine and Serotonin Systems Associated with Tourette Syndrome
Dean F Wong*, James Brasic, Hiroto Kuwabara, Yun Zhou, David Schretlen, Anil Kumar, Weiguo Ye, Mohab Alexander, Ayon Nandi, Albert Gjedde, Anthony Grace and Harvey Singer

Radiology, Johns Hopkins University, Baltimore, MD, USA

Dopamine (DA) and serotonin (5HT) systems are key in the pathology of Tourette Syndrome (TS). We will review imaging studies with DA and 5HT in TS. Wong et al. 1997 showed significant Bmax elevations in 25% of TS subjects. Wolf et al. demonstrated differences in D2 binding that predicted phenotypic severity. An elevation in DAT has been reported in both in vivo and postmortem TS brain. A negative correlation has been shown between vocal tics and SERT, and a recent preliminary report shows increased 5-HT2AR binding in selected cortico-striatal-thalamo-cortical circuits in TS subjects. There is a need to clarify the role of 5-HT in TS because it is a component of drugs used in the treatment of TS. The in vivo quantification of SERT and 5-HT2AR should shed light on this important pathophysiological question. Our recent studies consisted of 4 PET scans for DA measurements: Visit 1: high specific activity 11C raclopride (RAC) with saline, then with iv amphetamine (AMP) to measure DA release (DArel). Visit 2: 11C WIN 35,428 for measurement of DAT and low specific activity 11C RAC for D2. DA receptor density (Bmax) is measured from scans 1 and 4. 5HT and SERT parameters are measured with 11C McN5652 and 5HT2A with 11C MDL 100,907 (MDL). All scans involved bolus injections with radial arterial sampling and mathematical modeling. In addi-

tion, neurocognitive scales and behavioral ratings were conducted on all subjects, including the SCL-90, BPRS, and tic ratings. For DA, comparisons of 11 TS subjects, age 31 +/- 9 and 8 controls (CON), mean age 26 +/- 6, revealed significant elevations of DAR in both striata ($p<0.05$). DArel in TS was nearly twice that of controls: in left ventral striatum (VS), TS DArel was 14.4% vs. control DArel of 8.29%; in right VS, TS DArel was 15.9% vs. control DArel of 8.25%. There were trends for elevation of Bmax in the VS and decrease in the putamen (Pu); and trends for elevation of DAT in the basal ganglia (BG) and VS, in TS. Among TS subjects only (N=11), DArel correlated positively with SCL negative symptoms index (r=0.68), interpersonal sensitivity (r=0.66), and somatization (r=0.8) in the left VS. In the left anterior caudate, DArel in TS subjects correlated positively with SCL-90 psychotism (r=-0.73), negative symptoms index (r=0.68) and hostility (r=0.68).Lastly, D2 Bmax in left VS correlated negatively among TS subjects with the total time to complete trial making tests (r=-0.8) For 5HT, data from 7 TS and 7 controls (age matched at 32 +/- 6), reveal a reduction in SERT binding potential (BP) for TS in the midbrain, Pu, Ca, and thalamus. MT scores correlate with the BP of MDL on the temporal (r=-0.913) and occipital (r=-0.885) cortices, ($p<0.01$). BPRS scores correlate with the BP of MDL in the temporal (r=-0.808), occipital (r=-0.944) and orbitalfrontal (r=-0.854) cortices, $p<0.01$. For both DA and 5HT subject pools, there are significant differences between TS and controls for motor, tic, phonic tic and BPRS scores ($p<0.05$) Thus, using a higher resolution scanner (GE Advance), and improved volume of interest and modeling quantification, we could reproduce (57% increase in DAR for VS) our previous DAR findings (PET scanner GE4096+) (Singer et al, Am. J Psych, 159, 2002). Furthermore, we are observing intriging relationships between neuroreceptor dynamics and psychiatric symptoms. Future studies will allow simultaneous testing of allthe DA and 5HT parameters using multivariate comparisons. Grant support: NIH HS 38927, AA12839, DA00412, TSA.

Treatment of Tourette Syndrome and Co-Occurring Conditions

John T Walkup*

Psychiatry and Behavioral Sciences, Johns Hopkins Medical Institutions, Baltimore, MD, USA

Background: Since the 1960's when haloperidol was first found to be useful for reducing tic severity, treatment of Tourette syndrome (TS) has focused exclusively on somatic treatments. Many medications have been used for tic suppression, but very few have been rigorously evaluated. Methodological rigor is especially critical in TS as decreases in tic severity normally occur during mid to late adolescence, and the natural waxing and waning of tic severity confound the assessment of outcome and side effects. At this time based on the responsiveness of tics to dopamine blocking agents, the similarity of tics to other movement disorders and the presence of comorbid psychiatric disorders, TS is considered to be the result of an abnormality in the basal ganglia and frontal lobes, the circuitry which interconnects these areas and/or the neurotransmitter upon which they depend. Despite these clues there is yet no clearly defined pathophysiology for TS on which to develop new treatments. New treatments for TS are better considered new to TS as most treatments have first been established in another disorder then tried in TS. New somatic treatment strategies for tics include immunological treatments to address tics and OCD considered to be secondary to an autoimmune disorder (i.e. PANDAS) and treatments with direct stimulating effects on the brain - transmagnetc brain stimulation and deep brain stimulation. Although not a new treatment per se, new data supporting the benefit and lack of tic worsening with stimulants for ADHD in the context of tics may improve the outcome of many children seen in clinical settings. Behavioral treatments have been used for many years in TS by a very small group of clinicians/researchers. With the increase in empirical support for specific and targeted psychotherapeutic techniques in disorders considered to be associated with biological disturbance, it is not surprising that behavioral treatments would be tested in tic disorders now.

Method: Review of the treatment literature for Tourette syndrome and the tic disorders and discussion of the presenter's experience in behavioral treatment trials for TS.

Results: Results of current treatment trials suggest that dopamine blocking agents including typical and atypical antipsychotics can be effective in reducing tic severity. Alpha-2 agonists also appear to be effective for reducing tic severity and improving ADHD symptoms. There is little support for other agents for tic suppression. Stimulants for ADHD in the context of tics appear to a beneficial effect on ADHD symptoms and do not appear to worsen tics. Immunological treatment such as plasmapheresis and IV IG appear to be effective for both tics and OCD in children diagnosed with PANDAS, but not effective for chronic OCD. Antibiotics to prevent streptococcal infection-induced worsening of tics and OCD appears to be effective based on a recent report. Behavioral treatments have been effective in small pilot trials, large scale trials are underway.

Conclusion: Effective treatments exist for the TS and other tic disorders, however the mechanism of treatment response is poorly understood. Treatments of comborditity is limited to ADHD and tics. It is not clear if OCD in the context of TS is specifically responsive to traditional OCD treatment. Immunological and antibacterial prophylaxis for tics and OCD are still investigational, as is deep brain stimulation. Deep brain stimulation may offer great promise, but also warrants significant caution. Behavioral treatments show promise and federally funded trials are underway. Understanding the mechanism of behavioral treatments in the tic disorders i.e. how learning paradigms impact biological abnormalities may serve as a model for other neuropsychiatric disorders.

Panel Session
Drug Development: Advances in Neurotensin Neurobiology: Implications for Novel Treatments for Psychosis and Drug Abuse

Viral Vector-Induced Overexpression of the NT_1 Receptor in the Nucleus Accumbens: Further Evidence for Antipsychotic Effects of Increased NTergic Neurotransmission

Charles B Nemeroff*, Ricardo Caceda, Michael J Owens and Becky Kinkead

Psychiatry and Behav Sciences, Emory Univ Sch of Med, Atlanta, GA, USA

Background: A considerable literature has documented the close relationship between CNS dopamine (DA) circuits and those that utilize the tridecapeptide neurotransmitter neurotensin (NT). Specific attention has focused on NT-DA interactions in the nucleus accumbens (NAcc), a major terminal site of the mesolimbic DA system. Direct ICV or intraNAcc injection of NT blocks d-amphetamine-induced hyperlocomotion and rearing, effects similar to antipsychotic drugs. Additionally, typical antipsychotic drugs increases NT mRNA expression and NT concentrations in both the caudate nucleus and the NAcc whereas atypical antipsychotics increase these indices of NT biosynthesis only in the NAcc. Moreover NT receptor antagonists block the behavioral effects of certain atypical antipsychotics. In the present study, we sought to determine if virally mediated increases in NT_1 receptor expression in the NAcc accumbens produce antipsychotic drug like effects.

Methods: A lentiviral vector was chosen because of the crucial capability of integration of the transgene into the genome of non-dividing cells and the related properties of long-term expression of the transgene, low toxicity and immune host reaction, as well as the ability to infect neuronal cells. A bicistronic vector carrying green fluo-

rescent protein (GFP) as a reporter gene and the NT_1 receptor was constructed.
Results: HEK 293 cells (normally devoid of NT receptors) infected with NT_1-GFP vector exhibited increased expression of the NT receptor and GFP. NT-induced dose-dependent increases in cAMP and IP_3 formation in HEK 293 cells infected with the NT_1-GFP vector which was blocked by pretreatment with the NT receptor antagonist SR142948A, confirming functional coupling of the virally-overexpressed NT_1 receptor. NT receptor binding was significantly increased from 2 weeks up to four months after microinjection of the NT_1-GFP vector in the rat caudate nucleus and was restricted to an area of ≈ 1 mm from the site of injection. Further histological examination showed no signs of toxicity or proliferative vascular changes at the injection sites. We then evaluated the effects of viral-mediated overexpression of the NT_1 receptor in the NAcc shell on d-amphetamine- and dizocilpine (an NMDA receptor antagonist)-induced disruption of prepulse inhibition (PPI) of acoustic startle. Male Long Evans rats (n=64) were tested for PPI, ranked according to the average PPI response and distributed to four groups (n=16): control, clozapine, GFP virus, and NT_1-GFP virus. Rats from the virus groups received 1 uL of either GFP or NT_1- GFP virus bilaterally in the NAcc shell. Between the second and fourth weeks after surgeries, the effect of d-amphetamine (2.0 mg/kg, s.c.) or dizocilpine (0.1 mg/kg, s.c.) challenge on PPI was tested. Similar to clozapine, NT_1 receptor overexpression in the nucleus accumbens shall significantly reduce d-amphetamine- and dizocilpine-induced disruption of PPI. In addition, animals receiving the NT_1-GFP virus were protected against d-amphetamine (1.0 mg/kg)- and dizocilpine-induced hyperlocomotion. The NT receptor antagonist SR142948A had no effect on baseline response, but completely abolished the protective effect of NT_1 receptor overexpression against dizocilpine-induced hyperlocomotion.
Conclusions: Our results demonstrate that virally-mediated increased NT neurotransmission in the NAcc antagonizes the behavioral effects of excitation of the mesolimbic DA system induced by an DA receptor agonist and an NMDA receptor antagonist. Overexpression of the NT_1 receptor in the NAcc shell has behavioral effects similar to those of the atypical antipsychotic drug clozapine (NIMH MH-39415).

Neurotensin Regulation of Corticostriatal Function: Implications for Schizophrenia

Ariel Y Deutch*, K A Petrie, J Fadel and P R Dobner

Psychiatry, Vanderbilt Univ Med Ctr, Nashville, TN, USA

Background: Considerable attention has focused on the role of neurotensin (NT) in regulating dopaminergic function. This attention is based on the demonstration that central administration of the peptide has behavioral effects strikingly similar to those elicited by antipsychotic drugs (APDs) and by the demonstration that NT is expressed in some ventral tegmental area (VTA) dopamine (DA) neurons, particularly those that project to the prefrontal cortex (PFC). DA axons in the PFC synapse with both pyramidal cells, including those that project to the striatum, and GABAergic interneurons. We have examined the how neurotensin regulates both PFC and striatal function.
Results: Dialysis studies revealed that systemic or intra-PFC infusions of D2 DA agonists activate GABAergic interneurons. However, the coupling of the D2 receptor to potassium channels that hyperpolarize neurons would suggest an inhibitory effect of DA signaling through D2 receptors. We found that D2 agonists activate GABA neurons in the PFC by interacting with D2 autoreceptors on DA axons to increase release of the co-transmitter NT while decreasing release of DA. In turn, the released NT interacts with an NTR1 receptor expressed by parvalbumin-containing interneurons to activate these cells, culminating in an increase in GABA release. In parallel studies focusing on the striatum, we found that the ability of typical APDs to induce Fos were blunted by pretreatment with the NTR1 antagonist SR48692 and were similarly attenuated in NT null mutant mice; the actions of clozapine were not affected. Because there is a low level of extracellular neurotensin in the striatum that is thought to be derived from medium spiny neurons, the loss of NT signaling may decrease D2 heteroceptors tone on corticostriatal neurons to suppress the activation of NT-containing striatal neurons.
Discussion: Among the changes in the PFC in schizophrenia is a loss of GABAergic tone over pyramidal cells, particular GABA derived from parvalbumin-containing interneurons. Because there is a decrease in the DA innervation of the PFC in schizophrenia, which is thought to underlie the cognitive deficits, the ability of NT derived from these axons to activate GABAergic interneurons may be suppressed, contributing to the decrease in cortical GABAergic tone. Striatal changes regulated by NT may in turn be related to the motor side effects of typical APDs.

The Role of Neurotensin in Estrous Cycle Regulation of Prepulse Inhibition of Acoustic Startle

Becky Kinkead* and Charles B Nemeroff

Psychiatry and Behavioral Sciences, Emory Univ Sch of Med, Atlanta, GA, USA

Introduction: The neurotensin (NT) system is regulated by estrogen and previously, we demonstrated both sex- and estrous cycle-related regulation of the rat NT system, and in the effects of haloperidol administration on this system. In addition to regulation of the NT system across the estrous cycle, ovarian hormones also regulate prepulse inhibition (PPI) of the acoustic startle response in both rats and humans. Estrous cycle-related regulation of NT neurotransmission is one mechanism by which ovarian hormones may regulate PPI. This study was designed to examine whether blockade of NT neurotransmission disrupts PPI in female rats and whether the effects of antipsychotic drug administration on PPI differ depending on the stage of the estrous cycle.
Methods: The estrous cycle stage of adult female rats was determined by vaginal lavage and only rats demonstrating two complete estrous cycles were used in any experiment. PPI was measured in a San Diego Instruments startle chamber. Haloperidol (0.1 mg/kg, i.p.) was administered 30 minutes before PPI testing. The NT receptor antagonist SR142948A (0.1 mg/kg, i.p.) was administered one hour before PPI testing.
Results: Initial studies examined PPI in male rats, female rats in proestrus (P) and female rats in diestrus 1 (D1) at 4 time points (3 hours before or after lights on or off). Surprisingly, whereas PPI was stable across the day in male rats (68% inhibition at 12 dB prepulse intensity) and females in P (60%), PPI significantly decreased across the day of D1 (from a high of 67% to a low of 46%). PPI in males and females in P was significantly greater than females in D1 3 hours before and after lights off. Three hours after lights off, acute haloperidol administration significantly increased PPI during D1, but had no effect on PPI during P. Haloperidol had no effect on baseline PPI in adult male animals. The NT receptor antagonist significantly increased PPI during D1 and significantly decreased PPI during P. The NT receptor antagonist had no effect on baseline PPI in adult male animals.
Conclusions: The results with haloperidol support in part the hypothesis that during D1, PPI is disrupted in a manner similar to that seen in adult male rats after administration of DA agonists, PCP or isolation rearing. In contrast, although administration of the NT receptor antagonist decreased PPI during P (in support of the hypothesis that there is increased NT neurotransmission during P), the NT receptor antagonist also increased PPI during D1. These results fit well with our previous finding that NT concentrations are significantly higher in the VTA during D1 and significantly higher in the nucleus accumbens (NAcc) during P. Blocking NT neurotransmission in the NAcc during P would have the net effect of increasing DA transmission, and decreasing PPI. Blocking NT neurotransmission in

the VTA during D1 would have the net effect of decreasing DA transmission in the NAcc and increasing PPI. In an additional set of experiments, the effects of microinjection of NT in the VTA and NAcc on PPI during D1 and P were examined. Intra-accumbens injection of NT significantly increased PPI in rats during P, but not during D1. In contrast, intra-VTA injection of NT significantly decreased PPI in rats in D1, but not during P. Demonstration of the predicted estrous cycle-related differences in NT neurotransmission and its association with sensorimotor gating may yield an important clinical correlate and model explaining why female schizophrenia patients in general exhibit a better response to antipsychotic drugs, and why schizophrenic symptoms are exacerbated by low estrogen states (NIMH MH-39415).

NT69L: A Neurotensin Receptor Agonist for Treatment of Neuropsychiatric Diseases

Elliott Richelson*, Mona Boules and Paul Fredrickson

Mayo Clinic, Jacksonville, FL, USA

Background: For over 25 years, there has been an hypothesis that neurotensin (NT) is an endogenous neuroleptic [1], with its corollary being that a NT receptor (NTR) agonist would be an antipsychotic. This hypothesis has not been tested in humans. Because no one can make a non-peptide agonist of the NTR, researchers have focused on peptidic compounds. All known activities of NT(1-13) are mimicked by NT(8-13). Therefore, most NTR agonists under development are analogs of the 8 to 13 fragment. One widely studied of these analogs is our compound called NT69L, which has the sequence N(α)-me-Arg^{8},Lys^{9},Pro^{10},*neo*-Trp^{11},tert-Leu^{12}Leu^{13}. The amino acid *neo*-Trp is a regioisomer of Trp.
Methods: We reviewed the NT69L scientific literature, which dates back to the year 2000.
Results & Discussion: NT69L has nanomolar affinity for hNTR1 and hNTR2. Like neuroleptics, NT69L blocks the effects of psychostimulants (D-amphetamine [D-AMP] [2], cocaine [2], or nicotine [3]). NT69L does not cause catalepsy, but will block catalepsy caused by the typical antipsychotic drug haloperidol [4]. NT69L blocks the rotational behavior caused by either D-AMP or apomorphine [APO] in the unilaterally, 6-hydroxydopamine-lesioned rat model of Parkinson's disease [5]. NT69L blocks the initiation and expression of sensitization to nicotine [3, 6]. Repeated injections of NT69L by itself has no effect on locomotor activity. In an operant-conditioning paradigm in which rats were trained to self-infuse nicotine contingent upon pressing a lever, we found that when rats were injected with NT69L, the frequency of lever pressing for nicotine reinforcement (infused i.v.) was markedly reduced compared to that for rats injected with saline (Boules et al., unpublished data). All these data support the hypothesis that a neurotensin receptor agonist, specifically NT69L, can also treat addiction to nicotine. Of note, we have shown that NT69L is not reinforcing in rhesus monkeys [7], allaying concerns that it could itself be abused. Like neuroleptics, NT69L blocks climbing behavior caused by high-dose APO [4]. Like neuroleptics, NT69L blocks drug-disrupted prepulse inhibition caused by D-AMP [8], and dizocilpine (MK-801) [8, 9]. It also blocks the disruption of prepulse inhibition caused by a serotonin 5-HT$_{2A}$ agonist and an α_1-adrenergic agonist [10]. These results with NT69L suggest that it affects DA, glutamatergic, serotonergic, and adrenergic systems. Like neuroleptics, NT69L enhances turnover of dopamine in the nucleus accumbens and prefrontal cortex of rat brain, similarly to clozapine, but different from haloperidol (Boules et al., unpublished data). Furthermore, similar to clozapine, NT69L exhibits minimal c-fos induction in the striatum, with elevated c-fos in the nucleus accumbens shell, posterior olfactory nucleus, cingulate cortex and significant elevation in the paraventricular nucleus in the hypothalamus [11]. Finally, our studies with mice lacking NTR1 or NTR2 suggest that NTR1 is the key NTR subtype for the antipsychotic-like effects of NT69L. References: 1. Nemeroff, C.B., Biol Psychiatry, 1980. 15(2): p. 283-302. 2. Boules, M., et al., Eur J Pharmac, 2001. 426(1-2): p. 73-76. 3. Fredrickson, P., et al., Brain Res, 2003. 979(1-2): p. 245-8. 4. Cusack, B., et al., Brain Res, 2000. 856(1-2): p. 48-54. 5. Boules, M., et al., Eur J Pharmac, 2001. 428(2): p. 227-33. 6. Fredrickson, P., et al., Eur J Pharmacol, 2003. 458(1-2): p. 111-8. 7. Fantegrossi, W.E., et al., Pharmacol Biochem Behav, 2005. 80(2): p. 341-9. 8. Shilling, P.D., et al., Behav Brain Res, 2003. 143(1): p. 7-14. 9. Hedley, L.R., et al., in Society for Neuroscience,CD-ROM. Program no. 9.5, 2002: Washington, DC. 10. Shilling, P.D., et al., Psychopharmacology (Berl), 2004. 11. Ambrose, C., et al., Society for Neuroscience, 2003. 847.17.

Panel Session
Cortical Dopaminergic Neurotransmission and Cognition: Neuroimaging and Physiologic Studies

Dopamine Modulation of Glutamate and GABA Responses in the Rat Prefrontal Cortex

Kuei-Yuan Tseng, Nicolas Mallet, Kathy Toreson, Barbara Lewis, Catherine Lemoine, Francois Gonon and Patricio O'Donnell*

Center for Neuropharmacology & Neuroscience, Albany Medical College, Albany, NY, USA

Background: Although the physiological actions of dopamine (DA) in the prefrontal cortex (PFC) have been extensively studied, the field is plagued with inconsistent and often controversial results. As most of the in vitro pharmacological studies to date have been conducted in slices from very young, prepubertal animals, we decided to address this issue by studying the actions of endogenously released DA in vivo in parallel with pharmacological manipulations in slices obtained from adult animals, focusing on actions on local cortical circuits including pyramidal neurons and interneurons, and on the effects of different DA receptors on glutamatergic and GABAergic transmission within the rat PFC.
Methods: In vivo intracellular and yuxtacellular recordings were conducted from rats anesthetized with chloral hydrate, assessing the responses of pyramidal cells and fast-spiking interneurons to mesocortical activation. Neurobiotin injection allowed us to identify the cell type being recorded. In vitro recordings using whole-cell patch clamp were conducted in pyramidal neurons and fast-spiking interneurons, assessing the responses to a variety of DA agonists and antagonists. Emphasis was placed on the modulation of local synaptic activity and the responses to local administration of NMDA and AMPA.
Results: Electrical stimulation of the ventral tegmental area evoked a persistent depolarization along with a reduction in cell firing in PFC pyramidal neurons. The same procedure elicited an increase in cell firing in fast-spiking interneurons recorded yuxtacellularly, during a time window that coincided with the firing suppression in pyramidal neurons. This suggests that although the pyramidal cell depolarization may be a direct effect of DA (mediated by D1 receptors), the firing suppression involves activation of interneurons. In PFC slices obtained from adult animals, activation of D1 DA receptors resulted in an increase in pyramidal cell excitability. D2 activation reduced pyramidal excitability and increased fast-spiking interneuron excitability. In pyramidal neurons, D1 agonists potentiated the effects of NMDA, not AMPA, glutamatergic activation and D2 agonists attenuated both NMDA and AMPA responses. The effect of D2 on NMDA was blocked by a GABA-A antagonist, suggesting again it was mediated by an activation of local interneurons.
Discussion: This work suggests that DA modulation of fast signaling in the PFC is characterized by two main components: a D1 enhancement of NMDA activity in pyramidal neurons, which is balanced by a D2-dependent depression of both non-NMDA and NMDA responses, and an activation of local circuitry interneurons. In this way, a phasic increase in DA could reinforce ongoing activity at the NMDA

level (enhancing strong inputs) while attenuating weak inputs, thereby providing a contrast-enhancement mechanism.

The Cortical D1 Receptor: A Biomarker for Cortical Dopamine Transmission?

Anissa Abi-Dargham*, Jesper Ekelund, Baskar Kolachana, Gordon Frankle, Diana Martinez, Raj Narendran, Daniel Weinberger and Marc Laruelle

Columbia University, New York, NY, USA

Our group has recently documented that patients with schizophrenia exhibit increased D1 receptor availability measured with PET and [11C]NNC 112 in the dorsolateral prefrontal cortex (DLPFC), and that this increase correlates with deficits in working memory (Abi-Dargham, J of Neuroscience. 2002) and negative symptoms (unpublished data). In addition prefrontal dopamine depletion results in upregulation of D1 receptors and increased [11C]NNC 112 binding in a rodent model of dopamine depletion (Guo, J Neuropsychopharmacology, 2003). Furthermore, ketamine induced cortical dopamine dysfunction in chronic recreational ketamine users is associated with increased [11C]NNC 112 binding (Narendran, Am J Psych, in press). In this study we hypothesized that carriers of the Val allele of the catechol-O-methyltransferase (COMT) gene, a polymorphism associated with lower cortical DA availability, will show an increase in [11C]NNC 112 binding compared to the carriers of the met allele. Methods: We obtained COMT genotypes in patients with schizophrenia (n=15), substance dependence (n=7), schizotypal personality disorder (n=2) as well as their healthy controls (n=16) who had undergone PET imaging with [11C]NNC112. In this overall group of subjects (n=40) we had 13 val/val, 3 met/met and 24 val/met. We compared [11C]NNC112 in the val/val group versus the other two genotypes (met/met and met/val) pooled together. [11C]NNC 112 specific to nonspecific equilibrium partition coefficient, V3", was measured with kinetic analysis using the arterial input function. Results: No group differences were noted in plasma clearance, plasma free fraction, or nonspecific distribution volume of [11C]NNC 112. V3" was increased across frontal cortical regions in the val/val group reaching significance in the medial (p=0.04) and orbito-frontal (p=0.01) cortex, but only at a trend level in the DLPFC (p=0.08). No differences were detected in the striatal regions. Discussion: These results support a prominent role for COMT in determining dopamine tone in cortical but not striatal regions. D1 receptor elevations across cortical regions in the val/val group might represent an upregulation to compensate for lower levels of intrasynaptic dopamine in the val/val group. This finding supports the use of D1 receptor as a biomarker for cortical dopamine transmission. We will discuss the implications of such a biomarker for guiding the treatment in schizophrenia and other disorders by selecting both the patients to treat and developing targeted therapeutic approaches aiming at enhancing cortical dopamine transmission, such as D1 agonists, norepinephrine transporter inhibitors or COMT inhibitors. Supported by Lieber Center for Schizophrenia Research.

PET Imaging of Dopamine Metabolism and Receptors in Cortex: Relation to Cognitive Function in Parkinson's Disease

Robert Innis*

NIMH, Bethesda, MD, USA

Disruptions of dopaminergic (DA) neurotransmission in neocortex are likely to have an important pathophysiologic role in cognitive deficits of several disorders, including schizophrenia and Parkinson's disease. Two developing hypotheses posit that optimal DA transmission has a U-shaped response curve (with maximal performance ay a midpoint of neurotransmission) and that inverse pathophysiologic relationship exists between cortical and subcortical DA transmission. Both of these hypotheses are based primarily on neurochemical and physiological studies in animals. Despite the generally low level of DA transmission in cortex, rapidly developing PET technologies allow these hypotheses to be tested in healthy subjects and patients with neuropsychiatric disorders. Robert Innis (NIMH) will review studies in his lab and others to measure presynaptic DA metabolism and postsynaptic receptors in healthy subjects and patients with Parkinson's disease. Two studies will be presented: 1) [18F]Fallypride is a high affinity D2 receptor tracer with minimal nonspecific binding to allow quantitation the low densities of D2 receptors in neocortex. This tracer can be used in healthy subjects to measure increased DA transmission stimulated by amphetamine and depletion of synaptic DA with the synthesis inhibitor AMPT (alpha-methyl-para-tyrposine). Ongoing studies in healthy subjects show that amphetamine (0.3 mg/kg PO) caused a 5-10% decrease of D2 radioligand binding in striatum, thalamus, and medial temporal; cortex. 2) Cognitive dysfunction in Parkinson's disease has been associated with decreased prefrontal DA metabolism measured with [18F]FDOPA. An on-going study will be reported on the use of both the presynaptic marker [18F]FDOPA as well as the postsynaptic D1 receptor probe [11C]NNC-112 in Parkinsonian patients with and without dementia.

Interactions of Prefrontal Function and Dopamine: Multimodal Neuroimaging of Genetic Modulation

Andreas Meyer-Lindenberg*

GCAP, NIMH, Bethesda, MD, USA

Background: Regulatory interactions of cortical activity and midbrain dopaminergic function are critically involved in reward, working memory, substance abuse and schizophrenia. Animal models suggest a primary role of prefrontal cortex in this regulation, and recent postmortem data suggest modulation of this interaction by a common human val(108/158)met functional polymorphism of the Catechol-O-methyltransferase (COMT) gene, which acts at the level of prefrontal dopamine signalling. We report on a series of studies characterizing the impact of genetic variation on prefrontal function and prefrontal-midbrain interactions in humans in vivo, using an multimodal neuroimaging approach.
Methods: Multitracer PET neuroimaging was used in 24 healthy individuals to measure cerebral blood flow (with [15O]-H2O) during a working memory task, and presynaptic dopaminergic function using the tracer 6-[18F]-DOPA (6-FD). 124 participants were studied during the same working memory (n-back) task using fMRI. Individuals were genotyped for 4 SNPs in COMT and haplotypes were estimated using Bayesian estimation. The impact of genetic variation on brain function and midbrain dopamine synthesis-functional interactions was assessed using the general linear model as implemented in SPM2.
Results: In the subjects studied multimodally, midbrain dopamine synthesis was specifically coupled to prefrontal rCBF. Confirming post-mortem results, COMT val carriers had higher midbrain dopamine than met homozygotes. Genotype had a qualitative impact on prefrontal-midbrain interaction: in met-homozygotes, higher midbrain dopamine was predicted by higher task activation and lower rCBF during the control state, while the inverse was true for val-allele carriers. In 124 subjects in which the impact of complex variation in COMT on function was studied in fMRI, previously described risk variants in the functional Val158Met (rs4680) polymorphism, a 2-SNP haplotype composed of rs4680 and a P2 promoter region SNP (rs2097603), as well as a 3-SNP haplotype overtransmitted to schizophrenic patients in Ashkenazi Jews could be dissociated functionally based on the patterns of inefficient prefrontal working memory response.
Discussion: These data demonstrate a dopaminergic tuning mechanism in prefrontal cortex during working memory and suggest a systems-level mechanism for cognitive and neuropsychiatric associations with COMT. We have developed a method to use haplotype information in neuroimaging and provide evidence that the nonlinear response of prefrontal neurons to dopaminergic stimulation is a

neural mechanism of a nonadditive genetic interaction. This work illustrates an in vivo approach to functional validation in brain of the biological impact of complex genetic variations within a gene.

Panel Session
Protein Trafficking and Therapeutic Mechanisms for Mood Disorders

G Protein Trafficking and its Relevance to Depression and Antidepressant Therapy

Robert J Donati, Justin Brown, John Allen, Jiang-Zhou Yu, Ghanshyam N Pandey and Mark M Rasenick*

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

While the presynaptic effects of antidepressant drugs are well documented, chronic treatment of cultured cells reveals a possible post-synaptic mechanism for these agents. Antidepressant treatment mobilizes Gsalpha from a cytoskeletal-associated, cholesterol-rich membrane domain into a compartment where signaling is facilitated. This occurs not only in cultured neural and glial cells, but in rats treated chronically with drugs or ECS. All classes of antidepressants share this effect, suggesting a common mechanism of action. Although some G proteins, such as Gq, appear to use lipid rafts as a platform for signaling, Gsalpha moves to lipid rafts in the initial phases of the desentization process. At any given time, about 20% of the plasma membrane Gsalpha is ensconsed in those rafts, and chronic antidepressant treatment reduces this number by up to 70%. Acute treatment, or chronic exposure to a number of control compounds, do not have this effect. The above data predict that, in depression, Gsalpha would be more likely localized to lipid rafts, where it shows diminished signaling capabilities. Brain samples (Frontal cortex and cerebellum from 10 depressed suicides and 10 non-psychiatric controls obtained from the Brain Collection Program at the Maryland Psychiatric Research Center) were analyzed for the proportion of Gsalpha in lipid rafts and the ratio of the proportion of raft:non-raft Gsalpha was nearly twofold higher in the suicides. This suggests that Gsalpha (and signaling through Gsalpha coupled receptors) is muted in depression. It is also noteworthy that Gsalpha is more tightly associated with cytoskeletal proteins, such as tubulin, when in lipid rafts. Treatment of cells with microtubule-disrupting drugs mimics chronic antidepressant treatment in promoting the exodus of Gsalpha from lipid rafts and augmenting adenylyl cyclase stimulation. The two treatments are not additive in their effects. It is hypothesized that a complex formed between tubulin and Gsalpha prevents stimulation of adenylyl cyclase when Gsalpha is in lipid rafts and the abrogation of this complex allows a more facile stimulation of adenylyl cyclase. The complex has been modeled using molecular docking paradigms and efforts are currently underway to design probes to disrupt the interface, promoting more efficient signaling through Gs-coupled receptors. This may also provide a novel approach to antidepressant therapy.

Regulation of G Protein-Coupled Receptors by Endocytosis

Mark Von Zastrow*

UCSF, San Francisco, CA, USA

Many G protein-coupled receptors undergo rapid endocytosis following ligand-induced activation, including catecholamine receptors implicated in antidepressant function. Recent progress in elucidating biochemical mechanisms that regulate GPCR endocytosis, and determine the specificity with which receptors traverse divergent membrane pathways after endocytosis, will be discussed. Potential implications for pharmacotherapy will be suggested.

Modulation of AMPA Glutamate Receptor Trafficking by Antimanic Agents New Avenues for Drug Development

Jing Du*, Yanling Wei, Cynthia Falke, Neil Gray, Steve Szabo, Peixiong Yuan and Husseini K Manji

Laboratory of Molecular Pathophysiology, NIMH/NIH, Bethesda, MD, USA

Clinical and preclinical studies suggest that the glutamatergic system may represent a novel therapeutic target for severe recurrent mood disorders. Increasing data have shown 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. Further supporting data are recent findings that Ampakines have an antidepressant efficacy in animal depression models and AMPA receptor antagonist attenuate several "manic-like" behaviors produced by amphetamine administration. 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 and GluR2. Chronic treatment of rats with therapeutically relevant concentrations of lithium or valproate reduced hippocampal synaptosomal GluR1 and GluR2 levels. The reduction in synaptic GluR1 and GluR2 by lithium and valproate was due to a reduction of surface GluR1 and GluR2 distribution onto the neuronal membrane as demonstrated by three independent assays in cultured hippocampal neurons. By contrast, imipramine, an antidepressant, which can trigger manic episodes, increased synaptic expression of GluR1 and GluR2 in hippocampus in vivo. In addition, 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. GluR1p845 phosphorylation was also attenuated in hippocampus from lithium-or valproate-treated animals in vivo. Furthermore, Sp-cAMP treatment reversed the attenuation of phosphorylation by lithium and valproate and also brought GluR1 and GluR2 back to the surface, suggesting that phosphorylation of GluR1p845 is involved in the mechanism of GluR1 and GluR2 surface attenuation. To further confirm the mechanism, we found that a synthetic peptide Tat- p845, which specifically reduces the phosphorylation of GluR1 at the PKA site and surface expression of GluR1, also attenuated GluR2 surface expression in cultured hippocampal neurons. Therefore, we postulated that blocking insertion of GluR1/GluR2 hetero-tetramers is the mechanism for effects of lithium and valproate on both GluR1 andGluR2. Other new drugs possessing antidepressant efficacy were also studied on their role to regulate AMPA receptor trafficking. Recent studies have shown that anticonvulsants, lamotrigine and riluzole have efficacy in the treatment of bipolar depressed patients. Therefore, we sought to determine the role of two anticonvulsants lamotrigine and riluzole on AMPA receptor trafficking. Hippocampal neurons (10 DIV) were treated with lamotrigine (20uM) and riluzole (2uM) for 3 days. We found that the agents with a clinical antidepressant profile, namely lamotrigine and riluzole significantly enhanced surface expression of GluR1and GluR2 and phosphorylation of GluR1 at p845 in cultured hippocampal neurons. These studies suggest that GluR1/2 trafficking may confer antidepressant/antimanic profiles to antidepressant and antimanic agents and regulation of glutamatergically mediated synaptic plasticity may play a role in the treatment of bipolar disorder, and raises the possibility that agents more directly affecting synaptic GluR1/GluR2 may represent novel therapies for this devastating illness.

Leveling Glutamate Release: A Presynaptic Path Toward Antidepressant Action

Maurizio Popoli*, Giambattista Bonanno, Valentina Barbiero, Roberto Giambelli, Luca Raiteri, Laura Musazzi, Maurizio Raiteri and Giorgio Racagni

Center of Neuropharmacology-Dept. Pharmacol. Sci, University of Milano, Milano, Italy

Background: Glutamatergic neurotransmission has been implicated in pathophysiology of mood disorders. Recent neuroimaging and

histopathological studies in brain of patients with major depression or bipolar disorder revealed morphometric or functional modifications in regions where glutamatergic neurons and synapses predominate. Abnormal elevation of glutamate neurotransmission and glutamate levels was found in cortical/limbic brain areas. In animal studies, chronic stress, a major factor in pathogenesis of mood disorders, was found to induce atrophy of apical dendrites in CA3 hippocampal neurons; stress blocks long-term potentiation (LTP) and facilitates depression (LTD). Conversely, antidepressants (AD) were shown to overcome the effects of stress on synaptic plasticity. We studied the action of AD on glutamate/GABA release and related presynaptic molecular mechanisms, by using superfused synaptosomes freshly purified from drug-treated rats.
Methods: Rats were acutely or chronically treated with three different AD (reboxetine, fluoxetine, desipramine, 10 mg/kg, 2 weeks). Synaptic terminals (synaptosomes) were Percoll-purified from hippocampus, and basal as well as KCl- or ionomycin-evoked release of endogenous glutamate and GABA was assayed by using a superfusion apparatus and reverse-phase HPLC (Raiteri and Raiteri, 2000). Expression of presynaptic proteins and protein-protein interactions were studied by Western blot (WB) and coimmunoprecipitation coupled to WB, respectively (Bonanno et al., 2005).
Results: Chronic, not acute, treatment with all drugs markedly reduced depolarization-evoked release of glutamate from hippocampal synaptosomes (from 25-50%), stimulated by 15/25 mM KCl, without affecting GABA release. Basal release of the two neurotransmitters was not affected. In synaptic membranes of chronically treated rats we found a marked reduction of the protein-protein interaction between syntaxin-1 and Thr286 phosphorylated-αCaM kinase II (an interaction previously proposed to promote neurotransmitter release) and a marked increase of the interaction between syntaxin-1 and Munc-18 (an interaction proposed to reduce neurotransmitter release). These modifications were due to a selective change of αCaM kinase II phosphorylation in the readily releasable pool of vesicles associated to presynaptic membranes (Bonanno et al., 2005). Furthermore, the expression level of the three proteins forming the core SNARE presynaptic complex was reduced.
Discussion: These findings indicate that stabilizing glutamate neurotransmission in hippocampus is a common effect of antidepressants, likely representing a component of the therapeutic action, and suggest these drugs might work by limiting excessive release of glutamate when this is induced by stressful neuronal activation. This effect would be reinforced by the alteration of the balance between excitatory and inhibitory neurotransmission, due to the selective effect on glutamate vs. GABA. This seems due to selective AD-induced modifications in the protein presynaptic machinery, suggesting that these proteins could be interesting targets for novel drugs. References: Raiteri L, Raiteri M (2000) Synaptosomes still viable after 25 years of superfusion. Neurochem Res 25:1265-1274. Bonanno G., Giambelli R., Raiteri L., Tiraboschi E., Zappettini S., Musazzi L., Raiteri M., Racagni G., Popoli M. (2005) Chronic antidepressants reduce depolarization-evoked glutamate release and protein interactions favoring formation of SNARE complex in hippocampus. J. Neuroscience 25:3270-3279.

Panel Session
5-HT$_6$ Receptors: New Targets for Neuropsychiatric Disorders

The 5-HT$_6$ Receptor: Filled with Promise or Promises Unfilled?
David L Nelson*

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

This presentation will provide an overview and update on our understanding of the properties and potential roles of the 5-HT$_6$ receptor. The first reports of the discovery of the 5-HT$_6$ receptor (the result of homology cloning) occurred between 1993 (rat) and 1996 (human). Early interest in the receptor was generated because of its restriction to the central nervous system and because pharmacologic studies showed that a number of different antidepressants and antipsychotics had high affinity for the 5-HT$_6$ receptor. For example, the atypical antipsychotic agents olanzapine and clozapine both showed high affinity for this receptor with Ki values in the 5-20 nM range. This led to the hypothesis that certain of the therapeutic properties of these (and other) molecules might be due their 5-HT$_6$ receptor antagonist properties. However, confirmation of that hypothesis has been slow in coming, complicated by the subtle changes produced by altering 5-HT$_6$ receptor function and by possible species differences. Continuing work over the past decade has seen a gradual increase in understanding possible roles for this receptor within the CNS, which has been aided by the development of selective pharmacologic tools. The 5-HT$_6$ receptor appears to be restricted in distribution to the CNS, minimizing concerns about peripheral side effects of 5-HT$_6$ targeted drugs. Localization within the CNS has been effected using a variety of techniques, including the measurement of mRNA, radioligand binding, and immunohistochemistry. There seems to be general agreement that areas of highest expression include the striatum, nucleus accumbens, and the olfactory tubercles, while moderate expression levels occur in structures such as the cerebral cortices, hippocampus, and striatum.
Potential Disease Targets: There has been much speculation about the 5-HT$_6$ receptor as a target for treating CNS disorders, based on preclinical observations. Proposed therapeutic targets include cognitive disorders, obesity, anxiety, depression, and schizophrenia. Continued development of novel antagonists and the recent development of selective agonists should contribute to further understanding the actions of this receptor. However, the potential roles for this receptor in the pathogenesis and/or treatment of disease continue to be elusive and subject to much debate. The discovery of the C267T receptor polymorphism in humans has led to a number of studies attempting to link this to disease susceptibility or treatment outcome, but these do not yet appear to have generated conclusive results.
Memory/Cognition: This is the area with, perhaps, the most convincing data for involvement of the 5-HT$_6$ receptor, i.e., antagonism of the 5-HT$_6$ receptor leads to improvements in learning and/or memory in preclinical models. These studies range from the use of antisense to knock down receptor expression to the use of selective 5-HT$_6$ receptor antagonists. Antagonist studies have shown improvements by themselves in selected measures of memory and have also been shown to reverse scopolamine-induced deficits in specific memory tests. These latter effects, along with microdialysis studies, suggest a role for the 5-HT$_6$ receptor in regulating the release of acetylcholine, a neurotransmitter intimately associated with cognition and memory. Recently, Saegis Pharmaceuticals has reported pursuing the 5-HT$_6$ receptor antagonist, SGS518, for the treatment of cognitive impairment associated with schizophrenia. Their report of the successful completion of a phase I clinical trial with this drug suggests that the near future should hopefully provide insights into the effects of selective 5-HT$_6$ receptor antagonism in humans.

5-HT$_6$ Receptors in Striatum Inhibit Reward-Motivated Learning
John F Neumaier*

Psychiatry, University of Washington, Seattle, WA, USA

Background: 5-HT$_6$ antagonists have well described pro-cognitive effects in several rodent models of learning and memory. For example, we found that 14 days of 5-HT$_6$ antagonist treatment improved social and object recognition in 15-18 month old rats and acute antagonist treatment reversed scopolamine impairment of memory consolidation of social and object recognition. While a pro-cholinergic mechanism has been suggested for this cognitive enhancement, the neuroanatomical and neurochemical basis for this has not been determined.

Methods: Since 5-HT_6 receptors are heavily expressed in the neostriatum, we decided to focus on this brain region by examining the behavioral consequences of targeted expression of this receptor. We constructed a viral vector that expresses both hemagglutinin (HA) tagged 5-HT_6 receptors and GFP from separate transcriptional cassettes using a well-characterized HSV system.
Results: This transgenic receptor was fully functional in an in vitro luciferase assay system. Furthermore, HA-5-HT_6 receptors were rapidly expressed after injection of the vector into rat striatum and increased protein levels were stable for 1-2 weeks. In order to focus on a learning task that is strongly dependent upon striatal function, we use a simple operant learning task with sucrose pellets as rewards. Male Long-Evans rats received bilateral vector injections in striatum with either HA-5-HT_6/GFP or GFP-only control vectors via bilateral guide cannulas placed earlier. Some rats received either sham or no operation as additional controls. Three days after viral vector infusion the animals began three consecutive daily trials in the operant task. All control rats learned this task during this period as indicated by significantly increased successful lever presses over time. However, animals with increased expression of 5-HT_6 receptors showed no improvement in learning from day to day, whereas daily treatment with SB-258585 (5 mg/kg/d, a 5-HT_6 antagonist) reversed the effects of 5-HT_6 overexpression so that the animals acquired the task normally. There were no obvious nonspecific effects of increased 5-HT_6 expression; additional learning and behavioral tasks are currently being examined.
Conclusions: This is the first evidence that 5-HT_6 receptors in the rat striatum alter the consolidation of learning in an operant task. The neurochemical mechanism of this effect is not yet known, but is the target of current experimentation. One possible explanation is that 5-HT_6 receptors in medium spiny neurons increase inhibitory tone on cholinergic striatal neurons, thereby impairing cholinergic function. Rather than simply impairing cognitive function, this hypothesis would suggest that striatal 5-HT_6 receptors support the stability of learned behaviors at the price of decreased flexibility in learning new behaviors.

Selective Serotonin 5-HT_6 Receptor Antagonist(s) for the Treatment of Obesity

Patrizia M Caldirola* and Jan Svartengren

Project Management, Biovitrum AB, Stockholm, Europe, Sweden

Serotonin plays an important role in central feeding regulation. The serotonin 5-HT_6 receptor antagonists Ro 04-6790 and SB271046 have been shown to reduce body weight and food intake in rats (1,2). Our studies show robust effects on both food intake and body weight in animal by potent and selective 5-HT_6 receptor antagonists generated by Biovitrum internal program. The compound(s) reduced food intake in SD rat but did not affect general motor activity and water intake at threshold doses for effect on food intake. Meal pattern analysis of the feeding data indicated prolongation of the inter-meal interval, but not meal size. This effect on food intake occurred at doses which did not affect total locomotor activity, rearing, forward and peripheral activity, or kaolin intake indicating the specificity of the anorectic effect. The hypophagic effect results in a sustain reduction of body weight in high fat diet-induced rats in a 4 week treatment by repeated administration of compound(s). These results support the involvement of the serotonin 5-HT_6 receptor in the regulation of feeding and body weight in rats. Our working hypothesis together with in vitro and vivo results will be disclosed during the presentation. 1 Woolley ML et al. (2001); 2 Woolley ML et al. (2004); 3Hirst WD et al. (2003).

A 14 Day, Dose Escalation, Double Blind, Randomized, Placebo-Controlled Study of SGS518 in Adult Patients with Schizophrenia

Ann C Neale*, Helen Jenkins, Diane Amend and Michael Lesem

Clinical, Saegis Pharmaceuticals, Inc., Half Moon Bay, CA, USA

Introduction: SGS518 is a selective 5 Hydroxytryptamine-6 (5 HT_6) receptor antagonist being developed as a treatment for Cognitive Impairment Associated with Schizophrenia (CIAS). Cognitive dysfunction has been recognized as underlying the psychopathology of, and contributing to, impaired social and vocational function in schizophrenia. As a result, there is great interest in developing treatments for CIAS.
Methods: This study will evaluate a 5HT6 antagonist (SGS518) in humans for treatment of cognition impairment associated with schizophrenia. Twenty (20) patients with a DSM IV diagnosis of schizophrenia were enrolled in a Phase II, 14 day randomized, placebo (P)-controlled, double blind study to evaluate the safety, pharmacokinetic profiles and preliminary effect of SGS518 on cognitive function. Patients were randomized into 1 of 2 cohorts of 10 subjects each (8 active: 2 placebo) and remained in residence for 15 days. Patients randomized to SGS518 in Cohort 1 received 60 mg QD for 7 days and then escalated to 180 mg QD for 7 days. Patients randomized to Cohort 2 received 120 mg QD for 7 days and then escalated to 240 mg QD for 7 days. Cognition Testing using the Computerized Brief Assessment of Cognition Tests (BACS) was conducted prior to dosing, Day 6 prior to escalation and after receipt of the last dose on Day 13. The total duration of treatment was 14 days. A computerized version of the Brief Assessment of Cognition Scale (BACS) consists of tests for Verbal memory, Digit Sequencing, Symbol Coding, Semantic Fluency, Letter Fluency and Tower of London. Difference from Baseline z scores were used as the outcome variable. The assessment of the safety and tolerability of the study agent compared the incidence and severity of adverse events, and changes in laboratory, ECG, EEG or physical exam findings between the combined group of subjects receiving placebo (up to 4 subjects) and the cohorts receiving the different doses of SGS518. Pharmacokinetic samples were obtained daily with serial samples collected on Day 0, Day 6 and Day 13 of dosing.
Results: 20 male subjects (mean age 37.3 years) with schizophrenia were enrolled with 18 of the 20 subjects completing the study. Concurrent treatment with anti-psychotic medication included: Risperdal, Zyperxa, Seroquel and Abilify. There were 10 of the 20 subjects (2 in cohort 1; 4 in cohort 2; 4 subjects in the placebo group) reporting a total of 17 adverse events (2 in cohort 1, 10 in cohort 2 and 5 in the placebo group). The majority of the adverse events in cohort 2 were reported when subjects were receiving the 240 mg dose. All adverse events (AEs) were mild and resolved within 24 hours after onset. No subjects discontinued from the trial due to an AE, and there were no deaths or serious adverse events.The mean AUC/dose results ranged from 37.3 to 46.8 hr ng/mL in a dose responsive fashion. The mean tmax was 2 hours for all doses except 240 mg (3 hrs) and the mean terminal elimination half life after 14 days of dosing was 12.34 hrs (SD 2.19) for cohort 1 and 12.12 hrs (SD 5.08) and consistent with the data seen in a prior healthy volunteer study. The approximate steady-state of plasma concentration for SGS518 was reached after 3 days of dosing without evidence of accumulation. The analysis of the BACS data was ongoing at the time of the abstract deadline and will be presented at the meeting.
Conclusions: This study demonstrated that SGS518 administered orally over 14 days to patients with schizophrenia on concomitant antipsychotic treatment was well-tolerated. SGS518 represents a promising new therapy for treatment of cognition impairment associated with schizophrenia and further studies are warranted.

Panel Session
Stress in Addiction Relapse: Are There Viable Treatment Targets?

Novel Pharmacological Treatment Targets to Address Stress-Induced Relapse: Insights from Animal Models

Friedbert Weiss*

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

Background: Stress is a major factor implicated in relapse to drug use. Research on the neural basis of stress-associated relapse has tra-

ditionally focused on the corticotropin-releasing factor (CRF) system. Substantial evidence implicates dysregulation of the extrahypothalamic CRF system as a common factor in the anxiogenic and aversive consequences of withdrawal from drugs of abuse as well as in vulnerability to relapse. Moreover, stress can augment the effects of drug-related environmental stimuli (i.e., another important factor implicated in provoking relapse) on drug-seeking behavior.
Methods and Results: Efforts to elucidate the neurobiological basis of stress-induced relapse have revealed novel potential substrates, including the nociceptin/orphanin FQ (N/OFQ) opioid peptide system, Group II metabotropic glutamate receptors (mGluR) and nitric oxide (NO) signaling. In behavioral tests utilizing animal models of relapse, central administration of N/OFQ dose-dependently attenuated reinstatement induced by both footshock stress and drug cues. The attenuation of stress-induced reinstatement was traced to a functional CRF antagonist action of N/OFQ within the bed nucleus of the stria terminalis (BNST), a key extrahypothalamic CRF site for stress-induced drug-seeking. The peptide's inhibitory effects on cue-induced reinstatement on the other hand appear to depend on its functional opiate antagonist action in the nucleus accumbens, as measured by the reversal of morphine-induced Fos expression. With respect to the second target, activation of Group II mGluRs by LY379268 (a potent mGlu2/3 agonist) also dose-dependently reversed reinstatement induced by both stress and drug cues. Subsequent brain mapping studies, using FOS expression as a marker of neural activation, implicate increased inhibitory GABAergic output from the central nucleus of the amygdala (CeA) in the anti-stress actions of Group II mGluR activation, and attenuation of hippocampal contextual information processing in the reversal of cue-induced reinstatement. Lastly, nitric oxide (NO) signaling is thought to regulate aspects of the reinforcing actions of cocaine. We examined whether NO-dependent neurotransmission plays a similar role in the addictive actions of ethanol, as measured in terms of ethanol's primary reinforcing effects and the conditioned effects of ethanol-related contextual stimuli. L-NAME dose-dependently attenuated conditioned reinstatement induced by an ethanol cue without altering the drug's primary reinforcing effects. Follow-up studies with a neuronal NO-selective inhibitor (N-propyl-l-arginine) identified the paraventricular nucleus of the hypothalamus (PVN) as a critical site for the effects of NOS inhibition on conditioned ethanol-seeking.
Discussion: The results implicate Group II mGluRs, the N/OFQ system and NO signaling as promising targets for further scrutiny with respect to treatment drug potential. Moreover, considering that NO synthesis in the PVN suppresses HPA axis responses to stress, the data suggest that cue-induced alcohol-seeking requires HPA axis activation. This possibility is consistent with emerging evidence that environmental cues previously paired with drug availability acutely activate the HPA axis and result in elevated plasma levels of corticosterone, as well as with evidence that there is overlap between the effects of stress and drug cues in eliciting craving responses. (Supported by NIH/NIAAA AA10531, AA014351 and NIH/NIDA DA07348, DA017097).

The Role of Stress in Smoking Relapse: That's So Yesterday
Saul Shiffman* and Andrew Waters

Psychology, University of Pittsburgh, Pittsburgh, PA, USA

Background: Most efforts to stop smoking end in relapse. We studied the effect of stress and negative affect (NA) on smokers' initial lapses to smoking. Retrospective reports indicated that lapses occur in negative affect situations. We used Ecological Momentary Assessment to collect data just after a smoking lapse, and comparative data on other situations. We also prospectively analyzed stress and affect prior to lapses to assess the role of stress prior to the lapse and to contrast the role of slow-changing levels of day-to-day tonic stress and of fast changes in phasic stress in the hours and minutes preceding lapses.
Methods: Subjects were 215 smokers who quit smoking without pharmacological treatment. They carried a palmtop computer electronic diary (ED), monitoring smoking and affect for 4 weeks after quitting. Subjects used ED to report initial lapses and temptation episodes, identifying the triggering stimulus, distinguishing negative affect and stress from other triggers (e.g., smoking cues, positive affect). Additionally, ED assessed subjects at random times. At each time, ED assessed negative affect (NA). Each day, subjects completed a Perceived Stress Scale (PSS), and reported any negative life events (NLEs).
Results: We assessed whether lapse situations were associated with NA, compared to (a) temptation situations and (b) a randomly-selected occasion. In within-subject analyses, lapses were associated with significantly greater NA (57.2 T-score) compared to both randomly-selected occasions (50.0, $t=6.0$, $p<0.0001$) and temptations (52.8, $t=3.3$, $p<0.002$). 32% of lapses were said to be triggered by stress or negative affect. We used survival analysis to assess the effect of stress and NA on lapse risk, examining how each day's stress affects lapse risk the next day. Neither PSS (OR=0.72, ns), nor NLEs (OR=0.75, ns), nor average NA (OR=0.99, ns) was associated with lapse risk the next day. Among subjects who lapsed, there was no trend for PSS, NLEs, or NA to increase over the 4 days preceding the lapse, whether the lapse was triggered by stress or not. Thus, analyses consistently showed no effect of prior days' stress or NA on the risk of lapsing. Looking at more proximal influences, we assessed trends in NA on the lapse day itself, over the hours preceding the lapse. For stress-triggered lapses only, NA increased starting 6 hours before the lapse ($b=0.28$, $p<0.001$). (Non-stress-triggered lapses did not show this trend ($b=-0.02$, ns), and there was a significant interaction between time and the lapse trigger ($b=0.10$, $p<0.003$).) No similar trends were observed on the day prior to the lapse ($b=0.00$, ns). Individual subjects' time series showed that some subjects experienced gradual increases in NA leading up to the lapse, while others experienced precipitous spikes in NA.
Discussion: NA and stress have been proposed as influences on smoking lapses. Because recall introduces bias, we collected real-time and prospective data on stress and lapses. The data confirmed that lapse situations are marked by NA, even compared to temptation situations. However, stress and NA on prior days did not influence lapse risk. In contrast, NA was rising in the hours and minutes preceding the lapse. Thus, acute stressors and volatile affect - and not slowly-changing background stress - are important in precipitating lapses. This suggests that tonic withdrawal effects do not account for these effects. The role of nicotine withdrawal in enhancing reactivity to acute stressors should be explored, as should the role of pharmacotherapy in buffering smokers from acute stressors.

Lofexidine Attenuates Stress Induced Drug Craving and Arousal to Improve Stress-Induced Relapse Outcomes
Rajita Sinha*

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

Background: Stress is a major factor in addiction relapse. However, it has been difficult to identify specific responses that increase stress related relapse risk in clinical populations. The focus of our research is on the examination of stress related responses that increase alcohol and drug craving and relapse risk in addiction. Having identified measures that are associated with relapse risk, the goal is to develop new treatments that would attenuate the effects of stress-induced drug craving and reduce relapse risk in addiction.
Method: Human laboratory and functional brain imaging studies were conducted with recently abstinent, treatment-engaged alcohol and cocaine abusing individuals and matched healthy volunteers to examine subjective, physiological and hormonal responses during brief imagery exposure to personalized scripts of stressful, drug/alcohol cue-related and neutral-relaxing situations. Addicted samples were followed for 90 days after completion of treatment to assess relapse outcomes. A sample of naltrexone-treated opiate addicts were randomized to Lofexidine (alpha 2-adrenergic agonist) or placebo and their laboratory responses to stress-related opiate craving and stress related relapse were also assessed.

Results: Findings indicate stress and drug cues increase drug craving, negative emotions and psychobiological stress responses in addicted samples. Addicted individuals show a sensitized stress-induced craving and negative emotional responses and higher basal sympathetic responses. Stress-induced drug and alcohol craving predicts time to relapse in cocaine and alcohol dependent individuals. Furthermore, preliminary data suggests that Lofexidine attenuates stress-induced and drug cue-induced craving in the laboratory and improves opiate relapse outcomes.
Conclusion: The results from a series of studies suggest that stress-related changes in the motivation to use alcohol and drugs, and not anxiety or distress levels per se, accounts for the association between stress and time to drug relapse after a defined period of abstinence. Behavioral and pharmacological treatment development efforts to reduce stress-induced drug craving and regulate the distress state could be of benefit in improving addiction relapse outcomes. (Supported by NIH/NIAAA R01-AA013892 and NIH/NIDA P50-DA16556, R01-DA11077, R01-DA18219).

Stress and Drug Cues Motivate Smoking Relapse: Are There Neurobiological Substrates That Account for This Association?
Raymond Niaura*

Butler Hospital, Providence, RI, USA

Background: Stress, negative afftective states, and exposure to drug use cues are strongly asssociated with relapse to drug use. A variety of competing and complementary theories have sought to articulate the neubiological substrates of this association, with varying degrees of success.
Methods: These theories, and supporting evidence, will be selectively reviewed, with an aim toward integration. There will be a focus on nicotine dependence as an examplar of the myriad processes involved, and evidence that pharmacologic interventions can ameliorate the association between stress and relapse will be reviewed. Results from a large-scale placebo controlled randomized trial of bupropion will be presented. As will the results of laboratory studies examining whether nicotine administration and various nicotine replacement therapies reduce negative affective responses to smoking cues.
Results: Bupropion's effects on smoking cessation is associated with its ability to reduce negative affect (CES-Depression scale; withdrawal symptoms). However, at least for withdrawal symptoms, these effects are moderated by genetic variation in the D2 receptor gene (effect evident for those possessing the DRD2-Taq1 A2/A2 genotypes. Acute nicotine administration (smoking or nicotine patch) reduced craving and negative affect, but slower release formulations did not.
Discussion: Effects of different smoking cessation agents may operate through different mechanisms that may have to do with particular genetic vulnerability and modes of administration.

Thursday, December 15

Panel Session
Selecting Animal Models to Predict Pro-Cognitive Effects in Human Populations

Predictive Validity of Rodent Models of Learning and Memory
John G Csernansky*, Hongxin Dong, Maureen V Martin, Mark E Bardgett and Deanna Barch

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

Background: There is growing interest in the development of drugs to improve the cognition of patients with neuropsychiatric disorders. While there is an abundant literature on effects of psychotropic drugs on cognitive behaviors in rodents, especially behaviors related to learning and memory, the utility of data obtained in rodents for predicting the efficacy of such drugs in patients with neuropsychiatric disorders remains uncertain. In part, this uncertainty is due to 1) the difficulty of validating rodent models of neuropsychiatric disorders, and 2) species differences in the neuroanatomical structures that subserve certain cognitive behaviors.
Methods: Transgenic mice that overexpress amyloid precursor protein (Tg2576) were evaluated as a model of Alzheimer disease (AD), while administration of the non-competitive NMDA receptor antagonist, MK-801, to mice was evaluated as a model of the cognitive deficits of schizophrenia. Reversal learning in a water T-maze was used as test of working memory in rodents, and a fear conditioning paradigm was used to test contextual reference memory. In order to identify the neuroanatomical structures involved in working and reference memory in human subjects with and without schizophrenia, fMRI images were collected during a 2-back version of the n-back test and an intentional encoding task, respectively.
Results: In Tg2576 mice aged 9-10 months, three cholinesterase inhibitors, physostigmine, donepezil and galantamine, all improved reversal learning and contextual memory in a dose-dependent manner (Dong, et al, 2005). In mice with MK801-induced deficits in reversal learning and contextual memory, physostigmine and donepezil, but not galantamine, showed ameliorative effects (Csernansky, et al, 2005). fMRI studies in human subjects with and without schizophrenia engaged in tasks of working and reference memory revealed activation of an overlapping set of neuroanatomical structures that included the prefrontal cortex and medial temporal lobe (Barch, et al, 2002). In contrast to this neuroanatomical pattern, learning and memory in rodents has been shown to depend more narrowly on the integrity of medial lobe structures.
Discussion: The effects of the three cholinesterase inhibitors observed in Tg2576 mice are consistent with the demonstrated efficacy of such drugs in AD patients. The observed effects of the three cholinesterase inhibitors in mice with MK801-induced deficits in working and contextual memory suggests that such drugs should also have efficacy for ameliorating memory deficits in patients with schizophrenia. However, recent trials of such drugs in patients with schizophrenia have been disappointing. The shortcomings of current rodent models of the cognitive deficits of schizophrenia will be discussed, and recommendations for the improvement of such models will be made. References: Barch DM, et al. 2002 Working and long-term memory deficits in schizophrenia: Is there a common underlying prefrontal mechanism? J Abnormal Psychology 111:478-494. Dong HX, et al. 2005 Acetylcholinesterase inhibitors ameliorate behavioral deficits in the Tg2576 mouse model of Alzheimers disease. Psychopharmacol DOI: 10.1007/s00213-005-2230-6,. Csernansky JG, et al. 2005 Cholinesterase inhibitors ameliorate behavioral deficits induced by MK-801 in mice. Neuropsychopharmacol DOI: 10.1038/sj.npp.1300761. Supported by R01-MH60883 and P20-MH071616.

Neuropsychopharmacological Models of Attentional Dysfunction and Their Translation to the Clinic
Trevor W Robbins*

Experimental Psychology, University of Cambridge, Cambridge, United Kingdom

Background: Constructs of attention and vigilance are central to current attempts to define domains of cognitive dysfunction in disorders such as schizophrenia and initiatives like the NIMH MATRICS project. In rats and monkeys there are now several paradigms for assessing different aspects of attentional function which have promise in terms of translation to clinical application. This talk reviews some of the methods currently available, including one well-established task and one recently devised, with illustrative data, where appropriate.
Methods: Attentional set-shifting tasks are well-established in humans and monkeys, but have only recently been introduced for the

rat. Thus far however, this paradigms has been used effectively not only to produce data that parallels that obtained in primates, but also to provide potentially definitive new information. The 5 choice serial reaction time task (5CSRTT) in rats has affinities to continuous performance tests for humans and measures basic processes such as attentional selection, sustained attention and inhibitory control. Its neural basis is quite well-established, implicating defined fronto-striatal systems for various aspects of performance. In recent studies, the behavioral neuropharmacology of this task has been considerably extended, using central infusions of agents affecting gabaergic, glutamatergic, monoaminergic or cholinergic mechanisms, infused either into the prefrontal cortex (PFC) or nucleus accumbens (Nac). A novel task for rats for assessing inhibitory control, the stop signal reaction time (SSRTT), has also been validated using lesion studies and a pharmacological approach that compares effects of several agents currently used for medication of attention deficit /hyperactivity disorder (ADHD).

Results: Performance in long sessions on the 5CSRTT with fast event rates was particularly susceptible to ACh loss from the PFC produced by 192 IgG-saporin, whereas cortical NA loss produced by saporin dopamine beta-hydroxylase was associated with deficits early in the session. I will also describe new data showing effects of manipulations of GABA, NMDA, dopamine (DA) and 5-HT receptors in the PFC, and as well as (for DA and 5-HT) within the Nac. Behavioral deficits produced by PFC lesions were remediated by intra-accumbens infusions of the D2 receptor antagonist sulpiride. For the SSRTT, new comparative effects of a number of agents including methylphenidate and modafinil will be presented, showing selective dose-dependent speeding of the stop reaction time under certain conditions, as in human studies of both healthy volunteers and patients with attention deficit/hyperactivity disorder (ADHD).

Discussion: The problems of extrapolating across species when studying attentional functions will be discussed in the context of the paradigms discussed. Where possible, data from the tests in rats or non-human primates will be compared directly with the performance of human patients on analogous or identical tests, with a view to establishing criteria for task validation.

Evaluating Sensorimotor Gating Measures in Animals as Predictors of Pro-Cognitive Effects in Humans

Mark Geyer* and Jacob Ong

Dept Psychiatry, Univ California Sch Med, La Jolla, CA, USA

The NIMH-funded MATRICS program has ushered in a new era in the development of treatments for cognitive deficits in schizophrenia, independently of treating psychotic symptoms. Compounds to be used as co-treatments in schizophrenia patients already treated with antipsychotic drugs (APDs) may now be registered. Animal models having predictive validity for identifying existing APDs, including first generation compounds, would not appear to be useful here, as existing APDs do not ameliorate the cognitive deficits in schizophrenia and most patients will already be treated with APDs. Rather, models that identify compounds that specifically reduce cognitive deficits are needed. Animal paradigms exist to assess some of the major domains of cognitive deficits in schizophrenia: attention/vigilance; working memory; learning; speed of processing; and social cognition. Although prepulse inhibition (PPI) cannot be considered to be a cognitive process per se, abnormalities in pre-attentive information processing may be predictive of or even lead to complex cognitive deficits. Rodent models mimicking the deficits in PPI seen in schizophrenia, bipolar mania, and other disorders are used to identify APDs. Indeed, both first and second generation APDs reliably block the PPI deficits induced by dopamine agonists. Hence, the dopamine agonist PPI model is not useful in identifying cognitive enhancers of relevance to schizophrenia. In contrast, the PPI deficits caused by NMDA antagonists, like the exacerbation of symptoms they produce in patients, are insensitive to first generation APDs but are attenuated by clozapine. Similarly, PPI deficits in schizophrenia patients, like cognitive deficits, are not reversed by first generation APDs but may be attenuated by clozapine. Hence, treatment-induced reversals of deficits in PPI produced by NMDA antagonists may provide animal, and human, models to aid in the discovery of treatments of cognitive deficits in patients already treated with existing APDs. The mood stabilizer lamotrigine reduces both the psychological effects of ketamine in humans and the PPI-disruptive effects of ketamine in mice, and is being examined in bipolar mania and as a co-treatment with APDs in schizophrenia. To assess the effects of such anticonvulsant or mood stabilizing compounds in dopaminergic and glutamatergic PPI models, we compared the ability of phenytoin, carbamazepine, valproate, and lithium to reduce the PPI-disruptive effects of 10 mg/kg amphetamine or 100 mg/kg ketamine in 129SvPasIco mice. Standard procedures for PPI testing were used and yielded the following results: 1) 85 mg/kg lithium chloride prevented amphetamine- but not ketamine-induced PPI deficits in both 129SvPasIco and C57BL/6J mice; 2) 50 mg/kg carbamazepine prevented ketamine- but not amphetamine-induced PPI deficits; 3) 100 mg/kg valproate prevented neither amphetamine- nor ketamine-induced PPI deficits; 4) 30 mg/kg phenytoin prevented neither amphetamine- nor ketamine-induced PPI deficits but increased PPI on its own. If pro-cognitive effects in patients are predicted by differential effects in the dopamine (amphetamine) vs NMDA (ketamine) PPI models, further studies of carbamazepine and lamotrigine as possible co-treatments with APDs may be warranted. Given the absence of positive control compounds having known efficacy in the treatment of cognitive deficits in schizophrenia or bipolar disorder, especially when used as co-treatments with existing APDs, it is premature to establish the predictive validity of related animal models. Nevertheless, the NMDA antagonist PPI model may have heuristic value in this context due to its construct validity related to cognitive deficits in attention and information processing.

Serotonergic Neuromodulation of Behavioral and Cognitive Functions in Humans

Franz X Vollenweider*, Philipp A Csomor, Martin Paulus and Mark A Geyer

Psychiatry, University of Zurich, Zurich, Switzerland

Background: Recent preclinical and clinical studies suggest that hallucinogenic 5-HT2A/1A receptor agonists such as psilocybin may provide a useful model to investigate novel pathophysiologic hypotheses of schizophrenia (Aghajanian 2000, Vollenweider FX, 2001). Specifically, it was found 1) that psilocybin mimics certain positive symptoms of schizophrenia in normals, 2) alters brain activity in cortico-striato-thalamic pathways found to be implicated in the pathophysiology of schizophrenia, and 3) disrupts prepulse inhibition (PPI) a measure of sensorimotor gating in rodents (Vollenweider and Geyer 2001). However, virtually nothing is known about the cognitive effects of hallucinogenic 5-HT2A/1A agonists in normals. Given the increasing evidence that 5-HT2A and 5-HT1A receptors are critical in the modulation of cognitive processes such as working memory and learning, the aim of this study was 1) to investigate whether psilocybin produces comparable cognitive deficits as we recently found in unmedicated first episode schizophrenia and 2) whether the degree of cognitive deficits could be related to measures of psychopathology or alterations in sensorimotor gating.

Methods: The dose-response effects of psilocybin on cognitive performance and prepulse inhibition (PPI) of the startle reflex was investigated in healthy volunteers using double-blind, placebo-controlled study designs. In study 1 and 2, each subject received in counter-balanced order either placebo or psilocybin (115, 215, or 315 μg /kg PO) on 4 different occasions. Cognitive performance was assessed using a set of tasks of the Cambridge Neuropsychological Test Automated Battery(CANTAB). Startle measures and prepulse inhibition was assessed using various prepulse-pulse lead intervals (30, 60, 120, 240,

and 2000 ms). The data were analyzed using an ANOVA with repeated measurements. In study 3, subjects received placebo and psilocybin (215 μg/kg PO) and decision-making in the presence of uncertainty was assessed using a two-choice prediction task while regional brain activity was indexed by H2O-PET. Data were analyzed using SPM 99, PMOD and ANCOVA.
Results: In study 1 (n=16), psilocybin dose-dependently impaired sustained attention (RVP task), pattern recognition memory (PRM task), working memory (SP task), and paired associates learning (PAL task), but had no significant effect on planning (SOC task) compared to placebo. In study 2 (n=16), psilocybin was found to disrupt PPI at short lead intervals (30 msec), but had no effects at medium (120 msec) or opposite effects a long lead intervals (240 msec). In study 3 (n=10), psilocybin hampered brain activation bilaterally in inferior prefrontal, ventromedial and ventrolateral cortex, anterior cingulate, right insular and temporal cortex and concomitantly impaired decision-making in a two-choice prediction task.
Conclusion: The pattern of psilocybin-induced cognitive deficits including impairments in attention, pattern recognition, working memory, and decision-making is strikingly similar to those we recently found in unmedicated first episode schizophrenia patients showing comparable deficits on these tasks but virtually no impairments in planning functions (Vollenweider et al 2005, in prep.). Moreover, together with previous findings the present results also suggest that psilocybin-induced decision-making deficits depend on inferior prefrontal cortical functioning while working memory deficits may be linked to aberrant dorsolateral prefrontal activation. The data provide additional evidence that psilocybin may provide a useful probe to further investigate the neurobiology of cognitive deficits in psychotic states that are also relevant to schizophrenic psychoses.

Panel Session
Synaptic Plasticity: Focus on the Mechanisms and Clinical Implications of Long-Term Depression

Regulation of AMPA Receptor Function and Synaptic Plasticity

Richard L Huganir*

Department of Neuroscience, Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Neurotransmitter receptors mediate signal transduction at the postsynaptic membrane of synaptic connections between neurons in both the central and peripheral nervous systems. We have been studying the molecular mechanisms in the regulation of neurotransmitter receptor function. Recently we have focused on glutamate receptors, the major excitatory receptors in the brain. Glutamate receptors can be divided into two major classes: AMPA and NMDA receptors. AMPA receptors mediate rapid excitatory synaptic transmission while NMDA receptors play important roles in neuronal plasticity and development. Studies in our laboratory have found that both AMPA and NMDA receptors are multiply phosphorylated by a variety of protein kinases. Phosphorylation regulates several functional properties of these receptors including conductance and membrane targeting. For example, phosphorylation of the GluR1 subunit of AMPA receptors by multiple kinases including PKA, PKC and CaM kinase II regulates its ion channel function. The phosphorylation of AMPA receptors is regulated by neuromodulators such as dopamine, norepinephrine and serotonin as well as by neuroactive agents such as cocaine and Prozac. In addition, we have demonstrated that the phosphorylation of AMPA receptors is regulated during cellular models of learning and memory such as hippocampal long-term potentiation (LTP) and long-term depression (LTD). Moreover, phosphorylation of GluR1 is required for the expression of these forms of plasticity and for the retention of spatial memory. We have also recently shown that phosphorylation of the AMPA receptor GluR2 subunit is required for LTD in cerebellar Purkinje cells. We have also been examining the mechanisms of the subcellular targeting and clustering of glutamate receptors at synapses. We have identified a variety of proteins that directly or indirectly interact with AMPA and NMDA receptors. A novel family of proteins that we call GRIPs (Glutamate Receptor Interacting Proteins) directly bind to the C-termini of the GluR2/3 subunits of AMPA receptors. GRIPs contain seven PDZ domains, protein-protein interaction motifs, which crosslink AMPA receptors to each other or link them to other proteins. In addition, we have found that the C-termini of GluR2 also interacts with the PDZ domain of PICK1, a protein kinase C-binding protein that is found at excitatory synapses. Finally, the GluR2 subunit also interacts with the NSF protein, a protein involved in the regulation of membrane fusion events. These AMPA receptor interacting proteins are critical in the proper subcellular trafficking and synaptic targeting of these receptors. We have recently shown that the binding of PICK1 is required for cerebellar LTD. In summary, we have examined the molecular mechanisms underlying the regulation of neurotransmitter receptor function. Our studies have suggested that regulation of receptor function may be a major mechanism for the regulation of synaptic plasticity in the nervous system and may be an important determinant of animal behavior.

Distinct Triggering and Expression Mechanisms Underlie LTD of AMPA Receptor and NMDA Receptor Synaptic Responses

Robert Malenka*, Wade Morishita and Helene Marie

Psychiatry and Behavioral Sciences, Stanford Medical School, Palo Alto, CA, USA

Background: Although the mechanisms underlying long-term depression (LTD) of AMPA receptor-mediated postsynaptic currents (AMPAR EPSCs) have been extensively examined, little is known about the mechanisms responsible for LTD of NMDA receptor (NMDAR)-mediated EPSCs. This is particularly surprising given that NMDAR-dependent LTD in the CA1 region of the hippocampus is accompanied by a large decrease in NMDAR EPSCs.
Methods: Whole-cell voltage clamp recordings were made from CA1 pyramidal cells in acute hippocampal slices prepared from 3-4 week old Sprague-Dawley rats. Drugs and peptides were added to the whole cell pipette solution and allowed to diffuse into CA1 cells for 15-30 minutes. NMDAR EPSCs were recorded at -40 mV in the presence of AMPAR and GABA-A receptor antagonists. AMPAR EPSCs were recorded at -65 mV in the presence of NMDAR and GABA-A receptor antagonists. LTD was induced with a 5 Hz-3 minute train at -40 mV.
Results: LTD of NMDAR EPSCs was blocked by loading cells with the calcium chelator BAPTA or application of the NMDAR antagonist D-APV during the LTD induction protocol. These results indicate that like LTD of AMPAR EPSCs, this form of LTD requires an NMDAR-mediated rise in postsynaptic calcium. Similar to LTD of AMPAR EPSCs, loading cells with inhibitors of protein phosphatase 1 (PP1) blocked LTD of NMDAR EPSCs. However, in contrast to LTD of AMPAR EPSCs, loading cells with calcineurin (PP2B) inhibitors had no effect on LTD of NMDAR EPSCs. Because LTD of AMPAR EPSCs involves dynamin-dependent endocytosis of synaptic AMPARs, we examined the effects of inhibiting dynamin on LTD of NMDAR EPSCs. We found that three different inhibitors of dynamin, including in vivo viral mediated expression of a dominant negative version of dynamin, had no effect on LTD of NMDAR EPSCs while clearly inhibiting LTD of AMPAR EPSCs. In contrast, pharmacological manipulations of the actin cytoskeleton, which prevent actin depolymerization, blocked LTD of NMDAR EPSCs but did not affect LTD of AMPAR EPSCs.
Discussion: These results suggest that the same pattern of afferent activity elicits depression of AMPAR- and NMDAR-mediated synaptic responses by means of distinct triggering and expression mechanisms. LTD of AMPAR-mediated synaptic responses involves activa-

tion of a protein phosphatase cascade leading to endocytosis of AMPARs. In contrast, LTD of NMDAR EPSCs does not involve dynamin-dependent endocytosis of NMDARs but rather calcium-dependent depolymerization of the actin cytoskeleton, perhaps via dephosphorylation of the actin depolymerizing factor cofilin. Understanding the detailed molecular mechanisms by which AMPARs and NMDARs are independently regulated by activity holds great promise for the development of novel therapeutic agents, which will selectively modify different forms of synaptic plasticity.

Long-Term Synaptic Plasticity at Excitatory and Inhibitory Synapses on Dopamine Neurons of the VTA

Julie A Kauer*, Fereshteh S Nugent and Esther C Penick

Molecular Pharmacology, Physiology and Biotechnology, Brown University, Providence, RI, USA

The best characterized cellular mechanisms proposed to underlie information storage in the CNS are long-term potentiation and long-term depression (LTP and LTD). It is well established that excitatory glutamatergic synapses are strengthened or weakened in response to specific patterns of synaptic activation, and considerable evidence has accumulated suggesting that such changes in synaptic strength occur during learning or other adaptive responses to environmental stimuli. Drugs of abuse cause dopaminergic cells in the ventral tegmental area (VTA) to release dopamine in downstream target areas. Recent work has shown that glutamatergic synapses on dopamine neurons in this region undergo LTP, and multiple drugs of abuse trigger LTP at these excitatory synapses. We have characterized LTD at excitatory synapses on VTA dopamine neurons; we have also begun to examine a novel form of synaptic plasticity at GABAergic synapses onto VTA dopamine cells. Midbrain horizontal slices were prepared and dopamine neurons were recorded from using standard whole-cell patch clamp techniques. Excitatory synaptic currents (EPSCs) were evoked once every 10 seconds in the presence of a GABAA antagonist. After a stable recording period of at least 10 minutes, the neuron was depolarized to -40 mV and afferent stimulation increased to 1 Hz for six minutes. This induced long-term synaptic depression (71% of control EPSC amplitude). LTD was entirely blocked by bath application of amphetamine through mechanisms that involve D2 receptors and PKA. There are far fewer examples of synaptic plasticity of fast GABAergic synapses compared with those at excitatory synapses. GABAergic neurons comprise up to 35% of VTA neurons and act as local interneurons to inhibit VTA dopaminergic neurons and also provide important projections to the nucleus accumbens and prefrontal cortex. The normal functioning of GABAergic synapses in the ventral tegmental area is also altered as a result of in vivo exposure to drugs of abuse, and at least two major classes of addictive drugs, opioids and ethanol, exert potent, direct effects on GABAergic synaptic transmission. We therefore investigated long-term potentiation of GABAA mediated inhibitory synaptic currents (IPSCs), using whole-cell recordings from VTA slices. IPSCs were isolated by recording in the presence of strychnine (1 μM) and DNQX (10 μM). High frequency stimulation (100 Hz, 1 second, repeated twice; HFS) induced LTP of IPSCs onto DA cells (146 +/- 21% of baseline values 20 minutes after HFS, n=17). The paired pulse ratio (PPR) was significantly decreased 20 minutes after HFS, consistent with an increase in presynaptic GABA release. This is the first report of synaptic plasticity of GABAergic synapses in the VTA. Morphine has previously been reported to depress GABAergic synaptic transmission in the VTA (Johnson and North, 1992). We found that morphine (1 μM) not only depressed IPSCs but also blocked LTP induction at GABAergic synapses (n=10). The μ-opioid receptor agonist, DAMGO (1 μM) also blocked LTP (n=15). Thus, opioids promote the excitability of DA neurons by two mechanisms; a depression of GABA release onto DA neurons, and a blockade of IPSC-LTP that will further decrease the inhibition of DA neurons, a potentially important new mechanism by which opioid drugs alter the function of this brain area essential for addiction. Supported by DA11289, NARSAD and F32 DA 018023.

The mGluR Theory of Fragile X Mental Retardation

Mark Bear*

Brain & Cognitive Sciences, MIT, Cambridge, MA, USA

Many of the diverse functional consequences of activating group 1 metabotropic glutamate receptors require translation of pre-existing mRNA near synapses. One of these consequences is long-term depression (LTD) of transmission at hippocampal synapses. Loss of fragile X mental retardation protein (FMRP), the defect responsible for fragile X syndrome in humans, increases LTD in mouse hippocampus. This finding is consistent with the growing evidence that FMRP normally functions as a repressor of translation of specific mRNAs. A theory has been developed that can account for diverse neurological and psychiatric aspects of fragile X syndrome, based on the assumption that many of the protein-synthesis-dependent functions of metabotropic receptors are exaggerated in fragile X syndrome. The theory suggests new direction for basic research as well as novel therapeutic approaches for the treatment of humans with fragile X, the most frequent inherited cause of mental retardation and an identified cause of autism.

Panel Session
Advances in Functional Neuroanatomy and Neurochemistry of Resilience

Neural Substrates of Stress Inoculation Determined in vivo by Brain Imaging in Monkeys

David M Lyons*, Karen J Parker, Maor Katz, Christine L Buckmaster and Alan F Schatzberg

Department of Psychiatry and Behavioral Sciences, Stanford University Medical Center, Stanford, CA, USA

Background: Early exposure to severe forms of stress in childhood increases the incidence of mood and anxiety disorders in the aftermath of stressors in adulthood. Far less researched, but of equal importance, is the suggestion that mild early stressors instead of increasing vulnerability result in subsequent stress resistance. Variously described as inoculating, immunizing, steeling, toughening, or thriving, the notion that mild postnatal stress strengthens resistance to subsequent stressors has far-reaching implications for understanding the neuropathogenesis and prevention of affective disorders. Although the biology of stress inoculation in humans is largely unknown, important new insights have emerged from recent studies of monkeys.
Methods: Monkeys exposed early in life to brief intermittent social separations, mildly stressful foraging demands, or matched 'no-stress' control conditions were examined throughout adolescence and adulthood for differences in behavior, neurochemistry, and hypothalamic-pituitary-adrenal (HPA) axis physiology. In early adulthood, T1-weighted brain images were acquired at high resolution and processed for hippocampal and prefrontal volumetric analysis. Prefrontal white matter was also examined with diffusion tensor imaging (DTI) data acquired using a spiral readout trajectory that eliminates distortion artifacts.
Results: Prior exposure to mild early stressors that stimulate the HPA-axis induced diminished anxiety, increased exploration, and enhanced prefrontal-dependent cognitive control of behavior. Plasma levels of cortisol and cerebrospinal fluid levels of the norepinephrine metabolite MHPG were initially diminished for 8-12 months after stress inoculation compared to controls. Although differences in these baseline measures collected in undisturbed condi-

tions did not persist, enduring reductions in HPA-axis responsivity to subsequent stressors were evident in adolescence and adulthood. In contrast to studies of rats and mice, the development of stress resistance in monkeys was induced by postnatal stress exposure rather than postnatal differences in aspects of maternal behavior. Stress inoculation did not affect adult hippocampal volumes but resulted in larger prefrontal volumes in the right cerebral hemisphere. Asymmetric variation was expressed most clearly in ventromedial prefrontal cortex, and also occurred to a lesser extent in dorsolateral prefrontal cortex and prefrontal white matter volumes. Stress inoculation likewise induced changes in prefrontal white matter connectivity discerned in measures of fractional anisotropy determined in vivo by DTI.
Discussion: The long-term effects of postnatal stress inoculation in monkeys appear to reflect neuroadaptations in prefrontal circuits that mediate cognitive and neuroendocrine aspects of emotion regulation. Right frontal regions in studies of humans have been implicated in negative emotions, social withdrawal, cognitive control, sensitivity to stress, and regulation of the HPA-axis response. It is therefore intriguing that these same regions are altered by postnatal stress inoculation in studies of monkeys. Molecular and cellular research combined with brain imaging is needed to determine how stress inoculation shapes the development of prefrontal corticolimbic brain substrates of lifelong stress resistance. Supported by MH47573 and DA16902.

Neuroanatomic and Neuroendocrine Alterations in Trauma Survivors Associated with Coping and PTSD Risk Factors
Rachel Yehuda*, Julia A Golier, Lisa Tischler, Phil Harvey, Randall Newmark, Ren Kui Yang and Monte Buchsbaum

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

Rationale: Individual differences in parameters related to resilience are associated with structural and functional brain changes. Whereas there has been a tendency to focus on the negative or toxic effects of stress on brain, many persons emerge from traumatic situations positively transformed. This presents a mandate to distinguish among stress-activated changes that lead to negative outcomes vs. those associated with protection from these outcomes.
Methods: We report on data from a cohort of 17 veterans with PTSD and 16 veterans without PTSD who received an MRI scan followed by neuroendocrine assessment and cognitive testing. We examined hippocampal volume, 24-hr urinary cortisol excretion, glucocorticoid receptor responsiveness, neuropeptide Y, and DHEA and DHEAS levels in a group of middle-aged veterans with and without PTSD. A psychiatric evaluation was performed, which included an evaluation of coping and other resilience-related measures. Veterans were additionally subdivided on the basis of presence or absence of previous trauma exposure (prior to combat or the direct precipitant of symptoms).
Results: Though veterans with PTSD did not differ from those without PTSD in hippocampal volume, smaller left hippocampal volumes were observed in veterans who developed PTSD in response to their first reported traumatic exposure, compared to veterans who had first experienced a traumatic event to which they did not develop PTSD, prior to experiencing a subsequent event that led to PTSD. Left hippocampal volume was significantly associated with positive coping ($r=0.422$, $df=28$, $p=0.025$). In contrast, 24-hr urinary cortisol excretion and lysozyme IC50-DEX were associated with risk factors related to early trauma exposure ($r=0.506$, $df=27$, $p<0.001$; $r=0.37$, $df=25$, $p=0.059$ respectively).
Conclusion: Smaller hippocampal volumes in PTSD may be associated with specific risk and resilience factors. These may be distinct from vulnerability markers associated with increased responsiveness to glucocorticoids and/or other neuroendocrine measures that have been observed in combat-related PTSD.

Levels of Emotional Awareness and Alexithymia in Traumatized Subjects with and without PTSD: Implications for Resilience
Ruth Lanius*, Paul A Frewen and Richard D Lane

Psychiatry, University of Western Ontario, London, ON, Canada

Background: Lane and Schwartz (1987) articulate a developmental model of emotional awareness in which the capacity to be conscious of one's feelings, as well as to be aware of emotion in others, is an acquired skill that undergoes developmental progression, the basis of this learning being early attachment relationships. In the levels of emotional awareness framework, one moves from a rudimentary awareness of raw physical sensations through successively more sophisticated levels of emotional intelligence and empathy. This developmental process ultimately eventuates, in the most psychologically healthy and high functioning individuals, in the capacity to appreciate complexity in the emotional experience of self and others. It is possible, thus, that neglect and/or maltreatment during childhood may obstruct the normal development of emotional processing skills, leading to altered levels of emotional awareness, alexithymia, emotion regulation difficulties, and a vulnerability to developing PTSD in adulthood. The goal of this study was therefore to characterize functional brain correlates of emotional awareness and alexithymia in traumatized subjects with and without posttraumatic stress disorder (PTSD) using functional magnetic resonance imaging (fMRI) and to compare these brain correlates in subjects with positive adaptations to stress (resilience) to subjects with negative outcomes of stress (PTSD).
Methods: 4.0 Tesla fMRI was used to assess brain activation patterns during script-driven imagery of traumatic memories in traumatized subjects with (n=26) and without (n=16) PTSD. Subjects were assessed with a psychometric battery including the Clinician-Administered PTSD Scale (CAPS), Toronto Alexithymia Scale (TAS-20), Levels of Emotional Awareness Scale (LEAS), and Childhood Trauma Questionnaire (CTQ). Subjects' LEAS and TAS-20 scores were correlated with their SPM(t) maps; correlations were calculated separately in the PTSD and control groups. A region of interest approach was used, centered on identifying correlates of the psychometric scales with BOLD response in mPFC, ACC, PCC, thalamus, and insula.
Results: Individuals with PTSD (n = 26) exhibited less response in comparison with trauma-exposed non-psychiatric controls (n = 16) in medial prefrontal cortex (mPFC) and anterior cingulate cortex (ACC) during trauma script-driven imagery. fMRI Correlations with LEAS: In controls, significant correlations included bilateral ACC (BA 32), PCC (BA 23, 30, 31), and thalamic sites, in addition to right sided mPFC (BA 10). In PTSD subjects, greater levels of emotional awareness were associated with increased BOLD activity in right insula (BA 13, 22) and thalamus. fMRI Correlations with Toronto Alexithymia Scale (TAS-20) Scores: In controls, correlated regions of interest were specific to mPFC (BA 9, 10) bilaterally. In contrast, higher alexithymia in PTSD subjects was associated with increased BOLD activity bilaterally in thalamus, insula (BA 13, 47), and PCC (BA 23, 29, 31).
Discussion: Low levels of emotional awareness and alexithymia observed clinically in PTSD may be associated with altered neural processing in regions associated with episodic memory, self-referential processing, affective-bodily experience, and sensory-gating during symptom-provocation. The functional correlates of emotional awareness and alexithymia in traumatized subjects without PTSD suggest a role for the anterior cingulate gyrus and medial prefrontal cortex in the mediation of resilient adaptations to stress.

The Psychobiology of Human Resilience to Stress
Dennis Charney*

Mt. Sinai School of Medicine, New York, NY, USA

This presentation will review current concepts regarding the basis of human resilence to extreme stress both from a biological and psycho-

logical perspective.Psychological factors related to resilience include optimism, cognitive flexibility and reappraisal, faith/spiriuality, role models, moral compass, signature strengths,and fear modulation. Recent research has begun to identify the neurochemistry and functional neuroanatomy that may underlie resilience. An attempt will be made to integrate these two bodies of research in order to develop a "resilience prescription." The implications for the prevention of stress related pathology will be highlighted.

Panel Session
If You Prick Them, Do They not Bleed? Emotion Recognition and Schizophrenia

Hear No Evil: Early-Stage Auditory Processing and Emotion Recognition Deficits in Schizophrenia

Daniel C Javitt*

Nathan Kline Institute, Orangeburg, NY, USA

Background: Patients with schizophrenia have deficits in early auditory processing, involving dysfunction even at the level of primary sensory cortex. Patients also have deficits in emotion perception, which are related to social competence and functional outcome. The present studies investigated the relationship between deficits in basic auditory processing and ability to decode emotional content based upon vocal cues.
Methods: In Experiment 1 we examined relationships between emotion processing and early-stage auditory processing. Emotion processing was examined using the Kerr and Neale Voice Emotion Recognition and Identification tasks. Basic auditory processing was assessed using Tone Matching (TMT) and Distorted Tunes (DTT) tasks. As a comparator, Face Emotion Recognition and Identification were also measured. Relationships among measures was analyzed using principal components analysis. In Experiment 2, we examined structural bases of Voice Emotion Recognition and Identification. In Experiment 3, we assessed integrity of non-affective prosodic processing as well.
Results: For Experiment 1, patients with schizophrenia performed significantly more poorly than controls on both voice and face emotion recognition. Significant hierarchical correlations were found within, but not across, modalities, suggesting modality specific contributions to impaired emotion processing. Further, impairments in both DTT and VOICE-ID correlated significantly with proxy measures of impaired global outcome. In experiment 2, we observed significant correlations of voice emotion identification deficits with structural integrity of white matter tracts within the early auditory system. In experiment 3, deficits were observed in non-affective, as well as affective, forms of prosodic identification, suggesting significant generalization across higher-order processes.
Discussion: These results indicate that deficits in basic auditory processing contribute significantly to deficits in higher order cognitive functioning, including voice emotion recognition. Parallel deficits in visual processing may conspire to deprive patients of sensory processing capacities necessary for every-day social functioning.

See No Evil: Early-Stage Visual Processing and Emotion Recognition Deficits in Schizophrenia

Pamela D Butler*

Program in Cognitive Neuroscience and Schizophrenia, Nathan Kline Institute, Orangeburg, NY, USA

Background: Patients with schizophrenia have deficits in early visual processing, particularly involving the magnocellular visual pathway. Patients also have deficits in emotion perception, which are related to social competence and functional outcome. While deficits are particularly prominent in face emotion recognition, there is relatively preserved experience of emotion in evocative tests. Thus, rather than having a global emotion processing deficit, patients appear to have a specific deficit in emotion recognition. We explored the hypothesis that deficits in early visual processing may contribute to deficits in emotion processing.
Methods: In Experiment 1 we examined relationships between emotion processing and early-stage visual processing. Emotion processing was examined using the Penn Emotion Differentiation, Recognition, and Acuity tasks. Magnocellular and parvocellular visual pathway function were assessed using steady state visual evoked potentials (ssVEP) and psychophysically-determined contrast thresholds. Luminance contrast and stimulus size were utilized in the ssVEP and contrast threshold tasks to bias processing towards magnocellular vs parvocellular pathways. In Experiment 2, we examined the effects of decreasing the contrast in faces. This was done because intact contrast detection may be important for face emotion recognition and the magnocellular system is involved in detecting low contrasts.
Results: For Experiment 1, patients with schizophrenia (n=42) performed significantly more poorly than controls (n=33) on all three Penn emotion tasks ($p<0.001$). Failures of face emotion recognition correlated closely with visual processing deficits, particularly involving the magnocellular system ($p<0.003$). For experiment 2, patients (n=15) needed at least twice as much contrast as controls (n=20) to attain similar performance on face emotion identification.
Discussion: Taken together, these studies show significant relationships between visual pathway, particularly magnocellular, dysfunction and emotion processing performance. These results support the hypothesis that dysfunction in early-stage, low level processing in the visual system may upwardly generalize to erode higher-level processing.

Fear No Evil: Top-Down and Bottom-Up Processing of Emotions in Schizophrenia Investigated with Blocked and Event-Related fMRI

Ruben C Gur*, James Loughead, Mark Elliott, John D Ragland, Christian Kohler and Raquel E Gur

Psychiatry, University of Pennsylvania, Philadelphia, PA, USA

The results of 3 sequential fMRI studies will be reported. The first used a block design and showed reduced amygdala activation in SCZ for the top-down task of judging emotional valence on faces. The second study used a hybrid block and even-related design to look at the top-down activation (block analysis) and the bottom-up response to happy, sad angry and fearful faces (event-related analysis). This study showed that while the top-down activation in amygdala is reduced, as in the first study, the reason for that seems to be excessive bottom-up activation to fearful faces, which propagates throughout limbic and paralimbic regions and produces lower activation of inferior frontal cortex. amygdala bottom-up activation was especially strong for incorrect responses and associated with more severe flat affect. The third study used a sparse event-related design and focused on the amygdala and fusiform gyrus. It replicated the bottom-up increased amygdala activation for fearful (and this time angry too) faces in patients with SCZ, but the finer spatial resolution allowed us to look separately at ventral and dorsal amygdala and the effect was entirely in ventral aspects. The results suggest that the reason for difficulties in affect recognition in SCZ could be excessive bottom-up interference from threat related stimuli, to the point that it disrupts cortical evaluative processing even when the stimulus is relevant to the task. It is also noteworthy that bottom-up activation for non-emotional stimuli (eg with the p3a in an oddball task) is reduced in SCZ. The data illustrate the convergence of results across fMRI methodologies that can parse abnormalities to the point of revealing its mechanistic explanation.

To Thine Own Self Be True: Consummatory vs. Appetitive Pleasure in Schizophrenia

William Horan*

Psychiatry & Biobehavioral Sciences, UCLA, Los Angeles, CA, USA

Background: Anhedonia, the diminished capacity to experience pleasant emotions, is a common feature of schizophrenia that is associated with the impairments in daily functioning that characterize this disorder. Research using different methods to assess anhedonia and emotional experience has produced a seemingly paradoxical set of findings. Schizophrenia patients frequently report experiencing lower levels of pleasure than non-patients on interview-based and self-report trait anhedonia measures, yet also report experiencing levels of pleasant emotions that are similar to non-patients in laboratory studies that involve direct exposure to emotionally evocative stimuli. These findings suggest that schizophrenia patients are capable of experiencing a normal range and intensity of pleasant emotions but for some reason report experiencing low levels of pleasure more generally. A series of studies investigated two possible explanations for this pattern of findings. Study 1 examined whether anhedonia reflects faulty memory for pleasant emotional experiences. Building on neurobehavioral models that distinguish between different components of hedonic experience, Study 2 examined whether anhedonia is characterized by intact consummatory pleasure (liking) but impaired appetitive pleasure (wanting).
Methods: In Study 1, 30 schizophrenia outpatients and 31 healthy controls completed an emotional exposure and recall procedure. Participants first provided reports of their emotional experiences during exposure to a variety of pleasant and neutral foods and film clips, and then completed a surprise recall task for their original emotional responses after a 4-hour delay. In Study 2, 46 schizophrenia outpatients and 40 healthy controls completed a recently developed and validated self-report trait questionnaire that distinguishes between appetitive and consummatory pleasure as well as an assessment of functional outcome.
Results: Study 1 indicated that the schizophrenia patients did not significantly differ from controls in either their initial levels of reported pleasant emotional responses to evocative stimuli or in delayed recall accuracy for these experiences. Study 2 indicated that patients self-reported lower levels of appetitive pleasure, but similar consummatory pleasure, as compared to controls. In addition, among patients, lower levels of appetitive pleasure significantly correlated with worse social functioning in the community.
Discussion: The overall pattern of results suggests that the anhedonia commonly reported by schizophrenia patients reflects neither a global consummatory pleasure deficit nor faulty encoding and short-term memory for pleasant emotional experiences. However, schizophrenia may be associated with impaired appetitive pleasure. The distinction between consummatory and appetitive pleasure appears to have considerable explanatory value for reconciling the divergent findings across different methods of assessing anhedonia. On-going efforts to further clarify the nature of the hedonic deficit in schizophrenia will be discussed.

Panel Session
Clock Genes, Melatonin and Affective Disorders

Melatonin Receptors As Targets For Modulation Of Circadian Rhythms

Margarita L Dubocovich*

Mol Pharmacol Biol Chem, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Background: Melatonin (MLT) promotes sleep and modulates circadian rhythms through activation of two G-protein coupled receptors, the MT_1 and MT_2 (Dubocovich et al., Front. Biosc., 2003; Arendt and Skene, Sleep Med. Rev. 9:25, 2005). Psychiatric disorders are often associated with desynchronization of circadian and/or seasonal rhythms. Understanding the mechanisms by which endogenous oscillators are synchronized by non-photic stimuli such as MLT will allow the design of therapeutic strategies for the treatment of circadian disturbances of mood and sleep. This presentation will focus on the mechanism(s) of MT_1 and MT_2 MLT receptor-mediated regulation, signaling and clock gene expression in the suprachiasmatic nucleus (SCN), and their involvement in the modulation of circadian phase and in the antidepressant-like activity of the MLT ligand luzindole.
Methods:MLT-mediated phase shifts of spontaneous circadian rhythms of neuronal firing rate were recorded from SCN coronal brain slices using the single-unit recording technique. The onset of locomotor activity rhythms was assessed by monitoring wheel running activity in mice with genetic deletion of MT_1 (MT_1KO) and MT_2 (MT_2 KO) MLT receptors, or by actigraphy in non-human primates. Mice treated with luzindole (0.03 mg/ml) in the drinking water were tested in the forced swimming test. Cell proliferation and survival in the subgranular zone (SGZ) of the hippocampus was assessed following BrdU (75mg/kg x 4, ip) treatment using visualization with hydrogen peroxide.
Results: Activation of MT_2 receptors at CT 10 (Circadian Time 12: onset of activity) in rat or mouse SCN brain slice phase advanced circadian rhythms of neuronal firing as this effect is blocked by the selective and competitive MLT receptor antagonist 4P-PDOT and is absent in mice with genetic deletion of the MT_2 MLT receptor. In rat SCN brain slices, exposure to physiological concentrations of MLT for a period mimicking the nocturnal surge (300 pM, 8 h) desensitizes MT_2 receptors and precludes MLT-mediated phase advances of neuronal firing rhythms. In vivo MLT phase advances and phase delays circadian rhythm of motor activity when given at CT10 or CT2, respectively, to mice or non-human primates. These effects were receptor mediated as they were blocked by luzindole, a competitive MLT receptor antagonist. Paradoxically, administration of MLT to MT_1KO mice did not phase shift circadian rhythms of activity. These results suggest that activation of MT_1MLT receptors in vivo within and/or outside the SCN appears to be necessary to phase shift overt circadian rhythms of activity. Luzindole shows antidepressant-like activity in mice during forced swimming requiring expression of the MT_2 but not the MT_1 MLT receptor (Sumaya et al., J. Pineal Res., 2005). The effect of chronic treatment with luzindole on the time of immobility during swimming will be discussed in relation with its effect on cell proliferation and survival in the SGZ of the hippocampus.
Discussion: MLT phase shifts neuronal firing rhythms in the SCN brain slice via direct activation of MT_2 receptors within an SCN oscillator, while activation of MT_1 MLT receptors on an output pathway and/or an oscillator localized outside the SCN may be necessary to phase shift overt circadian activity rhythms. Results will be discussed in relation to the role of the MT_1 and MT_2 MLT receptors in modulating circadian responses, in normalizing disturbed circadian rhythms in psychiatric disorders, and as potential targets for the antidepressant action of MLT ligands. *Supported by USHPS Grants MH 52685, MH 42922, MH63466*

In Winter Depression (SAD), the Sweet Spot for the 10 pg/ml Plasma Dim Light Melatonin Onset (DLMO) is Six Hours Before Mid-Sleep

Alfred Lewy*, Jonathan Emens, Bryan Lefler and Vance Bauer

Psychiatry, Oregon Health & Science University, Portland, OR, USA

Introduction: The leading mechanistic understanding of SAD and the antidepressant effect of bright light treatment is the phase shift hypothesis (PSH). Accordingly, most patients with SAD have circadian rhythms that are phase delayed with respect to sleep, and morning bright light (which causes a phase advance) is generally more antidepressant than evening light (which causes a phase delay). The PSH also takes into account that not all SAD patients are phase de-

layed and too much of a phase shift could result in less of an antidepressant response. The present study was undertaken to test the PSH using melatonin administration: afternoon (PM) melatonin should be more antidepressant than morning (AM) melatonin in phase-delayed patients and AM melatonin should be more antidepressant than PM melatonin in phase-advanced patients. Low doses cannot be distinguished from placebo (there are no side effects, not even sleepiness); it would also be extremely unlikely that this chemical signal for nighttime darkness would be therapeutic in SAD for any reason other than causing corrective phase shifts (when appropriately timed).
Methods: In 69 patients in whom wrist actigraphy recordings of sleep times were done, subjects slept on consistent sleep times of their own choosing for four weeks. DLMOs and SIGH-SAD depression ratings were assessed at the end of a baseline week and after three weeks of treatment with either AM or PM melatonin (0.225-3 mg in 3-4 divided doses every two hours) or placebo.
Results: Before treatment, SIGH-SAD scores parabolically correlated with the phase angle difference (PAD) between DLMO and mid-sleep [R-square = 0.17, df = (2,65), P = 0.003] with a minimum at 5.88, as predicted based on the average (PAD= 6 hours) in healthy controls. This relationship held post-treatment, particularly in patients who had delayed DLMOs relative to mid-sleep at baseline (PAD<6). The linear regression for all phase-advanced and phase-delayed subjects who received PM melatonin — the treatment that caused the greatest phase shifts — between per cent change in depression ratings and shifts towards or away from PAD 6 was quite robust, despite the relatively small sample size (r = 0.59, r-square = 0.35, df = 20, P = 0.004). The parabolic fit of the data for the delayed subjects who received PM melatonin was impressive, particularly for such a small sample [R-squared = 0.65, df = (2,8), P = 0.01, minimum = 5.56]. The correct treatment (PM melatonin for delayed types and AM melatonin for advanced types) decreased depression ratings by 34%, compared to about 13-15% for the incorrect treatment and placebo groups or when these two groups were combined. The more conservative effect sizes based on per cent differences in change scores compared to the correct treatment were: 0.61 (incorrect treatment), 0.83 (placebo) and 0.69 (the latter two groups combined).
Discussion: Although this was not a melatonin treatment study, the clinical findings suggest that SAD and other circadian phase disorders might benefit from the use of low-dose melatonin taken at appropriate times during the day. The R-squares of 0.35-0.65 represent the range in the highest estimate of the circadian component of SAD and the mechanism of phase resetting by melatonin and presumably by light. It is hoped that the concept of the PAD 6 sweet spot, as well as the use of the DLMO and appropriately timed melatonin treatment, will help elucidate the circadian components of other sleep and mood disorders, particularly non-seasonal depression. SAD may be the first psychiatric disorder in which a physiological marker has been shown to correlate with symptom severity before and after treatment in the same patients.

The Role of Per Genes in Addiction and Depression

Rainer Spanagel*

Psychopharmacology, CIMH, Mannheim, Germany

New findings on the effects of drugs of abuse on clock genes and processes that underlie circadian rhythmicity will be reported. It has been shown that drug intake alters the circadian expression patterns of period (per) genes in various brain regions including the suprachiasmatic nucleus (SCN). Per2 gene activity regulates the glutamatergic system through glutamate re-uptake mechanisms and thereby affects drug intake and a variety of physiological processes that are governed by our internal clock. In summary, a new pathological chain has been identified that contributes to the pathological consequences of chronic drug intake. Similar pathological processes might be initiated by chronic stress and may thereby lead to a depressed-like state. References: Abarca C, Albrecht U, Spanagel R (2002) Cocaine sensitization and reward are influenced by circadian genes and rhythm. Proc Natl Acad Sci USA, 99:9026-30 Spanagel R, Rosenwasser AM, Schumann G, Sarkar DK (2005) Alcohol consumption and the biological clock. Alcohol Clin Exp Res in press Spanagel R, Pendyala G, Abarca C, Zghoul T, Sanchis-Segura, Magnone MC,Lascorz J, Depner M, Holzberg D, Soyka M, Schreiber S, Matsuda F, Lathrop M, Schumann G, Albrecht U (2005) The circadian clock gene Period2 alters the glutamatergic system and thereby modulates alcohol consumption. Nature Med 11, 35-42

The Molecular Clock as a Target in the Treatment of Bipolar Disorder

Francesco Benedetti*

Dept. Neuropsychiatric Sciences, Istituto Scientifico Ospedale San Raffaele, Milano, Italy

In the last few years light has been shed on the molecular mechanisms that regulate the functioning of the master clock of human body. At such molecular level several genes interact in intracellular autoregulatory transcriptional-translational feedback loops, with positive and negative components acting to regulate transcriptional events in order to produce a circadian rhythm. Since remission from both depression and mania is paralleled by a regularization of circadian abnormalities (a core symptomatological feature of mood episodes, independent from polarity) and given the proposed role of chronobiological abnormalities in the pathogenesis of mood disorders, it seems hypothesizable that treatments effective for bipolar disorder may share some action on gene expression in the SCN. Several lines of evidence support this hypothesis. It is now acquainted that both pharmacological antidepressants (SSRI, TCA, MAOI) and the rapid and powerful non-pharmacological treatments (e.g. sleep deprivation and light) can promote, through a cAMP-dependent signal transduction pathway, the CREB mediated gene transcription in the suprachiasmatic nucleus of the hypothalamus (SCN) (see Figure). Not surprisingly in this perspective, these are the same transductional and trascriptional pathways by which light stimuli synchronize every morning the molecular clock through the retino-hypothalamic tract. Moreover, growing evidence has been accumulated on the relatioship existing between pharmacodynamics of mood stabilizers and clock genes. Lithium salts, which are known to lengthen circadian rhythms, can inhibit GSK-3b, and probably valproic acid can do it as well. If GSK-3b is the mammalian homologue of the Drosophila enzyme SHAGGY, as it seems, its inhibition will ultimately results in an effect on the transcription of clock genes. In very recent studies we showed that polymorphisms in the promoter genes for clock genes (CLOCK, GSK-3b, hPER-3) can influence core features of the bipolar illness such as age at onset, rate of recurrence, lifetime prevalence of sleep disorders, and response to treatments. The size effect is similar to that already defined for gene polymorphisms regarding the brain monoaminergic systems, such as the promoter of the serotonin transporter (SERTPR): which, in turn, are known to influence the same core features and the antidepressant response to both drugs and chronotherapeutic treatments (sleep deprivation and light). An high field (3.0T) BOLD fMRI study performed at our research center showed that in bipolar depression the changes in neural response to emotional tasks caused by therapeutic repeated sleep deprivation are influenced by SERTPR. Such parallel findings point to an intimate relationship between the neurotransmitter systems targeted by drugs and the circadian rhythms targeted by chronotherapeutics. Finally, the genetic components of the molecular clock follow a circadian pattern of transcription, and may be influenced in different ways by external agents (such as light and drugs) depending on the time of administration. This raises the possibility that treatments of bipolar illness may exert different effects depending on phase-response curves. Findings at our center have shown this to be true for the sleep-inducing effect of benzodiazepines; studies on antidepressants drugs are ongoing. The defini-

tion of these mechanism will probably explain the mechanism of action of the chronotherapeutic techniques for bipolar illness, and will open a new field of research for both clinical and pre-clinical antidepressant pharmacology.

Panel Session
The Endocannabinoid System as Target for Drug Discovery

Overview of the Endocannabinoid System, CB1 Receptor Antagonists

Alexandros Makriyannis*

Center for Drug Discovery, Northeastern University, Boston, MA, USA

Background: The endocannabinoid system is an important target for drug discovery and development. This involves two families of endogenous ligands (endocannabinoids) represented by anandamide and 2-arichidonylglycerol (2-AG), both of which bind to at least two G-protein coupled cannabinoid receptors (CB1 and CB2). The endocannabinoid system is also modulated by the biochemical processes involved in the deactivation and the biosynthesis of the endocannabinoid ligands. Mechanisms for endocannabinoid deactivation include two known hydrolytic enzymes, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL) lipase, the oxidative enzyme cycloxygenase-2 (COX2) and a cellular anandamide reuptake mechanism (ANT) which remains to be fully characterized. Key among the biosynthetic enzymes are phophatidylethanolamine N-acyl transferase and a specific phospholipase D acting in tandem for the production of anandamide as well as a specific phospholipase A involved in the synthesis of 2-AG. A number of compounds that act on components of this system are under clinical and preclinical development.
Methods: Novel exciting approaches and technologies are being used to study interactions of ligands with cannabinoid receptors, to quantify endogenous ligands and to identify novel cannabinergic modulatory mechanisms. These include: a) mass spectrometry; b) molecular biological and genomics approaches; c) state of the art multidimensional NMR techniques, and, d) in vivo imaging modalities. Drug discovery based on the endocannabinoid system has been greatly assisted by our improved understanding of the structure and function of the target proteins which include the CB1 and CB2 cannabinoid receptors, fatty acid amide hydrolase (FAAH), an enzyme involved in the deactivation of the endogenous cannabinoid ligands, and the transport system(s) involved in the intracellular uptake of the endocannabinoids. Our research involves on the discovery of novel ligands for each of the proteins involved in the endocannabinoid system and their characterization. It requires a multifaceted approach involving ligand design and synthesis and biophysical and computational chemistry as well as biochemical methods. Information on the interactions of cannabimimetic ligands with their protein targets will reveal the molecular properties required for biological activity (pharmacophoric requirements) within the known cannabimimetic classes, and serve as the basis for the design of more effective ligands and, ultimately, therapeutic drugs.
Results: We have developed CB1 receptor inverse agonists and neutral antagonists that are under preclinical investigations in rodent and non-human primate models. These or similar compounds may have utility for indications including cocaine relapse and cannabis abuse.
Summary: Modulation of endocannabinoid tone can have profound physiological consequences and, thus, can serve as a basis for therapeutic drug discovery. A major success story in the field is the development of CB1 antagonists, one of which (Rimonabant, Accomplia) is currently in late clinical trials as an anti-obesity medication acting as an appetite suppressing agent and a modulator of lipid metabolism and is expected to become FDA approved during 2005. Rimonabant is also being developed for use in nicotine addiction, while other compounds in this class are under evaluation as medication for dependence on other drugs of abuse, such as alcohol, opioids and cocaine. They also show some promise for use in schizophrenia. We are currently developing CB1 antagonist-based medications for treatment of cocaine-, alcohol-, opiate- and cannabis abuse. Our novel ligands have improved pharmacological profiles and pharmacokinetic properties. Two of them are currently in preclinical development.

Neuroprotection Through Direct and Indirect Activation of Cannabinoid CB1 Receptors

Ben A Bahr*, Alexandros Makriyannis, Therese A Kosten and David A Karanian

Dept. Pharmaceutical Sciences, University of Connecticut, Storrs, CT, USA

Background: Drugs that target the cannabinoid system are being developed to treat MS, AD, PD, HD, ALS, and excitotoxic insults including stroke and traumatic brain injury. Protective effects are elicited by signaling pathways linked to cannabinoid CB1 receptors. We reported that blocking CB1 receptors causes disruption of neuronal maintenance and increases excitotoxic vulnerability (Karanian et al., Eur J Pharmacol 508:47-56, 2005). Endocannabinoid levels are elevated after injury, indicating a compensatory response that can be enhanced with specific inhibitors of anandamide transport and endocannabinoid hydrolysis. Previous reports have provided evidence that such inhibitors can treat pain, motor hyperactivity, parkinsonism, tumors, MS, and reduce the magnitude of kainate-induced seizures. Here, we tested whether dual modulation of endocannabinoid transport and fatty-acid amide hydrolase (FAAH) protects against the neurodegeneration associated with excitotoxicity in vitro and in vivo (see Karanian et al., J Neuroscience, in press, 2005).
Methods: The effects of transport inhibitor N-(4-hydroxyphenyl)-arachidonamide (AM404) and FAAH inhibitor palmitylsulfonyl fluoride (AM374) were compared to the direct CB1 action of R-methanandamide (AM356), using the excitotoxic hippocampal slice model (20-min AMPA insult) and adult rats injected unilaterally with AMPA into the dorsal hippocampus. CB1 responses of MAPK and FAK activation were monitored with anti-pERK and anti-pFAK. Protection against cytoskeletal breakdown, synaptic decline, and brain damage were assessed by immunoblot, histology, and behavioral tests.
Results: AM374 (100 nM) and AM404 (10 uM) produced additive effects on CB1-mediated MAPK and FAK activation. Correspondingly, post-insult application of the AM374/AM404 combination provided additive protection against cytoskeletal breakdown product (BDP; 96% reduction) and synaptic decline (60% recovery) in the hippocampal slice model; protection by AM356 was 72% and 68%, respectively. When injected with excitotoxin into the hippocampus of adult rats, the agonist reduced BDP by 78% as did AM374/AM404, and the latter provided complete cytoskeletal protection in 9 of 12 animals. Pre- (synapsin II and synaptophysin) and postsynaptic markers (GluR1) exhibited >90% protection as did the maintenance of FAK and ERK/MAPK pathways, and all protective effects were blocked by the CB1 antagonist AM251. AM374/AM404 also prevented pyknotic changes and death of CA1 pyramidal neurons in the excitotoxic hippocampus. In behavioral studies, a 4-6-fold increase in perseverative turning by excitotoxic rats was reduced to control levels by AM374/AM404 ($p=0.0017$); this reduction was more pronounced than that produced by AM356. In a fear-conditioning paradigm, AM374/AM404 also protected consolidation processes and, as a result, reduced the excitotoxic memory impairment ($p<0.0001$). The improved memory performance was blocked by AM251. The same animals with improved memory also exhibited reduction in BDP and recovery of synaptic markers. Cytoskeletal and synaptic protection in fact was found to correlate with functional protection ($r=0.73$, $p<0.01$).
Discussion: The data show that compensatory CB1 responses can be positively modulated by AM374 and AM404, leading to a sufficient level of signaling for protection against excitotoxic brain damage. The cellular and functional protection was evident 7 days post-insult and

correlated with the preservation of CB1-linked MAPK/FAK signaling. These results indicate that the transporter and FAAH are modulatory sites that may be exploited to enhance cannabinergic responses for therapeutic purposes.

Progress Towards the Development of a PET Radioligand for Cannabinoid CB1 Receptors

Samuel J Gatley* and Alexandros Makriyannis

Center for Drug Discovery, Northeastern University, Boston, MA, USA

The G-protein coupled cannabinoid CB1 receptor is responsible for the for the neuromodulatory effects of endocannabinoids such as arachidonyl ethanolamine (anandamide), and for the psychoactive effects of (-)-Δ9-tetrahydrocannabinol (Δ9-THC), the principal active ingredient of marijuana. A positron emission tomography (PET) radioligand for the CB1 receptor would allow quantitative imaging of this receptor in the living human brain. For this purpose, compounds with adequate affinity and binding site selectivity and ability to efficiently cross the blood brain barrier efficiently. The known families of compounds with high affinities for cannabinoids comprise: classical cannabinoids such as Δ9-THC; synthetic nonclassical cannabinoids with a clear structural relationship to Δ9-THC; aminoalkylindoles; anandamide and related compounds, and pyrazoles. Members of these families are highly lipophilic; log10(octanol/water) distribution ratios (logP values) of Δ9-THC, CP55940 (nonclassical cannabinoid) and WIN55212-2 (aminoalkylindole) in the literature are close to 7, 6 and 5, respectively. Pyrazoles, which unlike the other classes of CB1 receptor ligands are antagonists or inverse agonists, generally have logP values between 4 and 5. Aminoacid residues that are important for binding all these classes of ligands lie deep within the hydrophobic membrane-spanning domains of the CB1 receptor. We previously labeled the CB1 receptor ligand AM281 with iodine-123 (t1/2 = 13 h) and demonstrated in vivo specific binding in non-human primates using single photon emission tomography (SPECT). The first human SPECT studies with this tracer have recently been reported, but image quality was suboptimal and the ratio of specific to nonspecific binding low. However, a carbon-11 or fluorine-18 labeled ligand with equivalent or superior characteristics to [123I]AM281 would permit clinical research imaging studies of the CB1 receptor system because of the better sensitivity and spatial resolution afforded by positron emission tomographic (PET) imaging, as compared with SPECT imaging. We and others have synthesized a number of candidate PET and SPECT CB1 receptor radioligand and evaluated their in vivo binding in mouse brain. The main conclusions that can be drawn from the published studies to date are: (1) the pyrazoles as a group exhibit the poor brain uptake expected of compounds with logP values >4; (2) however, within the compounds tested a relationship between higher logP value and lower uptake in mouse brain is not apparent, indicating that other factors are the key determinants of uptake; (3) radiolabeled aminoalkylindoles may also have sufficient brain penetration for development as PET radioligands; (4) as shown by the case of the pyrazole AM251, which exhibited fair brain uptake in mouse brain but, unlike its close congener AM281, very poor uptake in baboon brain, there may be large species differences in brain penetration for a given compound; (5) the fact that AM281 has been used to image CB1 receptors in non-human primate and human brains is strongly indicative that a pyrazole PET radioligand useful in clinical research can be developed, despite the high lipophilicity of this group of compounds; (6) it is appropriate to continue to seek families of CB1 receptor ligands of lower lipophilicity, while also investigating the factors that affect brain uptake of compounds already available, and (7) it is appropriate to continue to evaluate brain uptake of pyrazoles and other compounds with high affinity for CB1 receptors in non human primates, since it is possible that rodents are poor model species for development of brain CB1 receptor PET radioligands.

"Gone to Pot": Emerging Pharmacological Evidence Implicating Cannabinoid Receptor Function in Cognition and Psychosis - Implications for Medications Development

Deepak C D'Souza*, Edward Perry, Daniel Mathalon, Ford Judith, Zoran Zimolo, Joel Gelernter and John Krystal

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

Emerging clinical and preclinical evidence suggest a role for exogenous and/or endogenous cannabinoids in cognition and psychosis. We first characterized the behavioral, cognitive and endocrine effects of 0, 2.5 mg and 5 mg intravenous delta-9-tetrahydrocannabinol (d-9-THC) in a 3 day, double-blind, randomized and counterbalanced study, in 22 healthy individuals who had been exposed to cannabis but had never been diagnosed with a cannabis abuse disorder. d-9-THC (1) produced schizophrenia-like positive and negative symptoms; (2) altered perception; (3) increased anxiety; (4) produced euphoria; (5) disrupted immediate and delayed word recall, sparing recognition recall; (6) impaired performance on tests of distractibility, verbal fluency and working memory (7) did not impair orientation; (8) increased plasma cortisol. In a parallel study of identical design, we studied the effects of d-9-THC in 13 stable, antipsychotic-treated schizophrenia patients. d-9-THC transiently increased (1) learning and recall deficits, (2) positive, negative and general schizophrenia symptoms; (3) perceptual alterations; (4) akathisia, rigidity and dyskinesia; (5) deficits in vigilance. Schizophrenia patients were more vulnerable to d-9-THC effects on recall relative to controls suggesting that CB-1R dysfunction contributes to the pathophysiology of the cognitive deficits associated with schizophrenia. Our initial study in healthy controls raised the question of why some, but not other individuals experienced clinically significant psychosis. In a pilot study (n=15) we show that healthy individuals with the polymorphisms of the COMT and GABRA2 genes may modulate vulnerablity to the psychotomimetic and amnestic effects of d-9-THC. More recently, we have attempted to study the mechanisms underlying the cognitive efefcts of cannabinoids in humans. Perceptual, memory and attentional functions are based on distributed processes that are believed to be bound together by synchronous high frequency oscillatory activity. Macroscopic neural synchrony can be evaluated noninvasively by entrainment of the EEG to auditory stimuli presented at various frequencies. Similarly, impairments in attention which are one of the most consistent effects of cannabinoids, can be studied using the P300 event related potential (ERP) associated with oddball task performance. In an ongoing pilot study d-9-THC transiently 1) reduced spectral power evoked by entrainment to 20 and 30Hz stimulation on an auditory entrainment task, 2) reduced both target P3b and novelty P3a amplitude, and 3) reduced N100 amplitude. Taken collectively these data raise the intrigiung possibility that CB-1R function may be a target for developing drugs to treat cognitive deficits, in particular those that are associated with schizophrenia, and general symptoms of psychosis. Unlike SR141617A, cannabidiol has been shown to possess antipsychotic efficacy as a stand alone treatment for schizophrenia. The availability of a wide range of CB ligands will now make it possible to test the therapeutic relevance of CB-1R function to cognition and psychosis.

Panel Session
A Noradrenergic Focus on Attention and Attention-Deficit Disorders

Norepinephrine Transporters: Genetic Variants and their Impact on Transporter Function and Regulation

Maureen K Hahn*

Pharmacology, Vanderbilt University Medical Center, Nashville, TN, USA

Norepinephrine (NE) plays an important role in attention, learning and memory and is hypothesized to play a role in Attention

Deficit/Hyperactivity Disorder (ADHD). NE released at synapses is inactivated through active transport into terminals by the presynaptically localized norepinephrine transporter (NET). NET is a target for tricyclic antidepressants, NET-selective reuptake inhibitors (NSRIs), and psychostimulants, including cocaine, methylphenidate and amphetamine. Stimulant drugs used to treat ADHD act on both the NET and dopamine (DA) transporter (DAT) whereas atomoxetine, also effective in treating ADHD, selectively targets NET. Single nucleotide polymorphisms (SNPs) that produce human NET (hNET) amino acid variants have been found and this genetic variation could contribute to risk for ADHD. Previously, our laboratory identified A457P in a familial form of Orthostatic Intolerance. A457P is a loss of function, dominant-negative transporter and is associated with increased heart rate and plasma norepinephrine levels. Interestingly, there is also a prominent psychiatric component to the phenotype of A457P heterozygote family members, who report memory difficulties and meet DSM-IV criteria for ADHD. These data suggest that other naturally-occurring hNET SNPs may also produce functional changes that might contribute to disease. We expressed in COS-7 cells a subset of the identified hNET variants and assayed protein expression and trafficking using cell-surface biotinylation and Western blot analysis, transport of radiolabeled substrate, antagonist interaction and regulation through protein kinase C (PKC) linked-pathways by the phorbol ester, β-PMA. The most dramatic effect was observed for A369P, which was devoid of the fully glycosylated 110 kDa form of protein, was retained completely within the intracellular compartment and lacked transport activity. N292T demonstrated an aberrant glycosylation pattern, with surface levels of the 110 kDa form decreased by 50%. N292T was similarly diminished in both [^{3}H]NE and [^{3}H]DA transport, with V_{MAX} values approximately 50% of hNET. That individuals are likely heterozygous carriers of hNET SNPs compelled us to examine the influence of coexpression of these hNET trafficking-compromised variants on hNET. A369P and N292T both produced a dominant-negative effect, impeding surface expression of hNET by 50 and 95%, respectively. The R121Q and F528C variants also dramatically affected several measures of hNET expression and function. F528C demonstrated a 35%, increase in surface protein levels. The V_{MAX} to K_M ratio of F528C transport of [^{3}H]NE was twice that of hNET, whereas that of [^{3}H]DA was not elevated, suggesting F528C yields more efficient transport of NE compared to DA. F528C also demonstrated an increase in the K_I value for desipramine competition of [^{3}H]NE uptake, losing potency by approximately 8-fold. R121Q produced a significant decrease in V_{MAX} for [^{3}H]NE transport to 65% of hNET and a V_{MAX} to K_M ratio decreased to half that of hNET. Thus, NE transport was more greatly diminished by the R121Q variant than DA transport. The changes in F528C and R121Q expression and substrate-specific transport suggested that there might be compromised regulation by PKC-sensitive pathways. Incubation of cells with β-PMA resulted in a 25% decrease in hNET [^{3}H]NE transport that was blocked by 1 μM staurosporine or 1 μM BIM. R121Q demonstrated an enhanced down-regulation compared to hNET in response to β-PMA treatment and F528C was resistant to the effects of β-PMA. These findings reveal multiple functional deficits of naturally-occurring hNET variants that may compromise NE reuptake and signaling in SNP carriers in the population.

Molecular Control of Noradrenergic Gene Expression and the Impact of Polymorphisms on Attention Disorders

Chun-Hyung Kim, Yoo-Sook Joung, Maureen K Hahn, Irwin Waldman, Bruce M Cohen, Randy Blakely and Kwang-Soo Kim*

Molecular Neurobiology Lab, McLean Hospital/Harvard Medical School, Belmont, MA, USA

The norepinephrine transporter (NET) is primarily responsible for re-uptake of norepinephrine (NE) into presynaptic nerve terminal, thus critically regulating both transmission and homeostasis of NE. Sine NE neurotransmission has a significant role in diverse brain functions, we hypothesize that promoter variations of the NET gene with altered promoter function may represent risk factor(s) for NE-related disorders such as attention deficit hyperactive disorder (ADHD), depression, and autonomic dysfunction. To address this, based on our previous study characterizing potentially important promoter domains (Kim et al., (2002) J. Neurosci.22: 2579), we sought to identify novel polymorphism(s) in the human NET (hNET) promoter. Toward this goal, we amplified several promoter domains including the distal enhancer domain between -4000 and -3100 bp from 100 independent genomic DNA samples and analyzed them by DNA sequencing. We have identified multiple, previously unidentified promoter polymorphisms in the 5' upstream and the 1st intron areas. To assess the potential functional effects of these polymorphisms, we have generated we generated hNET-reporter constructs containing each variation and examined their transcriptional activities using transient transfection assays. Among these novel polymorphisms, a common polymorphism was identified at -3081(A/T). We found that reporter gene expression driven by the construct containing allele -3081T displayed significantly diminished promoter function by approximately 30-50%, compared to the wild type construct. Furthermore, we found that a single major protein-DNA complex was formed with the -3081T allele probe but not with the wild type -3081A allele probe, suggesting that -3081T creates a new binding site for a transcription factor(s). Finally, we have initiated genetic association studies using DNA samples isolated from ADHD patients. Our preliminary analyses showed that the identified -3081T allele exists with a higher frequency in ADHD patients with a statistical significance. Supported by NARSAD Independent Award, MH48866, and DC006501.

Role for Norepinephrine in Attention, Learning and Memory: Insights from Knockout Mice

Steven A Thomas*, Charles F Murchison, Xiao-Yan Zhang, Ming Ouyang, Wei-Ping Zhang, Kevin Hellman and Ted Abel

Pharmacology, University of Pennsylvania, Philadelphia, PA, USA

Background: Adrenergic signaling likely acts as a stress response system in the CNS. Consistent with this, there is considerable evidence supporting roles for the adrenergic transmitters norepinephrine/epinephrine (NE/E) in promoting arousal and regulating attention. Evidence also suggests that NE/E mediates the enhanced memory consolidation that follows emotional events. To test of these hypotheses, we performed studies using mice (Dbh KO) harboring a null mutation in the dopamine β-hydroxylase gene. These mice are unable to synthesize NE/E.
Methods: To examine arousal, EEG and EMG were recorded from cerebral cortical and nuchal muscle surface electrodes and behavioral state was scored. To examine attention, learning and memory, fear conditioning and spatial navigation in the Morris water maze were assessed. Induction of immediate-early gene expression was quantified to examine neuronal activation following various behavioral epochs. NE was restored in Dbh KO mice by administering the synthetic amino acid precursor L-DOPS 5 h before testing.
Results: Relative to controls, Dbh KO mice sleep ~2 h more each day. The decrease in waking is due to a considerable decrease in the duration of waking bouts, in spite of an increase in the number of waking bouts and transitions from sleep to waking. In contrast, the amount of rapid-eye-movement (REM) sleep is only half that in controls due to a decrease in the number and duration of REM sleep bouts. Following 6 h of sleep deprivation, there is no rebound recovery of sleep time in the Dbh KO mice. One day after fear conditioning, Dbh KO mice are impaired in the expression of contextual but not cued fear. This deficit is present from 2 h to 4 d after training. Restoration of NE in Dbh KO mice before testing but not before training rescues contextual fear. Administration of β1-adrenergic receptor antagonists (but not other adrenergic receptor antagonists) mimics results from Dbh KO mice, as do results from β1 receptor KO mice. Similar time-

dependent impairments in retrieval were obtained for both contextual fear and spatial reference memory in rats treated with a β antagonist. Bilateral infusions of β1 antagonists into the dorsal hippocampus (DH) of mice impair retrieval but not consolidation of contextual fear. Administration of systemic or DH β1 antagonists in control mice and β1 agonists in Dbh KO mice indicates that contextual memory retrieval is required during and 3-4 h after context exposure for long-term extinction of fear. Finally, activation of CA1 pyramidal neurons is reduced following retrieval of contextual fear in the absence of NE/E, while activation of CA3 pyramidal neurons and dentate granule cells is not.

Discussion: The results provide genetic evidence that adrenergic signaling acts to maintain waking and is important for the regulation of REM sleep and sleep homeostasis. They also indicate that emotional memory formation and consolidation, and the attentional mechanisms needed to acquire the tasks above, do not depend critically on NE/E. Instead, β1-adrenergic signaling in the DH is required for the retrieval of an intermediate phase of hippocampus-dependent memory. β1 signaling may promote the transmission of retrieved information from CA3 to CA1, where it is compared to current contextual/spatial information coming directly from the entorhinal cortex. The results may be relevant to the recurrent, intrusive memories associated with post-traumatic stress disorder, and to the treatment of heart failure and hypertension with β blockers.

Noradrenergic Pharmacotherapies in Attention Deficit Hyperactivity Disorder

Thomas J Spencer*

Harvard Medical School, Boston, MA, USA

A variety of compounds with noradrenergic activity have shown documented anti-ADHD activity in clinical trials. In the last few years the first non-stimulant to be FDA approved for ADHD is the specific norepinephrine re-uptake inhibitor, atomoxetine. Prior and post approval a large data base has emerged on the clinical characteristics of atomoxetine in the treatment of ADHD across the lifecycle. In addition, there is a substantial body of literature documenting the efficacy of tricyclic antidepressants on ADHD in over 1, 000 subjects. Several formulations of the atypical antidepressant bupropion also have been documented to be effective in the treatment of ADHD in controlled clinical trials. In addition, there is evidence of the effectiveness of alpha adrenergic medications in ADHD. Despite the growing evidence of effectiveness of these agents, continued research is needed to increase our understanding of alternative pharmacologic treatments for the treatment of ADHD.

Panel Session
Plastic Changes in the Addicted Brain: A Glimpse at the Next Generation of Pharmacotherapies

Neuroadaptations in Proteins in the Postsynaptic Density and Cocaine Addiction

Peter Kalivas*, Shigenobu Toda, Hui Shen and Scott Mackler

Neurosciences, Medical University of South Carolina, Charleston, SC, USA

Repeated cocaine administration produces neuroplastic changes in the prefrontal cortical projections to the nucleus accumbens that are proposed to underlie cardinal features of addiction, such as the uncontrollable drive to obtain drug and relapse. Neuroplasticity in the brain involves changes in dendritic morphology and synaptic signaling. Two key features of neuroplasticity are the actin based organization of dendrite morphology and the postsynaptic density, and the ability of neurons to target proteins for destruction. Two new sets of data will be described that speak to each of these issues. Withdrawal from repeated cocaine favors the polymerization of actin in filopodia-like protein complexes. This adaptation is demonstrated by measuring levels of F- and G-actin and the associated actin binding proteins that regulate actin cycling and filopodia formation, including cofilin, lim-kinase, ena/Vasp, fascin, cortactin and Arp. Moreover, the restructuring of actin induced by cocaine is associated with a more labile regulation of glutamate receptor associated proteins in the postsynaptic density. Thus disruption of F-actin in rats treated with repeated cocaine induces a reduction in the membrane content of proteins such as GluR1, NMDAR2a and PSD-95. Finally, disruption of F-actin was shown to potentiate cocaine-seeking in a cocaine-priming reinstatement model, indicating that the restructuring of actin is a protective adaptation. A second adaptation produced by withdrawal from repeated cocaine that may be relevant to synaptic plasticity is the upregulation of the transcriptional regulator Nac1. In addition to being a transcriptional regulator, Nac1 undergoes a PKC- and activity-dependent translocation to the cytoplasm. Using a yeast two-hybrid strategy, it was found that Nac1 binds to Cul3, a subunit of E3 ligase responsible for protein ubiquitination, and to mov34, a subunit of the proteasome responsible for degrading polyubiquitinated proteins. The translocation of Nac1 from the nucleus to the cytoplasm also translocates its binding partners, Cul3 and the 20s proteasome. Thus, the machinery to degrade proteins is translocated by Nac1 to the cytoplasm in order to metabolize proteins internalized as a result of synaptic activity. Moreover, E3 ligase and the proteasome are translocated simultaneously, presumably creating a highly efficient degradatory protein complex. How cocaine withdrawal-induced upregulation of Nac1 alters this important cellular process is being investigated. In conclusion, protein organization, trafficking and degradation in the postsynaptic density is highly regulated to permit neuroplastic cellular and behavioral responses to important stimuli, including drugs of abuse such as cocaine. The cellular underpinnings of this plasticity are being elucidated and are strongly regulated by repeated cocaine administration. The proteins intimately involved in this process are potential pharmacotherapeutic targets for regulating cocaine-induced plasticity.

Role of Amygdala ERK in Incubation of Cocaine Craving

Lin Lu, Bruce T Hope and Yavin Shaham*

Behavioral Neuroscience, IRP-NIDA, Baltimore, MD, USA

Using a rat model of craving and relapse, we previously found time-dependent increases in cocaine seeking induced by exposure to drug-associated cues over the first months of withdrawal from cocaine, suggesting that drug craving incubates over time (Grimm et al. Nature 2001; Lu et al. Neuropharmacology 2004). In a recent series of experiments, we explored the role of amygdala extracellular signal-regulated kinases (ERK) signaling pathway in the incubation of cocaine craving (Lu et al. Nature Neuroscience 2005). As in our previous studies, we found that cocaine seeking induced by exposure to cocaine cues was substantially higher after 30 withdrawal days than after 1 day. More importantly, exposure to these cues increased ERK phosphorylation in the central, but not basolateral, amygdala after 30 days, but not 1 day, of withdrawal from cocaine self-administration. Furthermore, after 30 days of withdrawal from cocaine self-administration, inhibition of central, but not basolateral, amygdala ERK phosphorylation by a selective inhibitor (U0126) decreased cocaine seeking. Finally, after 1 day of withdrawal, stimulation of central amygdala ERK phosphorylation by NMDA increased cocaine seeking and this effect was reversed by U0126. The results of these experiments suggest that time-dependent increases in the responsiveness of central amygdala ERK signaling pathway to cocaine cues mediate the incubation of cocaine craving.

The Endogenous Brain Cannabinoid System and the Motivational Control of Reinforced Behavior
Steven R Goldberg*

Preclinical Pharmacology Section, Behavioral Neuroscience Research Branch, NIDA, IRP, NIH, DHHS, Baltimore, MD, USA

Background: Although endogenous cannabinoid systems have been implicated in neurobiological phenomena related to drug addiction, little is known about direct effects of endogenous ligands for cannabinoid receptors on brain reward processes. Indirect in-vivo evidence for a role of endogenous cannabinoid systems in the modulation of brain reward processes comes from behavioral studies of the effects of cannabinoid CB1 receptor antagonists on food consumption, electrical brain stimulation reward or intravenous self-administration of abused drugs such as heroin and cocaine.
Methods: Squirrel monkeys that had learned to intravenously self-administer either delta-9-tetrahydrocannabinol (THC), the endogenous cannabinoid anandamide or its metabolically-stable synthetic analog methanandamide, were pretreated with cannabinoid-CB1 or mu-opioid receptor antagonists. In other studies, rats were trained under a fixed-ratio schedule of food reinforcement to discriminate between intramuscular injections of either THC or morphine and injections of saline and different doses of various cannabinoid or opioid compounds were then tested by substitution or pretreatment. Finally, the ability of anandamide, methanandamide to increase levels of dopamine in the shell of the nucleus accumbens was studied using in-vivo microdialysis procedures in rats. These animal models allow evaluation of rewarding effects of synthetic and endogenous cannabinoids compounds acting at different levels on the brain cannabinoid system.
Results: Anandamide and its longer-lasting analog methanandamide, serve as effective reinforcers of drug-taking behavior when self-administered intravenously by squirrel monkeys, in a similar manner to THC, heroin and cocaine. In rats, anandamide and methanandamide produce THC-like discriminative effects and, like THC, heroin and cocaine, produce rapid increases in dopamine levels in the shell of the nucleus accumbens, a feature of most abused drugs. The reinforcing, discriminative and neurochemical effects of anandamide are potentiated by drugs that inhibit its inactivation and reduced by cannabinoid CB1 antagonists. Also, opioid antagonists reduce the reinforcing and discriminative effects of THC in monkeys and rats and cannabinoid CB1 antagonists reduce the reinforcing and discriminative effects of heroin in rats.
Discussion: These studies provide direct in-vivo evidence for a role of endogenous cannabinoids in the modulation of brain reward processes and suggest an additional direction for investigating functional interactions between endogenous cannabinoid, opioid and dopaminergic neurotransmitter systems that may mediate or modulate the rewarding and discriminative effects of various addictive drugs. This research approach provides an opportunity to search for drugs with the beneficial therapeutic actions of currently available cannabinoids (or opioids) but without their undesirable side effects such as abuse liability.

Why Are Drugs That Enter the Brain Rapidly Potentially the Most Addictive?
Terry Robinson*

University of Michigan, Ann Arbor, MI, USA

It is widely accepted that the more rapidly drugs of abuse reach the brain the greater their potential for addiction. This might be one reason why cocaine and nicotine are more addictive when they are smoked than when they are administered by other routes. Traditionally, rapidly administered drugs are thought to be more addictive because they are more euphorigenic and/or more reinforcing. However, evidence for this is not compelling. For example, variation in the rate of drug delivery has little effect on self-administration behavior. In this talk an alternative (although not mutually exclusive) explanation will be presented, based on the idea that the transition to addiction involves drug-induced plasticity in mesocorticolimbic systems. For example, rapidly administered cocaine or nicotine preferentially engage mesocorticolimbic circuits, as indicated by studies of drug-induced gene expression, and more readily induce plasticity in these circuits, as indicated by increased susceptibility to sensitization. Therefore, rapidly delivered drugs might promote addiction by promoting forms of neurobehavioural plasticity that contribute to the compulsive pursuit of drugs.

Panel Session
Neural Changes in Adolescence and Onset of Schizophrenia

Structural MRI of Typical Adolescent Brain Development
Jay N Giedd*

NIMH, Bethesda, MD, USA

Background: Characterization of anatomical changes in typical adolescent brain development provides a yard stick from which to assess deviations in clinical populations such as childhood onset schizophrenia.
Methods: MRI scans, neuropsychological testing, and DNA analysis is conducted on typically developing children and adolescents with longitudinal scans acquired at approximately 2 year intervals. The data base currently consists of approximately 1000 scans from 500 subjects. The images are analyzed by a variety of automated and manual techniques to yield measures of cortical thickness and regionally gray and white matter volumes.
Results: Adolescence is a time of particularly active reductions of cortical volumes (as assessed by slope of change over time compared to other ages). Speculatively, this reduction in cortical thickness may partly reflect pruning of synaptic connections. The developmental trajectories are regionally specific. Particularly late to reach adult levels of cortical thickness is the dorsolateral prefrontal cortex.
Conclusions: Comparison of developmental trajectories from typically developing populations to childhood onset schizophrenia suggest that the developmental deviations in the COS group may represent an exaggeration of the typical developmental changes.

Changes in Prefrontal Dopamine Function During Adolescence and in a Developmental Animal Model of Schizophrenia
Patricio O'Donnell* and Kuei-Yuan Tseng

Center for Neuropharmacology & Neuroscience, Albany Medical College, Albany, NY, USA

Background: Converging lines of evidence indicate that cortical areas including the prefrontal cortex (PFC) mature during adolescence. The onset of schizophrenia around this critical period may indicate that anomalies in such late developmental processes may be involved. Here we report changes in the electrophysiological effects of dopamine (DA) on prefrontal cortical pyramidal neurons and interneurons during adolescence. Also, as the neonatal ventral hippocampal lesion has been reported as an animal model causing periadolescent emergence of abnormal behaviors resembling phenomena observed in schizophrenia, we explored whether the periadolescent maturation of PFC DA actions was affected in these animals.
Methods: Both in vivo and in vitro recordings from PFC pyramidal neurons were conducted assessing the responses to DA agonists (in slices) and to electrical mesocortical activation (in vivo). The recordings were conducted in rats of different ages, both before puberty (postnatal day (PD) 28-35) and after adolescence (PD>50). Some recording were conducted in young and adult animals that had a neonatal (PD7-8) ventral hippocampal lesion (NVHL) or in sham-operated animals. In vivo intracellular recordings were conducted in

rats anesthetized with chloral hydrate using sharp electrodes, and in vitro recordings were conducted using the whole-cell technique.
Results: As we had previously studied the modulation of pyramidal cell responses to glutamate agonists in slices, we assessed the synergistic response to combined application of a D1 DA agonist and NMDA in slices from animals of different ages. In slices from adult rats, the combined activation of these receptors resulted in the emergence of membrane potential oscillations resembling in vivo up states, which were due to activation of a network of local neurons. This effect was not obtained in slices from prepubertal animals, indicating that PFC local network connectivity matures during adolescence. Similarly, the ability of D2 agonists to increase interneuron cell excitability was not observed before adolescence, suggesting that at least a population of interneurons acquires modulation by DA during that late developmental period. In neurons from adult animals with a NVHL, this D2 drive of interneurons was not observed, suggesting that absence of proper hippocampal innervation early in life may affect interneurons so they cannot mature during adolescence.
Discussion: The periadolescent changes in DA modulation of PFC circuits may be critical for the known maturation of PFC-dependent cognitive and executive functions. Previous work with NVHL animals revealed that although the impairment of hippocampal activity was carried out early, the behavioral symptoms (which resemble PFC deficits) did not emerge until after puberty. Although DA-glutamate and DA-GABA interactions in the PFC normally mature during adolescence, that maturation does not take place in NVHL animals. This could be responsible for the delayed emergence of symptoms in these animals, and it suggests that abnormal interneuron development during adolescence could be a critical component in schizophrenia pathophysiology.

Differential Expression in Schizophrenic Brain of a DISC1 Molecular Pathway and Association with DISC1 SNPs
Barbara K Lipska*, Thomas M Hyde, Tricia Peters, Daniel R Weinberger and Joel E Kleinman

NIMH, Bethesda, MD, USA

DISC1 has been identified as a schizophrenia susceptibility gene based on linkage and SNP association studies and clinical data suggesting that risk SNPs impact on hippocampal structure and function. In cell and animal models, C-terminus-truncated DISC1 disrupts intracellular transport, neural architecture and migration, perhaps because it fails to interact with binding partners involved in neuronal differentiation, such as NUDEL, FEZ1 and LIS1. We hypothesized that altered expression of DISC1 and/or its molecular partners may underlie its pathogenic role in schizophrenia and explain its genetic association. We examined the expression of DISC1 and these selected binding partners as well as reelin, a protein in a related signaling pathway, in the hippocampus and dorsolateral prefrontal cortex (DLPFC) of postmortem human brain of schizophrenic patients and controls. DISC1 mRNA was developmentally variable in the human brain, showing high levels of expression in early childhood (neonatal period, infancy) and then declining rapidly by teenage years and into adulthood. These data suggested that the overall pattern of DISC1 expression is similar across species and underscored its potential role in early human brain development, including the development of the hippocampal formation and the neocortex. We found no difference in the expression of DISC1 or reelin mRNA in schizophrenia and no association with previously identified risk SNPs. This may not be surprising given the fact that the risk alleles identified in our clinical study are common and that they are located far from the transcriptional start sites of the DISC1 variants. However, the expression of NUDEL, FEZ1 and LIS1 was each significantly reduced in schizophrenic tissue and expression of each showed association with high risk DISC1 polymorphisms. These data implicate genetically linked abnormalities in the DISC1 molecular pathway in the pathophysiology of schizophrenia.

Brain Development in Childhood Onset Schizophrenia and their Healthy Siblings
Judith L Rapoport*

DIRP, National Institute of Mental Health, NIH, Bethesda , MD, USA

Neurodevelopmental models dominate the study of schizophrenia but specifics of abnormal neurodevelopment are lacking in pediatric risk studies. Methods: Since 1989, prospective anatomic brain MRI scans and rescans have been obtained on healthy children (n=165, 450 scans), children with very early onset schizophrenia (COS) (n= 78, 220 scans) and their full siblings (n= 67, 168 scans). Regional cortical thickness and subcortical volumes and their developmental trajectories have been examined in relation to diagnosis and risk gene status. Results: The healthy subjects exhibit a back to front wave of cortical gray matter loss, and regionally heterogeneous change in hippocampal development across ages 5-26. The childhood onset schizophrenic subjects show an exaggeration of this normal developmental pattern. Healthy full siblings of childhood onset schizophrenic patients show an initial regional cortical thinning which ameliorates with age. Subjects with known risk alleles for the schizophrenia susceptibility genes such as GAD1, Dysbindin and Neuregulin show patterns of brain abnormality and developmental trajectories that differ from those for healthy controls and differ from each other.
Conclusions: Developmental trajectories provide clues to the underlying pathophysiology of schizophrenia implicating a general exaggeration of processes underlying adolescent brain development. In addition, protective factors may act via plastic brain developmental changes for healthy at risk subjects. It is expected that these developmental patterns will be useful endophenotypes for future genetic studies

Panel Session
Neuregulin 1 - ErbB4 Signaling in Schizophrenia

Neuregulin/ErbB Signaling in Schizophrenia: An Overview
Cary Lai* and Janet Weber

Molecular and Integrative Neuroscience, The Scripps Research Institute, La Jolla, CA, USA

The identification of the neuregulin-1 (NRG1) gene as a risk factor for a subset of patients with schizophrenia has led to considerable interest in the possible mechanisms by which it may be involved in the development of this disorder. The biological activities of the neuregulins have been studied for over 25 years and its reported roles are numerous and diverse, making it a challenge to determine which of these are relevant to the etiology of schizophrenia. As changes in the nucleotide sequence of the coding regions of the NRG1 gene have not been identified in schizophrenics, it is currently believed that the increased risk is related to changes in the level of expression of one or more of the NRG1 protein forms. In this overview, the molecular complexity and biological activities of distinct NRG1 isoforms and their signaling through their receptors, the protein-tyrosine kinases known as the ErbBs, will be summarized. As will be discussed in greater detail by the other members of this panel, there are multiple avenues by which alterations in NRG1 expression may contribute to the development of this disorder. First, NRG1 signaling appears to influence the migration of several neuronal populations, suggesting that changes in the final placement of neurons may lead to the formation of altered neuronal ciruitry in the brain. Second, NRG1 has been shown to influence the expression of multiple neurotransmitter receptors in neurons in vitro, suggesting that this factor could regulate the responsiveness of distinct populations of ErbB-expressing neurons in vivo. Third, ErbB4 is expressed at high levels in cortical and hippocampal interneurons, suggesting that NRG1 signaling may modulate the function of inhibitory cells and their interactions with pyramidal neurons. And last, NRG1 has been recently shown to influ-

ence the extent of myelination in the CNS, suggesting that changes in axonal conduction velocity may disrupt the timing of neuronal activity and alter the connectivity between brain regions. Our understanding of the relevant NRG1 isoforms and ErbB receptors underlying each of these biological activities will be evaluated.

Neuregulin 1- ErbB Signaling During Neuronal Migration in the Developing and Adult Cerebral Cortex

Eva S Anton*

Neuroscience Center, UNC, Chapel Hill, Chapel Hill, NC, USA

Normal development of the mammalian cerebral cortex requires coordinated migration of post mitotic neurons from the ventricular zone to the outermost layer of the developing cortical plate along radial glia. In the adult cerebral cortex, neural precursors migrate from the anterior subventricular zone (SVZ) to distinct forebrain regions and become interneurons. The mechanisms that determine how these processes are regulated in the embryonic and adult brains are not well understood. We found that neuregulin-1 (NRG-1)-erbB2 interactions exert a critical role in the establishment of radial glial cells needed to guide appropriate neuronal placement in the embryonic cortex. In contrast, ErbB4 activation helps to regulate the generation, migration, and differentiation of interneuronal precursors in the adult cortex. These results, together with recent studies identifying NRG1 as a susceptibility gene for Schizophrenia raise the intriguing possibility that impaired neuregulin-ErbB mediated neuronal precursor differentiation and migration, and the resultant changes in neural circuitry in the forebrain, may enhance the sensitivity for neurodevelopmental disorders such as schizophrenia.

Regulation of Synaptic Plasticity by the NRG-1/ErbB Signaling Pathway: Possible Implications for Schizophrenia

Andres Buonanno*, Oh-Bin Kwon, Detlef Vullhorst, Dax Hoffman and Marines Longart

National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA

Background: Neuregulin-1 (NRG-1), a trophic and differentiation factor that signals via ErbB receptor tyrosine kinases, has been identified genetically as a schizophrenia susceptibility gene. We previously hypothesized that the NRG/ErbB signaling pathway regulates plasticity at glutamatergic synapses based on our work showing that: NMDA and ErbB receptors co-localize at postsynaptic densities where they interact with PDZ domain proteins implicated in activity-dependent synaptic modifications, and that co-activation of both receptors regulate NMDA receptor (NMDAR) expression. Consistent with a role of the NRG/ErbB pathway in regulating synaptic plasticity and cognitive processes, NRG-1 was reported to regulate LTP in hippocampal slices. Moreover, NRG-1 and ErbB-4 mutant mice have lower NMDAR levels and manifest behavioral deficits that are reversed by clozapine treatment. To further explore the molecular mechanisms that regulate interactions between the NRG/ErbB and glutamatergic transmission, both reportedly altered in schizophrenia, our present studies have focused on how NRG-1 modulates plasticity at glutamate synapses, and how signaling via ErbB4 receptors is regulated during development.
Methods: The effects of NRG-1 on glutamatergic transmission and plasticity were analyzed electrophysiologically (field potentials and patch-clamp) in acute hippocampal slices. The effects of NRG-1 on glutamate and ErbB receptor trafficking were followed in hippocampal neurons using immunofluorescence cytochemistry, and in neurons transfected with receptors tagged with a pH-sensitive GFP that allow the specific visualization of surface receptors in live neurons utilizing spinning-disc microscopy.
Results and Discussion: We will present evidence that NRG-1 depotentiates (reverses) LTP at CA1 hippocampal synapses in an activity- and time-dependent fashion. NRG-1 selectively regulates AMPA receptor (AMPAR) postsynaptic currents in potentiated slices and their surface expression in dissociated hippocampal neurons. No effect of NRG-1 was observed on NMDAR currents or internalization. We will also show that ErbB receptor blockers prevent stimulus-dependent LTP depotentiation, consistent with a role of this pathway regulating homeostasis at glutamatergic synapses and with important implications in schizophrenia. Because of the importance of NRG/ErbB signaling for glutamatergic transmission and the interactions between both pathways, we analyzed the dynamic regulation of ErbB4 levels during neuronal development. Interestingly, we found that the proportion of ErbB4 surface receptors in neurons changes dramatically during development, and that receptor trafficking is differently regulated by NRG-1 during maturation. We will present evidence that suggests that ErbB4 surface levels are regulated by its interaction with PSD-95 (via its cytoplasmic C-terminal tail). Although we have not yet elucidated the precise mechanisms that regulate steady-state ErbB4 surface levels, our findings suggest that interactions between NRG-1 and its receptors may regulate the efficacy of this signaling pathway. In summary, our findings on the function and regulation of the NRG/ErbB signaling pathway have important implications for schizophrenia because they point to potential molecular mechanism that are altered in individuals carrying polymorphisms that modulate the levels of NRGs or their receptors. Our results also draw an important link between NRG signaling and glutamatergic transmission, the latter which is important for regulating cognitive function and is altered in schizophrenia.

ErbB4 Signaling is Altered in Schizophrenia

Chang-Gyu Hahn* and Hoau-Yan Wang

Psychiatry, University of Pennsylvania, Philadelphia, PA, USA

Background: Recent molecular genetics studies have implicated neuregulin 1 (NRG1) and its receptor, erbB, in the pathophysiology of schizophrenia. ErbB4 is of particular interest because of its crucial roles in modulating neurodevelopment and neurotransmitter receptor signaling, including that of N-methyl D aspartate (NMDA) receptors. In this study, we analyzed NRG1-induced erbB4 signaling in postmortem prefrontal cortex of 14 age- and sex-matched pairs of schizophrenia and control subjects.
Methods: To examine NRG1-induced erbB4 activation in postmortem brains, we employed the "ex vivo stimulation paradigm", in which slices of the prefrontal cortex were stimulated with NRG1. Synaptosomal extracts of stimulated tissues were then analyzed for tyrosine phosphorylation of erbB4 and other signaling mechanisms using immunoprecipitation. NMDA receptor activation in postmortem brain tissues was assessed similarly.
Results: Immunoblotting analyses showed no significant differences in NRG1 and erbB4 levels in the prefrontal cortex of schizophrenia cases compared to controls. In contrast, NRG1-induced erbB4 activation (measured by tyrosine phosphorylation of erbB4 in response to NRG1 stimulation) was dramatically increased in schizophrenia subjects compared to controls. NRG1 stimulation also led to enhanced activation of the downstream erbB4 signaling molecules, ERK2 and AKT in schizophrenia subjects compared to controls. Such hyper-responsiveness in erbB4 signaling might be due to altered association of erbB4 with PSD 95, since the association of erbB4 with PSD-95 has been shown to facilitate erbB4 activation. Schizophrenia subjects did, in fact, exhibit significant increases in erbB4 PSD-95 interactions compared to controls. As in previous studies in the rodent cerebral cortex, NRG1 was found to suppress NMDA receptor activation in the human prefrontal cortex. The suppressed activation was enhanced in schizophrenia, consistent with enhanced NRG1-erbB4 signaling observed in this illness.
Discussion: Results of our study demonstrate significantly enhanced erbB4 signaling in the prefrontal cortex of schizophrenia subjects, which might be due to altered association of erbB4 with PSD-95.

Since NRG1-induced suppression of NMDA receptor activation is more pronounced in schizophrenia subjects, we propose that enhanced NRG1-erbB 4 signaling may contribute to NMDA hypofunction in that disorder.

Panel Session
Drug Development: Novel Insights into the Neurobiology and Treatment of Autism Spectrum Disorders

Neuropeptides and the Social Brain: The Value of Comparative Studies in Autism Research

Larry J Young*

Psychiatry, Emory University, Atlanta, GA, USA

Autism spectrum disorders are a set of complex neurodevelopmental disorders characterized by a core deficit in social engagement and reciprocity, often accompanied by deficits in communication, repetitive or ritualistic behaviors, and cognitive impairments. The etiology of autism spectrum disorders is unclear, but likely arises from the interactions of dozens of genetic and environmental factors. Clearly, multiple approaches must be taken in the development of treatments for this devastating disorder. Research into the neurobiology regulating normal social cognition and attachment in animal models may prove useful for understanding the basis of the core social deficits in autism, potentially yielding novel targets for therapeutic interventions. Numerous studies in a variety of species have demonstrated that the neuropeptides oxytocin (OT) and vasopressin (AVP) play central roles in regulation social cognition, communication and attachment. Comparative studies using highly social, monogamous prairie voles, and asocial, nonmonogamous meadow voles further suggest that variations in these neuropeptides systems significantly influence social behavioral phenotype. Both OT and AVP facilitate social engagement and social bond formation in prairie voles, but not in meadow voles. The neuroanatomical distribution of the OTR and V1aR are strikingly different between the highly social, monogamous species, and the asocial, non-monogamous species. Of particular interest, OTR and V1aRs are concentrated in the nucleus accumbens and ventral pallidum, respectively, in monogamous species, and site-specific blockade of these receptor populations prevents social bonding. These regions are central components of the dopamine reward circuitry, suggesting that an interaction of OT and AVP with the brain's reward pathways is critical for normal social bonding. Using viral vector gene transfer, we have demonstrated that increasing the expression of V1aR in the ventral pallidum of the non-monogamous meadow vole results in the formation of social bonds similar to that of prairie voles. These studies suggest that variation in the expression of these neuropeptide receptors may significantly influence social behavior. We have identified a polymorphic microsatellite region in the promoter of the vole V1aR that likely results in the species differences in expression pattern. Since microsatellite DNA is inherently unstable, we investigated whether individual variation in microsatellite length between prairie voles might contribute to individual variation in V1aR expression in the brain as well as in social behavior. Male prairie voles with a longer than average microsatellite expressed higher levels of V1aR in specific brain regions, and displayed higher levels of social interaction and bonding, than males with shorter microsatellites. Humans may share some of these mechanisms and when disrupted, either by genetic, environmental or interactive causes, extreme phenotypes such as autism may be revealed. In fact, there are intriguing parallels between our studies in voles and studies in autistic patients. OT levels in the CSF of autistic children have been reported to be approximately half of that found in unaffected children. Furthermore, the human V1aR gene contains three polymorphic microsatellites in the promoter. Two independent studies have found a modest association between the number of repeats in one of these microsatellites and autism. These studies illustrate the power of the comparative neuroethological approach for understanding human neurobiology and suggest that examining the neurobiological bases of complex social behavior in divergent species is a valuable approach to gaining insights into human psychopathologies.

Molecular Mechanisms of Fragile X Syndrome Reveal Potential Therapeutic Approaches

Stephen T Warren*

Human Genetics, Emory University School of Medicine, Atlanta, GA, USA

Fragile X syndrome is an autistic-like syndrome that represents one of the most frequent forms of inherited mental retardation. The disorder is due a CGG trinucleotide repeat expansion in the untranslated region of the X-linked FMR1 gene. When the repeat length expands beyond 200 repeats in affected patients, the gene becomes heavily methylated and the chromatin shifts to a heterochromatic pattern silencing the gene. Thus the loss of the encoded protein, FMRP, is responsible for fragile X syndrome. FMRP is a selective RNA-binding protein that acts as a translational suppressor of its target mRNAs in a process involving endogenous microRNAs. The majority of cellular FMRP can be found associated with ribosomes, particularly at the base of or within dendritic spines, a location where local control of protein synthesis is established as critical in synaptic function. It has been found that mGluR5-stimulated protein synthesis-dependent LTD is exaggerated in the Fmr1 knockout mouse. Thus a consequence of FMRP loss is the enhanced translation of mRNAs normally associated with FMRP, such as that encoding MAP1B. This hypothesis has lead to rationale approaches for drug development for fragile X syndrome by exploring the utility of mGluR5 antagonists. Data will be presented that such antagonists do indeed moderate the phenotype caused by FMRP loss in model organisms. Moreover, using the Drosophila-model of fragile X syndrome responsive to mGluR5 antagonists, we have developed a moderate throughput platform for drug screens relevant to fragile X syndrome.

Clues From Basic Neurodevelopment For New Treatment Targets in Autism

Pat Levitt*, Daniel B Campbell, Kathie L Eagleson, Alexandre Bonnin, Antonio M Persico and James S Sutcliffe

Vanderbilt Kennedy Center for Research on Human Development, Vanderbilt University, Nashville, TN, USA

Background: Autism is a neurodevelopmental spectrum disorder that affects approximately 1 in 166 children. There has been a convergence of clinical and basic neuroscience evidence on the disrupted development of cortical circuitry. In particular, altered cortical and subcortical GABAergic function may be a source of vulnerability, as well as a potential target of intervention. Both animal models and human genetic studies can be integrated to address new opportunities. We have focused on c-met (MET), the tyrosine kinase receptor for hepatocyte growth factor (HGF), both of which have pleotropic biological activities. In addition, loci encoding these molecules co-localize with sites of linkage for autism vulnerability.
Methods: Genetic models in mice are used to probe developmental and behavioral disruption. Transgenic mice that misexpress genes controlling the migration and differentiation of GABAergic neurons were examined for alterations in cortical development and adult behavior. Family-based association studies in multiple samples were performed to determine a potential link between these molecular agents of interneuron development and autism spectrum disorders.
Results: Altered expression of both HGF and c-met in mice, via manipulation of an enzyme that activates pro-HGF, leads to reductions in GABAergic neurons in the cortex. The vulnerability for neuronal reduction is dependent upon genetic background, illustrating the role

of complex genetics in mediating the morphological phenotype. Animals with disruption of interneuron development exhibit increased anxiety and altered social behavior. In extrapolating these studies to potential vulnerability for autism, direct screening of the MET gene revealed functional polymorphisms. In addition to influencing brain development, a role for HGF/c-met signaling in peripheral organ functions that are disrupted in autism, such as the gastro-intestinal system, makes it an intriguing target.
Discussion: The data indicate that a unique basic-clinical approach to neurodevelopment disorders can be used to establish mechanisms that may underlie autism susceptibility. Detection of developmental changes requires detailed neuroanatomical analyses, and follow-up behavioral and bioinformatics studies may be applied to candidate biological systems potentially involved in autism. Such studies may facilitate the design of molecular diagnostic methods and to develop interventions that effectively modulate specific signaling systems to reduce the impact of genetic vulnerability. Supported by: NIMH R01MH67842 (PL) and NICHD P30HD15052 core grant to the Vanderbilt Kennedy Center (PL) and Telethon-Italy grant GGP02019, the Fondation Jerome Lejeune, and the Cure Autism Now Foundation (A.M.P.).

Pharmacological Treatment of Behavioral Symptoms in Autism
Christopher J McDougle*, Kimberly A Stigler and David J Posey

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

Background: To review the pharmacotherapy of autism.
Methods: Open-label and controlled drug studies have been conducted for the interfering symptoms associated with autism.
Results: For motor hyperactivity and inattention, studies have indicated that the alpha2 agonists, clonidine and guanfacine, are useful. A placebo-controlled study by the Research Units on Pediatric Psychopharmacology (RUPP) Autism Network found methylphenidate to be efficacious in 49% of subjects for these target symptoms. Preliminary data with the norepinephrine reuptake inhibitor atomoxetine are encouraging. Controlled studies of the selective serotonin reuptake inhibitor fluvoxamine found the drug to be more efficacious and better tolerated in adults than children with autism. A recent controlled study of low-dose liquid fluoxetine found the drug more effective than placebo for interfering repetitive behavior. A large placebo-controlled study of the atypical antipsychotic risperidone found the drug to be efficacious for reducing aggression, self-injury and tantrumming in 70% of children with autism and that the response was maintained for up to 6 months. Open-label studies of other atypical antipsychotics are generally encouraging. A small, single-blind study of the glutamatergic agent D-cycloserine showed significant benefit for the social withdrawal of autism.
Discussion: Significant advances have been made in treating interfering target symptoms associated with autism.

Panel Session
Neural Mechanisms of Extinction: Translating from Rats to Humans

Extinction Training Initiated Shortly after Trauma May Reduce Spontaneous Recovery, Renewal and Reinstatement: Implications for Exposure-Based Psychotherapy
Karyn M Myers and Michael Davis*

Psychiatry, Emory University, Atlanta, GA, USA

Behavioral evidence indicates that extinction of conditioned fear is an inhibitory learning process because extinguished fear reappears with the passage of time (spontaneous recovery), a shift of context (renewal), and unsignalled presentations of the unconditioned stimulus (reinstatement). However, there is also evidence to suggest that extinction is "unlearning" based on recruitment of phosphatases and suppression of fear-related gene expression following extinction training. Because extinction training was initiated relatively soon after fear acquisition in these latter studies it is possible that the mechanism of extinction varies with the time when extinction training is initiated, perhaps as a function of the consolidation state of the original fear memory. We examined this issue by comparing groups of rats extinguished 10 min, or 1, 24 or 72 hrs following acquisition on their susceptibility to spontaneous recovery, renewal and reinstatement. Rats extinguished 72 hrs after acquisition exhibited robust spontaneous recovery, renewal and reinstatement. In contrast, rats extinguished 10 min after acquisition did not show significant spontaneous recovery, renewal or reinstatement. Groups extinguished 1 or 24 hrs were intermediate depending on the measure. Calcineurin protein (measured via Western blot) increased in the amygdala following short interval (10 min) but not long interval (72 hrs) extinction whereas calcineurin mRNA (measured via in situ hybridization) in the basolateral nucleus of the amygdala did not differ among short and long interval extinction groups as compared to acquisition groups that did not get extinction training. This suggests a specific upregulation of calcineurin protein in the amygdala following short interval extinction, mediated through a translational but not a transcriptional mechanism, and may be consistent with the hypothesis that short interval extinction occurs via an erasure mechanism. Currently we are testing whether calcineurin inhibitors will block short interval but not long interval extinction. These data suggest that exposure-based psychotherapy given shortly after trauma may reduce relapse provided that a full therapeutic exposure is given.

Retention of Fear Extinction in Rats Depends on Infralimbic Prefrontal Cortex Plasticity
Gregory J Quirk*, Edwin Santini and James T Porter

Dept. of Physiology, Ponce School of Medicine, Ponce, Puerto Rico

While the role of the medial prefrontal cortex (mPFC) in behavioral inhibition is well established, recent work has focused on the mPFC mechanisms of extinction of conditioned fear. In extinction, fear responses are suppressed to a tone conditioned stimulus repeatedly presented in the absence of the shock unconditioned stimulus. Deficits in extinction are thought to be a predisposing factor in PTSD and other anxiety disorders. Converging evidence suggests that the infralimbic subregion mPFC (IL-mPFC) retains a memory of extinction and suppresses fear via feed-forward inhibition of the amygdala. IL neurons show potentiation of tone responses when rats are recalling extinction, and electrical stimulation of IL reduces fear and dampens amygdala output. Interfering with protein synthesis or NMDA receptors in IL impairs extinction retention, suggesting that extinction triggers plastic processes in IL necessary for consolidation of extinction memory. Using in-vitro slice recording techniques, we observed increased intrinsic excitability in IL neurons from rats given extinction training, compared to rats that received conditioning but no extinction. Depolarizing current caused neurons in the extinction group to fire almost twice the number of spikes as controls, suggesting that extinction alters membrane properties in IL. This increase in excitability was associated with an increase in Ih, a hyperpolarization-activated cation current. Finally, the rostral IL appears to be significantly larger in rats showing good retention of extinction, mirroring a similar finding in humans (Milad et al., 2005). This unusual convergence of findings suggests that retention of fear extinction requires potentiation in IL circuits. Studies of prefrontal extinction circuits in the rat serve as a useful model of PTSD, and the human mPFC may be an important target for future therapies aimed at strengthening extinction.

Neural Mechanisms of Long- and Short-Term Fear Extinction in Humans

Fred J Helmstetter*, Jennifer A Richards and Dominic T Cheng

Psychology, University of Wisconsin - Milwaukee, Milwaukee, WI, USA

Recent data from several laboratories support the idea that extinction after fear conditioning involves an active inhibitory learning process and potentially unique neural substrates. Prior work also suggests that mechanisms underlying decrimental behavioral change within an experimental session ('short-term extinction') may differ from those involved in retaining extinction memories over multiple days ('long-term extinction'). The present fMRI study examined learning-related changes in brain activity during three phases of differential fear conditioning: Initial fear acquisition, short-term extinction, and long-term extinction. In each phase we recorded whole-brain BOLD images, CS-UCS contingency estimates, and autonomic nervous system activity in real time. Contingency estimates reflect the subjects' trial by trial expectancy that a given visual stimulus will be followed by shock and thus serve as an index of 'explicit' or 'declarative' learning in fear conditioning. Healthy adult right-handed volunteers were presented with ten trials of two unique visual stimulus pairs in the acquisition phase. One stimulus in each pair was consistently followed by brief shock (CS+) while the other never signaled shock (CS-). Fifteen minutes after training one pair of stimuli was presented repeatedly without shock to assess brain metabolic changes during short-term extinction. A long-term extinction session was conducted 24 hours after acquisition using all four conditional stimuli. Analysis of variance comparing BOLD activity for CS+ and CS- trials during the acquisition phase and revealed significant contingency-related differences in a network of regions including prefrontal cortex, insula, and lentiform nucleus. Activity in these and other regions of interest was sampled during short-term and long-term extinction phases. During short-term extinction, differential activation to CS+, which often did not correspond to subjects' expectancies, was retained in a subset of regions. For long-term extinction, discrete areas of frontal cortex showed different patterns of activation including differential (CS+ vs CS-) responses to the stimuli that had not been previously extinguished as well as increased averaged (CS+/CS- combined) responses in long-term versus short-term extinction and acquisition. Anterior cingulate cortex was most active during the consolidation of new information regardless of experimental phase. These results suggest that changes in activation patterns within a network of brain regions facilitate new learning during short-term and long-term extinction.

Magnetic Resonance Imaging Studies of Extinction Retention in Human Subjects

Scott L Rauch*, Mohammed R Milad, Scott P Orr and Roger K Pitman

Psychiatry, Massachusetts General Hospital, Charlestown, MA, USA

Background: A popular neurocircuitry model of posttraumatic stress disorder (PTSD), initially motivated by the neural substrates of fear conditioning and extinction gleaned from animal research, focuses on the possible role of deficient top-down modulation of amygdala responses by territories of medial prefrontal cortex. Human neuroimaging data have provided some initial support for this working model. However, the homology between animals and man with respect to medial prefrontal regions and extinction functions has yet to be fully delineated. Here we present new structural as well as functional MRI data regarding the brain correlates of extinction retention in healthy human subjects.
Methods: Two novel extinction retention paradigms were used to study healthy adult human subjects in conjunction with magnetic resonance imaging (MRI); both involved conditioning and extinction training on day 1, with extinction retention testing on day 2. In experiment 1, the behavioral results, including skin conductance responses, were gathered offline, and subsequently, structural MRI data were gathered at 1.5T. Cortical thickness measures were made from the MRI and tested for correlation with measures of extinction retention using both region of interest (ROI) and vertex-based methods. In experiment 2, with an independent sample of subjects, conditioning extinction and extinction recall were performed during functional MRI acquisition in the context of an event-related design. Data were analyzed using voxelwise methods.
Results: Both experiments showed effective extinction retention on day 2. Data from experiment 1 showed that cortical thickness within medial orbitofrontal cortex correlates with extinction retention. Data from experiment 2 showed significant ventromedial prefrontal cortical activation associated with extinction retention.
Discussion: Convergent findings from our structural (cortical thickness) experiment and fMRI experiment, as well as fMRI results from other laboratories, implicate a specific territory within ventromedial prefrontal cortex in human extinction retention. Future studies using this approach are warranted to investigate the pathophysiology of PTSD and other anxiety disorders as well as the potential to guide behavioral treatment. Moreover, broader implications regarding the neural substrates of personality and anxiety vulnerability will be discussed.

Panel Session
Sex Differences in the Action of Opioids

Opioid Drugs: Where Sex Makes a Difference

Christopher J Evans* and Camron D Bryant

Psychiatry, UCLA, Los Angeles, CA, USA

The presentation will initially review a number of aspects of the opioid system. The potential for cellular environment and ligand interaction to create diversity in opioid agonist signaling has become increasingly evident in recent years. Receptors are now considered as just one component of large hetergenous protein complexes that are regulated by ligand occupation. Different ligands and different receptor environments result in different complexes formed, which subsequently influence both signaling and trafficking of the receptor. Knockout mice have become important tools in dissecting the roles of individual components of the endogenous opioid system in behaviors triggered by opioid ligands as well as other drugs of abuse and rewarding stimuli. Mu-opioid receptors appear to be critical for the analgesic and rewarding effects of most clinically used opioid analgesics as well as for triggering the opponent processes observed during withdrawal. Furthermore, an intact opioid system appears essential for the rewarding effects of other drugs of abuse such as nicotine, alcohol and THC. Interestingly intact cannabinoid and neurokinin systems appear to be required for opioid reward offering interesting new strategies for reducing the reward value for opioid therapeutics. There is a growing literature reporting differences in opioid responses between male and female rodents and many examples will be presented by other members of the panel. This presentation will focus on opioid tolerance. There are many potential contributions to opioid tolerance observed in analgesic assays. These include desensitization of receptor signaling processes, changes in synaptic efficacy, development of opponent processes and learning processes that adapt to the impact of the drug and performance in the analgesic assays. Here we present data that analgesic tolerance to morphine, measured by the hot plate and tail immersion assay, occurs both in male and female mice yet may recruit different circuitry. Male and female mice treated with ascending doses of morphine for 6 days show significant tolerance to a challenge dose of morphine on day 7 in both the hot plate and tail immersion assay. As has previously been shown, NMDA receptor antagonists such as MK-801 block the development of tolerance in male rodents. Here we show that in the hot plate assay, NMDA blockade of morphine tolerance does not occur in female mice. Using the tail immersion assay NMDA receptor blockade by MK-801 had no effect on morphine tolerance in either male or female mice.

Ovariectomy had no effect on the lack of MK-801 to block tolerance in female mice, suggesting this is not an effect of circulating hormones. Interestingly sex differences have also been observed in the role of NMDA receptors in stress-induced kappa-opioid analgesia. In males, but not females, NMDA receptor blockade results in disruption of kappa-mediated analgesia and stress-induced analgesia. Together these results suggest that there may be important sex differences in NMDA receptor interactions regulating opioid-mediated behaviors.

Sex Differences in Opiate Pharmacological Effects in Humans

James P Zacny*

Anesthesia & CC, University of Chicago, Chicago, IL, USA

Background: Sex differences in illicit use and abuse liability-related effects of opioids have been studied but certain questions remain. There appear to be sex differences in abuse of heroin: in the U.S. male heroin addicts outnumber female heroin addicts by a ratio of over 2:1. Prescription opioid abuse in the last seven years or so has become a significant problem in the U.S. but the existing epidemiological data does not indicate clear differences between males and females in non-medical usage. In terms of actual abuse liability of opioids, there are several nonhuman studies indicating that the rewarding or reinforcing effects of mu opioids may be greater in females than in males. Abuse liability testing of opioids in opioid or polydrug abusers has typically been done in males, so it is unclear whether there are sex differences between female and male abusers. The purpose of the present study was to examine opioid subjective effects, including those that are considered to be abuse liability-related (liking) in recreational drug users (users who do not meet psychiatric diagnostic criteria for substance use disorders) to determine if sex differences existed. In addition, psychomotor and physiological responses to opioids were also assessed to determine if sex differences existed in these measures.
Methods: Two 4-session experiments were conducted, one examining responses to the mu opioid agonists morphine, hydromorphone, and meperidine, and the other examining morphine and two mixed-action agonists, butorphanol and nalbuphine. In both experiments there was a saline control session. Sixteen males and sixteen females aged 21-39 years participated in each of the studies. A cumulative dosing design was used in which four injections were given at hourly intervals, followed by a 240 min recovery period. In Experiment 1, cumulative doses of morphine, hydromorphone, and meperidine were 17.5, 2.28, and 112.5 mg/70 kg, respectively; in Experiment 2, cumulative doses of morphine, butorphanol, and nalbuphine were 17.5, 3.5, and 17.5 mg/70 kg, respectively. Subjective effects (e.g., ARCI, VAS, drug liking), psychomotor performance (e.g., DSST), and physiological effects (e.g., miosis) were assessed at fixed time points.
Results: In both experiments the study drugs relative to placebo elevated peak ratings of liking as well as disliking, but there were no sex differences. There were no other abuse liability-related measures that showed sex differences. Strength of drug effect, a large number of other subjective effects, and miosis did not differ between the sexes in either experiment. There were Sex X Drug effects with some subjective and psychomotor effects measures, but they were few in number and not robust. The one measure that did show differential effects between the sexes was vomiting. Females vomited more, especially after morphine and nalbuphine, than males.
Conclusions: Although there were a few sex differences in these two studies, what stood out was the large number of opioid effects in both mu and mixed-action opioids in which there were no sex differences, including a key abuse liability-related measure, drug liking. This overall lack of sex differences is difficult to reconcile with the preclinical abuse liability literature. However, this is just one study and it only examined recreational drug users. It is possible that sex differences in abuse liability-related measures might emerge in opioid or polydrug abusers who are more sensitive to the euphorigenic effects of opioids.

Sex Differences in the Antihyperalgesic Actions of Morphine

Anne Z Murphy*

Biology, Georgia State Univ, Atlanta, GA, USA

Background: There are now well-established sex-related differences in the antinociceptive potency of opioids. Central or systemic administration of agonists directed at the mu or delta receptors generally produce a greater degree of analgesia in males versus females, however results to the contrary have also been reported. While morphine is generally prescribed for the alleviation of persistent or chronic pain, to date, studies examining sex based differences in opioid analgesia have exclusively employed acute noxious stimuli (i.e. tail-flick and hot plate test); thus, the potential dimorphic response of centrally acting opiates in the alleviation of persistent pain is not known.
Methods: All studies employed intact male or cycling female Sprague-Dawley (Zivic Miller) rats (250-300g). Cycle status in females was measured using vaginal lavage. Persistent inflammatory pain was induced by unilateral intraplantar injection of the inflammatory agent complete Freund's adjuvant (CFA). CFA-induced hyperalgesia was assessed using the paw thermal stimulator 24 hr, 7, 14 and 21d post-CFA in separate groups of animals. Following determination of baseline paw withdrawal latencies (PWLs), morphine was administered either systemically (0.5-12 mg/kg) or directly into the caudal ventrolateral periaqueductal gray (PAG) (0-15 μg/0.5 μl); PWLs were determined for 120 min post-administration.
Results: No sex differences were noted for baseline PWLs or in CFA-induced hyperalgesia (as reflected by a significant decrease in PWL). However, significant sex differences were noted in both the degree and duration of analgesia produced by either systemic or intra-PAG morphine; at all doses examined, the antihyperalgesic effects of morphine were significantly greater males in comparison to females. For example, systemic administration of the 8.0 mg/kg dose produced an 80.46 ± 12.17% maximum possible effect (%MPE) in males at 60 min post-administration; by contrast, in females, the %MPE was only 36.77 ± 11.44% (MWU; $p<.01$). Similar dimorphic responses were noted for the 12.0 mg/kg dose, where at 60 min post-morphine all males were at 100% MPE; in females, %MPE was 71.17 ± 13.10% (MWU; $p<.05$). A maximum antihyperalgesic response in females was not noted until the 15 mg/kg dose; this dose of morphine was fatal to all males tested. ED50 values, determined using sigmoidal dose-response function (variable slope) were 5.93 in males (95% confidence interval of 4.40-8.0) versus 9.4 in females (95% CI = 7.73-11.24; $p< .01$). Similar results were noted for intra-PAG morphine, in which morphine produced a significantly greater degree of analgesia in males in comparison to females at all doses tested. For example, intra-PAG morphine at 10μg/.5μg resulted in 100% MPE in males; in females, that dose of morphine was not significantly different from saline (0% MPE). Intra-PAG morphine was not effective in females until the 15μg/.5 μl dose (70% MPE). In males, the antihyperalgesic effects of morphine (either systemic or intra-PAG) were significantly increased as a function of time post-CFA; no significant shift in morphine potency was noted for females.
Discussion: The results of these studies are the first to demonstrate that morphine produces a significantly greater degree of antihyperalgesia in males in comparison to females in a model of persistent inflammatory pain. The results also demonstrate that the persistent pain-induced enhancement of opioid potency is restricted to males. These results are consistent with our anatomical data demonstrating reduced mu opioid receptor protein levels within the PAG of females, as well as sex differences in the anatomical and functional organization of the PAG descending pain modulatory circuit. This work is supported by NIH DA16272 and P50AR49555, and by NSF IBN9876754.

Sex Differences and Gonadal Steroid Influences on Mu-Opioid Receptor Neurotransmission in Humans

Jon-Kar Zubieta*, Mary M Heitzeg, Yolanda R Smith and Christian S Stohler

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

Background: Sex differences are being increasingly described in responses to pain and other forms of physical and emotional stress, as well in the prevalence of persistent idiopathic pain and other stress-associated disorders, including substance use disorders. Some of these differences are thought to be due to the effects of gonadal steroids on neurochemical circuits involved in stress regulation. However, the mechanisms underlying some of these sex differences are still poorly understood.
Methods: We have studied sex differences in the concentration of mu-opioid receptors and in the capacity to activate this neurotransmitter system in response to sustained pain, a model of physical and emotional stress. Studies were conducted in healthy males and females using PET and a mu-opioid receptor selective radiotracer. Activation of mu-opioid receptor mediated neurotransmission was determined as the reduction in the availability of mu-opioid receptors from a non-painful control state to deep muscular pain maintained at constant levels over 20 min.
Results: Sex differences and age effects in the concentration of mu opioid receptors were obtained in a number of cortical and subcortical regions, with higher concentrations in women, compared with men. Interactions with age were further observed in the thalamus and amygdala, whereby receptor concentrations declined after menopause in women to levels comparable to those of men. In studies conducted during follicular and luteal phases of young women, negative correlations between plasma levels of estradiol, but not progesterone and binding levels in the amygdala and hypothalamus were further observed. To further examine whether these effects were due to an influence of estradiol on mu-opioid receptor binding and endogenous opioid release, women were studied during the early follicular phase of the menstrual cycle before and after the administration of transdermal estradiol. Studies were conducted in the absence and presence of sustained pain, an stimulus known to activate endogenous opioid neurotransmission. Under low estradiol conditions women demonstrated significantly lower levels of endogenous opioid release in the thalamus, nucleus accumbens and amygdala. In the majority of these women, frank deactivation of basal levels of endogenous opioid activity were further observed, an effect associated with hyperalgesia and more pronounced negative affective states during the noxious challenge. After plasma levels were increased by the administration of estradiol to periovulatory levels, these sex differences in the capacity to activate mu-opioid receptor mediated neurotransmission were abolished or exceeded that of men. Baseline mu-opioid receptor concentrations were also increased in the same regions during the high estradiol state.
Discussion: These data demonstrate the presence of significant sex differences and effects of estradiol on mu-opioid receptors and endogenous opioid neurotransmission. These are stress suppressive circuits further implicated in the regulation of the HPA axis, responses to pain and the effects of opiate analgesics and a number of drugs of abuse interacting directly (i.e, opiates) or indirectly (psychostimulants, THC, nicotine) with endogenous opioid neurotransmission.

Panel Session
Whole Genome Association Studies: New Promise for Psychiatric Genetics

Linkage and Association Findings in Bipolar Disorder and Schizophrenia

Elliot S Gershon* and Chunyu Liu

Psychiatry, University of Chicago, Chicago, IL, USA

Linkage to a chromosomal region. Statistically strong findings have emerged from meta-analysis of all published whole-genome linkage scans. Badner and Gershon (2002) found evidence for susceptibility loci on 13q ($P < 6$ E-6) and 22q ($P < 1$ E-5) for bipolar disorder (BD), and on 8p ($P < 2$ E-4), 13q ($P < 7$ E-5), and 22q ($P < 9$ E-5) for schizophrenia (SZ). On chromosomes 13 and 22, the linkage regions overlapped for BD and SZ. Segurado, Lewis, and Levinson headed analyses that developed a rank-based meta-analysis method, and applied it to an overlapping but not identical data set of linkage reports (2003). They did not find genome-wide significant results for BD, and found one region significant for SZ on 2p. Position-based association. In non-isolate populations, association (linkage disequilibrium with illness) identifies a much smaller region than linkage, thus narrowing down the search for the causative gene. The linkage regions identified by Badner and Gershon's meta-analysis were each followed by replicated associations to a gene in a small segment of the region. These include G72 (DAOA) on chromosome 13, COMT and other genes on chromosome 22, and Neuregulin-1 on chromosome 8. Elsewhere in the genome, association based on linkage may be detected where linkage is significant only in one or a few data sets, because of the increased power of association vs. linkage. In addition, the statistical power to detect association is greater in a sample in which linkage is also detectable. Candidate genes and Whole-Genome Association (WGA). Despite the intrinsic appeal of WGA, exhaustive examination of SNPs in candidate genes is a useful alternative, even when there are hundreds of candidates, for several reasons. a. Only 71% of the genome, at most, is captured by haplotype blocks (Wall and Pritchard 2003). b. Costs are very high for each subject in WGA, and WGA requires larger sample sizes than candidate genes because of the very large numbers of SNPs. c. To reduce costs of 1.5 million SNPs WGA, Perlegen has proposed pooling for initial genotyping, which compromises detection of small frequency differences between groups. d. A focused candidate gene approach can be tailored to exhaust the SNP information in and around each gene, by achieving very high SNP density. The only commercially available WGA product, Affymetrix 500 K chip, has insufficient SNP coverage for most genes compared to a focused candidate gene study. e. SNPs derived from resequencing of candidate genes in a modest sized sample of patients and controls can enhance the SNP and haplotype map for studies of that disease.

Whole Genome Association: Technologies and Strategies for Application to Psychiatric Illness

John R Kelsoe*

Psychiatry, UCSD, La Jolla, CA, USA

Family based linkage studies have been successful in identifying several chromosomal regions for psychiatric illness, and a few specific genes. However, mapping studies to date make it increasingly clear that psychiatric illnesses are largely polygenic disorders in which numerous genes each make a small contribution to the overall risk for illness. Genetic association has been shown to have much greater power to detect such small gene effects, but markers must be much closer to the actual disease mutations in comparison to linkage. As a result, a very large number of markers are required in order to conduct association studies systematically across the entire genome. Two recent advances from the Human Genome Project now make whole genome association studies possible. First, over 10 million single nucleotide polymorphism (SNP) markers have been identified that cover the genome at high density. Secondly, a variety of high throughput genotyping methods have been developed that now make it possible to genotype hundreds of thousands of SNP markers quickly and inexpensively. The international HapMap project has provided data regarding the relationships among these identified SNP markers, thereby enabling the efficient selection of those markers providing the most information from specific populations. These SNPs can be considered together as haplotypes in order in increase the information for analysis. Genome based methods have also been developed to employ DNA based data in the matching of case and control populations. Microarray based genotyping technologies provide the highest

throughput and lowest cost of existing methods. These methods involve high density arraying of DNA oligonucleotides on microchips and a variety of detection methods. Such studies will generate a vast amount of data, > 1 billion genotypes, requiring high throughput bioinformatics and appropriate experimental design. Pooling of case and control samples has been employed as a faster and more economical approach to estimating population allele frequencies, however, a lower sensitivity to allele frequency differences and inability to employ haplotype methods will likely limit the utility of this approach. The very large number of comparisons present an important statistical challenge and require a replication sample for validation. In summary, whole genome association represents a powerful new approach to mapping genes for psychiatric disorders that is just now seeing its first application. Though serious technical and statistical challenges await, whole genome association methods promise the identification of numerous new susceptibility genes and the elucidation of new mechanisms and pathways of illness.

Dissecting Neuropsychiatric Traits Through Whole-Genome Association Analysis

Dietrich Stephan*

Neurogenomics, The Translational Genomics Research Institute (TGen), Phoenix, AZ, USA

The technical ability to identify the molecular underpinnings of common multigenic human disorders has only come of age in the past months. Ultra-high throughput genotyping of more than 500,000 single nucleotide polymorphisms (SNPs) in thousands of clinically affected individuals and matched controls can identify very small regions of the genome which harbor disease-predisposing nucleotide variants. This high throughput environment requires significant infrastructure, quality control monitoring, and data warehousing capabilities. The process for performing this type of genotyping will be discussed. Two general strategies are employed: pooling samples for genotyping and individual genotyping. Data describing each approach will be shown and discussed relative to progressive supranuclear palsy, Alzheimer's disease, and memory, a normal quantitative trait. Finally, we will discuss analysis strategies for the training sets. Each associated interval from these training sets must subsequently be validated using focused genotyping on an independent case/control cohort in order to ensure that the results are truly disease-associated and applicable to the disease population at large. The technology for scanning the three billion letter human genome is young, and here we present study design strategies, data acquisition pipelines, analysis strategies, and examples of successful implementation to uncover the molecular basis of several human brain disorders. Bipolar disorder will be discussed from this perspective.

Analysis Techiniques for Whole Genome Association Studies

David J Cutler*

Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Whole genome association studies present unique analytical challenges. A "typical" study using state of the art technology might seek to genotype 500,000 markers in a few thousand samples exhibiting variation for a trait of interest, say, schizophrenia or bipolar disorder. The challenges range from the mundane (How does one even format a couple billion genotypes to be analyzed?) to the relatively sophisticated (What are the most statistically efficient methods to analyze data at this scale?) to the truly subtle (Are population stratification, genotyping error, or unusual patterns of missing data confounding the results?). This talk highlights one possible set of solutions to these challenges, and focuses mainly on two aspects of the problem: finding an efficient solution to the "multiple test problem," and efficient utilization of the data even when the underlying disease alleles are relatively rare in the general population. The general solution presented involves exhaustively testing all markers and all adjacent combinations of markers (so-called haplotypes) and assessing statistical significance within a permutation framework. The challenges will be described in detail, the solutions described in a relatively general way, and expectations for such a study in somewhat greater detail.