Neuropsychopharmacology (2010) 35, S1–S77

Monday, December 6, 2010

Panel Session
DBS for OCD: Clinical Outcomes, Neuroimaging, Circuitry, and Fear Regulation

Long-Term DBS for OCD

Benjamin D. Greenberg*, Donald Malone, Wayne Goodman, Herbert Ward, Michael Okun, Kelly Foote, Andre Machado, Gerhard Friehs, Ali Rezai, Nicole McLaughlin, Paul Malloy, Cynthia Kubu, Steven Rasmussen

Butler Hospital, Providence, RI, Cleveland Clinic, Cleveland, OH, Mt. Sinai Medical Center, New York, NY, University of Florida, Gainesville, FL, Scott and White Hospital, Temple, TX, Ohio State University Medical Center, Columbus, OH, Butler Hospital/Brown University, Providence, RI

Background: DBS of the Ventral Capsule/Ventral Striatum (VC/VS) for highly refractory obsessive-compulsive disorder (OCD) has been studied since 2001 in the US. The FDA approved this therapy for humanitarian use in 2009 on the basis of open-label pilot data. Although 3 year followup data have been reported, longer-term outcomes are important 1) clinically in balancing risks, benefits, and burdens of this intensive treatment, and 2) for understanding behavioral phenotypes and associated neurocircuit effects related to benefit.
Methods: Pilot patients from three US centers (Butler Hospital/Brown Univ., Cleveland Clinic, Univ. Florida, N = 16) were followed longitudinally between three and eight years post implantation.
Results: Presurgical Yale-Brown OCD Scale (YBOCS) severity was 34.4 ± 0.6 (SEM, range 31-40), indicating severe illness in a population selected for surgery only after failure of aggressive behavioral treatment and medication trials. Mean YBOCS severity at 1 year was 22.4 ± 2.3 (range 3-30), and remained essentially unchanged on a group basis thereafter: year four, mean YBOCS = 22.1 ± 1.4 (range 14-33), year seven: 21.0 ± 0.25 (range 20-22). Individual means during long-term DBS (3-8 years) ranged from 11 to 31. Global Assessment of Functioning (GAF) scores were 37.1 ± 1.6 at baseline, consistent with severe impairment. GAF scores improved to a mean range of 52-59 over years one to seven. During followup, patients received continued pharmacotherapy and behavior therapy, although the number of medications prescribed decreased by one to two on average vs. persurgical baseline in patients who improved. Adverse events related to surgery infection, hemorrhage, a single seizure) had resolved by months after implantation. Mood, anxiety, and OCD symptoms worsened mildly, moderately, or severely when DBS was interrupted throughout chronic stimulation. Two of 16 patients died during followup of unrelated medical causes (in years one and eight). An additional 5/16 were not receiving continuous ongoing stimulation for reasons including suboptimal responses and/or barriers to receiving device replacements over time. Patients whose main OCD-related impairment were due to symptoms involving "incompleteness" (aimed at attaining a feeling that actions or the environment were "right") improved less overall than individuals whose symptoms focused on harm avoidance. But at least one incompleteness patient was a full responder (35% + decrease in YBOCS severity).
Discussion: Therapeutic effects DBS targeting a fronto-basal brain network implicated in OCD and related disorders appear sustained over years of continuing stimulation. Average severity changed from very severe to moderate, paralleled by improved functioning. However, about one-third of patients no longer received DBS three + years after implantation. Patients with symptoms based in avoidance of harm seemed to benefit most, suggesting that behavioral phenotypes related to fear and avoidance are important in the therapeutic response. These hypotheses are a focus of ongoing translational research by ourselves and collaborators. More long-term data are essential to optimizing patient selection. Some will be obtained in an ongoing NIMH-supported controlled trial of DBS for OCD. An essential role would be played by creation of a data registry for patient characteristics and outcomes, as suggested by investigators in the field and by NIMH.
Disclosure: B.D. Greenberg: Part 1; Medtronic, Inc.; Jazz Pharmaceuticals. Part 3; Medtronic, Inc.

Deep Brain Stimulation for OCD: Now Different Cortical Bundles Really Get to Their Targets

Suzanne Haber*

University of Rochester Rochester, NY

Background: Dysfunction of the ventral prefrontal cortex (vmPFC) and orbital cortex (OFC), strongly associated with the pathophysiology of OCD and part of the cortico-basal ganglia circuit, are connected to the thalamus, amygdala, and brainstem. DBS at the ventral capsule/ventral striatal site (VC/VS) targets this network. Each electrode has 4 contact sites spaced strategically to potentially involve different nodal points within the circuit. The goal of these experiments was to: 1. Determine organizational rules that govern the trajectory of ventral PFC efferent fiber bundles; 2. Determine which bundles are affected by DBS at each contact; and 3. Develop an electric field neurostimulation model around representative targets to determine likely volumes of effect for the commonly used clinical parameters.
Methods: 3-D reconstructions of the vmPFC and OFC fiber bundles were created from conventional anatomical tracings following tracer injections into the different prefrontal areas in primates. Human electrode representations were adjusted for size and imported into the primate 3-D model. Using neurostimulation modeling we visualized stimulus spread at the VC/VS site to determine how different parameter alters the inclusion of fibers at each contact. All experiments were conducted in accordance with the ILAR Guide for the Care and Use of Laboratory Animals and were approved by The University Committee on Animal Resources.
Results: Fibers from ventral prefrontal areas follow certain organizational rules. The route taken by fibers from different ventral prefrontal regions through the capsule depends largely on their medial-lateral location. Axons from medial regions remain in the most ventral parts, including the small fascicules embedded within the VS. In contrast, axons from lateral OFC areas travel more dorsal in the capsule. Within each group of fibers, those traveling to the brainstem are located ventral to those traveling to the thalamus. Electrode placements show which fibers are captured at each contact. Changing the stimulation parameters may or may not capture additional fiber destinations, depending on the contact.
Conclusions: These data illustrate the organization of each prefrontal cortical pathway through the internal capsule and the relationship between bundles arising from different cortical areas. Combining the fact that fibers traveling to the brainstem from each cortical area travel ventral to those going to the thalamus from that same cortical area, with the overall medial to lateral topographic arrangement of cortical fibers within the capsule, demonstrates a complex set of fibers associated each contact. Taken together these data show that each contact captures a different combination of brainstem and thalamic fibers from diverse prefrontal areas. This combination will vary according to the stimulation parameters used. These data also suggest that brainstem fibers may be a critical feature to the effectiveness of DBS for OCD.
Disclosure: S.N. Haber: Part 1; Eli Lilly Co., Medtronic.

DBS-Like High Frequency Stimulation of Fibers Within the Rat Ventral Striatum Strengthens Fear Extinction and Induces Plasticity in Extinction Circuits

Gregory J. Quirk*, Jose Rodriguez-Romaguera, Christian Bravo-Rivera, Fabricio H. M. Do Monte

University of Puerto Rico School of Medicine, San Juan, PR

Background: Deep brain stimulation (DBS) of the ventral capsule/ventral striatum is effective in reducing depression and anxiety symptoms in refractory obsessive compulsive disorder (OCD), but little is known about how DBS alleviates anxiety disorders. Persistent avoidance responses in OCD and other anxiety disorders may be due to a failure of extinction to reduce conditioned fear reactions. We therefore used a rat model to study the effects of DSB-like stimulation of the ventral striatum on fear expression and extinction in an auditory fear conditioning task. Following a recent study in anesthetized rats (McCraken and Grace, 2009), we targeted the nucleus accumbens core (Nac) with high frequency stimulation (130 Hz, HFS) similar to clinical DBS, or a low frequency stimulation (10 Hz, LFS) control condition.
Methods: On day 1, rats underwent fear conditioning by pairing tones (30 sec) with foot-shock, while pressing a bar for food. On day 2, rats received HFS, LFS, or no stimulation (NS) of the NAc 60 min prior to and during a 15 tone extinction session. On day 3, all rats were tested for extinction recall in the absence of stimulation. To determine the effect of stimulation on fear circuits, another set of rats underwent HFS and LFS for 60 min and were sacrificed 45 m later and processed for immunostaining for the plasticity marker pERK in cortico-amygdala circuits. Labeling in HFS and LFS groups was compared to a NS control.
Results: HFS rats, but not LFS, showed a reduction in fear expression during extinction. On the next day, HFS rats showed strengthened extinction memory, converting an ineffective extinction session to an effective one. In contrast, LFS resulted in exaggerated fear at test. There was no effect of HFS or LFS on rates of spontaneous bar pressing. HFS increased pERK-immunoreactivity in the amygdala intercalated cells and lateral division of the central nucleus (ITC/CeL), and area that exerts inhibitory control over amygdala output neurons in the medial central nucleus (CeM). In contrast, LFS increased label in the CeM, but not the ITC/CeL.
Discussion: The ability of HFS to reduce fear expression and strengthen extinction memory suggests that DBS may allow OCD patients to benefit from extinction-based therapies, which failed prior to surgery. The pattern of pERK label we observed suggests that DBS strengthens extinction by inducing plasticity in inhibitory circuits within the amygdala, known to be involved in extinction memory. Further experiments are underway to determine if HFS in the absence of extinction training is sufficient to reduce fear expression.
Disclosure: G.J. Quirk: None.

Examining the Neural Circuitry of Conditioned Fear Extinction in Obsessive Compulsive Disorder Using fMRI

Mohammed Milad*

Massachusetts General Hospital, Charlestown, MA

Recent mounting evidence gathered from human neuroimaging studies strongly supports the role of the medial orbitofrontal cortex (mOFC) and ventromedial prefrontal cortex (vmPFC) in fear extinction. These prefrontal regions are dysfunctional in anxiety disorders, including OCD. Most neuroimaging studies that examined the role of these prefrontal regions have employed neurocognitive or symptom-provocation paradigms. Direct tests of the functional integrity of the OFC/vmPFC in OCD during fear extinction remain to be conducted. Examining the integrity of the extinction circuitry in OCD patients is clinically important because extinction forms the basis for exposure therapy commonly used to treat anxiety disorders, including OCD. We began to fill this gap in the literature by examining the neural circuitry of fear extinction in OCD patients using fMRI and psychophysiological measures. OCD patients and matched healthy controls underwent a two-day fear conditioning and extinction protocol, previously developed and validated. All participants underwent the experimental protocol while they are in an fMRI scanner. Skin conductance response was monitored throughout the experiment and was used as the behavioral index of conditioning. On Day 1, subjects received conditioning followed by extinction, using photographs of colored lights as CSs. On day 2, extinction recall was assessed (test for extinction memory). Our preliminary data indicate that OCD patients showed intact fear acquisition and extinction training on day 1. On day 2, however, OCD patients showed exaggerated fear responses, indexed by SCR, suggesting impaired recall of the extinction (safety) memory. Functional MRI analysis shows failure to activate the vmPFC while OCD patients are recalling the extinction memory. These patterns of psychophysiological and brain dysfunctions observed in the OCD cohort examined herein resembles those recently reported in PTSD patients. Moreover, preliminary data from our collaborative team indicate that both acute and chronic deep brain stimulation (DBS) in OCD patients result in increased resting metabolic activity in the vmPFC. Collectively, therefore, our data suggest that OCD patients appear to exhibit impaired extinction recall that is associated with failure to activate the vmPFC. Current ongoing studies are aimed to examine the effects of DBS on rectifying the extinction deficits in OCD, which could provide a possible mechanism by which DBS could improve some of the clinical symptoms of OCD.
Disclosure: M.R. Milad: Part 1; Microtransponder.

Panel Session
Lost in Translation: Is It the Animal Models or Clinical Trial Designs That Are Responsible for the High Failure Rate in CNS Drug Development?

Increasing the Validity for Animal Tests of Depression by Genetic - Environment Interactions

Irwin Lucki*, Georgia E. Hodes, Gregory V. Carr

University of Pennsylvania, Philadelphia, PA

Background: Concerns have been raised about the predictive and face validity of some of the rodent tests for depressive behavior that have been used historically to measure the effects of antidepressant drugs. Behavioral tests for antidepressant activity have evolved from measuring drug responses in unperturbed "normal" animals to include genetic and environmental models of stress vulnerability and to include the need to examine chronic drug treatment. An overview, and specific examples from new research, will be presented on the use of specific strains of rats and mice to model the impairing effects of exposure to stress, the restorative effects of antidepressant drug treatment, and resistance to treatment with conventional antidepressants. Specific examples will be provided from new research.
Methods: Wistar-Kyoto (WKY) and Sprague-Dawley (SD) rats were tested for the effects of the SSRI fluoxetine and the kappa opioid receptor (KOR) antagonist DIPPA using the modified rat forced swim test (FST) and the novelty-induced hypophagia (NIH) test. Mice were tested for the behavioral effects of chronic treatment with fluoxetine or desipramine in the NIH test. Hippocampal cell proliferation was measured by incorporation of BrdU and levels of BDNF protein were measured using ELISA.
Results: WKY rats are a genetic model for exaggerated depression and anxiety behaviors. Despite resistance to the behavioral effects of SSRIs, WKY rats demonstrate a superior behavioral response to systemic administration of KOR antagonists in the forced swim test or novelty-induced hypophagia test. Inbred strains of mice also demonstrate dramatic differences in the response to acute and chronic antidepressant drug treatments. MRL/MpJ mice, a model for superior cellular

regeneration after injury, demonstrate a greater change in hippocampal neurogenesis, BDNF levels and behavioral responses to antidepressant drug treatments than other strains of mice, such as C57BL/6J mice. The large responses of MRL/MpJ mice allow exploration of underlying mechanisms. Although the response of C57BL/6J mice to antidepressants is smaller than other strains, these mice demonstrated robust increases in hippocampal neurogenesis, BDNF levels and respond behaviorally in the NIH test after exposure to stress induced by infusion of corticosterone or the chronic mild stress procedure.
Discussion: These studies illustrate ways that the face and predictive validity for antidepressant tests can be improved by using genetic and environmental interactions with stress. Genetic background and exposure to stress are major factors in determining the response of rodents to antidepressant drugs. These examples underscore the usefulness of considering these factors in drug discovery.
Disclosure: I. Lucki, Wyeth, Part 1; Astra-Zeneca, Part 4.

From New Drug to No Drug: Novel Mechanisms for Antidepressants in the Clinic
David Michelson*, Duane Snavely, Richard Hargreaves

Merck Sharp & Dohme Corp., North Wales, PA, PA

Candidate non-monoamine mechanisms to treat depression have generally disappointed hopes they could become therapeutics, even when effects in preclinical models have been demonstrated or initial clinical trials have been encouraging. As a result, despite continuing and considerable unmet medical need, the commitment within industry to drug discovery and development efforts for depressive illness is waning, and is unlikely to return without an understanding of why past efforts have failed, and how the probability of success can be improved going forward. One of the most disappointing failures of a putative treatment for depression was the NK-1 antagonist, which was extensively studied preclinically and in exploratory and confirmatory clinical trials, with the most comprehensive program involving the Merck drug candidate MK-0869. This program included 4 efficacy studies conducted as phase 2 exploratory trials, and 8 efficacy studies conducted as phase 3 confirmatory trials (this includes several studies stopped earlier than planned). In this presentation, we will provide an overview of the evidence that drove this development program forward, examine the translational strategies, and present analyses of the characteristics of the clinical studies at different phases of development, with specific attention to the type 1 statistical error early and loss of signal later to understand the lessons that can be drawn from this experience and the implications for the design of future antidepressant drug development programs.
Disclosure: D. Michelson: Part 1; Employee of Merck Reseach Laboratories. Part 5; Merck Reseach Laboratories.

Cross-Species Tests for Cognition Enhancement in Schizophrenia
Mark Geyer*, Jared Young

University of California, San Diego, La Jolla CA

Background: The NIMH-funded MATRICS Program (Measurement and Treatment Research to Improve Cognition in Schizophrenia) developed a broad consensus regarding the nature of the cognitive impairments in schizophrenia and how they might best be assessed and treated. There is an urgent need for improved translational tools to facilitate preclinical drug discovery and associated clinical proof of concept studies relevant to developing new treatments for cognition in schizophrenia. Hence, this presentation will focus on how preclinical scientists can develop and refine animal tests to identify novel procognitive agents having potential utility in the treatment of antipsychotic-treated schizophrenia patients. For example, attention/vigilance is commonly assessed in humans using the continuous performance test (CPT), which requires a response to signal events and an inhibition of response to non-signal events. Signal detection theory (SDT) is used to evaluate performance in the CPT. A recently developed rodent 5-choice (5C)-CPT requires both responses to target stimuli and the inhibition of responses to other stimuli, thereby enabling the use of SDT in assessing vigilance.
Methods: Initial validation of the 5C-CPT as a test of vigilance in mice was examined by comparisons of C57BL/6J and DBA/2J mice in an extended session challenge (Exp. 1). Further validation of the 5C-CPT was examined in dopamine D4 receptor wildtype (WT) and heterozygous (HT) mice, using challenges with: a variable stimulus duration (0.8, 1, and 2s; Exp. 2); and an extended intertrial interval (ITI) session, with vehicle or the 5-HT2C antagonist SB242084 (0.1 or 0.3 mg/kg) in a within subjects design (Exp. 3). Finally, the predictive validity of the 5C-CPT was assessed by assessing the effects of nicotine administration on vigilance in C57BL/6J mice (Exp. 4).
Results: In experiment 1, C57BL/6J mice exhibited higher levels of vigilance than DBA/2J mice and a less pronounced vigilance decrement despite comparable levels of accuracy. In experiment 2, shorter stimulus durations degraded performance by increasing false alarm responses and HT mice exhibited poorer vigilance performance. Here, no difference in premature responses was observed. In experiment 3, a 5-HT2C antagonist-induced increase in premature responses was observed, with no interaction or effect of genotype and no effect on false alarms. Finally in experiment 4 nicotine induced a significant improvement on performance of C57BL/6J mice, consistent with observations in humans.
Discussion: In these studies in mice, the use of target and non-target stimuli in the 5C-CPT enabled the: a) use of SDT to assess vigilance; b) identification of a vigilance decrement over time in two strains of mice; c) differentiation of impulsivity in response to non-target stimuli (false alarms) and motor impulsivity (premature responses); and d) identification of nicotine-induced improvements in vigilance in normal performing animals. These effects are consistent with human studies using the CPT. Hence, these studies suggest that the 5C-CPT enables vigilance testing in mice and is therefore available for use in efforts to develop and assess procognitive compounds having efficacy that may translate from rodent to human CPT testing. These studies were supported by NIH grant R21-MH85221 to Dr. Young and the Veteran's Administration VISN 22 MIRECC.
Disclosure: M.A. Geyer, San Diego Instruments, Part 1; Acadia, Part 1; Addex, Part 1; Chakra, Part 1; Medivation, Part 1; Omeros, Part 1; Johnson & Johnson, Part 1; Sepracor, Part 1; Takeda, Part 1; Wyeth-Ayerst, Part 1; Cenomed, Part 1; Wyeth-Ayerst, Part 2; San Diego Instruments, Part 2; Omeros, Part 2; IntraCellular Technologies, Part 4.

New Paths for Drug Development in Schizophrenia
Michael Egan*, Lyn Harper Mozley, Regina Gottwald, Duane Snavely, Ying Zhang, Xin Zhao, David Michelson

Merck Sharp & Dohme Corp., North Wales, PA

Background: Despite efforts to develop new treatments for schizophrenia, no drugs with a novel mechanism of action have been approved for decades. This failure may be related to target selection or execution of clinical trials. An analysis of recent drug trials suggests avenues that may increase the likelihood of developing effective, new drugs.
Methods: Results of clinical trials of antipsychotic and cognition drugs were collected from published studies, reviews, abstracts, and press releases. Trials were evaluated in terms of: a) support from animal models, b) evidence for CNS target engagement, c) dose selection, d) study design. Because many cognition trials have not yet been published, we also examine data from three recent, unpublished cognition trials to assess methodological issues. These trials were conducted to assess the feasibility of running cognition trials and used

an H3 inverse agonist, an NPY5 antagonist, and an ORL1 antagonist. Limited, preliminary, preclinical data suggested cognitive benefits for all three. Approximately 50 patients per study participated in randomized, double-blind, placebo-controlled studies using the Brief Assessment of Cognition in Schizophrenia (BACS) t-score.

Results: We identified >20 drug targets for psychosis and 10 for cognition tested over the last 30 years. Clinical trials showed a lack of efficacy for most mechanisms. Frequent problems with development paths included lack of efficacy in animal models, no evidence of CNS target engagement, unclear rationale for dose selection, poor power, lack of active comparator, and lack of replication. Recent trials that included approved antipsychotics also have shown lack of efficacy. Issues include high placebo response and concerns about PANSS ratings and inclusion of inappropriate patients. Relapse prevention designs may be more robust than trials of acutely psychotic inpatients. Regarding cognition studies, none included an active comparator. Cognition trials may have some advantages, in contrast to acute psychosis, as deficits are more trait-like and cognition measures are more objective, but concerns about patient heterogeneity and target selection remain challenges. Regarding three clinical trials of novel compounds, trial execution appeared adequate but all failed to demonstrate efficacy ($p > .2$). A practice effect was observed during placebo run in periods, suggesting changes in performance could be detected. The results highlight several additional issues including 1) dose selection; 2) duration of treatment; 3) the possibility that atypical antipsychotics may block pro-cognitive effects.

Discussion: Methodological refinements in clinical trial design and execution may improve the probability of success of future drug trails. More problematic are the paucity of validated drug targets. Preclinical cognition models in particular appear to be poor predictors of efficacy and markedly limit target validation efforts. The future of drug development may depend on identifying human biomarkers related to the pathophysiology of schizophrenia. Similar efforts in other diseases (e.g. Alzheimer's) have been successful in stimulating drug development. Fledging attempts to identify clinically measurable, molecular abnormalities (e.g. reduce prefrontal D1 density) suggest this may be a viable path forward in schizophrenia.

Disclosure: M. Egan: Part 5; Merck &Co, Inc.

Panel Session
Next Generation Sequencing and Neuropsychiatry

Rare and Common Variants in Neuropsychiatric Disease

David Goldstein*

Duke University, Durham, NC

There are now hundreds of confirmed common variants that associate with risk of common diseases, responses to infection, and responses to drugs. For most common diseases however all common variants identified to date explain only a few percent of the known heritabilities, and many of the signals emerging from genome wide association studies have yet to be tracked to a single common variant that has been shown to be responsible for the effect. Here I first contrast human traits that are and are not strongly influenced by common variants. I next review the role of rare copy number variants in schizophrenia and epilepsy, emphasizing the striking non specificity in the risk profiles of most of the variants implicated to date. Finally, I introduce an ARRA funded project which seeks to use whole genome sequencing to identify rare risk factors in each of 100 families that have at least one member with schizophrenia and multiple members affected with different neuropsychiatric conditions. Here I will report the first sequencing results in these families and our first efforts to evaluate patterns of co segregation between putative risk factors and affectation status in the families.

Disclosure: D. Goldstein, None.

Rare Variant Discovery in the Coding and Non-Coding Genome in Pervasive Developmental Disorders

Matthew State*

Yale University, New Haven, CT

Background: The recent flood of findings from genome wide association studies have confirmed the importance of common genetic polymorphisms for neuropsychiatric syndromes. At the same time they have underscored the surprisingly limited nature of this contribution as well as the need to consider the role of rare alleles. Next generation, massively parallel sequencing, for the first time, allows for unbiased genome wide identification of novel low frequency alleles. However, these new approaches have revealed a tremendous amount of rare variation in the normal human genome, making the separation of "signal" from "noise" in the study of clinical disorders a pressing challenge. This is particularly an issue for non-coding segments of the genome which are likely to be important for complex neuropsychiatric phenotypes, but where the functional consequences of this variation may be extremely difficult to interpret. We have taken several approaches characterizing novel rare mutations genome-wide in the study of Autism Spectrum Disorders (ASD) including: focusing on rare subsets of patients with extreme phenotypes, focusing on *de novo* variation among simplex families, and devising a strategy to study highly constrained non-coding sequences.

Methods: We focus on two patient samples: 1) 9 families ($N = 17$) with CDD, a very rare regressive ASD syndrome that displays apparently sporadic inheritance. In this sample we have applied whole exome sequencing using Nimblegen liquid capture and the Illumina GAIIx sequencing; and 2) 30 trios ($N = 90$) from the Simons Simplex Collection, which focuses on identifying simplex cases of autism. For this sample, we have devised a strategy to study the non-coding genome by focusing on the investigation of 15,000 highly conserved enhancers and empirically defined transcription factor binding sites. A custom Agilent array was used to capture these regions prior to sequencing. Both studies use whole blood derived DNA.

Results: Sequencing is currently underway. New data will be presented highlighting specific functional variations with a focus on recurrent *de novo* mutations. Our preliminary data to date has confirmed the sensitivity and specificity of whole-exome sequencing for the detection of *de novo* variation using solution based genomic partitioning and sequencing on the Illumina GAIIx instrument. We report a high false positive rate of *de novo* detection based on cell line derived DNA, demonstrating that value of whole blood DNA for these studies.

Discussion: Massively parallel sequencing is providing unprecedented opportunities for gene discovery in neuropsychiatric disorders. The identification of large amounts of rare, apparently, neutral variation in the normal human genome complicates the analysis of rare variant data. Leveraging extreme outliers, *de novo* mutations, and data on evolutionary constraint are promising approaches to addressing this problems.

Disclosure: M.W. State, None.

Using NextGen Sequencing to Interrogate Human Brain Evolution

Daniel H. Geschwind*

UCLA Department of Neurology, Los Angeles, CA

We have taken a multi-pronged approach to understanding the genetic basis of human higher cognition, including the assessment of gene expression differences between human and primate genes. The first generation of such studies involved microarray technology, but these studies, while illuminating, had several pitfalls including artifacts due to cross-species sequence differences. Next generation (NextGen) sequencing permits such analyses to be performed and to include analysis of splicing, all free from issues of hybridization, providing a far more complete view of evolutionary divergence in the brain transcriptome. We performed NextGen sequencing of hippocampus, cerebral cortex and caudate nucleus using 4-6 samples from each of

three primates: human, chimpanzee, and rhesus macaque using tag-based library generation on the Illumina Genome Analyzer and compared this to Affymetrix and Illumina whole genome microarray platforms. We found several times as many human specific changes using sequencing compared to microarrays using the rhesus macaque data as outgroup data. These genes fell into specific pathways that we could identify using Gene Ontology Analysis. Furthermore, we were able to adapt Whole Genome Co-Expression Network Analysis (WGCNA), which we have used for microarray gene expression (e.g. Oldham et al. 2008 Nat Neurosci.), to NextGen sequencing data. Here we were able to significantly enriched modules of co-expressed genes within the networks that were unique to human brain. We further expanded this initial study by performing RNA-seq, which provides more depth and additional information on splicing and miRNAs, in the same individuals and additional brain regions. This comprehensive analysis has allowed us to directly compare the methodologies and to determine expression and splicing differences between the species for the first time. These studies are the first of its kind to use sequencing to examine brain gene expression across multiple species. Additionally, these data provide important examples of the power of this emerging technology.
Disclosure: D.H. Geschwind: None.

High-Throughput DNA Sequencing in Autism Spectrum Disorders (ASD)
Christopher A. Walsh*, Tim Yu, Maria Chahrour, Christian Schubert, Sean Hill

Harvard University, Boston, MA

Although autism is among the most highly genetic of neuropsychiatric disorders, the precise genes that are causal are typically only known in 15-20% of patients. An increasing amount of data suggests that autism can arise from an increasingly heterogeneous collection of rare mutations, including syndromic forms (e.g., Fragile X or *MECP2* mutations), rare quasi-Mendelian forms (*NRXN1*, *SHANK3*, or *NLGN3/4* mutations), recurrent *de novo* copy number variants (CNV's, e.g., 22q11 deletions, 16p11.12 deletions or duplications, and 15p11 duplications) and other, diverse CNV's. Our work has focused on identifying recessive mutations associated with autism, on the hypothesis that recessive mutations may underlie additional quasi-Mendelian forms of autism, but that recessive mutations may also interact in more 'complex' fashion in other autistic children. We have recruited more than 240 families with one or more children affected with autism (>40 with 2 or more affected children) in which the two parents share common ancestry (typically as first cousins), focusing recruitment on countries where cousin marriage is conventional (including Kuwait, Saudi Arabia, Turkey, Pakistan, etc.). Analysis of the first 78 families (Morrow et al, *Science* 2008) identified a lower incidence of large, *de novo* CNV's (1/78) compared to comparably analyzed children whose parents were unrelated, consistent with an important role for recessive mutations in children with consanguineous parents. In contrast, 5/78 patients showed *homozygous* deletions of 18,000-321,000 bp of DNA, inherited through both parents from a common ancestor. Surprisingly, only one of these homozygous deletions removed the coding portion of a gene, suggesting that removal of noncoding DNA near genes, including predicted transcription factor binding sequences, might causes some cases of autism. At least one gene (*SCL9A9*, a.k.a. *NHE9*) near a large deletion seen in a patient with autism with seizures also showed heterozygous point mutations in American children with autism and seizures. Linkage analysis of larger families with 2-4 affected children suggested that recessive genes are heterogeneous, since different families showed linkage to diverse chromosomes and loci. We have now developed methods to identify recessive, homozygous point mutations from areas of linkage using array-capture with Nimblegen arrays followed by high-throughput Illumina sequencing. Initial analysis suggests that autistic children may have previously undescribed, "hypomorphic" mutations in genes previously associated with more severe Mendelian human disease, as well as mutations in novel genes not previously associated with disease. Supported by the NIMH, the Simons Foundation, the NLM Family Foundation and the Howard Hughes Medical Institute.
Disclosure: C.A. Walsh: None.

Panel Session
Oxidative Stress in Psychiatric Disorders: Implications for Pathophysiology and Pharmacotherapy

Novel Postmortem and *in vivo* Mitochondrial Association in Psychiatric Disorders
Marquis P. Vawter*, Maureen V. Martin, Brandi Rollins, P. Adolfo Sequeira, Emily A. Moon, William Bunney, Guia Guffanti, Steven Potkin, Fabio Macciardi

University of California - Irvine, Irvine, CA

Background: Alterations in expression of mitochondrial related genes are potential indicators of functional deficits and have emerged as a candidate pathway for bipolar disorder (BD) and schizophrenia (SZ). Multiple genes in the mitochondrial oxidative phosphorylation pathway are regulated by pH or strongly associated with pH regulation, thus disentangling cause and effect has been extremely difficult. Others have found an association of mtDNA polymorphisms with amygdala volume and pH, decreased postmortem pH in BD and SZ, increased oxidative stress indicators in BD and SZ, and mtDNA copy number alterations due to prolonged brain hypoxia. We studied mtDNA polymorphisms and association with brain pH and the common deletion of mtDNA in a postmortem study. In a separate *in vivo* study the mean blood oxygen level-dependent signal (BOLD) was measured during a cognitive task and differences between mtDNA allele groups were calculated.
Methods: Postmortem pH quantitative trait association with mtDNA SNPs was run in PLINK with permutation to obtain empirical p-values. The mtDNA common deletion (4,977 bp deletion) was measured by quantitative real time PCR assay (200 samples). Mean BOLD signal in regions of interest during the probe condition for a memory load of 3 items was compared to a memory load of 1 item by mtDNA allele.
Results: Three mtDNA SNPs define the super- haplogroup U, K, Uk matrilineages and haplogroup association of postmortem pH with U-K-Uk SNPs was robust ($p < 4 \times 10^{-4}$), and remained significant upon bootstrap permutations. The U-K-Uk mtDNA haplogroup had a significantly higher pH (mean 7.06 ± 0.18 SD) compared to the remaining haplogroup in European ancestry individuals (mean 6.86 ± 0.18 SD). Postmortem interval (PMI) was not different between the U-K-Uk super haplogroup and the remaining haplogroups, and including PMI in ANCOVA did not reduce the significance of the pH differences between U-K-Uk super haplogroup and the remaining haplogroups ($p = 0.0007$ with PMI as a covariate). MtDNA common deletion varied by 50 fold between brain regions, with age showing the strongest effect ($p < 10^{-12}$). The caudate, putamen, substantia nigra and amygdala showed the highest average deletion rates. The BD group shows a significant increase in deletion of mtDNA of 4,977 bp in DLPFC. This deletion normally accumulates with age, and was not increased in SZ subjects.Individuals within U-K-Uk haplogroup showed lowest BOLD activation levels on the cognitive task compared with subjects not in this haplogroup in multiple prefrontal regions and cerebellum.
Discussion: During hypoxia, individuals within the matrilineal haplogroup (U-K-Uk) maintain less acidification of brain pH compared to individuals in other haplogroups. This robust association was not altered by differences in PMI. In preliminary data analysis, the same matrilineal haplogroup (U-K-Uk) SNP that relates to higher brain pH was also associated to lower brain activation BOLD during a cognitive task. These results together might indicate decreased

coupling efficiency, such that alteration in pH could lead to lower brain activation as measured by the oxygen consumption while individuals with lower pH (more acidification) have higher use of molecular oxygen in brain. MtDNA stratification in other postmortem studies and genome wide association studies are needed to determine if SNPs that relate to brain pH and activation differences in this study are replicated.
Disclosure: M.P. Vawter, AbAStar MDx, Part 1; Pritzker Neuropsychiatric Foundation, Part 4; Penzner Foundation, Part 4.

The Role of Oxidative Stress in Anxiety-Like Behavior Revealed by a Functional Genomics Screen in Mice
Iiris Hovatta*, Jonas Donner, Sami Pirkola, Joseph D. Terwilliger, Carrolee Barlow

University of Helsinki, Helsinki, Finland, Helsinki University Central Hospital, Helsinki, Finland, BrainCells Inc., San Diego, CA

Background: Human anxiety disorders are complex diseases with largely unknown etiology. We have taken a cross-species approach to identify genes that regulate anxiety-like behavior using inbred mouse strains that differ in their innate anxiety levels as a model.
Methods: We carried out gene expression profiling of seven brain regions in 6 inbred mouse strains that differ in their innate anxiety levels to identify genes that regulate anxiety-like behavior. We then verified these results by qPCR, enzyme activity assays, and lentivirus-mediated gene transfer. To test whether genetic variants in any of these genes predispose humans to anxiety disorders, we carried out an association analysis in an anxiety disorder study sample derived from a Finnish population-based Health 2000 Survey. It consists of 321 patients and 653 carefully matched controls, all interviewed using CIDI to obtain DSM-IV diagnoses of panic disorder, generalized anxiety disorder, agoraphobia, social phobia, or phobia NOS.
Results: In the mouse study, we identified 17 genes with expression levels that correlate with anxiety behavior across six inbred mouse strains in at least one of the studied brain regions. The role of two genes, glyoxalase 1 (Glo1) and glutathione reductase 1 (Gsr), that are both involved in the regulation of oxidative stress was studied in functional analyses. Overexpression and/or silencing of these genes in the cingulate cortex of the mouse altered the anxiety-like behavior of the mice in the open-field test, indicating that both Glo1 and Gsr regulate anxiety-like behavior *in vivo*. In addition, pathway analysis of the most significantly differentially expressed genes revealed an over-representation of oxidative stress related genes, further providing evidence for the involvement of this pathway in the regulation of anxiety. In the human study, specific alleles and haplotypes of six of the examined 13 homologs of the genes identified from mouse revealed some evidence for association ($p \leq 0.01$), although GLO1 and GSR were not among the most significantly associated genes.
Discussion: Our findings suggest that oxidative stress is involved in the regulation of anxiety-like behavior in mice. Results from the human genetic association study illustrate the potential utility of cross-species approaches in identification of candidate genes for psychiatric disorders.
Disclosure: I. Hovatta, None.

Anxiety–Blame It on Oxidative Stress
Samina Salim*

University of Houston, Houston, TX

Background: Anxiety disorders affect an estimated 40 million people in the U.S. As far as the treatment for anxiety disorders are concerned, benzodiazepines and selective serotonin reuptake inhibitors are considered the "gold standard". Their chronic use, however, leads to tolerance, dependence and sedation. Better alternatives over existing anxiety treatments are needed. Since the focus of most anxiety research has been the traditional neurotransmitter systems including gamma amino butyric acid and serotonin receptor systems, a more broad understanding outside the classical theories of anxiety has been limited and has impeded discovery of novel interventions. A provocative concept away from these traditional theories is the involvement of oxidative stress in anxiety. Several recent reports support this concept. Our own studies attest to this concept.
Methods: We have employed biochemical and behavioral approaches to investigate direct involvement of oxidative stress in anxiety-like behavior of rats. Intraperitoneal injections of L-buthionine-(S,R)-sulfoximine (BSO) (300 mg/kg), an agent that increases oxidative stress were given to rats either for 3 or 7 days with or without antioxidant, tempol supplementation (1 mM in drinking water) and their effect on anxiety-like behavior was examined using light-dark and open-field anxiety tests. Oxidative stress was examined by malondialdehyde assay, nitrotyrosinylation and serum and urine 8-sioprostane levels.
Results: Our results suggest that, increasing oxidative stress by pharmacological intervention using BSO treatment increased anxiety-like behavior of rats. Interestingly, treatment with the antioxidant, tempol reduced brain oxidative stress and attenuated anxiety-like behavior of rats. These observations have prompted us to wonder about the mechanism(s) that enable the anxiolytic effect of tempol. Our data point towards a unique role for two antioxidant enzymes, Glyoxalase (Glo) 1 and Glutathione reductase (Gsr) 1 in this process, suggesting that regulation of antioxidant protein pool is potentially an important element of anxiety. Reduced Glo1 and Gsr1 proteins in the brain (hippocampus, amygdala and locus coeruleus) were observed in rats that exhibit anxiety-like behavior upon sub-chronic BSO treatment (7 day) and were recovered with tempol treatment. On the other hand, short-term BSO treatment (3 day) increased oxidative stress but did not produce anxiety but these rats had increased Glo1 and Gsr1 levels in hippocampus, amygdala and locus coeruleus, brain regions implicated in anxiety response. Two other antioxidant proteins, catalase and superoxide dismutase remained unchanged.
Discussion: These findings make the relationship between oxidative stress and anxiety even more intriguing. Earlier, overexpression of Glo1 and Gsr1 was reported to increase while inhibition of Glo1 expression to decrease anxiety-like behavior of mice. Another study has suggested a negative relationship between anxiety-like behavior and Glo1 expression. Regardless of different outcome of results due to genetic variation or differences in models between studies, regulation of the antioxidant protein pool posits as an important element of anxiety. Whether the observed differential expression of Glo1 and Gsr1 is simply associative with anxiety-like behavior or whether there is a causal role for these enzymes remains to be seen.
Disclosure: S. Salim, None.

Effects of Phosphodiesterase-2 Inhibition on Anxiety- and Depression-Related Behaviors Associated with Oxidative Stress
James M. O'Donnell*

West Virginia University, Morgantown, WV

The pathogenesis of several psychiatric disorders, including anxiety and depression, has been linked to oxidative stress, in part via alterations in cyclic nucleotide signaling. Phosphodiesterase-2 (PDE2), which is a component of the nitric oxide/guanylyl cyclase signaling pathway in neurons, may affect oxidative stress-induced behavioral changes by increasing cyclic GMP signaling. Experiments were carried out to examine the behavioral effects that result from increased oxidative stress. Mice were administered L-buthionine-(S,R)-sulfoximine (BSO), which altered indices of oxidative stress in brain regions associated with emotion and response to stress (i.e., amygdala, hypothalamus, hippocampus). This included increased lipid peroxidation, reduced antioxidant capacity, and increased NADPH oxidase subunits. Coincident with these neurochemical changes, an increase in anxiety-related behavior was observed using the elevated plus-maze, hole-board, and open-field tests. Administration of either the PDE2 inhibitor Bay 60-7550 or the NADPH oxidase inhibitor apocynin

reversed both the neurochemical and behavioral effects of increased oxidative stress; Bay 60-7550 exhibited a somewhat greater effect against the increased anxiety than did diazepam. Treatment of cultured neurons *in vitro* with BSO also altered neurochemical indices in a manner consistent with increased oxidative stress. Treatment with the PDE2 inhibitor Bay 60-7550 prevented the effects of BSO, while increasing cyclic GMP and phosphorylation of VASP by protein kinase G (PKG). Since PDE2 is a mixed cyclic GMP/cyclic AMP PDE, the effects of selective inhibitors of PKG and PKA were assessed. The PKG inhibitor KT-5823, but not the PKA inhibitor H89, blocked the ability of Bay 60-7550 to prevent the oxidative stress caused by BSO, suggesting cyclic GMP mediation. In order to assess the relationship between emotional stress and oxidative stress, mice were subjected to a repeated unpredictable stress (RUS) procedure. RUS produces anxiety-related behavior, demonstrated in the elevated plus-maze and hole-board tests, and depression-related behavior, demonstrated in the forced-swim and tail-suspension tests. These behavioral effects of RUS also were prevented by administration of the PDE2 inhibitor Bay 60-7550. Interestingly, RUS, in addition to altering behavior, also resulted in increased oxidative stress in amygdala, hypothalamus, and hippocampus, similar to that produced by BSO. Overall, the present findings suggest a relationship between oxidative stress and emotional stress, with its behavioral sequelae, consistent with increased anxiety and depression. The effects of the Bay 60-7550 in reversing both oxidative stress and subsequent behavioral changes suggests that PDE2 inhibitors and increased cyclic GMP signaling may have benefits in long-term psychiatric illnesses associated with oxidative stress.

Disclosure: J.M. O'Donnell, Lundbeck Pharmaceuticals, Part 1.

Panel Session
Plasticity of the Reward Circuitry in Depression and Stress-Related Disorders

Mechanisms of Stress-Induced Synaptic and Behavioral Plasticity in Nucleus Accumbens

Scott J. Russo*

Mount Sinai Medical School, New York, NY

Background: Major depressive disorder (MDD) is marked by a heterogeneous constellation of behavioral symptoms including both depressed mood and diminished interest in pleasurable stimuli (anhedonia), such as social interaction, sex and natural rewards. A detailed understanding of the neural substrates and molecular mechanisms that mediate these symptoms will provide us with novel and more selective targets for drug development and ultimately increase the efficacy of treatment.

Methods: Using chronic social defeat stress, a mouse model of stress-related mood dysfunction, we determined the molecular mechanisms controlling stress-induced synaptic plasticity of nucleus accumbens (NAc) medium spiny neurons (MSNs), which are key brain reward neurons that control mood.

Results: We found that chronic stress increases the formation of new immature spine structures with small postsynaptic densities (PSD) that contain the molecular machinery for glutamatergic neurotransmission. These measures highly correlate with social avoidance: Animals with the smallest PSDs on MSNs, showed the greatest degree of social avoidance. We next wanted to identify the underlying molecular mechanisms responsible for this neuronal restructuring. We found an increase in NFκB activity, which has been shown to regulate spineogenesis in the NAc. Using a herpes simplex virus (HSV) expressing a constitutively active I Kappa Kinase (IKKca) to activate NFκB or a dominant negative I Kappa Kinase (IKKdn) to inhibit NFκB, we show that stress-induced activation of NFκB in the NAc is both necessary and sufficient for social avoidance. In addition, blocking NFκB in susceptible animals blocks or reverses the formation of new spine structures on NAc MSNs, suggesting that this is a critical neuroadaptation driving social avoidance behavior. Although it is clear that NFκB regulates structural plasticity, the direct pathways downstream of NFκB that control the actin cytoskeleton are completely unknown. Here we find that intracranial injections of the HSV-IKK mutants into the NAc resulted in gross changes in Rac1-PAK1 signaling, a RhoGTPase pathway known to mediate actin cytoskeletal reorganization and the development of new spines. Inhibition of NFκB with IKKdn decreases activity within the Rac1-PAK1 pathway, whereas IKKca greatly increases its activity. Interestingly, social defeat also reduces activity of Rac1 and PAK1 in the NAc, and viral mediated repression of Rac1 and PAK1 increase susceptibility to social defeat, further highlighting the importance of these new spines in regulating stress-induced avoidance.

Discussion: These studies confirm an obligatory role of NAc NFκB in regulating cell morphology and social avoidance, providing fundamentally new information about the mechanisms of synaptic plasticity in depressive disorders.

Disclosure: S.J. Russo, None.

Two Faces of Stress: Chronic Cold and Restraint Produce Opposite Effects on VTA Dopamine Neuron Activity

Anthony Grace*, Ornella Valenti

University of Pittsburgh, Pittsburgh, PA

Introduction: Stress can have multiple effects on organisms, ranging from increasing responses to environmental stimuli in times of threat, to withdrawal during times of danger. Moreover, stress is known to be a context-dependent condition. Rats that are exposed to certain types of anxiogenic stressors (e.g., restraint) show a cross-sensitization between the stress and the behavioral response to amphetamine. However, other types of maintained stressors actually depress dopamine system function. We examined how two different stressors, chronic cold and restraint, affect dopamine neuron activity states and the behavioral responses to amphetamine.

Methods: One group of rats was exposed to two hours of restraint stress, and recorded 2 hours later. Another group of rats was shaved and exposed to 5 degrees C cold room for 14 days and recorded 24 hours later. Recordings were made from identified dopamine neurons in the VTA to assess: 1) population activity (DA neurons firing/track), firing rate, and burst firing. Locomotor activity was measured in an automated activity monitor after injection of saline or amphetamine (1.5 mg/kg).

Results: We found that rats exposed to restraint stress show an increase in the number of DA neurons firing (population activity) and an increased behavioral activation by amphetamine; both of these are reversed when the ventral subiculum is inactivated. Furthermore, this corresponded to increased c-fos staining in the ventral subiculum, a context-related area. In contrast, chronic cold stress leads to a decrease in DA neuron population activity, and a decrease in the behavioral response to amphetamine, with no c-fos activation in the ventral subiculum.

Discussion: These data suggest that stressors that are behaviorally activating tend to increase DA neuron drive in a context-dependent manner, whereas those associated with depressed conditions attenuate DA neuron drive. The population activity, or number of dopamine neurons firing, we propose reflects the responsivity of the DA system to external stimuli. Restraint stress, by causing a context-dependent increase in DA neuron population activity, renders the system hyper-responsive to potentially rewarding or threatening stimuli when in an environment reflective of these events. In contrast, constant inescapable cold stress leads to a decrease in DA neuron population activity and response to amphetamine, thereby diminishing the response of the organism to external stimuli. As a result, this latter condition would better reflect an anhedonic state more common of depression than of anxiety.

Disclosure: A.A. Grace, Johnson & Johnson, Part 1; Taisho, Part 1; AstraZeneca, Part 1; Lilly, Part 1; Lundbeck, Part 1; Abbott, Part 1; Galaxo Smith Klein, Part 1; Johnson & Johnson, Part 2; AstraZeneca, Part 2; Taisho, Part 2; Johnson & Johnson, Part 3; Taisho, Part 3; Abbott, Part 4; Lundbeck, Part 4; Galaxo Smith Klein, Part 4.

Long-Lasting Alterations in the Reward System After Chronic Mild Stress: The Potential of Localized Brain Stimulation

Abraham Zangen*

Weizmann Institute of Science, Rehovot, Israel

Background: Stress related disorders are likely to result from atypical processing and integration of information by several neural networks. Various types of neuroadaptations were identified within reward-related brain regions after chronic stress. Convergence of glutamatergic innervations from limbic and cortical structures under intense dopaminergic modulations, places the nucleus accumbens (NAc) as the major site for integration of emotional salience, contextual constraints and executive/motor plans. In this presentation I will describe our studies on long-lasting effects of chronic mild stress (CMS) on behavior, plasticity as measured by spontaneous and evoked responses in the NAc, and the potential of localized brain stimulation as a tool to investigate and affect plasticity in the reward system.
Methods: Rats were exposed during 4 weeks to either CMS or enriched environment, or a control condition. Behavioral analysis included measurements of sucrose preference and exploration of a novel environment. Electrophysiological recording in the NAc were used to measure long-lasting alterations in spontaneous activity (including spectral analysis) and evoked responses induced by stimulation of the ventral subiculum (vSub). In other groups of rats exposed to CMS we have measured the potential benefit of repeated electrical stimulation of reward-related brain sites on neuroplasticity and the behavioral impairments.
Results: CMS induced reduction in sucrose preference and exploration of a novel environment. These animals displayed an increase in spontaneous patterned network activity in the NAc and increased sensitivity of the vSub-NAc pathway. In contrast, the ability to sustain time-locked, hippocampally evoked, network response was strongly reduced. Repeated electrical stimulation of the NAc or the ventral (but not the dorsal) prelimbic cortex normalized sucrose preference after CMS and enhanced plasticity as indicated by brain-derived neurotrophic factor (BDNF) levels in the hippocampus.
Discussion: Our studies indicate that stressful life-experience induces depressive-like behavior and is associated with *in-vivo*, long-term functional adaptations in the reward system. CMS left a long-lasting mark on the NAc network activity, properties and response. These adaptations are suggested to reflect impaired plasticity in the reward system and are potentially affected by repeated localized electrical stimulation.
Disclosure: A. Zangen, Brainsway, Part 1; Brainsway, Part 2; Brainsway, Part 3; Brainsway, Part 4.

Major Depression and Childhood Adversity Are Associated with Dysfunctions Within the Reward Circuitry

Diego A. Pizzagalli*, Daniel G. Dillon, Dan Iosifescu, Maurizio Fava, Karlen Lyons-Ruth

Harvard University, Cambridge, MA

Background: Anhedonia (lack of reactivity to pleasurable stimuli) is a cardinal symptom of major depressive disorder (MDD) and has been associated with increased vulnerability to psychopathology and poor treatment outcome across disorders. Adverse, or stressful experiences encountered early in life have also been associated with the later development of psychopathology, including depression. Research in non-human animals has shown that adverse rearing environments and chronic stressors lead to dysregulated function within the brain reward circuitry and anhedonic behavior, but the etiological mechanisms and neurobiological underpinnings of anhedonia have not been well characterized in humans. In two studies, we tested the hypothesis that MDD participants and individuals exposed to childhood adversity would show reduced reward-related responses in basal ganglia regions implicated in incentive motivation, hedonic coding of stimuli, and reinforcement learning.
Methods: In both studies, functional magnetic resonance imaging (fMRI) was used in conjunction with a monetary incentive delay task that dissociates anticipatory and consummatory phases of reward processing. Between-group comparisons focused on neural responses to cues signaling possible monetary rewards (reward anticipation), as well as delivery of monetary gains (reward outcome).
Results: In Study 1, relative to healthy controls ($n = 31$), MDD participants ($n = 26$) showed significantly weaker responses to reward delivery in the left nucleus accumbens and bilateral caudate. In addition, the MDD group displayed reduced activation in response to reward-predicting cues in a small section of the left posterior putamen, although evidence for group differences during reward anticipation was weaker than in response to monetary gains. In the MDD group, severity of depressive and anhedonic symptoms were associated with reduced caudate volume bilaterally. In Study 2, individuals exposed to childhood adversity ($n = 13$) showed weaker responses to reward-predicting cues in the left global pallidus and putamen compared with healthy control participants ($n = 31$). Those who had experienced early adversity also reported elevated symptoms of anhedonia and depression, and rated reward cues less positively than controls. No group differences emerged in basal ganglia responses to reward delivery in Study 2.
Conclusions: Collectively, these findings indicate that MDD subjects and individuals with a history of early adversity are characterized by blunted responsiveness in mesolimbic pathways implicated in hedonic coding and reinforcement learning. Results of Study 1 specifically indicate that basal ganglia dysfunction in major depression may primarily affect the consummatory phase of reward processing, and that anhedonic symptoms in MDD are related to caudate volume. Findings from Study 2 suggest that childhood adversity in humans is associated with dysfunction in left basal ganglia regions involved in reward-learning and motivation, as well as blunted subjective responses to reward-predicting cues. These findings may have important implications for improving interventions for individuals with, or at increased risk for MDD.
Disclosure: D.A. Pizzagalli, ANT North America Inc., Part 1; AstraZeneca, Part 1.

Panel Session
Recent Advances in Glutamatergic Treatment of Schizophrenia

Pathophysiological Basis of NMDA Dysfunction in Schizophrenia

Daniel Javitt*

Nathan Kline Institute/New York University School of Medicine, Orangeburg, NY

Background: Negative symptoms and cognitive deficits are well established features of schizophrenia, but underlying mechanisms remain relatively obscure. This panel focuses on the role of N-methyl-D-aspartate (NMDA) dysfunction as a potential underlying pathophysiological feature of schizophrenia. NMDA theories of SZ were first developed based upon observations that NMDA antagonists such as phencyclidine (PCP) and ketamine induce symptoms and cognitive deficits closely resembling those of SZ, and have been supported since then by genetic findings and clinical studies. NMDA receptors are distributed in a widespread fashion throughout cortex and subcortical structures but within each cortical region are associated with only limited types of processing. As such, deficits in cognition in SZ should be widespread, but only involve NMDA-related processes. This presentation focuses on SZ as an NMDA deficiency disorder.

Methods: Data will be presented from representative datasets investigating contributions of specific NMDA-mediated processes such as non-linear gain, oscillatory entrainment and re-entrant processing to neurocognitive dysfunction and negative symptoms in SZ. Non-linear gain was investigated within sensory modalities using ERP and fMRI methods relative to findings in rodent and primate neurophysiological models. Symptom studies investigated potential underpinnings of negative symptoms using positive and negative valence stimuli along with direct behavioral observations.
Results: Patients showed decreased NMDA-related sensory responses within both visual and auditory modalities. In the visual modality, patients showed reduced non-linear gain of magnocellular response, with relatively intact linear gain components. A combined ERP/fMRI study showed relatively intact attention-related brain activation despite impaired early sensory response, suggesting preservation of specific top-down mechanisms despite early neurophysiological dysfunction. . In the auditory modality, patients showed impaired inter-trial coherence to repetitive stimuli along with impaired cross-frequency coupling, implicating impaired synchronization mechanisms. These findings are consistent with impaired local NMDA-dependent re-entrant processing within auditory cortex in SZ. In the behavioral studies, SZ showed no reduction in hedonia but did show increases in ambivalence, consistent with impaired NMDA-dependent non-linear gain and reciprocal inhibition within emotional brain regions as underpinnings of symptoms as well as cognitive dysfunction in SZ.
Discussion: These findings suggest that basic NMDA-related processes such as non-linear gain and phase synchronization are impaired throughout brain in SZ. These deficits are present in both sensory and higher cognitive brain regions and thus may underlie the overall pattern of neurocognitive dysfunction in SZ. On a symptom level, behavioral studies suggest that increases in ambivalence may be more critical than impairments in reward in understanding negative symptoms, consistent with NMDA hypotheses and findings in primate models of SZ. As with cognitive deficits, therefore, negative symptoms may be explained by failures of non-linear gain within underlying brain regions and may improve following NMDA augmentation in SZ.
Disclosure: D.C. Javitt, AstraZeneca, Pfizer, Schering-Plough, NPS, Sanofi, Solvay, Takeda, Sepracor, Glytech, AASI, Promentis, Pfizer, Roche, Jazz, Part 1; Pfizer, Glytech, Part 2; Pfizer, Glytech, Part 3; Jazz, Pfizer, Roche, Part 4; No, Part 5.

Modifying NMDA Receptor Function in Healthy Humans by Glycine, mGluR2/3 Agonists, and N-acetyl-cysteine

John H. Krystal*, Handan Gunduz-Bruce, Naomi Driesen, Deepak C. D'Souza

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

Schizophrenia appears to be associated with glutamatergic synaptic abnormalities that compromise NMDA glutamate receptor function. However, schizophrenia is a complex neurodevelopmental disorder whose component pathophysiologic mechanisms are not well understood. This presentation will focus on studies that have used ketamine infusion in healthy human subjects to model deficits in NMDA receptor function that might be associated with schizophrenia. In particular, this presentation will present findings from three studies. We will begin by briefly reviewing a published study of the mGluR2/3 agonist, LY354740. This study suggested that mGluR2/3 agonism attenuated the disruption of working memory produced by ketamine and tended to reduce its psychotigenic effects. Next, we will report new data on the impact of N-acetyl-cysteine (NAC) pretreatment on ketamine effects in healthy humans ($n = 16$). Preliminary analyses suggest that NAC may reduce ketamine's effects on P300 disruption. However, NAC pretreatment had mixed effects upon the behavioral effects of ketamine in healthy subjects. Lastly, we will report behavioral and fMRI data on the impact of glycine pretreatment upon ketamine effects in healthy human subjects. These data provide preliminary support for the hypothesis that prefrontal cortical deficits associated with ketamine administration might be attenuated by enhancing the stimulation of the glycine site of the NMDA receptor complex.
Disclosure: J.H. Krystal, *Part 1*; Abbott Laboratories, Aisling Capital, LLC, AstraZeneca Pharmaceuticals, Brintnall & Nicolini, Inc., Bristol-Myers Squibb, Eli Lilly and Co., F. Hoffman-La Roche, Ltd., Forest Laboratories, GlaxoSmithKline, Janssen Pharmaceuticals, Lohocla Research Corp., Merz Pharmaceuticals, Pfizer Pharmaceuticals, SK Holdings Co., Ltd.. *Part 2*; Society of Biological Psychiatry, Pfizer Pharmaceuticals, AstraZeneca, Takeda Pharmaceuticals. *Part 4*; Johnson and Johnson (grant to VA but not personally to my laboratory), AstraZeneca.

Effects of the Glycine Transporter Typ1 Inhibitor RG1678 in Schizophrenic Patients with Predominant Negative Symptoms

Daniel Umbricht*, Kisook Yoo, Eriene Youssef, Ernest Dorflinger, Meret Martin-Facklam, Bausch Alexander, Richard Arrowsmith, Daniela Alberati, Luca Santarelli

F. Hoffmann-La Roche Ltd., Basel, Switzerland, F. Hoffmann-La Roche Ltd., Nutley, NJ

Background: Deficient signaling through the N-methyl-D-aspartate (NMDA) receptor has been hypothesized to be a key factor underlying many signs and symptoms of schizophrenia. Targeting the allosteric glycine site of the NMDA receptor has been proposed an approach to enhance NMDA receptor functioning. Studies with full and partial agonists at the glycine site and sarcosine, a naturally occurring inhibitor of the glycine transporter type 1 (GlyT1), have provided initial support for this hypothesis. However, some of these studies have been hampered by the need to administer huge doses of agonists, or by the relatively unselective nature of GlyT1 inhibition. RG1678 is a potent and non-competitive inhibitor of the glycine transporter type 1 (GlyT1) with more than 1000 fold selectivity for the human GlyT1 versus the GlyT2 transporter and at least 300 fold greater selectivity over other targets. Preclinically, it shows efficacy in assays of NMDA receptor functioning and schizophrenia models and increases CSF glycine levels in a dose-dependent fashion.
Methods: The effects of RG1678 on negative symptoms of schizophrenia were investigated in a phase IIb proof-of-concept study. Patients with prominent negative symptoms who were stable on antipsychotic treatment were randomized equally to 8 weeks of treatment with three doses of RG1678 (10 mg, 30 mg, 60 mg) or placebo once daily. Efficacy parameters were: Change from baseline in the PANSS negative symptom factor score (NSFS; *Marder SR, Davis JM, Chouinard G; Clin Psychiatry 1997;58: 538-546*), proportion of responders defined as showing $\geq 20\%$ improvement in NSFS and CGI-Improvement of Negative Symptoms. Functional effects were assessed with the Personal and Social Performance (PSP) Scale.
Results: 323 patients were randomized (Male: 64%; Caucasian: 66%; age: 39.9 ± 10.1 (SD) y; duration of illness 11.7 ± 8.9 y; PANSS total: 79.2 ± 9.3; NSFS 26.1 ± 3.9; Positive Symptom Factor Score: 17.7 ± 3.7). RG1678 demonstrated a robust and clinically meaningful effect on negative symptoms. Compared to placebo significantly greater improvements were observed on negative symptoms as measured by change in the NSFS, percentage of patients meeting response criteria and CGI-Improvement of Negative Symptoms. In addition a positive effect on functioning as assessed by the change in PSP score from baseline was observed in patients treated with RG1678. RG1678 was well tolerated. The percentage of patients withdrawn for AEs ranged from 1% to 9%. The percentage of withdrawals for any reason was similar in all four treatment groups (13-20%).
Discussion: RG1678 demonstrated a robust, consistent and clinically meaningful reduction of negative symptoms that was accompanied by an emergence of positive functional effects. This proof-of-concept study is the first to provide clinical support for glycine reuptake inhibition as a therapeutic approach for negative symptoms in schizophrenia in patients whose symptomatology was specifically characterized by predominant negative symptoms.
Disclosure: D. Umbricht, F. Hoffmann-La Roche Ltd., Part 1.

Modulation of Glutamate Signaling at Multiple Levels for Treatment of Postitive Symptoms and Cognitive Disturbances in Schizophrenia
Jeffrey Conn*

Vanderbilt University Medical Center, Nashville, TN

Background: The glutamate hypothesis of schizophrenia has led to a major focus on development of that could ameliorate the symptoms of schizophrenia by regulating glutamatergic transmission. One approach that has been employed with some success in proof of concept studies is the use of modulatory site agonists at the NMDA receptor such as glycine and D-cycloserine. However, it has not been possible to develop direct ligands at the glycine site or other direct modulators of the NMDA receptor that are suitable for development as therapeutic agents. We and others have taken alternative approaches to modulate glutamatergic signaling in a manner that may provide efficacy in treatment of schizophrenia.
Methods: We have used molecular pharmacology, electrophysiology, behavioral, and imaging approaches to assess the effects of compounds that regulate glutamatergic function in brain circuits that may be important for treatment of schizophrenia patients. For instance, we have developed highly selective positive allosteric modulators of the metabotropic glutamate receptor mGluR5 and mGluR2 subtypes as well as the M1 muscarinic acetylcholine receptor. M1 and mGluR5 potentiate currents through NMDA receptor channels in cortical, limbic, and basal ganglia regions. In contrast, mGluR2 modulates thalamocortical pathways in a manner that may offset effects of NMDA receptor hypofunction. In addition to developing selective activators of GPCRs that regulate NMDA receptors, we have evaluated effects of novel highly selective inhibitors of the GlyT1 glycine transporter.
Results: Selective positive allosteric modulators (PAMs) of mGluR2, mGluR5 and M1 are all effective in modulating specific aspects of glutamatergic signaling in forebrain regions in brain slices and *in vivo*. In addition, modulators of each of these receptors has efficacy in animal models that have been used to predict antipsychotic effects and mGluR5 and M1 PAMs have efficacy in improving different domains of cognitive function. New selective inhibitors of GlyT1 also have efficacy in animal models that are commonly used to predict efficacy in treatment of schizophrenia. Interestingly, newer competitive inhibitors of GlyT1 that are not based on the sarcosine scaffold do not have the adverse effects that have been observed with earlier compounds.
Discussion: The finding that highly selective agents that target different aspects of glutamatergic signaling provides strong support for the hypothesis that modulation of glutamatergic signaling in limbic and forebrain regions may provide an exciting approach for treatment of multiple symptom clusters that are observed in schizophrenia patients.
Disclosure: P. Conn, Puretech Ventures, Part 1; Genentech, Part 1; Abbott, Part 1; Eli Lilly, Part 1; Solvay, Part 1; Millipore, Part 1; Bristol Myers Squibb, Part 1; EPIX, Part 1; Metastatix, Part 1; Evotech, Part 1; Merck, Part 1; AMRI, Part 1; Merck Serono, Part 1; Adalor, Part 1; Johnson and Johnson, Part 1; Johnson and Johnson, Part 2; Johnson and Johnson, Part 3; Johnson and Johnson, Part 4; Seaside Therapeutics, Part 4.

Panel Session
Social Stress Models for Depression and Drug Abuse

Individual Differences in Stress Reactivity During Social Stress May Predict Susceptibility and Resilience to Depression and Cardiovascular Co-Morbidity: Role of Corticotropin-Releasing Factor
Susan K. Wood*, Seema Bhatnagar, Rita Valentino

Children's Hospital of Philadelphia, Philadelphia, PA

Background: Affective disorders increase the risk of cardiovascular disease. However, the neurobiological systems involved in this co-morbidity are poorly understood. In rodents, social stress increases depressive-like behaviors and elicits robust noradrenergic stimulation. We previously reported the emergence of two phenotypic populations during social defeat in Sprague Dawley rats; one population exhibited passive behaviors and short latencies to exhibit a supine defeat posture (SL, <300 s) while the other exhibited active behaviors (eg., upright posture) and longer defeat latencies (LL, >300 s). We concluded that the SL subset was more susceptible to a depressive phenotype. In the present studies, because active coping strategies result in greater noradrenergic stimulation, we hypothesize that the LL phenotype may be more susceptible to stress-induced cardiovascular dysfunction. The stress-related neuropeptide, corticotropin-releasing factor (CRF), is implicated in affective disorders and CRF1 activation within the brain elicits both central and peripheral noradrenergic activation and therefore represents a putative mediator of co-morbid cardiovascular and affective disorders.
Methods: The resident-intruder model was used whereby Sprague Dawley intruder rats were placed into the cage of an aggressive Long Evans resident during 7 daily defeats (30 min/day). Rats were treated with vehicle or the centrally-active CRF1 antagonist NBI-30775 (10 mg/kg) 1 hr prior to defeat on days 1-7. The latency for an intruder to exhibit a supine, submissive posture was recorded daily. *In vivo* ECG telemetry was conducted on days 1, 4, and 7 at rest and during defeat and the time-domain index of heart rate variability (HRV), SDNN, was calculated to identify shifts in sympathetic-parasympathetic balance. The Porsolt forced swim test (FST) was conducted under drug-free conditions 24 hrs after the 7th defeat.
Results: Thirty-eight percent of vehicle-treated rats exhibited the SL phenotype, while only 9% of NBI-30775 treated rats exhibited an SL phenotype. Immobility was increased during the FST in vehicle-treated SL and LL rats compared with controls, while this effect was blocked in rats treated with NBI-30775 during defeat. Increased cardiac:body weight, suggestive of maladaptive cardiac hypertrophy, was positively correlated with defeat latency ($r = 0.62$, $p = 0.0007$). A reduction in heart rate variability (SDNN), indicative of a shift towards sympathetic dominance, occurred in both SL and LL rats at rest on the 7th day of defeat compared with controls. Interestingly, administration of NBI-30775 inhibited the development of reduced HRV.
Discussion: Decreased HRV is observed in depressed patients and is associated with a greater risk of cardiovascular disease. In the present study, a reduction in resting HRV occurred in all vehicle-treated defeated rats but LL rats were more susceptible to maladaptive cardiac hypertrophy, whereas treatment with NBI-30775 inhibited the cardiovascular repercussions of defeat. These data also suggest that passive stress coping and development of depressive-like behaviors is, in part, mediated by CRF1 activation. These studies reveal a role for CRF1 to mediate the development of stress-induced depressive-like behavior and exaggerated sympathetic activity and implicate CRF1 as a novel therapeutic target for co-morbid depression and cardiovascular disease. MH 40008, AHA 0825572D, MH06751 to SB.
Disclosure: S.K. Wood, None.

Optogenetic Stimulation of the Medial Prefrontal Cortex Has Antidepressant-Like Effects
Herbert E. Covington III*, MaryKay Lobo, Ian Maze, James M. Hyman, Subroto Ghose, Carol Tamminga, Rachael Neve, Karl Deisseroth, Eric Nestler

Duke University, Durham, NC

Human deep brain stimulation and imaging studies have highlighted a key role for the prefrontal cortex in clinical depression, however, it remains unknown whether excitation or inhibition of prefrontal cortical neuronal activity is associated with antidepressant responses. The objective of the current series of experiments is to examine the

relationship between chronic stress-induced molecular modifications of the mPFC and depressive-like behaviors in mice. The functional activity of infralimbic and prelimbic cells were inferred from zif268 expression, CREB activity, and c-fos expression after chronic social defeat stress. Here, we report that cellular indicators of functional activity, including the immediate early gene *zif268* and the transcription factor *creb*, are reduced in prefrontal cortex of depressed human brains obtained postmortem and after chronic social stress in mice. Deficits in mPFC functional activity persist long after social stress, and these deficits are corrected via stimulation of the mPFC using virally-mediated expression of channel rhodopsin 2 (ChR2) which when activated with a blue laser (470 nm) will result in neuronal firing. Laser stimulations mimicking patterns of mPFC "burst" firing, not only restored functional activity, but also corrected behaviors impaired by social stress, indicating strong antidepressant-like effects of mPFC stimulation.
Disclosure: H.E. Covington: None.

HDAC6 in Raphe-Efferent Pathways Modulate Resiliency to Social Defeat Through Chromatin-Independent Mechanisms
Julie Espallegues, Sarah Teegarden, Sheryl Beck, Olivier Berton*

University of Pennsylvania, Philadelphia, PA

Background: The HDAC6 protein that we examine in this study is a novel therapeutic candidate emerged from the burgeoning field of epigenetics. HDAC genes encode a family of lysine deacetylases targeted by a novel class of anticancer therapy called HDAC inhibitors (HDACis). Non-selective HDACis, that promote protein hyper-acetylation in the brain, have demonstrated consistent behavioral activity across various rodent models of affective disorders and antidepressant response, including the chronic social defeat (SD) paradigm. Although most studies, thus far, have interpreted this antidepressant-like activity in the context of chromatin-remodeling mechanisms (ie the canonical role of HDACs) it is now well established that the influence of HDACs extends far beyond histones and transcriptional regulation. In fact, certain HDACs, such as HDAC6, are involved primarily in "chromatin-independent" functions. These include the regulation of chaperone proteins such as HSP90, an important component of steroid receptor signaling. Previous tissue culture studies, in non neuronal systems, have shown that inhibition of HDAC6 blunts glucocorticoid Receptor (GR) signaling, an important determinant of stress vulnerability. In the present studies we asked whether this regulatory role extends to neuronal tissues in culture, and to the mouse social defeat model *in vivo*.
Methods: The SD paradigm in mice was used to assess individual vulnerability to behavioral effects of social stress, as well as GR receptor function. HDAC6 pattern of expression was mapped in the mouse and human brain using immunohistochemistry and *in situ* hybridization, and regulation of HDAC6 after SD and antidepressant treatments was examined using qPCR. To test the causal role of HDAC6 in stress resiliency we have generated 2 lines of mutant mice lacking HDAC6 in distinct neuronal populations.
Results: Our mapping data indicates that HDAC6 protein is found in several brain areas in the mouse and human brain, but the densest clusters of HDAC6-positive cells are encountered in the raphe nuclei where HDAC6 co-localizes with serotonergic markers and GR protein. In serotonergic cell line, pretreatment with HDAC6 inhibiting compounds blunts dexamethasone-induced GR translocation. Serotonin-selective KO of HDAC6 promote resilience to social defeat an effect associated with HSP90 hyperacetylation in serotonin neurons, and blunted GR-mediated gene regulation. The effects SD on electrophysiological and morphological properties 5-HT neurons are being examined in HDAC6 KO.
Conclusion: Our data support the view that HDAC6 is a candidate target for antidepressant and pro-resilience interventions through targeted inhibition of GR signaling in the serotonergic system.
Disclosure: O. Berton, None.

Early Life Stress Affects the Development of Vasopressin-Regulated Social Behavior
Alexa H. Veenema*, Michael Lukas, Remco Bredewold, Inga Neumann

Boston College, Chestnut Hill, MA, University of Regensburg, Regensburg, Germany, University of Massachusetts, Amherst, MA

Background: Early life stress (child neglect, child abuse, child trauma) is a major risk factor for the development of pervasive social deficits that are a key feature of several psychiatric disorders, including depression and aggression disorders.
Methods: Early life stress is modelled in rodents by separating the pups from their mother for 3 h per day during the first two weeks of life (maternal separation). It is well known that maternal separation causes long-lasting changes in emotional and neuroendocrine responses. We investigated whether maternal separation alters the development of social behavior in male Wistar rats and whether this is associated with changes in the brain vasopressin system. Vasopressin is an important regulator of diverse aspects of social behavior.
Results: Maternal separation increased aggressive behaviors during juvenile play-fighting and during adult resident-intruder aggression, which was accompanied by increased vasopressin mRNA expression in the hypothalamic paraventricular nucleus of both juvenile and adult rats. Moreover, maternal separation impaired social recognition (lack of discrimination between a familiar and unfamiliar rat), which was associated with a diminished capacity to show a rise in vasopressin release within the septum, as was found in control male rats. Application of synthetic vasopressin during the acquisition phase restored social recognition in maternal separated rats.
Discussion: These findings demonstrate that early life stress changes several aspects of social behavior which are caused by alterations in the brain vasopressin system.
Disclosure: A.H. Veenema, None.

Study Group Session

Has Drug Development in Psychiatry Hit a Road Block? An RFP Pro Con Panel (Pro - Yes, There Is Roadblock; Con - No, There Is No Roadblock)
David Pickar*, Charles Nemeroff, George Koob and Michael E. Thase

Gabriel Sciences, LLC, Chevy Chase, MD

Although the past decade has seen more new drug approvals than any other prior decade, no drug has indication of superiority to another in its class. Charles Nemeroff and David Pickar will take the Pro position – "Yes, there is a roadblock." Nemeroff will present genetic and imaging data that support the biological heterogeneity of depression. Nemeroff asserts that the resistance by industry to target subtypes of depression is a roadblock for drug development. Pickar will argue that continuing emphasis on monotherapy for schizophrenia rather than building on and enhancing antipsychotic drug effects mechanisms holds back clinical advance. Pickar will present data of alpha2 antagonist augmentation of antipsychotic drugs in conjunction with genetic predictors of outcome. George Koob and Michael Thase will take the Con position – "No roadblock." Koob will discuss the use of animal models for medication development in the domains of anxiety, depression, schizophrenia, and substance use disorders with a focus on face and predictive validity. He will argue that a process termed the "Rosetta Stone" approach where existing pharmacotherapies are used to validate and improve animal models holds promise to directly advance new drug development. Thase will argue that the apparent roadblock in developing novel treatments for depression and related disorders is more of

a perceptual illusion than an actual obstruction, partly a consequence of several decades of relative inattention to the methodological factors that ensure the internal validity of clinical trials. He will also argue that multi-stage clinical trials that begin with a course of SSRI therapy have shown sufficient design sensitivity to demonstrate the effectiveness of several adjunctive medication strategies.
Disclosure: D. Pickar, Gabriel Sciences, LLC, Part 1.

Study Group Session

Institutional Review Board Conundrums in Neuropsychiatry: Case Examples and Resolutions
Katherine L. Wisner*, Paul S. Appelbaum and Thomas Kosten

University of Pittsburgh, Pittsburgh, PA

Neuropsychiatry researchers frequently identify problems in obtaining IRB approval of research proposals. ACNP Council charged the Human Research Committee with conducting a survey of members to identify common IRB problems and solutions, and approved posting of case examples on the website as a member service. Two cases derived from the ACNP survey, Solutions for Common IRB Issues in Neuropsychiatry, will be presented with recreation of the process of objections, rebuttals, and iterative compromises that resulted in resolution and IRB approval. ACNP members were divided in their opinions about the appropriateness of IRB approval, which makes these cases likely to stimulate a lively debate. Dr. Wisner will present the dilemma of an NIMH-funded R01 that was denied IRB approval due to inclusion of a placebo control group in a randomized trial comparing a novel antidepressant agent with a standard antidepressant drug for postpartum depression. The IRB's primary concern was that it is not ethical to withhold efficacious treatment from depressed individuals and directed removal of the placebo group. The investigator's view was that removing the placebo group risked finding that a novel agent is as efficacious as a standard drug without knowing whether either is more efficacious than placebo in postpartum women. In the survey, 18% of ACNP respondents said their IRB would not approve, 31% said their IRB would approve, and 51% thought their IRB might approve the inclusion of a placebo control with conditions imposed. Dr. Kosten will present the second case, which involves conducting an ethnographic interview on drug dependent adolescents attending a juvenile probation outpatient treatment program in three states and including adolescents who refuse to allow contacting their parents for consent. The IRB concerns include concealing information from parents, state law variability about age of independent consent, coercion of these adolescent ''prisoners'', whether a certificate of confidentiality protects disclosure of the illegal drug activities, and conditions for mandatory reporting of drug activity, sexual activity, AIDS risk, child abuse and suicidality. In the ACNP survey, 15% of ACNP respondents said their IRB would not approve, 42% said their IRB would approve, and 43% thought their IRB might approve with parental consent. Dr. Appelbaum will moderate the study group and summarize the theoretical underpinnings of the issues to close the discussion of each case. He will highlight the ethical issues the cases raise, and potential approaches to balancing the principles involved. The floor will be opened to the audience for their comments about each case and questions raised by the IRBs. This study group will also serve to obtain feedback about the model format for posting cases on the ACNP website.
Disclosure: K.L. Wisner, Eli Lilly Co., Part 1; Novartis, Part 1.

Study Group Session

The Alcohol Clinical Trials Initiative (ACTIVE): Progress Report and Feedback
Raymond Anton*, Raye Litten, Daniel E. Falk, Henry R. Kranzler, Raymond Bartus, Celia Winchell, Amy M. Duhig and Joseph M. Palumbo

Medical University of South Carolina, Charleston, SC

The ACNP sponsored Alcohol Clinical Trials Initiative (ACTIVE) was conceived as a process whereby ACNP members from academia and representatives from the FDA, NIAAA, and the pharmaceutical industry could work together to identify and clarify clinical trials methodology to evaluate medications to treat alcohol dependence. To that end, a group of 26 individuals met three times over a one-year period to develop a consensus on some key issues in the conduct of clinical trials for alcoholism that might be addressed using data from completed multi-center clinical trials. A steering committee that has directed the effort will present a description of the framework of the effort and the progress that has been made to address these issues to date. Dr. Raymond Anton (Chair of ACTIVE) will present an overview of the perceived need that led to this effort, the overall organization of the initiative, and the key questions that ACTIVE has begun to address. These questions include the drinking outcomes that are meaningful and acceptable to all parties, including practitioners, the definition of a "treatment responder," how to handle missing data in clinical trials, the role of patient self-reported outcomes, and the need for biological markers of drinking and treatment response. Drs. Joseph Palumbo (Johnson & Johnson) and Raymond Bartus (Cergene) will highlight challenges within the pharmaceutical industry to support development of medications to treat alcoholism and other addictions. Dr. Celia Winchell (FDA) will detail the history of clinical trials and the need for a better definition and analytic approach to treatment response in alcohol dependence. Dr. Amy Duhig (Eli Lilly & Co.) will describe the process of development of Patient Reported Outcomes (PRO's), the FDA requirements to validate PRO's, and work being done by her and the ACTIVE group in the area of PRO drinking-related consequences. Drs. Raye Litten and Daniel Falk (NIAAA) will present the results of data analyses that are beginning to inform the choice of an appropriate trial length and primary drinking outcomes. Dr. Henry Kranzler will moderate the presentations and discussions while encouraging audience participation. The goal of this study group is to describe to the ACNP membership the work that has been done so far by the ACTIVE group and to solicit input from the membership and other participants to guide subsequent efforts. These efforts will include the publication of one or more consensus papers on key methodological considerations in the conduct of clinical trials for alcohol dependence.
Disclosure: R.F. Anton, Eli Lilly, Part 1; Alcohol Clinical Trials Initiative (ACTIVE) is supported by Lilly, Janssen, Schering Plough, Lundbeck, Alkermes, GSK, and Abbott. Receives support from ACTIVE., Part 1.

Study Group Session

Where Is the Big Payoff of Psychiatric Genetics: GWAS, Nextgen Sequencing, or Biology-Based Studies?
David Rubinow*, Patrick Sullivan and Daniel Weinberger

UNC School of Medicine, Chapel Hill, NC

The power and promise of genomics for uncovering the roots of psychiatric illnesses are indisputable. More controversial, however, is how the various possible genetic investigations that should be prioritized. Genomewide association studies (GWAS) occupy a central role in this controversy. On one hand, GWAS are the coin of the realm - large scale studies that, as Patrick Sullivan has argued, avoid many of the pitfalls of

candidate gene association studies (i.e., small n, selective post-hoc hypotheses, high false positive rate, infrequent replication of findings [failure to find either the same disease:gene associations or associations with the same specific structural variants]. On the other hand, as Daniel Weinberger has argued, the GWA model is based on a linear, predictable relationship between risk and illness that belies the pathophysiological complexity and heterogeneity of psychiatric syndromes. This Pro-Con study group will promote discussion of the strengths and weaknesses of GWAS v. targeted gene association studies as strategies to understand the genetic predispositions to psychiatric illnesses.
Disclosure: D. Rubinow, Azevan Pharmaceuticals, Part 1; Dialogues in Clinical Neuroscience (Journal underwritten by Servier Pharmaceuticals) Editorial Board.

Tuesday, December 7, 2010

Panel Session
Habenula Session 1: Role of the Habenula in Addiction and Emotional States

Role of the Lateral Habenula in Motivational Control of Animal Behaviors
Masayuki Matsumoto*

Kyoto University, Inuyama, Aichi, Japan

Background: Animals learn to obtain rewards and to avoid punishments. The lateral habenula (LHb) is a good candidate for the neural mechanism underlying such learning. Indeed, our recent study suggested that neurons in the LHb are inhibited by reward and excited by reward omission, and that these reward-related signals are transmitted from the LHb to midbrain dopamine neurons which are involved in learning and motivation (Matsumoto & Hikosaka, 2007). However, it was unclear whether the reward-related signals of the LHb actually influence animal behaviors. In this talk, I will present our recent data showing the role of the LHb signals in behaviors.
Methods: We first examined what kind of information is signaled by neurons in the LHb. For this purpose, we recorded the activity of LHb neurons in monkeys during a Pavlovian procedure with an appetitive unconditioned stimulus (liquid reward) and an aversive unconditioned stimulus (airpuff directed at the face). This Pavlovian procedure consisted of two blocks of trials, a reward block and a punishment block. In the reward block, three conditioned stimuli were associated with reward, with probabilities of 100%, 50% and 0%. In the punishment block, three conditioned stimuli were associated with airpuff, with probabilities of 100%, 50% and 0%. Thus, this Pavlovian procedure had two distinct contexts: one in which reward was available and another in which aversive airpuff was feared. This Pavlovian procedure enabled us to examine whether LHb neurons encode punishment-related signals as well as reward-related signals. To examine whether the LHb signals influence animal behaviors, we next artificially activated the LHb by electrical stimulation while monkeys were performing a visually guided saccade task. The visual target was presented randomly on the right or left and the monkey had to make a saccade to it immediately. Saccades to one direction were followed by electrical stimulation of the LHb while saccades to the other direction were not.
Results: In the first experiment, we found that LHb neurons were most strongly excited by a conditioned stimulus associated with the most unpleasant event in each block. Thus, the LHb neurons showed the strongest excitation to the conditioned stimulus associated with 0% reward in the reward block and the conditioned stimulus associated with 100% airpuff in the punishment block. The magnitude of the excitation decreased as the reward probability increased and the airpuff probability decreased. These results suggest that LHb neurons encode negative value signals induced by both reward and aversive airpuff. In the second experiment, we found that the latency of the saccade associated with the post-saccadic electrical stimulation of the LHb increased gradually as the saccade was repeatedly followed by the stimulation. The latency of the saccade that was not associated with the LHb stimulation decreased gradually. These gradual changes in saccade latency suggest that the post-saccadic LHb stimulation suppresses saccadic eye movements by influencing a learning mechanism, rather than a motor execution mechanism.
Discussion: Together with the recording experiment showing the activation of LHb neurons by the unpleasant conditioned stimuli, the electrical stimulation experiment suggested that the activity of LHb neurons contributes to learning to suppress behaviors which would lead to unpleasant events. Whether the LHb stimulation effect is mediated by dopamine neurons remains to be determined.
Disclosure: M. Matsumoto, None.

Genetic and Molecular Studies to Dissect the Contribution of the Habenular Circuit to Brain Function and Behavior
Ines Ibanez-Tallon*

Max-Delbrueck-Centrum, Berlin, Germany

The habenular complex is a crossroad for descending projections from the forebrain to nuclei in the mid and hindbrain. Such feedback relay could be key in the modulation of a variety of behavioral functions including cognition, reward and social behaviors. We are investigating the contribution of specific receptors, ion channels and transporters in the control of neurotransmission from medial habenula. Given the dense concentration of nAChR in habenular neurosn and the recent genome wide association studies linking the CHRNB4-CHRNA3-CHRNA5 gene cluster to nicotine addiction, we are investigating the role of the β4 subunit to nicotinic function using transgenic mice (Tabac mice) expressing elevated levels of β4 in the habenula. Targeted overexpression of β4 results in strong potentiation of nicotine-evoked currents and surface expression of functional a3b4-containing receptors in these mice. Behaviorally, Tabac mice displayed decreased social interaction and aggression, as well as increased sensitivity to nicotine and pronounced aversion to nicotine. Using a combination of bioinformatics analysis and *in vitro* functional mapping experiments we have mapped this potentiation property to a single domain unique in β4. These data establish that the β4 is rate-limiting for nAChR activity and nicotine responsiveness *in vivo* and provides a novel mechanistic insight into the involvement of the CHRNB4-CHRNA3-CHRNA5 gene cluster in nicotine consumption. In addition to approach the molecular complexity of the habenula we have collected TRAP (Translational ribosome affinity profiling) data and discovered and characterized a number of ion channels and transporters in this population (i.e. BK channels, ASIC channels, R-type voltage-gated calcium channels) that may work in combination with nAChRs. Detailed electrophysiological and biochemical studies are in progress to test the idea that one of these molecules forms a direct interaction with nAChRs and contributes to nicotine dependence. Furthermore we have optimized a series of lentivirus tethered toxin constructs that block neurotransmission and are well suited for functional dissection of neural circuits (Auer et al. 2010. Nature Methods). Some of these are Cre dependent, and they are now being introduced into an habenula specific Cre-recombinase transgenic line for functional analysis of the habenula. In conclusion, we are employing a variety of genetic approaches to dissect the function of the habenula in the mammalian brain and to determine its molecular properties.
Disclosure: I. Ibanez-Tallon, None.

Stronger Excitatory Synapse onto Neurons That Signal Disappointment in Depressed Rats
Bo Li*

Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

The cellular basis of depressive disorders is poorly understood. Recent studies in monkeys indicate that neurons in the lateral habenula

(LHb), a nucleus that mediates communication between forebrain and midbrain structures, increase their activity when an animal fails to receive an expected positive reward, i.e. these neurons provide a disappointment signal. LHb neurons project to and modulate dopamine-rich regions such as the ventral-tegmental area (VTA) and substantia nigra that control reward-seeking behavior and participate in depressive disorders. Here we show in two rat models of depression that excitatory synapses onto LHb neurons projecting to the VTA are potentiated. Synaptic potentiation is due to an enhanced presynaptic release probability. Depleting transmitter release by repeated electrical stimulation of LHb afferents, using a protocol that can be effective on depressed patients, dramatically suppresses synaptic drive onto VTA-projecting LHb neurons in brain slices and significantly reduces depression-like behavior in rats. Our results suggest that increased presynaptic action onto LHb neurons contributes to depression by enhancing disappointment signals leading to reduced reward-seeking behavior.
Disclosure: B. Li, Takeda Pharmaceuticals North America, Inc, Part 1; Dana Foundation, Part 1; Dana Foundation, Part 4.

α3β4 Nicotinic Receptors in the Habenulo-Interpeduncular Pathway: Site of Action for Novel Anti-Addictive Agents
Stanley D. Glick*

Albany Medical College, Albany, NY

Background: Many new agents, including dopamine agonists and antagonists, GABA agonists, glutamate antagonists, opioid partial agonists, and catalytic antibodies, are being studied and/or developed as potential treatments for drug abuse. Most of these treatments are targeted at a specific drug or drug class of abuse. However, we discovered that 18-methoxycoronaridine (18-MC), an iboga alkaloid congener, has the potential to be useful in treating multiple forms of drug abuse. In rats, 18-MC decreased the self-administration of morphine (Glick et al. 1996), cocaine (Glick et al., 1996), methamphetamine (Glick et al., 2000a), nicotine (Glick et al., 2000a) and alcohol (Rezvani et al., 1997) while having no effect on responding for water (Glick et al., 1996a, 1998). Subsequent studies showed that 18-MC's primary mechanism of action is to selectively block α3β4 nicotinic receptors (Glick et al., 2002; Pace et al., 2004). These results led us to hypothesize that the α3β4 receptor was a viable as well as novel target around which to develop treatments for drug addiction (Maisonneuve and Glick, 2003). The mechanisms of action of virtually all drugs of abuse appear to involve the dopaminergic mesolimbic system, and new anti-addictive medications are usually designed to affect this system. Although 18-MC also affects this system, it does so indirectly via other pathways (cf. Maisonneuve and Glick, 2003). It was in fact the distribution of α3β4 nicotinic receptors in the brain that suggested a role of these other pathways in mediating 18-MC's behavioral effects, since α3β4 nicotinic receptors are preferentially localized in the medial habenula and interpeduncular nucleus (e.g., Klink et al., 2001; Quick et al., 1999).
Methods and Results: The interpeduncular nucleus receives its main input from the medial habenula, forming the habenulo-interpeduncular pathway in the fasciculus retroflexus. While there are multiple avenues for interaction between this pathway and the mesolimbic pathway in the medial forebrain bundle, it has been known since the 1980's that the habenulo-interpeduncular pathway can function as a reward system separate from the mesolimbic pathway (e.g., Sutherland and Nakajima, 1981), although it appears that the two pathways modulate each other. Based on its α3β4 nicotinic antagonist action, we postulated that 18-MC might act in the habenulo-interpeduncular pathway to dampen the activity of the mesolimbic pathway. Accordingly, we found that local administration of 18-MC into either the medial habenula or interpeduncular nucleus decreased both morphine (Glick et al., 2006) and methamphetamine (Glick et al., 2008) self-administration in rats and also blocked sensitization of morphine-induced dopamine release in the nucleus accumbens (Taraschenko et al., 2007).
Discussion: Nicotinic α3β4 receptor subtypes are thought to be located on the soma of the medial habenula efferents and on axon terminals of afferents to the interpeduncular nucleus (Clarke et al., 1986; Mulle et al., 1991). Cholinergic activation of nicotinic receptors in the interpeduncular nucleus has been shown to enhance glutamate (Girod and Role, 2001) and GABA release (Lena et al., 1993), thereby regulating the excitatory and inhibitory output of this structure. Interference with neuronal activity in this system may be the basis for the putative "broad spectrum" anti-addictive activity of 18-MC and
Disclosure: S.D. Glick, None.

Panel Session
Hippocampal-Prefrontal Interactions: A Genetic Risk Mechanism for Schizophrenia

Fronto-Temporal Connectivity in Psychosis
Philip McGuire*

Institute of Psychiatry, Kings College London, London, United Kingdom

Background: For over a century, theoretical models have proposed that psychosis results from a disruption of normal connectivity between brain regions. Data from a range of neuroimaging studies using different techniques are broadly consistent with this hypothesis, and have particularly implicated fronto-temporal dysconnectivity in psychosis.
Methods: In a series of studies, patients with first episode psychosis, subjects at ultra high risk of psychosis, and controls were compared using functional MRI, volumetric MRI, diffusion tensor imaging, MR Spectroscopy and PET. Data were analysed using voxel-based approaches, tractography, functional connectivity and dynamic causal modeling.
Results: Both first episode patients and high risk subjects showed altered fronto-temporal functional connectivity and altered anatomical connectivity relative to controls. The severity of the findings in the high risk group was often intermediate to that in first episode patients. Within the temporal lobe, findings were particularly evident in the parahippocampal region, where there were also local changes in glutamate levels, and altered functional connectivity with the striatum, in association with elevated striatal dopamine function.
Discussion: Data from several studies across a range of imaging modalities point to an alteration in the normal relationship between prefrontal and temporal cortex in subjects with psychosis. The presence of similar findings in subjects with prodromal symptoms indicates that these changes are not secondary to the disorder or its treatment, but predate its full clinical expression. These human data are consistent with findings from contemporary animal models of psychosis.
Disclosure: P. McGuire, None.

Genome-Wide Significant Risk Variants for Psychosis and Hippocampal-Prefrontal Interactions
Andreas Meyer-Lindenberg*

Central Institute of Mental Health, Mannheim, Germany

Background: Disturbed interactions between prefrontal cortex and hippocampus have been found in manifest schizophrenia, but the relevance of this finding for genetic risk for psychosis was unclear. The recent identification and confirmation of genome-wide significant risk variants for schizophrenia, combined with imaging genetics, permits a strong inference on the contribution of limbic-prefrontal connectivity to the genetic risk architecture.

Method: In 115/117 healthy German participants from the general population genotyped for common SNPs in ZNF804A and CACNA1C, we used functional magnetic resonance imaging and a well-validated executive cognition task probe, the n-back task, shown to be abnormal in schizophrenia, related to heritable risk, and sensitive to genetic variation in candidate genes, as well as a newly developed episodic memory paradigm. We employed conservative analysis statistics by applying family-wise error and Gaussian random field theory previously shown to exert strong control of type I error over multiple comparisons in imaging genetics. We studied regional brain activation of DLPFC and HF during task performance as well as coupling between these structures using functional connectivity, a well-established correlative measure. We also used the same method to study connectivity during an emotional face matching task and resting.
Results: For ZNF804A, during the n-back task, no regional activation effects were observed, but the HF was uncoupled from DLPFC in non-risk allele homozygotes, showing dose-dependent increased connectivity with DLPFC risk allele carriers ($p < .0001$, uncorrected; $p < .01$, corrected for multiple comparisons, coordinates of maximum effect [-36 -21 -18]). This finding was present only during working memory, but not during face matching or at rest. For CACNA1C, strong regional activation deficits were observed in HF together with abnormal coupling between left and right HF.
Discussion: Abnormal functional coupling of the DLPFC with HF found here for carriers of the ZNF804 variant mirrors findings in overt schizophrenia, implicating this pathomechanism for the disorder as a mediator of genetic risk. The finding was tied to working memory and not found in control conditions. Our data therefore show a degree of disease-related and cognitive specificity on the systems level that corresponds closely to the genetic association with the psychiatric-behavioral phenotype and imply that DLPFC-HF coupling contributes to genetic risk for psychosis in the broader sense. For CACNA1C, a more local effect in HF was identified that corresponds closely to finding in knockout mouse models of this variant. Even here, connectivity of HF was disturbed. Taken together, our data support a key role of HF and DLPFC connectivity in schizophrenia neurogenetics.
Disclosure: A. Meyer-Lindenberg, Astra Zeneca, Part 1; Eli Lilly, Part 1; GlaxoSmithKline, Part 1; Johnson and Johnson, Part 1; Novartis, Part 1; Servier, Part 1; Roche, Part 2.

Impaired Hippocampal-Prefrontal Synchrony in Genetic Mouse Models of Schizophrenia
Joshua Gordon*

Columbia University, New York, NY

Background: Abnormal functional connectivity between brain areas has been postulated as an important pathophysiological mechanism underlying schizophrenia. In particular, neuroimaging and electroencephalographic studies suggest that functional connectivity between the prefrontal cortex and the temporal lobe is altered in schizophrenia patients. However, it is unclear how such dysconnectivity is related to the etiology of the illness. Furthermore, because patient studies are based on macroscopic measurements of brain activity, it is not known how abnormal functional connectivity is manifest at the level of neuronal circuits. Because schizophrenia has a strong genetic component, mouse models of genetic risk factors are likely to play an important role in clarifying these issues.
Methods: We therefore studied neural activity in Df(16)A+/- mice, which model a microdeletion on human chromosome 22 (22q11.2) that constitutes one of the largest known genetic risk factors for schizophrenia. We recorded activity in the hippocampus and prefrontal cortex of these mice while they performed a task requiring working memory, one of the cognitive functions disrupted in the disease.
Results: In wild-type mice, hippocampal-prefrontal synchrony increases during performance the discrete-trial T-maze task, a test of spatial working memory. Df(16)A+/- mice, which are impaired in the acquisition of this task, show dramatically reduced synchrony, measured both by phase-locking of prefrontal cells to hippocampal theta oscillations and by coherence of prefrontal and hippocampal local field potentials. Furthermore, the magnitude of hippocampal-prefrontal coherence at the onset of training was correlated with the time it took animals to learn the task. Phase locking of prefrontal cells to the local theta rhythm was unaffected in Df(16)A+/- mice, as was theta power in the prefrontal cortex and hippocampus, suggesting a specific impairment in synchrony between the two structures.
Discussion: These data suggest how the deficits in functional connectivity observed in patients with schizophrenia may be realized at the single neuron level. Our findings further argue that impaired long-range synchrony of neural activity is one consequence of the 22q11.2 deletion and may be a fundamental component of the pathophysiology underlying schizophrenia.
Disclosure: J.A. Gordon, None.

Altered Prefrontal-Hippocampal Coupling: A Potential Intermediate Phenotype for Schizophrenia and Association with *ZNF804a*
Daniel Weinberger*

Clinical Brain Disorders Branch, NIMH, Bethesda, MD

Background: Abnormal dorsolateral prefrontal cortex (DLPFC) activation has consistently been found in patients with schizophrenia (PTS) and in genetically high risk subjects, implicating it as a risk associated intermediate biologic phenotype. Recent studies have shown patterns of abnormal DLPFC functional connectivity with other brain areas in schizophrenia and strong association of these patterns with a potential susceptibility gene (*ZNF804a*). However, whether DLPFC connectivity is a trait phenomenon linked to genetic risk for illness is not known and without this knowledge, the association of *ZNF804a* with this physiologic measure cannot be taken as evidence of a neural system mechanism of clinical genetic risk. We performed an fMRI study to test the hypothesis that altered connectivity is a heritable feature of genetic risk for schizophrenia.
Method: We investigated the functional connectivity of DLPFC in healthy siblings (SIBS) of PTS compared with normal controls (NCS). Four-hundred-two subjects (153 NCS, 171 SIBS, and 78 PTS) participated in this study. Each participant underwent BOLD fMRI (3T) while performing the 2-back working memory task. NCS were also genotyped for rs1344706 in ZNF804A, a SNP that showed genome wide significance in an earlier clinical association study of psychosis (O'Donovan et al 2008) and also showed significant association with measures of prefrontal functional connectivity in an imaging genetic study of normal subjects. (Esslinger et al, 2009). The effects of familiality and of genotype were explored both on DLPFC activity and connectivity. Task-independent and task-dependent functional coupling analyses- between DLPFC and other potential brain 'target' regions- were performed with 1) the traditional seeded connectivity, and 2) psycho-physiological interaction (PPI) analysis to investigate the interaction between task load (2 back versus 0 back) and connectivity.
Results: Consistent with prior reports, SIBS showed greater PFC activation than NCS despite normal performance (i.e. greater inefficiency). Between group comparisons in the seeded connectivity analysis showed a significant increase in the negative coupling between right DLPFC and right hippocampus in PTS and SIBS when compared to NCS. The same groups (SIBS and PTS) also showed a decrease in the positive functional coupling between right DLPFC and right IPL. In the PPI analysis, SIBS showed an intermediate pattern of diminished modulation of the positive coupling between right DLPFC and hippocampus compared to NCS. Notably, connectivity measures and prefrontal activation measures within individuals did not correlate. *ZNF804A* genotype significantly modulated DLPFC coupling but not DLPFC activity.

Conclusions: The coupling and its task related modulation between DLPFC and other brain regions is compromised in SIBS of PTS /and is independent of DLPFC engagement *per se*, suggesting a novel independent neural system intermediate phenotype related to genetic risk for schizophrenia. The selective association of *ZNF804a* with this phenotype and not with prefrontal efficiency *per se* suggests that this intermediate phenotype proxies a distinct neural system mechanism related to genetic risk for schizophrenia and the biology of this gene.
Disclosure: D.R. Weinberger: None.

Panel Session
Mitochondrial Dysfunction in Neuropsychiatric Disorders: Neuropharmacological Strategies

Energyomics-Energenomics: Insights into Common Diseases
Douglas C. Wallace*

University of California, Irvine, Irvine, CA

Bioenergetic dysfunction is being found to be a common factor in a wide range of rare and common diseases. Rare diseases associated with more severe mutations in nuclear DNA (nDNA) and/or mitochondrial DNA (mtDNA) encoded bioenergetic genes now constitute a well established class of diseases. These mitochondrial diseases encompass phenotypes ranging from lethal childhood Leigh syndrome to adult-onset diabetes and neuro-psychiatric disorders. Because bioenergetics requires the interaction of over a thousand nDNA genes as well as the maternally-inherited and multi-copy mtDNA oxidative phosphorylation (OXPHOS) genes, the inheritance patterns of mitochondrial diseases can be highly complex. Characterization of the genetics, biochemistry, and physiology of mitochondrial diseases has produced concepts that are applicable to a wide spectrum of metabolic and degenerative diseases, cancer, and aging. The importance of bioenergetic dysfunction in the common diseases initially escaped the attention of the Western medical community because Western medical thinking was based on a predominately anatomical perspective of disease and a Mendelian perspective of genetics. However, the search for common disease variants in the nDNA has been relatively unproductive. An important clue as to why this is true comes from the fact that the environment pays a major role in the etiology of common diseases. The primary component of the environment is the availability of and demands on energy (calories), which varies both regionally and seasonally. Adjusting to regional bioenergetic differences requires changes in cellular energetics that are stable over thousands of years. This is most readily achieved by the accumulation of functional variants in the mtDNA bioenergetic genes, the mtDNA having a high mutation rate, with the highly deleterious mutations being eliminated via an intra-ovarian selective system. Environmental energetic fluctuations are managed by epigenomic regulation, mediated by mitochondrial high energy intermediates. Finally, the mtDNAs in post-mitotic organs like the brain accumulate age-related somatic mtDNA mutations which result in the progressive decline in mitochondrial function, providing the aging clock. Since the tissue most reliant on mitochondrial energetics is the central nervous system, subtle changes in mitochondrial bioenergetics can result in changes in mood and behavior. Therefore, predisposition to psychiatric disorders should be influenced by variation in mtDNA and nDNA bioenergetic genes, the age-related accumulation of brain somatic mtDNA mutations, and environmental stressors which alter the regulation of bioenergetic genes and precipitate symptoms.
Disclosure: D.C. Wallace, None.

Novel Brain Heteroplasmy and Association of Mitochondrial DNA in Psychiatric Disorder
Marquis P. Vawter*, P. Adolfo Sequeira, Maureen V. Martin, Brandi Rollins, Emily A. Moon, William Bunney, Erin Smith, John Kelsoe

University of California, Irvine, Irvine, CA

Background: Theories of mitochondrial deficiency in schizophrenia and bipolar disorder have been proposed due to bio-energetic alterations found in brain imaging and postmortem studies of oxidative phosphorylation pathways. This presentation will address the potential role of somatic mtDNA variants and increase in heteroplasmy in different vulnerable brain regions and to what extent normal aging can account for the rates of mutations in brain. Risk for psychiatric disorders might be increased in certain mtDNA haplogroups. Unique data on ethnic specific haplogroup mtDNA SNPs and risk for schizophrenia and bipolar disorder will be presented using datasets from the Genetic Association Information Network.
Methods: The amount and number of somatic mutations in mtDNA (homoplasmic and heteroplasmic mutations) across 11 brain regions was studied with Illumina GAII resequencing (80 samples). The hypothesis that specific risk variants such as heteroplasmy accumulates in brain and are either not present or present at lower levels in germ line tissue such as blood was tested. BD association was tested in a case-control analysis of the Genetic Association Information Network (1743 subjects) mtDNA SNP data from GAIN bipolar working group for European ancestry (EA) using Cochran-Mantel-Haenszel test with multidimensional scaling cluster stratifications. The mtDNA genotype calls were made in batches to balance cases and controls using Birdseed analysis. The GAIN schizophrenia cases ($n = 1161$) and controls ($n = 1372$) were also tested for the same mtDNA allele procedure for association and majority of controls overlap in BD and SZ analyses.
Results: Brain MtDNA genome resequencing (average coverage 1250x) showed novel homoplasmic mutations in coding regions while the rate of homoplasmic mutation was greater in non-coding mtDNA regions. Over 20 novel mtDNA coding variants were found in brain. The GAIN-BD-EA analysis was highly significant particularly at two loci (p-value before correction for CMH test stratified by 4 PCA components: T7028C and G12308A) which are ethnic specific haplogroup markers. The GAIN SZ-EA analysis was nominally significant for one SNP before correction (T7028C) but G12308A did not show a haplogroup association ($p = 5\ x10^{-1}$).
Discussion: Resequencing mtDNA with next generation sequencing gives high coverage and allows homoplasmy detection in brain for somatic mutations. This preliminary GAIN mtDNA association analysis requires caution, as it cannot be excluded that BD subjects were genotyped on separate plates from controls so that a bias in calls might explain the results. Given this caveat, however the current analysis suggests that matrilineal descendants may have lower prevalence or risk of BD that carry SNP T7028C and higher risk for individuals with G12308A. These relative risks between these two groups require further replication in independent samples. At this time it is not possible to exclude somatic accumulation of mutations and haplogroup risk as mechanisms that contribute to neuropsychiatric disorders.
Disclosure: M.P. Vawter, None.

Neural Basis of Bipolar Disorder-Like Phenotypes in Mice Accumulating Deleted Mitochondrial DNA
Tadafumi Kato*

Brain Science Institute, Saitama, Japan

Bipolar disorder is characterized by recurrent manic and depressive episodes and genetic factors contribute to its pathophysiology. However, it is still not known how bi-directional behavioral changes are caused by genetic factors. Neuroprotective effects of mood

stabilizers suggest that vulnerability of neurons is involved in its pathophysiology. Multiple lines of evidence suggest the role of mitochondrial dysfunction in the pathophysiology of bipolar disorder: altered energy metabolism in the brain detected by magnetic resonance spectroscopy, comorbidity of mood disorders in mitochondrial diseases, altered expression of mitochondria-related genes, and accumulation of mitochondrial DNA (mtDNA) mutations in the postmortem brains. We developed mutant polymerase gamma (Polg) transgenic (Tg) mice with neuron-specific accumulation of mutant mtDNAs. The mice showed bipolar disorder-like phenotypes such as periodic change of wheel running activity and distorted diurnal activity rhythm, which is improved by lithium treatment. By searching for the genes commonly altered in the Tg mice and the postmortem brains of patients with bipolar disorder, we found that Ppif, encoding cyclophilin D, was commonly altered. We tested the effect of a brain blood barrier-permeable cyclophilin D inhibitor, and found that it was effective for the behavioral phenotype of the Tg mice. To further search for the neural basis of the bipolar disorder-like phenotypes in the Tg mice, we searched for the brain regions accumulating partially deleted mtDNAs, by developing a method for quantitative mapping of deleted mtDNA. Using this method, we found that deleted mtDNAs are specifically accumulated in several brain areas related to the regulation of emotion. Deleted mtDNAs are reported to be preferentially amplified due to its shorter length, and dominate in cells after mtDNA depletion. We now speculate that deleted mtDNAs accumulate in the neural systems that undergo active neuroplastic changes, and dendrites accumulating deleted mtDNAs become more vulnerable to stress, resulting in viscous cycle.
Disclosure: T. Kato, Asahi Kasei Corporation, Part 1; Astellas Pharma Inc, Part 1; Dainippon Sumitomo Pharma Co., Ltd, Part 1; Eli Lilly Japan K.K., Part 1; GlaxoSmithKline, Part 1; Kyowa Hakko Kirin, Co., Ltd., Part 1; Meiji Seika Kaisha, Ltd., Part 1; Pfizer Inc., Part 1; Taisho Toyama Pharmaceutical Co., Ltd., Part 1; Yoshitomiyakuhin Corporation, Part 1; Takeda Science Foundation, Part 1; Mitsubishi Pharma Research Foundation, Part 1; NARSAD, Part 1; Takeda Science Foundation, Part 4; Mitsubishi Pharma Research Foundation, Part 4; NARSAD, Part 4.

Setting the Balance: Mitochondrial Dysfunction in Postmortem Brain from Patients with Bipolar Disorder

L. Trevor Young*, Ana C. Andreazza, Jun-Feng Wang

University of British Columbia, Vancouver, BC, Canada

Background: Accumulating evidence suggests that mitochondrial dysfunction and oxidative damage underlies the pathophysiology of bipolar disorder (BD) and schizophrenia (SCZ). Previous studies in our lab have shown that the mRNA levels of NADH dehydrogenase ubiquinone Fe-S protein 7 (NDUFS7), a subunit of mitochondrial electron transport chain complex-1, was significant lower in postmortem prefrontal cortex of BD subjects. In addition, BD patients have shown increased lipid peroxidation and serum levels of the 3-nitrotyrosine in cingulate cortex and serum, respectively. The propose of this study is evaluate whether decreased NDUFS7 levels result in decreased complex I activity and increased oxidative damage to proteins in prefrontal cortex of subjects with BD, SCZ or MDD.
Methods: Post-mortem prefrontal cortex from subjects with BD, major depressive disorder (MDD) or SCZ, and from non-psychiatric comparison controls was generously provided by the Stanley Foundation Neuropathology Consortium. Assays were carried out in mitochondrial particles extracted from prefrontal cortex of subjects. NDUFS7 protein and carbonyl groups (protein oxidation) levels were measured using immunoblotting analysis. The activity of complex-1 was measured by following the decrease in absorbance of NADH at 340 nm with a diode-array spectrophotometer. The tyrosine-nitration induced damage was evaluated by measuring the levels of 3-nitrotyrosine with a competitive enzyme immunoassay.
Results: NDUFS7 levels and complex I activity were decreased significantly in prefrontal cortex from individuals with BD, but were unchanged in MDD and SCZ patients, compared to non-psychiatric controls. Protein oxidation levels were increased significantly in BD whereas levels for MDD and SCZ patients did not differ significantly from control. In tissue from both BD and SCZ we observed increased levels of tyrosine-nitration induced damage.
Conclusions: These findings suggest that impairment of complex I may lead to increased protein oxidation in BD. Therefore, mitochondrial dysfunction and oxidative damage may be potential biomarkers for the illness or may help guide terapeutics.
Disclosure: L. Young, Eli Lilly, Astra Zeneca, Pfizer, Bristol Myers Squibb, Part 1.

Panel Session
mTOR Signaling: At the Crossroads of Synaptic Plasticity and Brain Disorders

mTOR Signaling: Synaptic Plasticity, Memory, and Developmental Disability

Eric Klann*

New York University, New York, NY

Background: A requirement for *de novo* protein synthesis is one of the hallmarks of long-lasting synaptic plasticity and long-term memory. Recent studies, including several from our laboratory, have identified signaling cascades, including the mTOR signaling pathway, that couple neurotransmitter and neurotrophin receptors to the translation regulatory machinery in the hippocampus during synaptic plasticity and memory. Interestingly, mutations in negative upstream regulators and downstream effectors of mTOR are associated with certain types of developmental disability and autism. Synaptic plasticity and memory phenotypes in genetically engineered mice that display altered mTOR signaling will be discussed.
Methods: Mice with a conditional deletion for tuberous sclerosis complex 2 (TSC2) and transgenic mice with a mutation in the GAP domain of TSC2 were used to model tuberous sclerosis, and transgenic mice that overexpress eIF4E were used to model non-syndromic autism in these studies. Molecular studies were conducted in the hippocampus and cortex of the mutant mice to examine mTOR signaling and rates of protein synthesis. Protein synthesis-dependent forms of hippocampal synaptic plasticity also were examined in the mutant mice. Finally, a battery of behavioral tests were conducted to determine whether the mice displayed altered phenotypes consistent with human patients with tuberous sclerosis and autism.
Results: We have found that the mouse models of tuberous sclerosis have multiple molecular, synaptic plasticity, and behavioral phenotypes. Studies with eIF4E transgenic mice were begun recently and the results will be discussed at the meeting.
Discussion: These studies have revealed interesting links between mTOR signaling, synaptic plasticity, memory, and behavior. These studies also have provided insight into the molecular basis of certain types of developmental disability and autism.
Disclosure: E. Klann: None.

AMPA Receptor- and BDNF-Mediated Regulation of Local Protein Synthesis in Hippocampus

Michel Baudry*

University of Southern California, Los Angeles, CA

Background: Brain-derived neurotrophic factor (BDNF) stimulates local dendritic mRNA translation and is involved in formation and consolidation of memory. CX614, one of the best studied positive AMPA receptor modulators (a.k.a. ampakines), increases BDNF

mRNA and protein and facilitates LTP induction. We were therefore interested in determining whether positive AMPA receptor modulation could also rapidly stimulate local dendritic mRNA translation in a BDNF-dependent manner. Moreover, we previously showed that CX614 could rapidly activate the calcium-dependent protease calpain, and we determined whether calpain activation could participate in CX614-mediated increase in local protein synthesis.

Methods: We used primary neuronal cultures as well as acute hippocampal slices to study the effects of BDNF and CX614 on the protein synthesis machinery (mTOR and associated proteins, 4EBP1, and p70S6 K). Activation of local protein synthesis was determined by western blots and immunohistochemistry. It was also determined by using a green fluorescent (GFP) reporter cDNA flanked by the 5′ and 3′ untranslated regions (UTR) from the CaMKIIα subunit. Finally, we used TrkB-Fc in primary cultured neurons to trap released BDNF in combination with western blotting, in order to determine the effect of CX614 treatment on BDNF release.

Results: CX614 treatment of primary neuronal cultures and acute hippocampal slices rapidly activated the translation machinery and increased local dendritic protein synthesis. The acute effect of CX614 on translation was mediated by increased BDNF release as demonstrated with a BDNF scavenging assay using TrkB-Fc during CX614 treatment of cultured primary neurons and was blocked by nifedipine, ryanodine, and by lack of extracellular Ca^{++} in acute hippocampal slices. Finally, CX614, like BDNF, rapidly increased dendritic translation of an exogenous translation reporter. We previously showed that both CX614 and BDNF activated the calcium-dependent protease calpain in primary neuronal cultures and acute hippocampal slices. Interestingly, the stimulatory effects of BDNF on local protein synthesis required the activation of m-calpain through ERK-mediated phosphorylation.

Discussion: Our findings that positive modulation of AMPA receptor function by ampakines results in BDNF release and activation of local protein synthesis machinery and local protein synthesis, in particular ARC and possibly CaMKIIα, shed new light on the cellular/molecular events implicated in synaptic plasticity. Furthermore, since calpain inhibition reduced BDNF-mediated mTOR activation, our results provide unique links between several elements that have long been proposed to participate in synaptic plasticity, BDNF, calpain, ERK and local protein synthesis.

Disclosure: M. Baudry: None.

BDNF/mTOR Pathway Mediates Ketamine Stimulation of Synaptogenesis in Medial Prefrontal Cortex (mPFC)

George Aghajanian*

Yale University School of Medicine, New Haven, CT

Background: Previous studies in mouse models have shown that a genetic deficiency in brain-derived neurotrophic factor (BDNF) leads to constitutive loss of synaptic spines and excitatory postsynaptic potentials (EPSCs) in the apical dendritic tuft of mPFC layer V pyramidal cells. There are several reports that a single sub-anesthetic dose of ketamine, which induces a robust increase in cortical BDNF, has a rapid antidepressant effect in treatment-resistant depressed patients. Based on its BDNF releasing properties, we hypothesized that ketamine might have an effect on synaptic function opposite to that of BDNF deficiency.

Methods: The influence of ketamine on synaptic physiology and structure was examined by means of whole cell patch clamp recording and 2-photon laser scanning in rat and mouse mPFC brain slices.

Results: Twenty-four hrs after a single dose of ketamine (10 mg/kg, i.p.), we found a large increase in the frequency of EPSCs induced by serotonin (5-HT) or hypocretin/orexin (hcrt), suggesting enhancement of both cortico-cortical and thalamic inputs. In both cases, EPSC amplitude was increased, corresponding to the peak period of ketamine-induced elevation in pre- and postsynaptic-synaptic proteins (e.g., AMPA/GluR1, synapsin I, and PDS-95) (Duman, this panel). In parallel with the increase in EPSC frequency and synaptic proteins, imaging of the recorded cells revealed an increase in spine density in proximal and distal apical tuft segments of of layer V cells. In addition to increased spine density, there was an increase in spine size and EPSC amplitude, indicating greater spine maturity. The above effects of ketamine were diminished in knock-in mice with the reduced-function Val/66/Met BDNF allele or after pre-injection (i.c.v.) with rapamycin, which blocks mTOR downstream from BDNF.

Discussion: These results indicate that the increase in mPFC synaptogenesis induced by ketamine is mediated via the BDNF/mTOR pathway. We suggest that enhanced synaptic connectivity in mPFC and possibly other regions may underlie ketamine's ability to produce a rapid antidepressant effects both in animal models and clinically.

Disclosure: G. Aghajanian: None.

Rapid Antidepressant Actions of Ketamine Require Stimulation of Mammalian Target of Rapaymicin (mTOR) Signaling and Synaptic Protein Synthesis

Ronald Duman*

Yale University School of Medicine, New Haven, CT

Background: The rapid antidepressant response seen following ketamine administration in treatment resistant depressed patients demonstrates a powerful new approach for treating mood disorders compared to the weeks or months required for standard medications. Ketamine is a non-selective glutamate N-methyl-D-aspartic acid (NMDA) receptor antagonist. However, the molecular and cellular mechanisms underlying the rapid and efficacious therapeutic actions of ketamine are likely more complicated than simple NMDA receptor blockade, and so far have not been identified. We carried out a series of studies to examine the cellular signaling pathways that mediate the behavioral actions of ketamine, focusing on cascades known to rapidly influence synaptic plasticity, most notably the mammalian target of rapamycin (mTOR) pathway.

Methods: Levels of the phosphorylated and activated forms of mTOR and related signaling proteins, 4E-BP1 and p70S6K, were determined by western blot analysis of synaptoneurosome preparations of prefrontal cortex (PFC). Levels of postsynaptic density 95 (PSD95), glutamate-AMPA receptor R1 (GluR1), and synapsin I, were also determined. The role of mTOR signaling in the behavioral actions of ketamine in the forced swim test (FST), learned helplessness (LH), and chronic unpredictable stress (CUS)/anhedonia model was determined by pre-infusion with rapamycin (ICV). The role of mTOR signaling in the antidepressant actions of an NMDA receptor 2B (NR2B) subtype selective antagonist, Ro 25-6981, was also examined.

Results: Here we report that a low, behaviorally active dose of ketamine rapidly (1 hr) stimulates mTOR signaling, including increased levels of phosphorylated mTOR, 4E-BP1, and p70S6K. Ketamine administration also produced a rapid (2-6 hr) increase in levels of the synaptic proteins GluR1, PSD95, and synapsin I in synaptoneurosome preparations of PFC. Pre-infusion with the mTOR inhibitor, rapamycin, completely blocked the induction of these synaptic proteins. Moreover, rapamycin pre-infusion completely blocked the rapid antidepressant actions of ketamine in the FST, LH, and CUS/anhedonia model. The NR2B selective antagonist, Ro 25-6981, also rapidly increased mTOR signaling, and its antidepressant behavioral responses were blocked by rapamycin.

Discussion: Fast activation of mTOR signaling, elevation of synapse associated proteins, and increased density and function of spines (see G Aghajanian) represents a novel mechanism for the rapid antidepressant actions of ketamine. These effects provide a mechanism for rapid reversal of stress- and/or depression-mediated atrophy of PFC neurons, reported in studies of rodent models and in postmortem tissue of depressed subjects. Identification of the signaling pathways underlying the rapid induction of synapses in the PFC provides novel targets for drug development, including NR2B selective compounds, but also agents that could directly influence mTOR signaling, such as

metabotropic glutamate receptor 5 (mGluR5), and other as yet unidentified receptors that represent novel and promising drug targets.
Disclosure: R.S. Duman: Part 1; Lilly. Part 2; Lundbeck. Part 3; Taisho. Part 4; Organon. Part 5; Repligen.

Panel Session
Sex, Stress, and Environmental Modulation of Neurodevelopment and Adult Behavioral Susceptibilities

Sex-Specific Transgenerational Programming of Stress Dysregulation
Tracy L. Bale*

University of Pennsylvania, Philadelphia, PA

Background: Sex-biased neurodevelopmental disorders, including depression, anxiety, autism and schizophrenia, have been associated with maternal stress or perturbations experienced during pregnancy. The mechanisms through which fetal antecedents contribute to disease development are not well understood, though likely involve a complex interaction between maternal environment, placental changes, embryo sex and epigenetic programming.
Methods: We have recently identified a sensitive period of early gestation where stress has sex-specific long-term programming effects on offspring stress pathway neurodevelopment. Dams were exposed to chronic, variable stress for the first 7 days pregnancy. Male and female offspring were examined as adults in tests of stress coping, including a tail suspension test, forced swim test, and Barnes maze. HPA axis stress response was also measured following a 15-min restraint stress. Gene expression patterns were determined by Taqman RT-PCR from micropunches of specific brain regions. Mechanistic examination was conducted for changes in inflammatory cytokines and growth factors in male and female placentas from prenatally stressed dams. Second generation animals were generated to determine the heritability of these outcomes and to identify potential epigenetic targets regulating genes involved in programming stress pathways and neurodevelopment.
Results: Male offspring showed increased stress sensitivity as adults in behavioral and physiological stress measures including tests assessing learning and memory, anhedonia, and coping strategies and a heightened HPA stress axis response. CRF expression is significantly increased in the amygdala and glucocorticoid receptors are significantly reduced in the hippocampus in these mice. Fitting with their behavioral and physiological stress phenotype, these males also appear demasculinized as adults with reduced testosterone levels, smaller testis weight, and shorter anogenital distances suggesting a disruption in normal masculinization during development. Mechanistically, we have assessed the involvement of the placenta in transmitting information as to stress-mediated changes in the maternal milieu to the developing embryo. We have identified sex-specific effects of maternal stress on placental inflammatory cytokines, growth factors and epigenetic machinery. Further, recently generated second generation offspring also show a stress-sensitive phenotype in males produced from the first generation paternal lineage, supporting a transgenerational epigenetic mode of transmission. These males also demonstrate a potential disruption in normal perinatal masculinization similar to first generation males. Analyses of gene expression patterns during early brain development suggest alterations in GABA signaling in these mice.
Discussion: These results may provide critical insight into the mechanisms contributing to sex biased disease vulnerability to prenatal stress during early pregnancy. As many neurodevelopmental disorders have a sex bias in presentation, these studies may identify novel gene targets and contributing mechanisms in the etiology of these diseases.
Disclosure: T.L. Bale: Part 4; AstraZeneca.

Enduring Consequences of Maternal High Fat Diet for Brain Inflammation and Behavior of Male and Female Offspring
Staci Bilbo*

Duke University, Durham, NC

Background: Maternal obesity is an increasing public health concern, and is associated with a number of adverse outcomes for both mother and baby. Increased adipose tissue in obese individuals is characterized by low-grade inflammation, which likely contributes to insulin resistance, dyslipidemia, and hypertension. Obesity and peripheral inflammation are also each independently associated with cognitive disorders such as Alzheimer's and dementia, although a direct link among these factors is lacking. That is, whether peripheral inflammation in obesity contributes directly to inflammation in the brain, and thus cognitive disruption, remains unclear. Moreover, whether maternal obesity has any influence on inflammation of offspring, particularly in brain regions important for cognition (e.g., hippocampus), is completely unknown.
Methods: Female breeders were fed one of 3 diets: 1) 60% saturated high fat diet (SFD), 2) 60% high trans fat diet (TFD), or 3) 10% saturated low fat diet (LFD) for 4 weeks prior to mating, and remained on the diet throughout pregnancy and lactation. A subset of pup brains and peripheral tissues were collected 1 day (P1) or 20 days (P20) after birth. A second group of pups were analyzed for anxiety and spatial cognition in adulthood, and brains were also collected at P60, after being placed on standard lab chow at weaning. For tissue collection at P20 & P60, rats were injected with saline or bacterial lipopolysaccharide (LPS) and brains were collected 4 h later, in order to assess the response to a specific inflammatory challenge.
Results: SFD/TFD exposure significantly increased body weight and leptin in both moms and pups compared to controls. Several inflammatory markers were increased at P1 in both males and females, including CD11b & toll-like-receptor (TLR) 4 (markers of microglial activation) in the hippocampus, and C-reactive protein in the liver. At weaning and in adulthood, pro-inflammatory cytokine expression was strikingly increased in the periphery and hippocampus following a bacterial challenge (lipopolysaccharide; LPS) in the SFD/TFD groups compared to controls, but the influence of each diet differed by sex. Microglial activation within the hippocampus was also increased basally in SFD rats, suggesting a chronic priming of the cells. Finally, there were marked changes in anxiety and spatial learning in SFD/TFD groups.
Discussion: These effects were all observed in adulthood even after the pups were placed on standard chow at weaning, suggesting these outcomes were programmed early in life. Taken together, we have demonstrated marked, enduring effects of maternal saturated and trans fat diets on brain inflammation and cognitive outcomes in the offspring.
Disclosure: S. Bilbo: None.

Non-Genomic Transmission of Maternal and Paternal Effects
Frances A. Champagne*

Columbia University, New York, NY

Background: Individual differences in anxiety-like and social behavior have been observed in laboratory rodents and we have previously demonstrated the role of variations in early-life mother-infant interactions in shaping the molecular and neurobiological substrates of these behaviors. More recently, we have explored the interaction between maternal and paternal influences on development and the mechanisms through which a transgenerational impact of social experiences can occur.
Methods: In the current series of studies, we used both a genetic and environmental approach to the study of the inheritance of parental effects. In mice lacking a functional copy of the paternally expressed imprinted genes *Peg1* or *Peg3*, we assessed response to

novelty, maternal behavior, and the development of non-mutant (WT) offspring and grand-offspring of knockout (KO) females. To study the parental transmission of environmental effects, we exposed male Balb/c mice to postnatal and juvenile environments characterized by social/physical isolation (ISO), enrichment (ENR), or standard rearing conditions (STD). In adulthood, males were mated with STD reared females, separated from the female 1 week prior to parturition, and mother-infant interactions with offspring were observed.
Results: Transgenic mice lacking a functional copy of *Peg3* or *Peg1*exhibit heightened inhibition of novelty and impairments in social/reproductive behavior. In female knockout mice, there are also gross impairments in prenatal and postnatal maternal care. KO females display significant reductions in gestational food intake ($p < .05$), increased latencies to retrieve pups on postnatal Day 0 ($p < .01$), and exhibit reduced nursing and licking/grooming (LG) of pups during the first week postpartum ($p < .05$). Non-mutant offspring of these females were found to display behavioral inhibition and decreased motivation to retrieve pups and grand-offspring were observed to have elevated latencies to explore a novel environment. Amongst Balb/c males, ENR rearing lead to reduced anxiety-like behavior (increased exploration of a novel environment; reduced fecal boli) compared to ISO males. Females mated with ISO males exhibited reduced nursing and LG of pups ($p < .05$) during the early postnatal period compared to females mated with ENR males. This paternally-induced change in maternal behavior was not associated with the level of anxiety-like behavior displayed by the male.
Discussion: These studies illustrate a non-genomic transmission of paternal effects. In the transgenerational studies of *Peg1* and *Peg3*, data indicate that despite the lack of transmission of the genetic perturbation (*Peg1* and *Peg3* mutations are only expressed when inherited from knockout males) there is an inheritance of the phenotypic characteristics associated with the knockout. We have also observed the influence of paternal social experiences in mice on maternal behavior with implications for the development of subsequent generations of offspring. Taken together, these studies illustrate the interplay that can occur between maternal and paternal influences and the possibility of maternally mediated paternal genetic and environmental effects. We are currently exploring the neuroendocrine and molecular mechanisms through which these within and across generation influences on behavior are achieved.
Disclosure: F.A. Champagne: None.

Age at Migration and Risk of Psychoses in Immigrant/Ethnic Minority Groups

Ezra Susser*, Wim Veling, Jean-Paul Selten, Hans Wijbrand Hoek

Columbia University Medical Center, New York, NY

Background: Many previous studies have demonstrated increased risk of psychotic disorders in specific immigrant/ethnic minority groups in the UK and Holland. Studies from The Hague have shown an increased risk in both first and second generation immigrant/minority groups compared with Dutch. They have also shown that the increased risk in immigrant/minority groups is associated with a low density of own ethnic group in the neighborhood of residence, which suggests that social context may play a role. Thus far, however, neither UK nor Dutch studies have ruled out selective migration as an explanation. Nor have they determined the relevant timing of exposure.
Methods: Data will be presented from a large ongoing study of incident psychoses in The Hague. This study identifies the individuals aged 15-54 making contact with a physician for a suspected psychotic disorder in The Hague; administers diagnostic interviews to these individuals; and assigns DSM IV diagnoses by consensus of two psychiatrists. A comprehensive municipal registration system provides the denominator. The study size is sufficient to examine incidence rates in immigrants from Morocco, Surinam, Turkey, Netherlands Antilles, other Non-Western countries, and in Dutch. For the present analysis, we obtained additional data on age at migration in order to address selective migration and timing of exposure. The data for this analysis includes 358 non-Western immigrants and 226 Dutch with first onset psychoses.
Results: Risk was most elevated (about three fold) among immigrants who arrived in Holland before age 5. This was similar to the increased risk in second generation immigrant/minority groups. These results were consistent across each of the immigrant/minority groups. They were also similar in men and women.
Discussion: From the increased risk in immigrants who arrived before age 5, we infer that selective migration cannot explain the increased risk of psychotic disorders in immigrant/ethnic minority groups in The Hague. From the series of findings in The Hague, taken together, we infer that the social experience of immigrant/ethnic minority status during early childhood years may play an important role in their increased risk of psychotic disorders. It has previously been established that early social experience can influence neurodevelopment and we discuss some of the pathways that may pertain to these results.
Disclosure: E. Susser: None.

Panel Session
Stimulant Pro-Drugs as Potential Treatments for Cocaine Dependence

Neurochemistry of Stimulant Prodrug Medications

Michael H. Baumann*

IRP, NIDA, NIH, Baltimore, MD

Background: The abuse liability of stimulant medications could be reduced by using prodrug formulations. Prodrugs are inactive entities that are converted to active medicines after systemic administration. Here we describe the neurochemistry of stimulant prodrugs, and present unique *in vivo* data with phendimetrazine (PDM) and diethylpropion (DEP).
Methods: *In vitro* assays measuring transporter-mediated uptake and release of dopamine (DA) and serotonin (5-HT) were conducted in rat brain synaptosomes. *In vivo* neurochemical effects were examined in rats undergoing microdialysis in n. accumbens. Dialysate concentrations of DA and 5-HT were measured by HPLC-ECD.
Results: PDM and DEP displayed no activity in assays measuring uptake and release. The *N*-demethylated metabolite of PDM (i.e., phenmetrazine) was a potent releaser of DA ($IC50 = 125$ nM) and a less potent releaser of 5-HT ($IC50 = 7765$ nM). The *N*-deethylated metabolite of DEP caused weak inhibition of DA uptake ($IC50 = 1000$ nM) and 5-HT release ($IC50 = 2120$ nM). PDM and DEP had no effect on extracellular transmitter levels when administered locally into the n. accumbens, whereas the *N*-dealkylated metabolites of both drugs afforded dose-related elevations in DA and 5-HT.
Conclusions: PDM and DEP appear to be prodrugs which are converted to active medicines after systemic administration. Prodrugs may be desirable agonist medications for cocaine dependence, since slower rates of active drug entering the brain would be predicted to reduce addictive properties of the prescribed formulations.
Disclosure: M.H. Baumann, None.

Evaluation of Cocaine Analogs as Cocaine Medications in Nonhuman Primates

Leonard Howell*

Yerkes National Primate Research Center, Emory University, Atlanta, GA

Background: Given the important role of the dopamine transporter (DAT) in the addictive properties of cocaine, the development and use

of compounds that target the DAT represents a reasonable approach for the pharmacological treatment of cocaine abuse.
Methods: The present report describes a series of studies conducted in nonhuman primates that evaluated the effectiveness of phenyltropane DAT inhibitors in reducing cocaine self-administration. In addition, drug substitution studies evaluated the abuse liability of the DAT inhibitors. PET neuroimaging studies quantified DAT occupancy at behaviorally relevant doses, characterized the time-course of drug uptake in brain, and documented drug-induced changes in cerebral blood flow as a model of brain activation. Lastly, *in vivo* microdialysis quantified drug-induced changes in neurochemistry associated with drug effects on behavior.
Results: Selective DAT inhibitors were effective in reducing cocaine use but high (>70%) levels of DAT occupancy were associated with significant reductions in cocaine self-administration. The selective DAT inhibitors were reliably self-administered but rates of responding were lower than those maintained by cocaine even at higher levels of DAT occupancy. A profile of slow rate of drug uptake in brain accompanied by a gradual increase in extracellular dopamine may account for the more limited reinforcing effectiveness of the DAT inhibitors. Selective serotonin transporter (SERT) inhibitors were also effective in reducing cocaine use and blocked cocaine-induced brain activation and increases in extracellular dopamine. Co-administration of SERT inhibitors with a selective DAT inhibitor was more effective than the DAT inhibitor administered alone, even at comparable levels of DAT occupancy.
Discussion: The results indicate that selective DAT inhibitors seem promising as cocaine medications based on preclinical evaluations. However, their reinforcing effects in animal models indicate that they may exhibit high abuse liability that could limit their clinical utility. In addition, the effects of high levels of DAT occupancy that are required to reduce cocaine use are unknown. Alternatively, development of medications involving dual actions at DAT and SERT could lead to compounds with cocaine-like properties appropriate in substitute-agonist pharmacotherapy but with limited abuse liability. Hence, combined inhibition of DAT and SERT may be a viable approach to treat cocaine addiction.
Disclosure: Howell, None.

Effects of Monoamine Releasers on Cocaine Self-Administration by Rhesus Monkeys
S Stevens Negus*

Virginia Commonwealth University, Richmond, VA

Background: Amphetamine and other monoamine releasers (MARs) serve as one class of target active metabolites for prodrugs that may serve as candidate agonist-medications for the treatment of stimulant dependence. The pharmacologic selectivity of MARs for promoting release of dopamine (DA), serotonin (5HT) and norepinephrine (NE) may influence the behavioral selectivity of these compounds to safely reduce stimulant self-administration. This talk will review data from a series of experiments that have examined effects of chronic treatment with a range of MARs on responding maintained by a stimulant (cocaine) and a non-drug reinforcer (food) in rhesus monkeys.
Methods: Rhesus monkeys implanted with chronic intravenous double-lumen catheters had access to cocaine (0-0.1 mg/kg/inj) or food (1 g banana-flavored pellets) under two schedules of reinforcement. Under one schedule, cocaine injections or food pellets were available under second-order schedule during alternating daily components, and the primary dependent variables were the numbers of injections and pellets delivered per day (maximum of 80 injections and 100 food pellets). Under the other schedule, cocaine injections and food pellets were available simultaneously under a concurrent-choice schedule, and the primary dependent variables were the % cocaine choices and overall number of choices. Monoamine releasers were delivered by continuous infusion through one lumen of the double-lumen catheter for up to 28 consecutive days. Monoamine releasers varied in their selectivity for releasing dopamine vs. serotonin.
Results: Under the second-order schedule, the cocaine dose-effect curve displayed an inverted-U shape with peak self-administration rates at doses of 0.01-0.032 mg/kg/inj. Under the concurrent-choice schedule, cocaine maintained a dose-dependent increase in cocaine choice, with peak levels of cocaine choice at doses of 0.032-0.1 mg/kg/inj. Amphetamine produced a dose-dependent, selective and sustained decrease in cocaine self-administration under the second-order schedule for periods up to 28-days. Tolerance developed within one week to any effects of amphetamine on food-maintained responding. Amphetamine also produced a dose-dependent decrease in cocaine choice and an increase in food choice under the concurrent-choice schedule. Similarly selective effects were produced by other monoamine releasers with DA vs. 5HT selectivity of 30- to 40-fold (e.g. phenmetrazine).
Discussion: These findings suggest at least three general conclusions. First, in agreement with clinical and other preclinical findings with amphetamine, these results indicate that chronic amphetamine treatment can produce sustained, selective and robust decreases in cocaine self-administration in nonhuman primates. Second, compounds such as phenmetrazine, which have 30- to 40-fold selectivity for releasing DA vs. 5HT may be especially effective in producing selective reductions in cocaine self-administration. Finally, these results support the hypothesis that compounds such as amphetamine (metabolite of lisdexamphetamine) and phenmetrazine (metabolite of phendimetrazine) may be useful target metabolites for prodrug agonist medications. Studies are underway to further examine conditions under which monoamine releasers may be optimally effective in combination with environmental manipulations.
Disclosure: S.S. Negus, Alkermes (Consultant), Part 1; Grunenthal (Consultant), Part 1; Limerick Biopharma, Part 1.

Agonist-Like Medications for Cocaine Dependence Treatment: Clinical Studies
David V. Herin*

Dept. of Psychiatry, University of Minnesota, Minneapolis, MN

Background: Cocaine dependence remains a significant problem in the United States, with no universally-effective medications. Several approaches have been used to pursue pharmacotherapeutic agents to treat this disorder. One approach utilizes antagonist medications to block monoamine receptors and thus reduce the desired effects of cocaine. Preclinical and human laboratory studies have identified some potential antagonist compounds for cocaine dependence treatment, however clinical trials have generally found few changes or even increases in drug use. In contrast, substantial evidence supports the agonist-like "replacement" medication strategy. This approach posits that medications with properties similar to the abused drug, but possessing lesser abuse liability, will normalize neurochemistry and stabilize behavior, thus reducing drug use. Several agonist-like agents have been investigated including L-dopa/carbidopa, bupropion, modafinil, methylphenidate, and amphetamine analogues, with safety and some efficacy demonstrated in preclinical and clinical studies. Of these medications, there is considerable promise for amphetamine analogues including sustained release (SR) d-amphetamine and methamphetamine. Additionally, recent advances in drug delivery technology have resulted in amphetamine prodrug preparations with even greater abuse-resistant properties.
Methods: First, the presentation will detail our published and recent data demonstrating reductions in cocaine use with d-amphetamine SR (0, 30, 60 mg/day) plus cognitive behavioral therapy (CBT). Additionally, rationale for use of the prodrug Lisdexamfetamine (LDX) will be discussed, plus data from our current double-blind, placebo-controlled clinical study investigating changes in cocaine use with LDX (0, 70 mg/day) plus CBT. Further, a case study from a new

pilot trial utilizing greater doses of LDX (70-140 mg/day) will be presented.
Results and Discussion: Collectively, these clinical data support the safety and efficacy of agonist-like medications such as d-amphetamine SR for treatment of cocaine dependence and suggest potential for the novel prodrug medication LDX.
Disclosure: D.V. Herin, None.

Panel Session
The Influence of Higher-Order Reward, Interoceptive, and Inhibitory Neural Processes on Disturbances of Appetite and Weight

Convergence of Limbic and Interoceptive Information in the Anterior Insula: Anatomy of How the 'Gut' Can Inform Emotional States and Eating Behaviors
Julie L. Fudge*

University of Rochester Medical Center, Rochester, NY

Background: The insula, or 'fifth' lobe of the primate brain, begins on the caudal orbital surface and extends caudally along the lateral surface of the brain. The insula is activated by intense emotional states, including experiences linked to emotional or physical pain, and pleasure. Gut sensations contribute to experiences ranging from fullness/satiety to their emotional analogue, 'contentment'. Other visceral experiences include nausea, and its emotionally-laden analogue 'butterflies', or anxiety. Because the insula appears to integrate visceral experience with emotional experience, we examined the anatomy of this system in Old world monkeys. Specifically, we asked how inputs from subcortical gustatory centers interfaces with inputs from the amygdala in the anterior insula. We then examined output from the insula to the striatum, to understand how this information might modulate goal-directed behaviors such as food consumption.
Methods: Injections of bidirectional tracers were placed in the insula and amygdala of Old World primates. We examined the distribution of labeled cells in the gustatory thalamus, and amygdala following insula injections, and projections from the amygdala to the insula. We also charted the distribution of afferently labeled fibers in the striatum resulting from insula tracer injections.
Results: The anterior insula receives input from the amygdala and the 'gustatory thalamus' based on both anterograde and retrograde studies. Both the agranular and dysgranular insula receive inputs from the gustatory thalamus and the amygdala, and project to the 'limbic striatum', mainly in the ventromedial putamen.
Conclusions: The interface between visceral information (from gustatory thalamus) and emotionally tagged external cues (from amygdala) occurs in the anterior insula. Emotional experiences that are colored by 'gut sensations' in the insula are transmitted to the ventral striatum, specifically in the region of the ventromedial putamen. This pathway suggests a way that emotional associations can converge with taste and visceral sensations to influence ingestive behavior through striatal circuits.
Disclosure: J.L. Fudge, None.

The Influence of Adiposity, Genotype and Phenotype on Caudate Response to Food in Humans
Dana M. Small*

The John B Pierce Laboratory and Yale University, New Haven, CT

Background: In healthy weight humans the caudate nucleus responds during the consumption of palatable foods, and eating a favorite meal results in decreased [C^{11}]raclopride binding potential, thus implicating dopaminergic mechanisms in the human dorsal striatum in food reward. In overweight individuals caudate response to milkshake is reduced and there is an inverse relationship between response and future weight gain. Moreover, both of these relationships depend upon genotype, with stronger associations observed for people who carry one or two copies of the taq IA A1 allele, which is associated with compromised striatal dopamine signaling. What is unknown is 1) whether this response is associated with anhedonia, impulsivity, or eating style; 2) whether it is a better predictor of weight gain compared to traditional (and less expensive) measures; and 3) whether it precedes or follows weight gain. The aim of the current study was to address these questions.
Methods: Perceptual ratings and fMRI BOLD response to the taste and smell of milkshake flavors and aromas was measured in 44 healthy and overweight individuals (BMI range 19.2 to 42.3). Participants also completed several questionnaires assessing eating style, and impulsivity, and completed a measure of food reinforcement. Neuroimaging data were pre- and post-processed with SPM5 and 26 individuals returned one year later to have their body mass index (BMI) re-assessed.
Results: Replicating prior work we found an inverse relationship between BMI and response in the caudate to milkshake (18, 15, 9; $z = 3.7$; $p = 0.02$). This response correlated with impulsivity ($r = -.59$; $p = 0.008$), but not with the milkshake pleasantness or intensity rating, food reinforcement, nor with any measure of eating style. Response here also predicted future weight gain and the strength of this relationship was stronger in A1 carriers ($r = -.87$ in carriers and -.45 in non carriers). Moreover, a stepwise regression analyses showed that caudate response was the best predictor ($R^2 = .24$; $p = 0.04$) of future weight gain (better than initial BMI, eating style, perceptual ratings, food reinforcement, and impulsivity). No differential caudate responses were observed as a function of genotype or phenotype, when groups were matched for BMI.
Discussion: These findings replicate prior work in showing a strong relationship between caudate response to milkshake and adiposity, especially in those who carry the A1 allele. The data also extend prior work. First they show that caudate response is a better predictor of future weight gain than many traditional measures. Second, they show that response in the caudate is related to trait impulsivity, but not to food reward or to eating style. Thus, the results do not support the conclusion that reduced caudate responses are associated with anhedonia. Finally, we show that the influence of the gene on caudate response is dependent on BMI, since no differential caudate effect is observed in carriers vs. noncarriers who are matched for BMI. This suggests that the reduced caudate response results from an interaction of genotype and adiposity, which is in turn consistent with the possibility that the response reflects a neural adaptation to increased adiposity.
Disclosure: D.M. Small, None.

Functional Neuroimaging of Food Motivation and Monetary Reward Processing in Obese and Healthy-Weight Groups
Cary R. Savage*

University of Kansas Medical Center, Kansas City, KS

Background: Obesity arises from chronic imbalances between energy intake and expenditure. Health-related decisions impacting energy balance are influenced by a convergence of processes in the brain, as individuals weigh the perceived balance between the rewarding and punishing aspects of behavioral choices, and whether gratification is immediate or delayed. Functional magnetic resonance imaging (fMRI) studies are identifying brain mechanisms contributing to energy intake and expenditure in obese and healthy weight groups. Previous neuroimaging work in obesity has focused on food reward, but there is no reason to believe that reward centers of the brain distinguish between reward types. This presentation will summarize findings from a previous fMRI study of food motivation and present new data examining brain processes underlying non-food (monetary) reward processing.

Methods: The food motivation study used fMRI to examine brain activation in obese (n = 10) and healthy weight (n = 10) adults while they viewed food and nonfood images in pre-meal and post-meal states. Participants were scanned in two food motivation states (order counterbalanced): 1) Pre-Meal, after fasting at least 4 hours; and 2) Post-meal, immediately after eating a 500 Kcal meal. During scanning, visual images of food and nonfood stimuli were presented in counterbalanced blocks interspersed with blurred baseline images. The monetary reward study is ongoing and consists of presentation of cues that predict the delivery of monetary reward or punishment with 75% probability. Following a short delay, obese (n = 10 to date) and healthy weight (n = 12 to date) participants receive feedback on how much they won or lost on each trial. This event-related design allows separation of brain responses during anticipation and delivery of monetary rewards and punishments. Groups in both studies were matched for age, handedness (all R), and sex and all fMRI data were analyzed using GLM analysis (random effects) through BrainVoyager.
Results: In the food motivation study, obese participants showed increased activation, compared to HW participants, in anterior cingulate cortex (ACC) in the pre-meal condition, and medial prefrontal cortex (MPFC) in both conditions. Self-report measures of hunger were positively correlated with Pre-Meal MPFC activations. In the monetary reward study, both groups showed larger activations to anticipation of reward (vs. punishment) in the striatum bilaterally (x,y,z = 15,5,-2 and -12,5-2) and MPFC (-6,25,-2). Further, a region in ACC (0,11,25) showed greater response to anticipation of monetary reward in healthy weight controls and monetary punishment in obese participants.
Discussion: Functional neuroimaging studies of food motivation show hyperactivation among obese in cortical regions rich in dopaminergic projections from ventral tegmental reward circuits, especially the medial prefrontal cortex and anterior cingulate cortex. These findings add to growing evidence of increased sensitivity to food reward in obese groups. Preliminary findings using nonfood, monetary, rewards also show differences between obese and healthy weight groups, with evidence that ACC plays a differential role processing monetary rewards and punishments in the two groups. Results from these studies will be discussed in the context of previous fMRI studies showing modified brain responses during reward processing in obese groups.
Disclosure: C.R. Savage, Merck & Co, Part 1; Merck & Co, Part 2.

Insula Response to Sweet Taste and Anticipation of Food in Anorexia and Bulimia Nervosa: Is Altered Sensory-Interoceptive Function a Biomarker That Reflects the Urge to Eat?

Walter Kaye*, Tyson A. Oberndorfer, Guido Frank, Julie L. Fudge, Alan Simmons, Angela Wagner, Martin Paulus, Amanda Grethe

University of California, San Diego, La Jolla, CA

Background: How are individuals with anorexia nervosa (AN) able to consume a few hundred calories per day and maintain an extremely low weight for many years, when most people struggle to lose a few pounds? And why do individuals with bulimia nervosa (BN) binge on thousands of calories per day? Several lines of evidence suggest that pathological eating behaviors in AN and BN may be related to aberrant 'top-down' neural processes that modulate the sensory, interoceptive, rewarding, and cognitive aspects of food ingestion. This circuit includes the anterior insula, as well as the amygdala, the ventral anterior cingulate cortex, the orbital frontal cortex (OFC), and ventral-central striatum.
Methods: In order to avoid the confounding effects of malnutrition, female subjects that were recovered from AN (RAN) and BN (RBN) were compared to healthy control women (CW). Functional magnetic resonance imaging (fMRI) was used to examine (1) response to tastes of sucrose (1 cc 10% solution) vs an artificial sweetener and (2) response to pictures of palatable foods vs color-matched neutral objects.
Results: Study 1: A significant group by condition difference was found in the superior sulcus of the right AI ($F(2,51) = 10.72$, $p < 0.001$) masked by *a priori* regions of interest. A *post hoc* analysis showed sucrose elicited significantly decreased right AI activation in 14 RAN ($p = 0.01$) and increased activation in 14 RBN ($p = 0.02$) compared to 14 CW. Study 2: A whole brain group by condition ANOVA revealed one region of significant interaction in Brodmann's Area 13 of the right anterior insula ($p < 0.001$) (Volume: 1344 μL, XYZ = 38,11,-8). This interaction was driven by greater insula activation in 14 RAN versus 12 CW while anticipating images of food ($p < 0.001$), and by deactivation of the insula in RAN while anticipating images of neutral objects ($p = 0.015$).
Discussion: Study 1 suggests that individuals who undereat or overeat have an altered set-point, and/or altered sensitivity, when consuming sucrose that is neutrally represented in the AI as well as other related regions that modulate sensory-interoceptive-incentive signals. These findings replicate previous findings in RAN (Wagner 2008). Moreover other studies show obese subjects or obese binge eating subjects (Rothemund, 2007. Schienle, 2008, Stice, 2008, Stoeckel, 2008) tend to have increased insula activation in response to pictures or tastes of food. The AI plays a critical role in interoceptive awareness of internal body states and, in concert with the overlying operculum and OFC represents the sensory experience of food in the brain. Thus, changes in food hunger, or other body states creates signals in the AI that drive behaviors that subsequently resolve the altered signal. The set-point for under-eaters may be biased toward a basal level of satiety (diminished AI signals), whereas the set-point for overeaters may be biased toward a basal level of hunger (increased AI signals). In study 2, increased right AI activation during anticipation may suggest amplified emotional reactivity to the upcoming 'aversive' interoceptive stimulus, similar to findings in pathological anxiety (Simmons, 2008, Simmons, 2006). These results support a model of "interoceptive disconnection" between signals related to anticipating and consuming palatable foods in AN. In theory, such a disconnect may reflect distorted reward based learning. Whether such disconnects occur in BN remain to be determined.
Disclosure: W.H. Kaye, Lundbeck, Part 1; Merck, Part 1; Astra-Zeneca, Part 4; NIMH, Part 4; Price Foundation, Part 4.

Panel Session
Developmental Trajectories and Mechanisms Underlying the Phenotypic Risk for Anxiety and Depression: Cross Species Perspectives from Infancy to Adolescence to Adulthood

Developmental Differences in the Extinction of Learned Fear: Preclinical Studies

Rick Richardson*

University of New South Wales, Sydney NSW 2052, Australia

Background: Fear typically protects us from danger. However, an anxiety disorder emerges when fear becomes pervasive and interferes with normal functioning. The most utilized treatment for anxiety disorders is exposure-based therapy, which relies on the process of extinction. Although preclinical research on the extinction of learned fear is a widely used model preparation for exposure-based therapy a conspicuous omission in this area of research has been the systematic study of extinction during early development.
Methods: In all experiments, rats receive Pavlovian fear conditioning where a neutral conditioned stimulus (CS) is paired with an aversive shock unconditioned stimulus. Extinction involves repeatedly presenting the CS by itself, and leads to a loss of fear (i.e., as measured by freezing). We have studied developmental differences in extinction of learned fear behaviorally, pharmacologically, and neurally, but in this talk I will focus on the pharmacological experiments. Rats were injected with MK-801 (Dizocilpine), a non-competitive antagonist of the NMDA receptor, prior to extinction to assess the role of NMDA in learning extinction or FG7142, a partial inverse agonist of the

benzodiazepine receptor, prior to testing to assess the role of GABAergic inhibition in expression of extinction.
Results: If extinction of learned fear occurs after the age of weaning (e.g., postnatal day 23), then both NMDA and GABA are critically involved, just like in adults. In contrast, if extinction occurs earlier in life (i.e., prior to postnatal day 18) then neither NMDA nor GABA are involved. This pharmacological evidence supports the conclusion that there is a fundamentally different system mediating extinction early in life. Additional studies show that the transition between these two extinction systems occurs at younger ages in rats exposed to early life trauma (maternal separation). That is, NMDA and GABA are involved in extinction of learned fear early in life if the rats had experienced early life trauma. Finally, the adult extinction system is unstable during adolescence (i.e., postnatal day 35), leading to marked impairments in extinction retention. Importantly, these deficits in extinction retention in adolescent rats can be alleviated by the partial NMDA receptor agonist D-Cycloserine (DCS).
Discussion: Studies on extinction in immature animals have significant implications for elucidating the developmental neurobiology of anxiety disorders and may have significant implications for the development of effective treatments for anxiety disorders. Our results show that a fundamentally different system mediates extinction of learned fear early in life compared to later in life. The early extinction system is relapse resistant and does not involve NMDA or GABA receptors. In contrast, the later maturing extinction system critically relies on both NMDA and GABA receptors and is relapse prone. Further, the later maturing system is markedly impaired during adolescence, with rats this age exhibiting profound deficits in retaining extinction. This deficit, however, can be attenuated by injecting DCS at the time of extinction. Finally, preliminary data on maternally-separated rats suggest that early life trauma causes a premature transition to the "adult type" extinction system which may render individuals more vulnerable to relapse and chronic anxiety.
Disclosure: R. Richardson, None.

Differential Heritability of Hippocampal and Amygdala Function in Relation to the Development of Anxious Temperament

Ned H. Kalin*

University of Wisconsin, Department of Psychiatry, Madison, WI

Background: Studies in rhesus monkeys demonstrate a valid and reliable model to study the factors mediating the development of anxious temperament and the risk to develop anxiety and depression.
Methods: Phenotypic (anxious temperament) and functional brain data (FDG-PET) were collected from a large cohort of preadolescent monkeys ($n > 200$) that belonged to a single multigenerational pedigree. A voxel wise analysis was performed examining the relation between individual differences in anxious temperament and glucose metabolic activity. Heritability analyses were performed on behavioral and functional brain data. Animals were also genotyped for the serotonin transporter polymorphism (short and long alleles).
Results: Results demonstrated that metabolic activity in the CeA amygdala region and anterior hippocampus was predictive of individual differences in anxious temperament. Heritability analyses revealed that anxious temperament is significantly heritable as is threat-induced function in the anterior hippocampal region that is predictive of anxious temperament. Interestingly, the CeA region predictive of anxious temperament was not significantly heritable. No significant effects of the serotonin transporter polymorphism on the phenotype or brain activity that was predictive of anxious temperament were detected.
Discussion: These results characterize the neural circuit associated with primate anxious temperament. The data further show differential heritability of metabolic activity in this circuit suggesting that anterior hippocampus is more affected by heritable influences whereas the CeA appears to be more affected by environment. These data provide a frame work, at the neural circuit level, to test hypotheses regarding mechanisms underlying the interactive influences of genes and environment on the development of anxious temperament.
Disclosure: N.H. Kalin, Astra Zeneca; Bristol-Myers Squibb; Cenerx BioPharma; Corcept Therapeutics; Eli Lilly & Co.; Novartis; Otsuka American Pharmaceuticals; Sanofi Aventis; Wyeth Pharmaceutical, Part 1; Elsevier, Letters & Sciences, Part 2; Cenerex, Corcept, Promoter Neurosciences, Part 3.

Impact of a Human Infant Phenotype on Brain Development and Function in Late Adolescence and Young Adulthood: The Circuitry of Anxiety and Mood

Carl Schwartz*

Massachusetts General Hospital & Harvard Medical School, Boston, MA

Background: One of the central thrusts of research in developmental psychopathology over the past 50 years has been the quest to identify increasingly early developmental markers of elevated risk for anxiety and depression, in the hope of improving primary and secondary prevention. Longitudinal studies provide a unique perspective on the development of circuitry mediating the regulation and dysregulation of anxiety and mood.
Methods: A single laboratory-based behavioral assessment of human infants at four months of age is described that identifies two contrasting infant phenotypes. 135 subjects from this infant cohort were studied at 18 years of age with high resolution structural MRI, three fMRI protocols, and diffusion tensor imaging.
Results: We found alterations in brain structure and function, including patterns of connectivity, associated with these risk phenotypes. Both published and to-be published data will be presented that identifies variations in cortical architecture (including cingulate and prefrontal cortex) and volumetric differences in subcortical structures in circuits subsuming regulation of mood and anxiety. Variations in the patterns of cortical activation and connectivity revealed by the fMRI paradigms (affective facial perception, novelty facial perception and discrepancy) that are related to both infant phenotypes and gender will be detailed.
Discussion: The footprint of these infant phenotypes in the brains of late adolescents and young adults brain provides insight into the origin of individual differences in both baseline trait anxiety and the high incidence of anxiety and mood disorder in young adults. Unexpectedly, several of these measures showed marked sex differences; analyses to date suggest that these differences are not hormonally mediated. Developmental mechanisms that might underlie these temperament and gender differences will be discussed. These phenotypes may represent one substrate for the individual differences that have been observed in fear learning, extinction learning, and extinction retention. Experimental approaches to further exploring and understanding these phenomena are outlined.
Disclosure: C. Schwartz, None.

Phenotypic Risk for Anxiety and Depression in Adolescence: Insights from Human Imaging to Mouse Genetics

BJ Casey*

Sackler Institute, New York, NY

Background: Adolescence is often described as a period of intense and frequent negative emotions. This emotional dysregulation has been hypothesized to explain the increased rates of anxiety, depression and suicide during this time of life. Yet for some teens, experiences during this period are unremarkable and they emerge from this period with a healthy, positive outcome. Findings from human imaging and mouse genetic studies are provided as examples of genetic factors that enhance risk for psychopathology during this period of development.
Methods: We used human functional imaging to examine responses to fear-related probes in 60 typically developing children, adolescents

and adults. Second we examined parallel phenotypes in 68 mice and 72 humans resulting from a common single-nucleotide polymorphism in the brain-derived neurotrophic factor (BDNF) gene, implicated in emotional and anxiety-related behavior. We used an inbred genetic knock-in mouse strain expressing the variant BDNF that recapitulated the phenotypic effects of the human polymorphism.
Results: Our normative developmental imaging data showed inflections during adolescence in the amygdala relative to the ventromedial prefrontal cortex in response to fear related probes, not observed in pre- and post-adolescence. Our genetic data showed that both mice and humans with the BDNF variant were impaired in extinguishing a conditioned fear response, which was paralleled by atypical frontoamygdala activity in humans.
Conclusions: Together, these studies provide converging methods for understanding the highly variable positive and negative experiences of adolescence and factors that may increase the risk for anxiety and depression during this period of life. Each of these approaches (behavioral genetics, imaging genetics and mouse models of behavior) alone provides limited information on gene function in complex human behavior, but together, they are forming bridges between animal models and human psychiatric disorders. Moreover, our findings have implications for the efficacy of treatments that rely on extinction mechanisms such as exposure therapy in treatment of anxiety.
Disclosure: B. Casey, None.

Panel Session
Discovery of Small Molecules for Research on Neuropsychiatric Disorders: Target Validation and Drug Discovery

Compound Screening for Small Molecule Modulators of the Transcription Factor, ΔFosB
Eric Nestler*

Mount Sinai School of Medicine, New York, NY

Depression remains one of the leading causes of morbidity worldwide. Although there are many effective treatments for depression, more than half of all depressed patients today are incompletely treated with available approaches. Data from our animal models now provide substantial support for the view that stress induction of Delta-FosB represents a positive, adaptive mechanism that helps an animal cope with stress. First, Delta-FosB induction in several brain regions in response to chronic stress correlates with animals that are relatively resistant (or resilient) to deleterious effects of the stress. This has been documented in both the learned helplessness and chronic social defeat stress paradigms. Second, using viral-mediated gene transfer and inducible and brain region-specific bitransgenic mouse models, we have shown that overexpression of Delta-FosB in nucleus accumbens or in periaqueductal gray is sufficient to render animals resistant to subsequent stress and to reverse behavioral abnormalities induced by chronic stress. Studies of other brain regions are currently underway. Third, and conversely, overexpression of a dominant negative antagonist of Delta-FosB (termed Delta-cJun or -JunD) in these brain regions makes animals more vulnerable to the deleterious effects of chronic stress. Fourth, we have found that chronic administration of standard antidepressant medications (e.g., fluoxetine, imipramine) also induces Delta-FosB in most of these same brain regions, and that overexpression of the dominant negative antagonists of Delta-FosB block the antidepressant-like behavioral effects of these medications in several behavioral assays, including social defeat. Fifth, in collaboration with Carol Tamminga (UT Southwestern), we have demonstrated that depressed humans have lower levels of Delta-FosB in the nucleus accumbens compared to extensively matched control subjects. The availability of high affinity, small molecule ligands for Delta-FosB could potentially be used for clinical investigations in depression and perhaps as a novel tool to diagnose depression or other stress-related disorders or track a patient's progress during treatment. This presentation will report the progress made in the identification of small molecule modulators of Delta-FosB in collaboration with Dr. Gabby Rudenko (U Michigan).
Disclosure: E.J. Nestler, None.

Chemical Genomic Studies of the Role of Wnt/GSK-3 Signaling in Neuropsychiatric Disease
Stephen J. Haggarty*

Harvard Medical School/Massachusetts General Hospital, Boston, MA

Background: Wnt/GSK-3 signaling plays a fundamental role in CNS through the regulation of diverse processes ranging from neurogenesis to behaviors relevant to cognitive and neuropsychiatric disorders. Our recent studies have shown a direct interaction of schizophrenia risk gene Disrupted-in-Schizophrenia 1 (DISC1) with GSK-3 and its regulation of β-catenin-dependent neurogenesis and mood-related behavioral response in mice. Additionally, previous biochemical, cellular, and behavioral findings have implicated a role for direct and indirect inhibition of GSK-3 signaling by mood stabilizers, antidepressants, and antipsychotics. However, the molecular nature of mechanisms and the downstream consequences of GSK-3 inhibition, including the relevance of altered synaptic and transcriptional effects, remain poorly understood.
Methods: With the long-term goal of gaining insight the function of disease-associated genetic variation and developing novel small-molecule probes to target Wnt/GSK-3 signaling in the CNS, we have developed a panel of biochemical and cell-based assays that are capable of supporting high-throughput small molecule and RNAi screens.
Results: Preliminary results from the use of this panel of assays to identify and characterize novel inhibitors of GSK-3 and to identify new targets within the Wnt/GSK-3 pathway will be presented. We will also discuss advances in the use of patient-specific induced pluripotent stem (iPS) cells that now enable probing of Wnt/GSK-3 signaling in a human neurodevelopmental context using small-molecules and functional genomic approaches.
Discussion: Genetic and pharmacological findings suggest that dysregulation of Wnt/GSK-3 signaling may play an important role in the pathophysiology of neuropsychiatric disorders. The small-molecule probes we are developing will provide a means to better understand the cellular and circuit level consequences of altering Wnt/GSK-3 signaling in the CNS and may provide new avenues for therapeutic development.

Discovery of Novel Allosteric Modulators Muscarinic Receptors for Treatment of CNS Disorders
Jeffrey Conn*

Vanderbilt University Medical Center, Nashville, TN

Background: Previous animal and clinical studies with suggest that agonists of the M1 and/or M4 muscarinic acetylcholine receptor (mAChR) subtypes may provide a novel approach to treatment of schizophrenia and may provide efficacy in enhancing cognitive function. In addition, the M5 mAChR has been implicated in regulating firing of midbrain dopamine neurons and may provide a potential target for treatment of a range of CNS disorders, including schizophrenia, ADHD, and addictive disorders. Unfortunately, previous attempts to develop highly selective antagonists or agonists of individual mAChR subtypes have failed to achieve high selectivity for a single mAChR subtype. Previous ligands also have activity at M2 and M3, which leads to adverse effects that have prevented full development and marketing of mAChR agonists. Failure to develop selective traditional (orthosteric) ligands of individual mAChR subtypes is likely due to the fact that the ACh binding site is highly conserved.

We have taken a novel approach to develop highly selective antagonists and activators of M1, M4, and M5 by targeting allosteric sites on the receptor rather than developing agents that act as traditional orthosteric agonists.
Results: We have used a combination of high throughput screening and technology-enabled medicinal chemistry approaches to discover novel compounds that are highly selective as allosteric agonists or positive allosteric modulators of M1, M4, or M5 and have no activity at other mAChR subtypes. Highly selective allosteric activators of M1 include allosteric agonists, that directly activate the receptor, and positive allosteric modulators (PAMs). These latter compounds do not activate M1 when added alone but potentiate the response to acetylcholine (ACh) or other orthosteric agonists. For M4 and M5, we have focused exclusively on PAMs. Interestingly, these mAChR PAMs have no detectable affinity at the orthosteric binding site but increase the affinity of ACh. In addition, our data suggest that the M4 potentiators also increase coupling of the receptor to GTP-binding proteins. We have now optimized allosteric activators of both M1 and M4 to a point at which they have robust CNS activity and allow systemic administration for *in vivo* studies. Interestingly, both M1 and M4-selective compounds have robust electrophysiological effects in limbic and forebrain regions that provide important new insights into the respective roles of these two mAChR subtypes. In addition, both M1 and M4 PAMs have efficacy in animal models that predict both antipsychotic and cognition-enhancing efficacy. However, M1 and M4 PAMs have distinct profiles across animal models. We are now assessing the effects of M5 PAMs in regulating dopamine cell firing.
Discussion: These studies provide an exciting advance in demonstrating a novel approach to development of small molecule ligands that are highly selective for individual mAChR subtypes and suggest that selective allosteric activators of M1 and/or M4 may provide a novel approach to treatment of schizophrenia and other disorders that lead to impaired cognitive function. It will be important to fully characterize the *in vivo* effects of these compounds to help establish the relative contributions of M1 and M4 to different behavioral effects of non-selective mAChR agonists. *Supported by NIMH and NINDS, Vanderbilt is a site in the NIH-supported Molecular Libraries Probe Center Network (MLPCN).*
Disclosure: P. Conn, Puretech Ventures, Part 1; Genentech, Part 1; Abbott, Part 1; Eli Lilly, Part 1; Solvay, Part 1; Millipore, Part 1; Bristol Myers Squibb, Part 1; EPIX, Part 1; Metastatix, Part 1; Evotech, Part 1; Merck, Part 1; AMRI, Part 1; Merck Serono, Part 1; Adalor, Part 1; Johnson and Johnson, Part 1; Johnson and Johnson, Part 2; Johnson and Johnson, Part 3; Johnson and Johnson, Part 4; Seaside Therapeutics, Part 4.

New Molecular Targets for Neuropsychiatric Drug Discovery

Bryan L. Roth*

University of North Carolina School of Medicine, Chapel Hill, NC

Background: The 'druggable genome', defined as "the genes in the human genome that express proteins able to bind drug-like molecules" (Hopkins and Groom, Nature Rev Drug Discv 2002), is thought to contain 2000-3000 molecular targets. At most, 5% of the druggable genome has been exploited for the purposes of psychiatric drug discovery (Roth et al, Nature Rev Drug Discov 2004; Allan and Roth, Ann Rev Pharmacol, in press) leaving a vast universe of potential molecular targets for neuropsychiatric drug discovery. In this talk, I will highlight how our integrated chemical biology approach facilitates the discovery of new molecular targets for neuropsychiatric drug discovery.
Methods: We utilize both cheminformatics and physical screening approaches (see for instance Keiser et al, Nature 2009) to identify drug targets on a 'druggable genome'-wide fashion. Newly created technologies suitable for screening the entire druggable GPCR-ome (which represents 360+ molecular targets) in a massively parallel fashion will also be highlighted.
Results: I will first present new findings related to antipsychotic drug discovery. In this first portion of the talk I will show results from a screen of more than 2500 distinct molecular targets in an effort to uncover new molecular targets for atypical antipsychotic drug discovery. I then show how we validated a family of targets using a battery of antipsychotic drug behavioral tests in wild-type and selected knock-out mice. Next I will present new and currently unpublished findings showing how our integrated chemical biology approach has facilitated the discovery of new targets for neuropsychiatric drugs. For this second part of my talk I will show how a dedicated screen of the orphan GPCR-ome has revealed the hidden pharmacology of neuropsychiatric drugs. None of the findings that I will present have been published at the time of abstract submission.
Discussion: These results validate the unbiased chemical biology approach for the discovery and validation of new molecular targets for neuropsychiatric drug discovery.
Disclosure: B.L. Roth, Consultant in past 2 years: AMRI, Inc; Merck; Invitrogen; Medivation, Part 1; Listed as inventor or co-inventor on several US and International Patents for serotonergic and opiatergic compounds to treat neuropsychiatric disorders. Patents are owned by either Case Western Reserv, Part 1; Expert witness in patent litigation, Part 2.

Panel Session
Dissecting the Heterogeneity of Treatment Response in First Episode Schizophrenia

Meta-Analysis on the Effects of Second-Generation Antipsychotic Drugs in First Episode Schizophrenia

Stefan Leucht*

Technische Universitat Hospital, Munich, Germany

Background: The first episode of schizophrenia is a critical phase of the illness. It has been claimed that second-generation antipsychotic drugs (SGA) should be preferred for the treatment of first episode patients to allow for a soft first contact with treatment by avoiding the disturbing extrapyramidal side-effects of first-generation antipsychotic drugs (FGA), namely as dystonia, akathisia or parkinsonism. However, in a Cochrane review published in 2003 we identified only 2 randomised controlled trials that compared risperidone and olanzapine with haloperidol in first episode schizophrenia, but did not find any clear superiority (Rummel et al. 2003). As since then a number of further studies have been published, we will present an update of the earlier meta-analysis.
Methods: We produced meta-analyses of randomized controlled studies that compared SGAs with placebo, SGAs with FGAs or SGAs with SGAs head-to-head in first-episode schizophrenia. The trials were selected from update searches made for our systematic reviews that had included any participants with schizophrenia irrespective of the number of episodes (Leucht et al. 2009a,b,c). The results of the single studies will be combined in meta-analyses using relative risks for dichotomous outcomes and standardized mean differences (Hedges's g) for continuous outcomes as effect size measures, all calculations based on a random effects model.
Results: According to a preliminary analysis eight further RCTs from Western countries that compared SGAs with FGAs and five studies that compared SGAs head-to-head have been published. This much larger dataset now allows for a more thorough investigation of efficacy and tolerability differences between these compounds. We will present data on overall efficacy (PANSS/BPRS total score), trial discontinuation, positive and negative symptoms, EPS and weight gain.
Discussion: The meta-analysis will present the up-to-date randomized-trial evidence on the effects of second-generation antipsychotic drugs in first episode schizophrenia.

References: Rummel C, Hamann J, Kissling W, Leucht S. New generation antipsychotics for first episode schizophrenia. Cochrane Database Syst Rev 2003;CD004410.
Leucht S, Corves C, Arbter D, Engel RR, Li CB, Davis JM: Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis. Lancet 2009; 373(9657):31-41.
Leucht S, Arbter D, Engel RR, Kissling W, Davis JM: How effective are second-generation antipsychotic drugs? A meta-analysis of placebo-controlled trials. Molecular Psychiatry 2009; 14(4):429-447.
Leucht S, Komossa K, Rummel-Kluge C, Corves C, Hunger H, Schmid F, Lobos CA, Schwarz S, Davis JM: A Meta-Analysis of Head-to-Head Comparisons of Second-Generation Antipsychotics in the Treatment of Schizophrenia. American Journal of Psychiatry 2009; 166(2):152-163.
Disclosure: S. Leucht, SanofiAventis, Part 1; Bristol-Myers Squibb, Part 1; EliLilly, Part 1; EssexPharma, Part 1; AstraZeneca, Part 1; GlaxoSmithKline, Part 1; Janssen/Johnson and Johnson, Part 1; Lundbeck, Part 1; SanofiAventis, Part 2; EliLilly, Part 2; EliLilly, Part 3; EliLilly, Part 4.

Clinical Predictors of Response/Remission in Antipsychotic-Naive First Episode Patients

Wolfgang Fleischhacker*

Medical University Innsbruck, Dept. of Biological Psychiatry, Innsbruck, Austria

Background: Given the importance of the success of early treatment interventions in patients with first episode psychosis, early predictors of response are of considerable clinical interest. The prompt identification of responders/non-responders will help to provide optimal services to this group of patients, which will have a major impact on long term outcome.
Methods: Close to 500 patients with a first episode of schizophrenia, schizophreniform, or schizoaffective disorder were studied in the European First Episode in Schizophrenia Trial (EUFEST) (Kahn et al., 2008). About one third of the investigated patients were naive to antipsychotics. All cause discontinuation, defined as loss of retention on treatment, was the primary outcome variable of this clinical trial, in which the effectiveness of 4 new generation antipsychotics (amisulpride, quetiapine, olanzapine, ziprasidone) was compared to that of a low dose of haloperidol over a one year period. Next to other secondary outcome variables the study sample was investigated with respect to factors predicting treatment response and remission.
Results: Patients initially randomized to haloperidol met the primary outcome criterion significantly more often than those treated with any of the newer antipsychotics. Treatment adherence and higher PANSS total scores at baseline were positive predictors of response and remission, while akathisia, male gender and concurrent substance abuse proved to be negative predictors (Boter et al. 2009). In addition, a positive attitude towards treatment, as measured with the Drug Attitude Inventory, predicted retainment in the study (Gaebel et al. 2010). These results were generally upheld when restricting statistical analyses to the subsample of patients which had not been pretreated with antipsychotics.
Discussion: These analyses provide clinical guidance for the treatment of patients suffering from a first episode of psychosis.
References: Boter H, Peuskens J, LibigerJ, Fleischhacker WW, Davidson M, Galderisi S, Kahn RS, EUFEST study group: Effectiveness of antipsychotics in first-episode schizophrenia and schizophreniform disorder on response and remission: an open randomized clinical trial (EUFEST′). Schizophr Res 2009, 115, 97-103.
Gaebel W, Riesbeck M, von Wilmsdorff M, Burns T, Derks EM, Kahn RS, Rössler W, Fleischhacker WW, EUFEST Study Group: Drug attitude as predictor for effectiveness in first-episode schizophrenia: Results of an open randomized trial (EUFEST). Eur Neuropsychopharmacol 2010, 20, 310-316.
Kahn RS, Fleischhacker WW, Boter H et al.: Effectiveness of antipsychotic drugs in first-episode schizophrenia and schizophreniform disorder: an open randomised clinical trial. Lancet 2008, 371, 1085-97.
Disclosure: W. Fleischhacker, Janssen-Cilag, Pfizer, BMS/Otsuka, Astra Zeneca, Elí Lilly, Lundbeck, UBC, MedAvante, Part 1; BMS/Otsuka, Alkermes, Pfizer, Janssen, Eli Lilly, Part 4.

Predictors of Outcome in Schizophrenia: Neuroanatomical and Symptomatic Markers

Rene Sylvain Kahn*

Rudolf Magnus Institute of Neuroscience, Utrecht, Netherlands

Background: Schizophrenia is a heterogeneous disease and so is its outcome. It is clear that even early in the disease a large number of subjects does not respond satisfactorily and a majority goes on to experience frequent relapses anyhow. Despite several decades of research no clear markers have been found that predict outcome.
Methods: In a large dataset of over 4,000 subjects, including patients with schizophrenia, their siblings and healthy subjects, all assessed with the same diagnostic instrument (Comprehensive Assessment of Symptoms and History, CASH) a combination of latent class analysis and factor analysis was used to delineate subgroups.
Results: Consistent with the literature several subgroups were found of which one group, closely resembling the original depiction of schizophrenia by Kraepelin and characterized by psychotic, disorganized and negative symptoms were delineated. This "Kraepelinian" group was also characterized by significantly lower IQ than the other 4 subgroups. Most interestingly, it was this group of patients that showed the largest decline in brain volume over time.
Conclusion: This study was able to delineate a subgroup of schizophrenia patients characterized by the core-symptoms of schizophrenia as described by Kraepelin i.e. cognitive decline, disorganization, positive and negative symptoms. These patients showed, as has been predicted by previous studies, the poorest outcome. Moreover, it was this subgroup of patients that showed the largest brain volumes over a five year follow-up period. This study therefore suggests that the subgroup of schizophrenia characterized by lower IQ, disorganized symptoms and brain loss over time can be delineated. It may be this subgroup that forms the core of the schizophrenic illness and may show specific genetic characteristics.
Disclosure: R.S. Kahn, Lilly, Johnson&Johnson, Astra-Zeneca, Otsuka, Dainippur, GSK, Part 2.

Pharmacogenomics of Treatment Response in First Episode Schizophrenia

Anil Malhotra*

The Zucker Hillside Hospital, Glen Oaks, NY

Background: Pharmacogenomic studies of first episode schizophrenia may provide a novel means to identify biological predictors of antipsychotic drug response. In particular, the lack of substantial prior treatment histories may enhance genetic studies of side effects associated with treatment, in which prior treatment may confound precise phenotype-genotype relationships. Moreover, first episode cohorts more closely approximate the full range of potential response characteristics, as compared to chronic patient cohorts in may be biased towards less responsive and side-effect prone subjects.
Methods: We have conducted a pharmacogenetic study of over 100 first episode schizophrenia subjects enrolled in a comprehensive clinical trials of the second generation antipsychotics, olanzapine and risperidone, as well as a meta-analysis of over 700 patients treated with antipsychotics. Moreover, we have conducted a genome-wide association (GWAS) study of a large cohort of antipsychotic-naive children and adolescent patients undergoing their initial antipsychotic drug treatment.
Results: Candidate gene analysis of the first episode cohort revealed that the dopamine DRD2 receptor -141C Ins/Del polymorphism

predicted antipsychotic response to olanzapine and risperidone, a result extended by meta-analytic results suggesting an overall effect that was more pronounced in first episode subjects. Moreover, the same polymorphism was associated with weight gain in the first episode cohort, such that patients with the Del allele were less likely to clinically respond and more likely to gain weight than Ins/Ins subjects, regardless of antipsychotic drug and/or dosage. Finally, preliminary GWAS results in the antipsychotic-naive cohort are consistent with these results, as well as indicate a new genome-wide significant result in a gene linked to obesity in the general population.
Discussion: Candidate gene, GWAS, and meta-analytic strategies each provide evidence for association of the DRD2 gene with antipsychotic drug response and antipsychotic drug-induced weight gain. Moreover, GWAS results reveal a potential new locus associated with substantial weight gain in patients undergoing their first antipsychotic drug treatment.
Disclosure: A. Malhotra, Merck, Part 1; PGx Health, Part 2; Eli Lilly, Part 3.

Panel Session
Habenula Session 2: Role of the Habenula in Addiction and Depression: Worse than Expected

The Anatomical Connections of the Habenular Nuclei, with a Historical Perspective

Miles Herkenham*

National Institute of Mental Health, Bethesda, MD

Background: The medial and lateral habenular nuclei are two paired, phylogenetically conserved structures lying at the top of the thalamus. They and the pineal gland are embryologically derived from the epithalamus. In fact, in cold-blooded vertebrates, the pineal and the parietal "third" eye are neurally connected to the medial habenula in a striking asymmetric fashion and deliver information about solar radiation to endocrine and behavioral centers controlling basking behavior. In mammals, major afferent habenular inputs are from the septo-preoptico-hypothalamic continuum, and efferent outputs are largely to the "limbic midbrain area," a term coined by Nauta in 1958. The "dorsal diencephalic conduction route," with its prominent afferent stria medullaris and efferent fasciculus retroflexus, got its name from the fact that largely ventrally disposed sources of inputs relay descending limbic influences to the midbrain limbic area after taking a detour to the dorsal side of the thalamus.
Methods: Historically, classical silver and Golgi stains have provided details of highly conserved and distinctive unusual architecture. Tract-tracing methods showed that the medial and lateral nuclei have precise inputs and outputs. Histochemical and molecular markers have shown striking neurochemical specificity and subnuclear compartmentalization.
Results: The habenular nuclei provide segregated and precise relays from septal and diencephalic limbic areas to paramedian midbrain limbic areas. The medial habenula is dominated by septal inputs and projects almost exclusively to the interpeduncular nucleus. Its rigid anatomy is starkly contrasted by the plethora of unusually and sometimes nearly uniquely expressed genes in this nucleus, suggesting that isolated forms of molecular evolution occur in this otherwise highly conserved structure. The lateral habenula has the striking property of integrating inputs from limbic (hypothalamic) and basal ganglia (pallidal) sources. It in turn relays these inputs to the paramedian midbrain, notably the serotonergic dorsal and median raphe nuclei and the GABAergic rostromedial pontine tegmental area (RMTg). The lateral habenula projects also to the dopaminergic ventral tegmental area and substantia nigra pars compacta, but this direct projection is light compared to the strong projection relayed to the tegmental dopamine areas via the RMTg.
Discussion: By these connections, convergent limbic and basal ganglia inputs can influence the major serotonergic, GABAergic, and dopaminergic centers of the midbrain. The lateral habenula especially strongly controls the physiological properties of neurons in both the serotonin and dopaminergic nuclei of the tegmentum. These connections form the basis for the unique functional properties of the habenula described in the Panel.
Disclosure: M. Herkenham, None.

"You Can't Always Like What You Want" - Role of the Lateral Habenula in the Encoding of Motivational Salience

Paul Shepard*

Maryland Psychiatric Research Center, Baltimore, MD

Background: Projections from the lateral habenula (LHb) to the ventral midbrain and reticular formation provide an important source of inhibitory control over dopamine and serotonin neurons, respectively. Transient increases in LHb activity are associated with phasic decreases in DA cell firing elicited by the unanticipated absence of an expected reward. LHb neurons also respond to direct nociceptive stimulation and thus appear to encode the motivational value of a variety of aversive stimuli. Sustained increases in LHb activity are associated with reduced brain serotonin levels and the emergence of depressive mood and behavior in humans and in animal models of major depressive disorder.
Methods: This presentation will review recent advances in our understanding of the habenula-mesencephalic pathway and its role in functional modulation of monoaminergic neurons with an emphasis on dopamine-containing neurons.
Results: Electrophysiological studies have shown that stimulation of the lateral habenula is capable of suppressing the activity of dopamine and serotonin neurons at a population level. The powerful inhibitory effects exerted by the LHb on DA neurons will be contrasted with the relatively sparse innervation of the ventral tegmental area (VTA) and substantia nigra (SN) by the LHb. The role of GABA neurons in mediating the inhibitory effects of LHb activation on DA neurons will also be discussed with an emphasis on recent data suggesting that a newly identified brain region, the rostromedial tegmental nucleus, serves as the principal target of LHb efferents and the source of feed-forward inhibition of DA cell activity. A novel circuit will be proposed that offers a potential explanation for the predominantly inhibitory response of midbrain DA neurons to aversive stimuli as well as the paradoxical excitation elicited by identical stimuli in a subpopulation of VTA DA neurons.
Conclusions: Having only recently emerged from relative obscurity, the habenula and its connections with monoaminergic and GABAergic neurons are providing new insights into the circuitry responsible for encoding motivational salience. These perspectives have provided a new conceptual framework for understanding the basis of clinical syndromes ranging from major depressive disorder to cue-induced relapse of drug seeking behavior.
Disclosure: P. Shepard, None.

The Habenula as a Target of Deep Brain Stimulation for the Treatment of Depression

Fritz Henn*

Brookhaven National Laboratories, Upton, NY

We have used an animal model of depression, learned helplessness; to attempt to define the circuit mediating helplessness, determine the cellular basis underlying these circuit changes and the pathology leading to these changes and treatment approaches to the circuit dysfunction. Once this was established we have begun translational studies in man. Using the learned helpless model two rat lines were created by selectively breeding rats either sensitive or resistant to helplessness training selectively with one another. After over 60 generations the helpless line has the characteristics of a treatment

resistant depression not responding to normal antidepressants or ECT, while the non helpless line is resistant to the effects of uncontrollable stress. Using micro PET we determined differences in baseline metabolic rates between the strains. This pointed to an area in the medial thalamus including the habenula as overactive and suggested the VTA was underactive. Electrophysiological studies showed the l. habenula was 15 fold more active in the helpless line and these cells project in part to the VTA inhibiting DA release. We will show preliminary evidence that the l. habenula appears to receive increased glutaminergic input driving the over activity, and that this may be on the basis of astrocytic dysfunction. Using the helpless line we investigated the effects of bilateral deep brain stimulation. We showed that DBS resulted in depressed cellular activity and an improvement in the helplessness. Opticogenetic studies are underway to define the exact mechanism of the cell silencing. To determine if the changes seen in the animal model applied to human depression we looked at brain changes in patients subjected to tryptophan depletion (Roiser et al 2009). This revealed activation of the habenula, confirming an earlier PET study by Morris et al (1999). Finally we have utilized DBS in a patient with a long history of treatment resistant depression (Sartorious et al 2010). This resulted in a full recovery which reverses within 24 hours when the stimulator is off. These data suggest that the l. habenula, which receives cortical and limbic input, has strong reciprocal connections with the hypothalamus and alters the activity in the midbrain nuclei, including the VTA, DRN and LC, may be a critical point in the circuit mediating major depression.
Disclosure: F.A. Henn, None.

Functional Role of the Habenula in Cognition and Nicotine Addiction: From Mice to Men
Ramiro Salas*, Philip R. Baldwin, Mariella De Biasi, P Read Montague

Baylor College of Medicine, Houston, TX

Background: Nicotine, the main addictive component of tobacco, acts at nicotinic acetylcholine receptors (nAChRs) in the brain. There is an enormous variety of nAChRs and through pharmacological and genetic experiments in mice, we have shown that although β2 subunit-containing receptors are important for the rewarding effects of nicotine, receptors comprising the β4, α5 and α2 subunits mediate withdrawal symptoms. These subunits are highly expressed in the habenula (Hb) and one of its major targets, the interpeduncular nucleus (ipn), and we next showed that blocking cholinergic activity in the Hb or the ipn can precipitate withdrawal in nicotine-treated mice. Genome-wide association studies showed that single nucleotide polymorphisms in the β4, α5 and α3 nAChRs are important mediators of tobacco addiction risk in humans. In monkeys, the Hb has been shown to signal negative reward prediction errors: when habenular cells activate due to the non-delivery of expected reward, dopamine cells shut down. In addition, upon unexpected reward, habenular cells showed small decreases while dopaminergic cells showed large increases in activity.
Methods: We studied habenular activation during negative prediction errors using fMRI in humans. In humans, the habenula's extremely small size has prevented direct assessments of its function. We developed a method to functionally locate and study the habenula in humans using fMRI, based on the expected reward-dependent response phenomenology of habenula and striatum. Broadly, the logic of our approach had two basic components: (1) Identify habenular voxels by seeking anti-correlations with striatal responses to unexpected reward delivery during early conditioning. This identifies the habenula using a small negative-going BOLD response in specific voxels contained in a region large enough to contain the habenula. (2) In a separate session, probe the habenular voxels using the unexpected non-delivery of reward. In addition, to be able to image such a small area, we used a large number of subjects (50), a manual co-registration technique, and avoided pre-processing steps such as smoothing and normalization to a template brain.
Results: We provide conclusive evidence for activation in human habenula to negative reward prediction errors. When expected reward was delivered with a 4 second delay, striatal activity decreased while habenular activity significantly increased. Once reward was finally delivered, striatal activity markedly increased, while habenular activity returned to basal levels. We are currently repeating this experiment in non-smokers and in sated and abstinent smokers.
Discussion: We have shown that by using a large number of subjects, small voxel size and a manual coregistration technique coupled to a correlational approach to identify the habenula, we can assess the activity of the habenula in humans undergoing a passive learning task. Our approach may provide a way to image activity in analogously small brain structures, given that activity in those areas correlates in a systematic fashion with large easier-to-image brain regions.
Disclosure: R. Salas, None.

Panel Session
New Functional Insights into Sleep and Their Implications for Psychiatry

Dynamics of Human Neurobehavioral Functions During Chronic Sleep Restriction and Recovery: Critical Contributions from Sleep Duration, Slow Wave Sleep Homeostasis, and Phenotypic Vulnerability
Mathias Basner*

University of Pennsylvania, Philadelphia, PA

Background: The daily integrity of waking neurobehavioral functions (cognitive, physiological, and subjective) depends on sleep. However, most studies this relationship have involved acute total sleep deprivation, instead of chronic partial sleep deprivation, which is what modern humans commonly experienced through life style and pathologies. Therefore what is known about sleep-wake dynamics is based largely on acute or short time constants in sleep neurobiological processes mediating wake neurobehavioral functions. This has led to the view that sleep responds to deprivation by intensification of nonREM slow wave activity (the putative marker of sleep homeostasis), and the assumption that as long as slow wave activity intensifies, neurobehavioral functions can be recovered more quickly than they are diminished by sleep loss.
Methods: For the past 15 years we have studied longer time constants in human sleep-wake dynamics by systematically investigating the dose-response effects of repeated days of chronic sleep restriction on accumulation of neurobehavioral deficits, and the recovery of these functions. Thus far, our experiments have involved more than 500 healthy adults (21-50y; 50% female; 50% minorities) continuously monitored physiologically and behaviorally in a medically secure, environmentally controlled laboratory, and evaluated longitudinally on a range of neurobehavioral functions during 9 experiments involving randomization to sleep dose (for a total of $>$7,500 days).
Results: These experiments have established that chronic restriction of sleep results in neurobehavioral deficits in cognitive processes involving attention, perception and memory that escalate (1) at a rate inversely proportional to the dose of sleep provided, and (2) as a function of the chronicity of the restriction. NonREM slow wave responses_the putative marker of sleep homeostasis_were less marked under chronic sleep restriction than total sleep deprivation, but slow wave energy in the sleep EEG had a strong relationship with the extension of sleep duration during recovery from chronic sleep restriction. It also appeared to be critical for the recovery of speed of cognitive throughout. Our experiments also identified marked inter-individual differences (i.e., differential vulnerability) to the neurobehavioral effects of chronic sleep restriction within those healthy adults who habitually sleep 6.5-8.5 h per night. While the

majority of healthy adults developed cumulative cognitive deficits, with some manifesting very rapid and severe deficits in response to chronic sleep restriction, a subset manifested no deficits as sleep loss progressed. We have investigated the neural basis of these phenotypic responses using fMRI and by examining variability in genetic polymorphisms known to be involved in regulation of arousal.
Discussion: This body of work has demonstrated longer time constants are present in the neurobehavioral manifestations of sleep-wake dynamics, and that slow wave sleep energy has a role in apparent recovery from chronic sleep restriction, but the basis of the phenotypic differences in neurobehavioral responses to chronic sleep restriction are not yet understood.
Disclosure: F. Henn: Bristol-Myers Squibb.

Sleep Disturbances in Psychiatric Illness: Metabolic Implications
Eve Van Cauter*

University of Chicago, Chicago, IL

Background: Reduced sleep duration and quality are nearly universal correlates of psychiatric illness.
Methods: Healthy normal subjects are submitted to experimental manipulations of sleep duration (bedtime restriction) or sleep quality (selective suppression of deep slow-wave sleep) for 3-4 days under carefully controlled laboratory conditions, mimicking the sleep disturbances of psychiatric illness. State of the art metabolic measures including total body insulin sensitivity and insulin action at the level of the adipocyte are assessed.
Results: Both total body insulin sensitivity and insulin signaling in peripheral adipocytes are impaired by reduced sleep duration or quality.
Conclusions: Sleep disturbances in psychiatric illness are likely to be associated with metabolic disturbances that are evident in peripheral molecular signaling pathways.
Disclosure: E. van Cauter, None.

Sleep and Brain Energy Levels: ATP Changes During Sleep and the Role of Nitric Oxide in Sleep
Robert McCarley*

Harvard/VAMC, Brockton, MA

Sleep is one of the most pervasive biological phenomena, but one whose function remains elusive. Although many theories of function, indirect evidence, and even commonsense suggest sleep is needed for an increase in brain energy, brain energy levels have not been directly measured with modern technology. Our previous extensive research showing an increase in adenosine during wake, acting to inhibit wake-active neurons, suggested that the increase in adenosine might be a result of utilization of Adenosine TriPhosphate (ATP) in waking neuronal activity. As ATP is utilized, a chain of coupled reactions would lead to an increase in intracellular adenosine, which would be transported across the membrane by equilibrative transporters, leading to feedback inhibition on the wake active cells. These data prompted us to examine directly ATP levels during sleep and wake. Using a validated luciferin - luciferase ATP assay and rapid regional brain dissections and freezing in rat, we here report that ATP levels, the energy currency of brain cells, show a surge in the initial hours of spontaneous sleep in wake-active brain regions of frontal and cingulate cortex, hippocampus and basal forebrain. This surge does not occur in the sleep-active brain region of ventrolateral preoptic hypothalamus. The surge is dependent on sleep but not time of day, since preventing sleep by gentle handling of rats for 3 h or 6 h also prevents the surge in ATP. A significant positive correlation was observed between the surge in ATP and EEG NREM slow wave sleep (delta activity, 0.5-4.5 Hz) during spontaneous sleep. Inducing sleep and SWS (delta activity) by adenosine infusion into basal forebrain during the normally active dark period also increases ATP. Taken together, these observations suggest that the surge in ATP occurs when the neuronal activity is reduced, as occurs during sleep. The levels of phosphorylated AMP-activated protein kinase (P-AMPK), well known for its role in cellular energy sensing and regulation, and ATP show reciprocal changes. P-AMPK levels are lower during the sleep-induced ATP surge than during wake or sleep deprivation. These results suggest that sleep-induced surge in ATP and the decrease in P-AMPK levels set the stage for increased anabolic processes during sleep and provides insight into the molecular events leading to the restorative biosynthetic processes occurring during sleep. This would be consistent with recent findings on the necessary role of sleep in memory consolidation, synaptic plasticity and remodeling, including an increase in the transcription of genes involved in protein synthesis and synaptic plasticity. Our data significantly recast the old speculation that sleep is for energy restoration. We now restate the energy hypothesis as "sleep is for an energy surge", an ATP surge that nourishes the anabolic, restorative biosynthetic processes occurring during sleep. We then looked further into mechanisms leading to adenosine production during sleep, focusing on the role of the gaseous neuromodulator, nitric oxide (NO). We developed a novel assay for cellular NO, DAF, a dye taken up by cells and, in the presence of NO, became fluorescent and was trapped in the cell, allowing precise measurement of NO. Our studies indicate that forced wakefulness activated NO as part of the sleep homeostatic cascade and that NO promoted recovery sleep following sleep deprivation by the production of adenosine.
Disclosure: R.W. McCarley: Part 1; Merck consultant, Janssen consultant, Sanofi-Aventis consultant.

Optogenetic Control of Sleep and Waking
Luis De Lecea*

Stanford, Palo Alto, CA

Background: The hypocretins (Hcrts), also known as orexins, are two neuropeptides produced by a few thousand neurons in the lateral hypothalamus. Genetic studies have demonstrated that Hcrt function is necessary for wake stability, as lack of function leads to narcolepsy. Here we have tested whether Hcrt activity is sufficient to promote arousal stability by manipulating the activity of the Hcrt system using optogenetics.
Methods: We targeted Channelrhodopsin-2 (ChR2), a light-activated cation channel that enables temporally and spatially precise activation of transfected neurons2 to hypocretin (Hcrt)-expressing cells. Phasic activity over 5 Hz dramatically increases the probability of behavioral state transitions. Using the same optogenetic strategy we developed a novel method to fragment sleep without compromising total sleep amounts. We transduced Hcrt neurons in C57Bl/6 mice using a Hcrt::ChR2-YFP or control Hcrt::YFP lentivirus. We induced micro-fragmentation of sleep by delivering trains of light pulses 10 sec at 20 Hz using a blue laser diode (20 mW; 477 nm) using three different protocols: 30, 60 or 120 sec intervals over 4 h during the light phase. We used Novel Object Recognition (NOR) task to study the effects of sleep on memory consolidation in rodents.
Results: We found that all stimulation protocols significantly increased the frequency of transitions from sleep (non-rapid eye movement (NREM) and rapid eye movement REM) to wake and shortened NREM sleep episodes. The mean NREM sleep episode in the control animals was 62 + /-2.9 sec. In the 30 sec, 60 sec, and 120 sec stimulation protocols, NREM sleep episode durations were 34 = + /-2.8 sec, 38 + /-3.5 sec, and 45 + /-4.3 sec respectively. Rapid eye movement (REM) sleep was not significantly affected. Immediately after the training session mice were stimulated for 4 h in different intervals. We found that stimulations every 60 sec resulted in a significant reduction in the performance on the test session 24 h later. Stimulation at a longer interval, every 120 sec did not affect the performance. These effects were further validated by the use of more frequent stimulation protocol (30 sec intervals), which impaired memory performance and

a more spares stimulation protocol (240 sec intervals) that did not affect the performance. Sleep fragmentation by gentle handling every 60 sec also impaired performance on the NOR task.
Discussion: Our data reveal a minimal quantum of uninterrupted sleep required for memory consolidation Optogenetic methods allow us to demonstrate a prominent role for the hypocretinergic system in orchestrating arousal circuits and in cognitive function with unprecedented temporal precision.
Disclosure: L. de Lecea, None.

Panel Session
Omega-3 Fatty Acids and Depression from Cellular Mechanisms to Clinical Care

Omega-3 Essential Fatty Acids in Depression and Illnesses

John M. Davis*, Joseph R. Hibbeln, Brian P. Hallahan

University of Illinois at Chicago, Chicago, IL, NIAAA, Rockville, MD, National University of Ireland Galway, Galwa, Ireland

Since omega-3 fatty acids are essential fatty acids (EFA) that cannot be synthesized to a large degree in the body from other dietary substance, the limiting factor in determining tissue concentrations is the amount of omega-3 fatty acids in the diet. Omega-3 and omega-6 are both constituents of many tissues and, in some sense, compete for incorporation into tissue. In the last 100 years or so, there has been a large increase in the dietary consumption of the omega-6 fatty acids due to alteration of farming and animal feed. The increase in the incidence of depression over these years parallels the increase of omega-6, so that much more omega-6 is consumed relative to omega-3 EFA's. Hence, an omega-3 EFA dietary deficit may be a risk factor for depression. We will present a meta-analysis of randomized, double-blind-placebo-controlled studies testing the administration of omega-3 EFA for the treatment of depression. We preformed a systematic literature search, subdividing studies as those which used an EPA predominant or a DHA formulation. Omega 3 is more effective than placebo in treating depressive illness, although there is some variability in results. We explored whether the composition of the omega-3 administered is important, finding that EPA predominant formulation is necessary for the full therapeutic antidepressant action with a large effect size, with little variability. The DHA predominant formulation has little antidepressant efficacy. We conclude that omega-3 does have antidepressant properties. We will also discuss the role of omega-3 in depression or other disorders, in the broader range of ecological, epidemiological and experimental context, focusing on findings that have implication on cellular mechanism of antidepressant action or in disease.
Disclosure: J.M. Davis: None.

Phospholipase A2 and Cyclo-Oxygenase 2 Genes Influence the Risk of Interferon-Alpha-Induced Depression by Regulating Polyunsaturated Fatty Acids Levels

Carmine M. Pariante*, Kuan-Pin Su

Psychological Medicine, London, United Kingdom, China Medical University and Hospital, Taichung, Taiwan

Background: Phospholipase A2 (PLA2) and cyclo-oxygenase 2 (COX2) are the two key enzymes in the metabolism of polyunsaturated fatty acids (PUFAs), which in turn play an important role in cytokine-induced depression and sickness behaviour.
Methods: Patients with chronic hepatitis C viral (HCV) infection (n = 132) were assessed to examine the effects of seven single nucleotide polymorphisms (SNPs) in COX2 and PLA2 genes on the development of depression during interferon-alpha treatment; a subsample (n = 63) was assessed for the erythrocyte levels of the three main PUFAs, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA). An independent "replication" sample of patients with major depression unrelated to cytokine treatment (n = 82) was also examined.
Results: 28% of participants developed interferon-alpha-induced depression. Participants with the PLA2 BanI GG or the COX2 rs4648308 AG genotypes had a higher risk of IFN-alpha-induced depression (odds ratio = 3.1 and 3.5, respectively). The "at risk" PLA2 genotype was associated with lower EPA levels, and the "at risk" COX2 genotype was associated with lower DHA levels, during IFN-alpha treatment. The PLA2 BanI GG polymorphism was also associated with more somatic symptoms of depression, both in patients with interferon-alpha-induced depression and in the replication sample of patients with major depression.
Conclusions: Genetic variations in the COX2 and PLA2 genes increase the risk of IFN-alpha-induced depression, possibly by affecting the levels of EPA and DHA. Moreover, PLA2 genotype is associated with somatic symptoms in depression. Our study confirms the role of inflammatory mechanisms in major depression.
Disclosure: C.M. Pariante, None.

Increased Brain DHA Enhances Neurogenesis, Neuritogenesis and Spatial Learning Performance

Jing X. Kang*

Harvard Medical School, Charlestown, MA

Background: Docosahexaenoic acid (DHA), an n-3 long chain polyunsaturated fatty acid (LC-PUFA) highly rich in nerve system, is critical for brain development and function. It has been shown that DHA deficiency impairs cognitive performance, while DHA supplementation improves the condition. However, the mechanisms underlying the roles of DHA in neural development and brain function still remain to be elucidated. The objective of this study was to investigate the effects of DHA on neurogenesis and neuritogenesis, as well as their relation to cognitive function.
Methods: We used the fat-1 transgenic mouse model instead of dietary supplementation to create the difference of DHA content in brain tissues to eliminate confounding factors of diet. BrdU was used to label the newly generated cells by immunohistochemisty staining with specific antibody. Dendritic spine density of hippocampus was measured by Golgi-Cox staining. Animal performance of learning and memory was assessed by using Morris Water Maze. *in vitro* experiments were also performed to examine neural differentiation and proliferation, and neurite outgrowth of neural cells.
Results: Increased brain DHA enhances significantly hippocampal neurogenesis as shown by increased number of proliferating neurons and neuritogenesis as evidenced by increased density of dendritic spines of CA1 pyramidal neurons in hippocampus. Concurrently, the fat-1 mice exhibit better performance of learning and memory tested by Morris water maze when compared with the control wild-type animals. *In vitro* experiments further demonstrate that DHA promotes differentiation and neurite outgrowth of neuronal cells derived from mouse embryonic stem (ES) cells and also increases the proliferation of cells undergoing differentiation into neuronal lineages from the ES cells.
Discussion: The present study is the first to look at the effect of DHA on both structural and functional aspects in the brain under a dietary confounding factors free system, and demonstrate that increased brain DHA enhances neurogenesis and neuritogenesis in hippocampus and thereby improves learning and memory performance. The results of this study not only provide new insight into how DHA can influence cognitive function but also suggest a role for DHA in prevention and treatment of nerve injury and neurodegenerative diseases.
Disclosure: J.X. Kang, None.

Fish Oil Treatment Translocates Gsα from Lipid Rafts: A Possible Mechanism for the Antidepressant Effects of n-3 PUFA

Mark Rasenick*

University of Illinois Chicago College of Medicine, Chicago, IL

Background: Several clinical studies suggest that decreased dietary fish oil is associated with increased incidence of depression and that that dietary n-3 PUFA decreases symptoms of depression, either as monotherapy or in conjunction with antidepressant drugs. One suggestion as a mechanism for n-3 PUFA actions is the antiinflamatory effects associated with fish oil. Another possible mechanism relates to the association of DHA and EPA with membrane microdomains and the mobilization of the G protein, Gsα, our of cholesterol-rich lipid rafts and into a membrane domain where Gsα more closely associates with adenylyl cyclase increasing signaling through 5HT 4.6.7, D1 or β2 receptors. It is this latter mechanism that this study addresses.
Methods: C6 glioma cells were treated with the n-3 PUFAs, DHA and EPA (50 μg/ml) or citalopram (10 μM) for 1-4 days. Some cells were treated with a combination of n-3 PUFA and SSRI. Cells were harvested and lipid raft fractions prepared and assayed for Gsα content. Fluorescence recovery after photobleaching (FRAP) was determined using a Zeiss 510 Meta confocal system in C6 cells that had been transfected with GFP-Gsα as well as treated with citalopram and/or DHA.
Results: As had been previously established, Gsα content in lipid raft fractions was reduced about 50% by chronic citalopram treatment and Gsα[[Unsupported Character - Symbol Font ]] was redistributed into non-raft membrane fractions. No change was seen in content of Gsα. Furthermore, AlF4- - activated adenylyl cyclase, an indication of the coupling between Gsα and adenylyl cyclase, was increased subsequent to citalopram treatment in a dose- and time-dependent manner. DHA and EPA treatment had a similar effect, while EPA showed a somewhat lower efficacy. Furthermore, both n-3 PUFAs potentiated citalopram actions on Gsa translocation and adenylyl cyclase. Finally, chronic n-3 PUFA treatment decreased recovery time of a photobleached fluorescent GFP-Gsα fusion protein. Chonic antidepressant treatment had a similar effect.
Discussion: The effects of n-3 PUFA and SSRIs in translocating Gsα from lipid rafts may require different molecular targets. It has been suggested that n-3 PUFA alter membrane properties of non-raft domains. It has also been suggested that n-3 PUFA might modulate acyl transferase activity, modifying prenylation of G proteins and changing their membrane association or compartmentalization. Should this prove to be the case, it would suggest a clear molecular rationale for both antidepressant properties and supplemental antidepressant effects for n-3 PUFA. Experiments to ascertain this are underway.
Disclosure: M.M. Rasenick, Eli Lilly, Part 1; Lundbeck, Part 1; StrokeMed, Part 1; Sepracor, Part 1; Pax Neuroscience, Part 1; Eli Lilly, Part 2; Eli Lilly, Part 4; Lundbeck, Part 4.

Panel Session
The Insula–A Therapeutic Target for Nicotine Dependence?

Role of the Insula in Drug Addiction: Imaging Inhibitory Control in Adolescent Smokers

Edythe London*, Adriana Galván

UCLA, Los Angeles, CA

Background: Drug addiction is a complex disorder, characterized by maladaptive responses to environmental and interoceptive stimuli that can trigger craving for the abused drug. Brain imaging studies have shown that craving for both cocaine and cigarettes, induced by drug-relevant visual stimuli, is correlated with changes in glucose metabolism in the insula, which is thought to have an important role in drug craving. Among human smokers who suffered strokes that lesioned the insula, an unexpectedly large proportion quit smoking and endorsed a loss of cigarette craving. This finding suggested that modulating insula activity may provide a useful approach to the treatment of nicotine dependence. This presentation will focus on the importance of the insula with regard to inhibitory control, a cognitive function that is important to addiction, and how the contribution of the insula to self-control may be compromised in adolescent smokers.
Methods: Participants were twenty-five adolescent smokers (15-21 years of age) who reported daily smoking for at least 6 months before their participation in the study, and twenty-five matched nonsmokers (16-21 years of age) who reported smoking fewer than 5 cigarettes in their lifetimes. The subjects performed a response inhibition task (Stop-signal Task) while undergoing a functional magnetic resonance imaging (fMRI) scan. The primary outcome measure was blood oxygen level-dependent (BOLD) signal change during successful response inhibition.
Results: As observed before in healthy nonsmokers, inhibition during the Stop-signal Task involved activation of a network of regions, including the inferior frontal gyrus, anterior cingulate cortex and insula. Task-related activity in these regions was correlated with stopping ability (greater activity with smaller stop-signal reaction time). In our sample, there were no significant group differences in activation; but during inhibition, activity in cortical areas, including the insula, anterior cingulate cortex and inferior frontal gyrus of the adolescent smokers, was positively correlated with time from waking to the first cigarette of the day. In addition, activity during inhibition, particularly in the insula and anterior cingulate cortex, was negatively correlated with number of cigarettes smoked per day.
Discussion: These findings in adolescent smokers suggest that activity in the insula, related to inhibitory control of a motor response, is either directly related to the ability to resist the urge to smoke or inversely related to the magnitude of that urge. They also suggest that insula activity during inhibition either is negatively affected by exposure to cigarette smoke, or alternatively, that individuals who smoke more may have less activation of the insula during inhibition even before they initiate cigarette smoking. In any case, the observed link between insula activation and successful inhibition along with evidence for an important contribution of the insula to decision-making, provides a basis for controversy regarding the potential value of using TMS to attenuate insula activity for the treatment of nicotine dependence.
Author Disclosure: Work described in this presentation was supported in part by a grant from Philip Morris USA, which had no involvement in the design, collection, analysis or interpretation of the data.
Disclosure: E.D. London, Philip Morris USA, Part 4.

Damage to the Insula Disrupts Addiction to Cigarette Smoking

Nasir H. Naqvi*

Columbia University, New York, NY

Background: A large number of functional imaging studies have shown that cue-induced drug urges are associated with activity in a cortical network that involves the insula. Despite this fact, the insula has received relatively little attention for its role in addiction. Here, we sought to test the role of the insula in addiction by examining the effects of focal brain damage in this region on human cigarette smoking behavior.
Methods: We identified patients who sustained damage to the insula (N = 19) at the time that they were smoking regularly (mean # of cigarettes per day = 27; SD = 13.9), and compared them with patients with damage in non-insula regions (N = 50) who were smoking a similar amount when they sustained brain damage (mean # of cigarettes per day = 27, SD = 14.6). We first compared the rate of quitting smoking between these two groups. We then compared the rate of "disruption of smoking addiction" between the two groups, defined as quitting smoking immediately after brain injury, with great ease, without urges, and without relapsing.

Results: We found that smokers who sustained damage to the insula were more likely than smokers with damage in non-insula regions to quit smoking after their brain injury, though this difference was not significant (odds ratio = 2.94, x2 = 2.74, and $P = 0.10$). Smokers with insula damage were significantly more likely to undergo a "disruption of smoking addiction" after their brain injury compared to smokers with non-insula damage (odds ratio = 22.05, x2 = 16.64, and $P = 0.0005$). Effects of both right and left insula damage were found. A whole-brain, region of interest analysis demonstrated that this effect was not due to damage in regions surrounding the insula.
Conclusions: The results demonstrate that damage to the insula is associated with a profound disruption in the addiction to smoking, evidenced by an ability to quit easily, immediately, without relapsing and without an urge to smoke. We discuss these findings in light of a theoretical framework for the role of the insula in conscious interoception, emotional feelings and decision-making. We also present preliminary behavioral data showing that the interoceptive effects of smoking contribute to conscious feelings that may promote addiction, including smoking pleasure and cue-induced smoking urges.
Disclosure: N.H. Naqvi, None.

Granular Insular Cortex Inactivation as a Novel Therapeutic Strategy for Nicotine Addiction
Bernard Le Foll*, Abhiram Pushparaj, Benoit Forget

Centre for Addiction and Mental Health, Toronto, ON, Canada

Background: Nicotine is the principal component of tobacco smoke that leads to addiction and recent evidence suggest that damage to the insular cortex (insula) disrupt tobacco addiction in human smokers. Our aim was to investigate the effect of an inactivation of this structure in an animal model of nicotine addiction.
Methods: We have investigated the effect of reversible inactivation of the granular insula through local bilateral infusions of the GABA agonists (0.3 nmol baclofen + 0.03 nmol muscimol) on nicotine self-administration under fixed and progressive ratio and on reinstatement of nicotine seeking induced by nicotine priming or nicotine-associated cues in rats. We have also evaluated the effect of insula inactivation on food self-administration and relapse as a control. Only rats with correct bilateral placement of the cannulaes in the granular insula have been included for data analysis.
Results: Infusion of the balofen-muscimol mixture into the insula significantly reduced nicotine self-administration compared to vehicle administration in the insula ($P < 0.05$) under both fixed and progressive ratio schedules of reinforcement. In contrast, insular cortex inactivation did not modify responding for food (NS). Significant reinstatement of nicotine seeking was obtained by cues presentation and nicotine priming (0.15 mg/kg). Both reinstatements ($P < 0.05$) were attenuated by insular cortex inactivation, whereas reinstatement for food seeking was not affected (NS).
Conclusions: Our study indicated that the integrity of the granular insula is necessary to the rats to exhibit motivation to take nicotine and to relapse to nicotine seeking, but not for their motivation to consume food pellets or to relapse for food seeking. Indeed, methods those are able to modulate the activity of the insula, such as repetitive transcranial magnetic stimulation or deep brain stimulation, may represent a new therapeutic way to treat tobacco addiction and relapse in humans.
Disclosure: B. Le Foll, Pfizer, Part 1.

Can Repeated Stimulation of the Prefrontal or Insular Cortices Affect Addictive Behavior?
Abraham Zangen*

Weizmann Institute of Science, Rehovot, Israel

Background: Repeated administration of nicotine and other addictive drugs induces neuroadaptations in several brain regions resulting in altered cortical neurotransmission and excitability. Electrical stimulation of specific brain regions can be used in animal models and humans to induce local activation or disruption of specific circuitries or alter neuronal excitability and cause neuroadaptations. Non-surgical stimulation of specific brain regions in human addicts can be achieved by transcranial magnetic stimulation (TMS). Repetitive TMS (rTMS) is used for transient stimulation or disruption of neural activity in specific cortical regions, allowing investigation of the acute effect of stimulation on drug craving, while repeated sessions can induce long-lasting neuroadaptations and thereby become a potential therapy.
Methods: We have investigated the effects of repeated stimulation of the prefrontal cortex in animal models and humans on glutamatergic neuroadaptations, drug craving and consumption. Recently, we have begun to study the effects of repeated stimulation of the insular cortex (using a unique TMS coil) on cigarette craving, consumption and dependence in heavy smokers.
Results: Ten daily stimulation sessions of the prefrontal cortex reduced drug seeking and consumption in animals trained to self-administer cocaine. The treatment also normalized some of the neuroadaptations in glutamatergic receptors that were induced by repeated consumption of cocaine in the ventral tegmental area and nucleus accumbens. Similarly, ten rTMS (but not sham) sessions over the prefrontal cortex in heavy smokers reduced cigarette craving, consumption and nicotine dependence, however, these effects were quite modest and tended to dissipate over time. Preliminary data on the effects of repeated rTMS sessions over the insular cortex using a unique deep TMS coil indicate that this treatment is well tolerated does not induce significant side effects and can cause reductions in nicotine craving and consumption.
Discussion: The potential use of localized electromagnetic stimulation in the study and treatment of addictive behaviors is promising, but requires additional basic research and combination with imaging techniques in order to identify optimal stimulation parameters that may induce long-lasting therapeutic effects.
Disclosure: A. Zangen, Brainsway, Part 1; Brainsway, Part 2; Brainsway, Part 3; Brainsway, Part 4.

Wednesday, December 8, 2010

Panel Session
Developmental Etiologies of Degenerative Disorders: Lessons from Psychiatric Diseases

Impairment of Developmental Stem Cell-Mediated Striatal Neurogenesis in a Knock-In Model of Huntington's Disease: Implications for the Pathogenesis and Treatment of Neurodegenerative Diseases
Mark F. Mehler*

Albert Einstein College of Medicine, Bronx, NY

Background: The pathogenesis of Huntington's disease (HD) and other neurodegenerative diseases remains elusive. HD is caused by mutation in exon 1 of the gene that codes for huntingtin and represents the prototypical example of the expansion repeat subclass of neurodegenerative diseases. Although huntingtin is pan-neuronal, pathological changes in HD are selective, targeting the medium spiny projections neurons of the striatum. Research initiatives in pathological brain aging have traditionally focused on defining the pathobiological processes mediating neuronal dysfunction and death during adult life. However, there is increasing evidence that huntingtin has selective functions in the developing striatum. These observations suggest a potential mechanism to explain the selective cellular vulnerability present in HD as well as in other neurodegenerative diseases. The identification of increasingly early pathophysiological abnormalities in HD therefore suggests the possibility that

impairments of striatal medium spiny neuron (MSN) specification and maturation may underlie the etiology of HD.
Methods: To examine these important issues, we utilized combined detailed *in vivo* and *in vitro* analysis of regional stem cell-mediated self-renewal, expansion, proliferation, lineage restriction, striatal neurogenesis, neuronal subtype specification, maturation and the progressive elaboration of the functional organization and associated compartmentalization of the striatum in an HD knock-in mouse model that most faithfully recapitulates the molecular genetics of human HD.
Results: We demonstrate that HD knock-in (Hdh-Q111) mice exhibited delayed acquisition of the early striatal cytoarchitecture with aberrant expression of progressive markers of MSN neurogenesis (Islet1, DARPP-32, mGluR1, and NeuN). Hdh-Q111 striatal progenitors also displayed delayed cell cycle exit between embryonic day (E) 13.5-15.5 (BrdU birth-dating) and an enhanced fraction of abnormal cycling cells in association with expansion of the pool of intermediate progenitors and over expression of the core pluripotency (PP) factor, Sox2. Clonal analysis further revealed that Hdh-Q111 neural stem cells (NSCs) displayed: impaired lineage restriction, reduced proliferative potential, enhanced late-stage self-renewal, and deregulated MSN subtype specification. Further, our analysis revealed that in addition to Sox2, the core PP factor, Nanog is expressed within the striatal generative and mantle regions, and in Hdh-Q111 embryos the fraction of Nanog-expressing MSN precursors was substantially increased. Moreover, compared to Hdh-Q18 embryos, the Hdh-Q111 striatal anlagen exhibited significantly higher levels of the essential PP cofactor, Stat3.
Discussion: These findings suggest that Sox2 and Nanog may play roles during a selective window of embryonic brain maturation, and alterations of these factors may, in part, be responsible for mediating the aberrant program of Hdh-Q111 striatal MSN specification and maturation. We propose that these HD-associated developmental abnormalities might compromise neuronal homeostasis and subsequently render MSNs and their associated corticostriatal and striatonigral as well as stratopallidal functional networks more vulnerable to late life stressors, culminating in progressive neuronal and neural network dysfunction and ultimately neurodegeneration.
Disclosure: M.F. Mehler, None.

Developmental Etiology of a Neurodegenerative Disorder: SCA1
Harry T. Orr*

University of Minnesota, Minneapolis, MN

Background: Spinocerebellar ataxia type 1 (SCA1) is one of nine inherited, typically adult onset, polyglutamine neurodegenerative diseases. Besides an expanded glutamine tract, disease requires phosphorylation of S776 in ATXN1 (the SCA1 encoded protein). We used a conditional transgenic mouse model of SCA1 to delay the postnatal expression of mutant ATXN1 until after completion of cerebellar development. Mice in which expression of ATXN1[82Q] was delayed during postnatal development had a much less severe form of disease than mice in which mutant ATXN1 was expressed prior to the completion of cerebellar postnatal development. These results indicate that compromising cerebellar development contributes to severity of neurodegeneration in an adult. During the first 2-3 weeks after birth, climbing fiber (CF) terminals translocate from the Purkinje cell (PC)s bodies and form synaptic contacts on the primary and secondary branches of the PC dendritic tree.
Methods: To examine if CF terminals fail to develop properly and extend fully along PC dendrites in SCA1 mice, we used VGLUT2 immunostaining and anterograde labeling of olivocerebellar projections following injection of biotinylated dextran-amine (BDA) into the contralateral inferior.
Results: In both unaffected lines there was a progressive increase in the extension of CF terminals along PC dendrites from p14, reaching a maximum at five weeks of age and remaining constant to 12 weeks of age. In ATXN1[82Q] mice, extension of CF terminals remained constant between p14 and p17. By five weeks of age the CF terminals in ATXN1[82Q] mice increased significantly but to a level less than seen in wt FVB animals. Like ATXN1[82Q] CF terminals, CF terminals in ATXN1[30Q]-D776 mice, with a phospho-mimicking residue at position 776, showed no signs of increasing their extension along PCs between days p14 and p17. This failure of CF terminals to course along PCs remained out to five weeks of age in ATXN1[30Q]-D776 mice. These data are consistent with an alteration in CF development in SCA1 affected mice. This compromise in CF synapse localization was more pronounced in ATXN1[30Q]-D776 mice than in ATXN1[82Q]-S776 mice.
Discussion: The decreased ability of CF terminals to develop normally in the SCA1 affected mice raises the possibility that this process is an important component of the developmental aspect of SCA1. In both SCA1 affected lines transgene expression was restricted to PCs. Thus, the affect on CF terminal differentiation reflects a property intrinsic to PCs. We further suggest that the ability of mutant ATXN1 to affect CF development is linked to the degree that phosphorylation of S776 is misregulated.
Disclosure: H.T. Orr, Athena Diagnostics, Part 1.

Abnormal Brain Structure in Children at Risk for Huntington's Disease: Evidence for Abnormal Brain Development
Peggy Nopoulos*

University of Iowa, Iowa City, IA

Background: Huntington's Disease (HD) is conceptualized as a neurodegenerative disease. However, several lines of evidence support the notion that prior to degeneration, there may be abnormal brain development. This study was designed to evaluate brain structure in children at risk for HD, thereby examining brain structure decades prior to the onset of symptoms.
Methods: A total of 21 children (ages 7-18 years) at risk for HD were enrolled in the study. For research purposes only, genetic studies divided these subjects into 13 gene expanded and 8 gene non-expanded participants. As CAG length is related to age of onset, estimated 'years to onset' can be calculated. For the gene expanded group, the mean estimated years to onset was 34.4 years. Thus, these subjects were, on average, greater than three decades from manifesting significant disease symptoms (onset of disease). All subjects underwent MRI brain scanning. The gene non-expanded subjects were merged with a data base of healthy controls and brain structure was evaluating comparing the 13 gene-expanded subjects with a total of 136 healthy control subjects (ages 7-18). Methods included volume of brain tissue, cortical thickness mapping, and Fractional Anisotropy (FA) from Diffusion Tensor Imaging (DTI).
Results: The gene-expanded subjects had significant abnormalities in brain structure - compared to controls, an excess of white matter and a decrease in cortical gray matter. The increased volume of white matter was localized to the sub-cortical region and frontal lobes. This was also supported by a significantly elevated FA in the sub-cortical region and frontal lobes. Cortical thickness maps indicate the areas of thinning occur mostly in the parietal and occipital lobes. The caudate had a significant group by age interaction such that the trajectory of volume change over time was different in the gene-expanded subjects compared to controls: young gene-expanded subjects were enlarged but the older subjects (teenagers) had reduced volume of the caudate.
Discussion: Subjects who are estimated to be greater than three decades from diagnosis have abnormalities of brain structure supporting the notion that abnormal development plays an important role in the pathoetiology of HD. Although HD is a rare disease, it can serve as a model for other degenerative brain disorders such as Parksinson's Disease, other polyglutamine expansion diseases (such as the Spinal Cerebellar Ataxias), and Alzheimer's Disease.
Disclosure: P.C. Nopoulos, None.

Mouse Model of Inducible Expression of Mutant Disrupted-In-Schizophrenia-1: Towards Developmental and Adult Functions of the Candidate Gene
Mikhail V. Pletnikov*

Johns Hopkins University School of Medicine, Baltimore, MD

Background: Major psychiatric disorders have a neurodevelopmental origin. New mouse models with cellular, spatial and temporal manipulations in candidate genes will help advance our knowledge of the specific functions of those genes in the developing and mature brain. Genetic evidence implicates mutations and polymorphisms in the gene Disrupted-In-Schizophrenia-1 (*DISC1*) as risk factors for both schizophrenia and mood disorders. We have generated a transgenic mouse model of forebrain neurons-restricted inducible expression of mutant human DISC1.
Methods: In order to gain insight into the roles of DISC1 at various stages of neurodevelopment, we examined the effects of mutant hDISC1 expressed during 1) only prenatal period, 2) only postnatal period, or 3) both periods. We evaluated a set of mouse behaviors, volumes of the lateral ventricles, cortex and hippocampus, cortical regional quantities of GABA neurons, the linear density of dendritic spines in the hippocampus and cortex, regional levels of monoamines, expression of endogenous DISC1 and its interacting proteins in adult male and female mutant and control mice.
Results: All periods of expression similarly led to decreased levels of cortical dopamine and fewer parvalbumin-positive neurons in the cortex. Combined prenatal and postnatal expression produced increased aggression and enhanced response to psychostimulants in male mice along with increased linear density of dendritic spines on neurons of the dentate gyrus of the hippocampus, and lower levels of endogenous DISC1 and LIS1. Prenatal expression only resulted in smaller brain volume, while selective postnatal expression gave rise to decreased social behavior in male mice and depression-like responses in female mice as well as enlarged lateral ventricles and decreased dopamine content in the hippocampus of female mice, and decreased level of endogenous DISC1.
Discussion: Our data show that mutant DISC1 exerts differential effects on neurobehavioral phenotypes, depending on the stage of development at which the protein is expressed. The multiple and diverse abnormalities detected in mutant DISC1 mice are reminiscent of findings in major mental diseases.
Disclosure: M.V. Pletnikov, None.

Panel Session
How Does Anxiety Take Hold: Anatomical and Functional Connectivity in Adolescents and Adults

Specific Regions of Prefrontal Cortex and Insula Differentially Influence Amygdala Output Paths: Implications for Brain Development and Adolescent Anxiety
Julie Fudge*

University of Rochester Medical Center, Rochester, NY

Background: Hyperactive amygdala responses are a well-established finding in anxiety disorders. Developmental fMRI studies indicate that the amygdala is relatively more 'sensitive' to emotional information in adolescents compared to adults. While this relative 'receptiveness' to emotional cues has survival value, it may also be a substrate for the development of anxiety disorders in this age group. One hypothesis is that underdeveloped inhibitory control between the prefrontal cortex (PFC) and amygdala in adolescents contributes to this vulnerable window. To better define this question for human studies, we examined specific PFC-amygdala paths in nonhuman primates.
Methods: We placed injections of bi-directional neuronal tracers into the basal nucleus in Old world monkeys using stereotaxic techniques. We analyzed the distribution of retrogradely labeled cells throughout the PFC and insula for each injection site. We also compared the distribution of labeled fibers in the striatum, a key output for the basal nucleus.
Results: Preliminary results indicate two patterns of connections: the ventral basal amygdala receives restricted and discrete PFC inputs and influences specific striatal regions, while the dorsal amygdala receives broad PFC inputs, and has a much more widespread influence on the striatum. Specifically, the ventromedial basal nucleus receives its main inputs from the insula, while the ventrolateral basal nucleus receives its main inputs from the subgenual cingulate (sgACC). Output paths from these ventral basal nucleus regions are also relatively segregated: the insula- medial basal nucleus path is directed to ventromedial nucleus accumbens, while the sgACC- lateral basal nucleus path is connectd to the ventrolateral nucleus accumbens. In contrast, broad PFC and insula regions project to the dorsal basal nucleus, which in turn projects broadly to the ventral striatum, including large regions of the ventral striatum posterior to the anterior commissure.
Discussion: These differential PFC-amygdala-striatal paths suggest specific modulatory controls that can be tested in adolescent and adult humans using functional neuroimaging.
Disclosure: J.L. Fudge, None.

The Development of Amygdalo-Cortical Connectivity in Animal Models: Implications in Pathogenesis
Miles G. Cunningham*

McLean Hospital, Harvard Medical School, Belmont, MA

Background: Adolescence is a period of rapid biopsychosocial growth and is a critical stage for the development of emotional maturity and diverse forms of psychopathology. In animal models, the basolateral nucleus of the amygdala (BLA), known to mediate fear and anxiety and for assigning emotional valence to cognitive processes, densely innervates the medial prefrontal cortex (mPFC), a homologue of the human anterior cingulate cortex, mediating emotional, attentional, and motivational behaviors. Because these two corticolimbic regions appear to contribute to the integration of emotion and cognition during the adolescent period, we sought to characterize the development of their connectivity, with particular interest in the role of the GABAergic interneuron. We then altered activity of this limbic relay with sustained activation of the BLA during a critical window of amygdalo-cortical innervation. This procedure may serve as a model for adolescent anxiety, and it also has implications for the development of schizophrenia.
Methods: Using the rapid anterograde tracer, biocytin, delivered stereotaxically to the posterior division of the BLA, amygdalofugal fiber density was analyzed within the mPFC at successive postnatal time points through development (postnatal days 6-120). We also analyzed the interaction of BLA fibers with GABA-immunoreactive (-ir) elements within the mPFC using light, confocal and electron microscopy. A novel, minimally-invasive, chronic infusion method was then devised and implanted to deliver the GABAA receptor antagonist, picrotoxin, within the BLA over a critical two-week post-weanling period. The electrophysiologic effects of this developmental picrotoxin infusion (DPI) on mPFC GABA currents were studied using slice preparations. A rapid Golgi method was also used to investigate changes in neuronal morphology, and the effects of DPI on GABAergic interneurons was studied with immunocytochemistry.
Results: The density of labeled [[Unsupported Character - ﬁ]]bers originating from the BLA increases dramatically within layers II and V of the mPFC during the late postweanling period. Light and confocal microscopy revealed that BLA fibers interacted with virtually every GABAergic neuron observed, having an 8-fold curvilinear increase in axodendritic contacts and a 3-fold increase in axosomatic contacts through development. Ultrastructural analysis demonstrated that the greatest proportion of BLA appositions were with GABA-negative spines (30.8%) and GABA-positive dendritic

shafts (35.5%). DPI was associated with a dramatic reduction in parvalbumin-ir neurons within the mPFC, and electrophysiological recordings from pyramidal neurons revealed significantly decreased GABA currents. Golgi staining demonstrated increased neurite sprouting for both stellate and pyramidal neurons in the BLA with a significant increase in spine density.
Discussion: Late development of amygdalo-cortical connectivity may provide an anatomical basis for the integration of normal as well as abnormal emotional behavior during maturation. The GABAergic interneuron is a primary target for amygdalar afferents and positioned to modulate limbic processing. Loss of GABAergic function may result in dysregulation of amygdalo-cortical function and can occur with increased activation of the amygdala during a critical period of corticolimbic development, such as with increased arousal states during adolescence and early adulthood.
Disclosure: M.G. Cunningham, None.

The Impact of Anxiety on Developmental Trajectories of Amygdala Functional Connectivity in Adolescents: A Resting State fMRI Study
Amy K. Roy*, Kristin Gotimer, Erica L. Dixon, Adriana Di Martino, Monique Ernst, Daniel Pine, Francisco Castellanos, Michael Milham

NYU Child Study Center, New York, NY

Background: Neurodevelopmental theories suggest that adolescence is characterized by a significant gap between the developmental trajectories of prefrontal and limbic regions. As such, the emotional and behavioral changes observed during this developmental period may emerge from interactions between regions such as the amygdala and prefrontal cortex that have been implicated in emotion regulation processes. Anxiety disorders, including generalized anxiety disorder and social phobia, that show an increased onset during adolescence are characterized by hyperresponsivity of the amygdala as well as dysfunction of related prefrontal regions. While there is some evidence that amygdala functional connectivity (FC) is altered in adolescents with anxiety disorders, this has not been evaluated systematically within a developmental context. Therefore, the aim of the current work was twofold: (1) to evaluate the intrinsic FC of the amygdala across development; and (2) to examine differences in amygdala FC between adolescents with anxiety disorders and typically developing age-matched controls. Measures of the intrinsic FC of the amygdala were obtained using novel resting-state functional magnetic resonance imaging (fMRI) methods.
Methods: Participants were 90 typically developing children (ages 7-12; $n = 31$), adolescents (ages 13- 19; $n = 26$), and adults (ages 20-40; $n = 33$) and 13 adolescents diagnosed with a primary anxiety disorder (ages 12-17). During the MRI session, participants were scanned for 6 minutes at rest (e.g., lie still with eyes open) and a high resolution anatomical scan was obtained for registration purposes. Data preprocessing and functional connectivity analyses were based on those used in our previous study of amygdala FC in adults (Roy et al., 2009). Regions of interest (ROIs; total amygdala and basolateral, centromedial, and superficial subdivisions) for each hemisphere were determined using stereotaxic, probabilistic maps implemented in FSL's Juelich histological atlas. To address our first aim, analyses of variance were used to evaluate differences in amygdala FC across the three age groups. To address the second aim, we examined group differences in amygdala FC between adolescents with and without anxiety disorders.
Results: (1) Significant differences in amygdala FC were observed across the three developmental groups. In particular, children showed greater total amygdala FC than adolescents and adults across several cortical and subcortical regions including the inferior frontal gyrus, precuneus, and thalamus. No differences between adolescents and adults were observed. (2) Adolescents with anxiety disorders showed altered FC between the total amygdala and the dorsolateral prefrontal cortex, suggestive of altered emotion regulation capacity. Further analyses showed specific disruptions in the FC of individual amygdala subdivisions as well.
Discussion: These results suggest significant developmental changes in the intrinsic FC of the amygdala that may be altered in adolescents with anxiety disorders.
Disclosure: A.K. Roy, None.

Patterns of Functional Activation and Co-Activation During Fear and Reward Processing in Adolescent Health and Anxiety
Amanda Guyer*

University of California Davis, CA

Background: Neurodevelopmental models of psychopathology suggest that altered subcortical reactivity and protracted cortical development renders adolescence a vulnerable period for anxiety. One hypothesis suggests that an imbalance in cognitive input from cortical regions relative to emotion-driven input from subcortical regions alters the processing of salient stimuli that correlates with information-processing symptoms consistent with anxiety (e.g., attentional biases to threat). Considerable work implicates abnormal neural activation and disrupted attention to facial-threat cues in adult anxiety disorders. We extended this past work to adolescents with and without anxiety disorders by examining functional activation in "fear circuity" (e.g., amygdala and ventral-lateral prefrontal cortex (vlPFC)) during social threat processing and in "reward circuitry" (e.g., striatal and orbitofrontal cortex (OFC)) during reward processing.
Methods: Two event-related functional magnetic resonance imaging (fMRI) tasks were used. Fear-circuitry response to anticipated social evaluation from peers was assessed with an ecologically-valid Chatroom task. Participants were led to believe they would interact online with a study participant, viewed photographs of these "peers," and rated their interest in chatting with each. Participants were photographed and told the peers would also rate them. During a subsequent fMRI scan, participants rated the peer's interest in chatting with them. Reward-circuitry response to anticipated reward cues was assessed with the Monetary Incentive Delay task, where participants responded to incentives of varying magnitude to be gained or lost. AFNI was used for between-group and psychophysiological interaction (PPI) connectivity analyses to assess hypothesized patterns of event-related functional activations and co-activations.
Results: Anxious vs. healthy adolescents showed amygdala hyperactivation when anticipating peer evaluation from undesirable peers, $t_{26} = 3.53$ (right amygdala peak: 27, −3, −21). PPI analyses revealed a significant positive association between amygdala and vlPFC activation in anxious vs healthy adolescents in response to these stimuli, $t_{26} = 3.79$ (BA47: -32, 44, −24). Anxious vs. healthy adolescents showed caudate ($F(2,55) = 3.52$, $p = .036$) and putamen ($F(2,55) = 3.94$, $p = .025$) hyperactivation when anticipating large incentives. Guided by these between-group differences in striatal response, PPI analyses of striatal-OFC co-activation patterns are underway.
Discussion: Past research found amygdala-vlPFC dysfunction in adolescents with anxiety disorders when viewing negatively-valenced social stimuli in threatening contexts. The current findings suggest that fear-circuitry dysfunction also manifests in anxious adolescents who misperceive anticipated social evaluation from positively-valenced peers as threatening. In addition, the present results indicate striatal hyperactivation in anxious adolescents to non-social reward cues of high salience that may co-activate with orbitofrontal regions involved in regulating behavior associated with reward sensitivity. Mapping patterns of functional connectivity in fear and reward circuitry can enrich neurodevelopmental models of anxiety and inform pediatric anxiety treatments regarding specific symptom-relevant perturbations in cognitive processing of salient stimuli.
Disclosure: A.E. Guyer, None.

Panel Session
Interaction Between Adult Hippocampal Neurogenesis, Hypothalamic-Pituitary-Adrenal Axis Regulation and Mood-Related Behavior

Role of the Hypothalamic-Pituitary-Adrenal Axis and Cytokine Signaling in the Antineurogenic and Behavioral Responses to Stress

Ronald Duman*

Yale University, New Haven, CT

Background: Brain imaging studies have consistently reported that stress-related psychiatric disorders, including depression and PTSD, result in atrophy of limbic structures implicated in these illnesses, most notably the hippocampus. Studies in rodents report that repeated stress causes retraction of apical dendrites of pyramidal neurons, as well as decreased neurogenesis in the adult hippocampus. Investigations of the mechanisms underlying the reduction of hippocampal neurogenesis have focused on the HPA axis, and demonstrate a role for elevation of glucocorticoids. In addition, more recent studies provide evidence for that elevation of cytokines play a significant role in the actions of stress. Studies of the interactions of the HPA axis and cytokines and related signaling cascades will be presented and discussed.
Methods: Rats or mice were exposed to chronic unpredictable stress (CUS), and sucrose preference was determined. Behavior was also tested in novelty suppressed feeding (NSF), and other models of anxiety. The role of cytokines, specifically interleukin-1β (IL-1β) was assessed in rat by administration of an inhibitor IL-1Ra and in mutant mice by deletion of the IL-1R. Levels of neurogenesis were assessed by injection and labeling newborn cells with BrdU. *In vivo* and *in vitro* studies were conducted to examine related signaling pathways including NFκB. The expression of IL-1R1 and NFκB in neural progenitor cells was assessed by immunohistochemistry and in NFκB reporter mice.
Results: Administration of an IL-1β antagonist completely blocked the decrease in sucrose preference and reduction in hippocampal neurogenesis caused by exposure to CUS, and a similar blockade was observed in IL-1R deletion mutant mice, which also displayed reduced anxiety in several models. Administration of an NFκB inhibitor also blocked the actions of CUS on sucrose preference and neurogenesis. Cellular localization studies demonstrate that IL-R1 is expressed on neural progenitor cells and that CUS increases NFκB signaling in these same cells. *In vitro* studies confirm that IL-1β directly inhibits the proliferation of neural progenitor cells and that these effects are mediated by NFκB signaling.
Discussion: The results demonstrate that IL-1β and NFκB signaling underlie the anhedonic and antineurogenic effects of CUS. The data also demonstrate that inhibition of neurogenesis occur via direct effects of IL-1β on neural progenitor cell. This contrasts with the results of another study, which reports that the effects of CUS are blocked by removal of adrenal-glucocorticoids, indicating an indirect effect of IL-1β with activation of the HPA axis as an intermediary. Further studies are needed to examine cytokine and HPA axis interactions. In either case, the results indicate that IL-1β and NFκB are novel targets for the treatment of stress related disorders, including depression and anxiety. Studies are being conducted to identify and validate these and related targets that either block or regulate cytokine release and signaling.
Disclosure: R.S. Duman: Part 1; Lilly. Part 2; Lundbeck. Part 3; Taisho. Part 4; Organon. Part 5; Repligen.

Impact of Hippocampal Neurogenesis on Mood and HPA Axis Function

Amar Sahay, Kimberly Scobie, Alexis S. Hill, René Hen*

Columbia University, New York, NY

Background: Adult hippocampal neurogenesis is a unique form of plasticity that generates new neurons in the dentate gyrus throughout life. Adult-born neurons have been implicated in both cognitive functions and in mediating the behavioral effects of antidepressants. However, it is not known whether stimulation of adult hippocampal neurogenesis is sufficient to improve cognition and mood.
Methods: Here we use an inducible genetic gain-of-function strategy to cell autonomously augment adult neurogenesis. Specifically, we take advantage of the fact that a significant fraction of adult-born hippocampal neurons undergo apoptotic cell death during the first weeks after their birth. Therefore we reasoned that a blockade of apoptotic cell death in young neurons would result in an increase in neurogenesis.
Results: We show that mice in which the pro-apoptotic gene, *Bax*, is deleted specifically in adult progenitors have increased survival of adult-born dentate granule neurons, exhibit enhanced neurogenesis-dependent synaptic plasticity and discriminate between similar contexts more efficiently than controls. In addition, increasing the number of adult-born neurons produced an antidepressant-like behavioral response in mice that were exposed to a chronic stress paradigm. Furthermore we show that mice with increased neurogenesis have a reduced response to stress as measured by lower levels of circulating corticosterone. These results together with ablation studies suggest that young hippocampal neurons contribute to the modulation of the HPA axis.
Discussion: Our findings suggest that strategies aimed at stimulating hippocampal neurogenesis may impact mood as a consequence of their effect on HPA axis function. Furthermore, our results suggest that inhibitors of cell death may be used in antidepressant therapies.
Disclosure: R. Hen, Brain Cells, consultant; AstraZeneca, consultant.

The Impact of Suppression of Adult Hippocampal Neurogenesis on Hypothalamic Neuroendocrinology

Keri Martinowich*, Robert Schloesser

National Institute of Mental Health, Bethesda, MD

Background: The hippocampus shows remarkable structural and functional plasticity. An important component of adult structural plasticity is the unique capacity of the hippocampus for lifelong neurogenesis. Decreases in hippocampal neurogenesis have been identified in various animal models of stress and after glucocorticoid (GC) administration. Stress and hypothalamic pituitary adrenal (HPA) axis dysregulation are key factors in the pathophysiology of mood disorders. The hippocampus has been recognized for its capacity as a potential negative feedback regulator of the HPA axis. Whether generation of newly born granule cells in the adult hippocampus is important for hypothalamic control and normal endocrine functioning has not been well studied.
Methods: To study the impact of newly born granule cells on hypothalamic neuroendocrinology we conducted experiments using transgenic mice that allow conditional ablation of newborn neurons. In these mice, herpes-simplex virus thymidine kinase (HSV-tk) is expressed under control of the human glial fibrillary acidic protein (GFAP) promoter. This promoter restricts HSV-tk expression to astrocytes and neural progenitors and administration of the antiviral drug valganciclovir (VGCV) ablates actively dividing GFAP+ cells. Transgenic animals undergoing prolonged VGCV treatment (NG-) completely lack hippocampal neurogenesis while wild-type animals undergoing prolonged treatment (Ctrl) retain intact hippocampal neurogenesis. To study the stress response and neuroendocrine function after suppression of neurogenesis, we measured GC levels, mapped hypothalamic neuronal activation patterns, analyzed hypothalamic gene expression and used *in situ* hybridization and immunohistochemistry to map cell populations driving changes in patterns of expression.
Results: We measured plasma concentrations of corticosterone (cort) in Ctrl and NG- mice in the home cage and after mild stress exposure. As expected, stress exposure resulted in significantly increased cort in both groups. However, comparison of groups after stress revealed that

NG- mice show significantly higher cort. Importantly, we verified that NG- animals were not chronically stress by showing no difference between Ctrl and NG- animals in total body weights or in weights of adrenals, thymus and testes. It has been shown that differences in levels of hippocampal glucocorticoid receptor (GR) expression are correlated with GC feedback sensitivity and the HPA axis response. We examined hippocampal GR expression in Ctrl and NG- mice and found no differences suggesting that suppression of neurogenesis affects HPA axis inhibition through an alternative mechanism. To better understand the HPA axis dysregulation following stress we carried out c-fos labeling in the hypothalamus of Ctrl and NG- mice and identified significantly different patterns of neuronal activation. Genome-wide analysis of hypothalamic gene expression provided further evidence that systems involved in neuronal activation were significantly changed. Expression changes for several candidate genes pointed to changes in specific neuronal sub-populations within the hypothalamus.
Discussion: Our studies provide strong preliminary evidence that newborn neurons play a functional role in the hippocampal circuitry and are particularly important for the hippocampal output capacity that provides normal inhibitory control over the hypothalamus and its normal endocrine functioning.
Disclosure: K. Martinowich, None.

Is Hippocampal Neurogenesis Involved in the Regulation of the HPA Axis?
Alexandre Surget, Tanti Arnaud, René Hen, Catherine Belzung*

Université François Rabelais, Tours, France, Columbia University, New York, NY, Université François Rabelais de Tours, Tours, France

Background: Hippocampal neurogenesis, if required for the therapeutic effects of monoaminergic-acting antidepressants (ADs) such as fluoxetine or imipramine, is not necessary to the effects of antidepressant-like compounds acting via the hypothalamus-pituitary-adrenal (HPA)-axis such as a CRF1 antagonist or the V1b antagonist. This suggests that another mechanism might underlie the therapeutic effects of the hypothalamic-pituitary-adrenal (HPA)-acting drugs. It is well known from the literature that the HPA axis is disrupted in depression, and that the restoration of this dysfunction is necessary to observe ADs effects. As it is well known that the hippocampus participates to the negative feedback over the HPA axis, we thus hypothesized that one of the functions of neurogenesis in the AD's effects may concern its ability to restore this feedback when it has been disrupted. We will present experimental evidence supporting this hypothesis.
Methods: BALB/c mice were subjected to unpredictable chronic mild stress (UCMS) and chronically treated with fluoxetine. Dexamethasone suppression test was applied using either ip injection -to assess the integrity of the HPA-negative-feedback- or using intra-hippocampal administration, to evaluate the hippocampal participation in this feedback. Plasmatic corticosterone was measured via RIA. Suppression of hippocampal neurogenesis was achieved via focal irradiation. Brain activation was done using fos immunohistochemistry.
Results: We demonstrate that UCMS reduces the number of new hippocampal neurons and decreases the negative feedback on the hypothalamo-pituitary-adrenal (HPA) axis measured by the dexamethasone suppression test. This involves the hippocampus, as intra-hippocampal dexamethasone also elicits corticosterone suppression. This suppression is absent after UCMS, an effects that is reversed by fluoxetine. This is achieved via a pathway going from the hippocampus to the paraventricular nucleus of the hypothalamus, via a relay in the bed nucleus of stria terminalis (BNST), as intra-hippocampal dexamethasone induces an activation of the BNST, that is reduced by UCMS and counteracted by fluoxetine. While ablation of hippocampal neurogenesis has no effect on its own in the intra-hippocampal dexamethasone test, UCMS elicits a deficit in corticosterone suppression. This deficit is reversed by fluoxetine in non irradiated mice, but not in mice having abolition of hippocampal neurogenesis.
Discussion: These results suggest ADs act through neurogenesis to re-establish hippocampal regulation of the HPA axis.
Disclosure: C. Belzung, None.

Panel Session
Intersecting Neurobiology and Epidemiology of ADHD and Drug Addiction

Stimulant Treatment History, Assigned and Self-Selected, as Predictor of Late Adolescent Substance Abuse in ADHD
Brooke S.G. Molina*

University of Pittsburgh, Pittsburgh, PA

Background: A controversial issue in research on ADHD is the effect of the CNS stimulant treatments on risk for substance use disorders (SUDs). The small literature that has examined this question has yielded mixed results with some studies reporting worsening, and others reporting attenuation, of SUD risk. A limited number of large longitudinal studies of children with ADHD exist, and none provide prospectively gathered treatment data that follow an initial period of random assignment to evidence-based treatments. The longitudinal follow-up of the children in the multisite Multimodal Treatment of ADHD study (MTA) provides this opportunity.
Methods: The original MTA sample consists of 579 children who were diagnosed with DSM-IV Combined Type ADHD and randomly assigned to one of 4 treatment groups for 14 mos: intensive medication management, intensive behavior therapy (Beh), the combination (Comb), or referral to community care. The sample was interviewed at immediate post-treatment, 2, 3, 6, 8, 10, and most recently 12, yrs post baseline. A local normative comparison group (LNCG; $n = 289$) of classmate controls was recruited at the 2-year assessment and has been interviewed in parallel. This abstract describes results from the 8-year assessment (80% study retention; M age = 17).
Results: Substance use/SUD was examined as a function of 1) childhood ADHD diagnosis; b) randomly assigned treatment in childhood and past year stimulant treatment; and c) cumulative stimulant treatment since baseline. Use of any substance beyond selected levels was the primary outcome. Random effects regression indicated a main effect of ADHD group on substance use overall, $p < .0001$, and at all time points, $p = .0025$ to.0394. Using GEE models, neither treatment assignment nor past year stimulant treatment were significant predictors of substance use with one exception: children who received Beh or Comb were less likely to report substance use at 3 yrs than were the other children, $p = .038$. Propensity score matching analyses were used to create matched pairs of subjects with minimal differences in potential confounders (e.g., ADHD symptom severity, treatment group assignment) but large differences in cumulative stimulant treatment. Using these matched pairs, no significant associations were found between cumulative medication treatment and substance use or SUD, all p-values $> .25$ (up to.73).
Discussion: Our results provide confirmation of substance use risk in adolescence for children diagnosed with combined type ADHD. Our results extend findings of potential behavior therapy benefit on the initiation of substances in early adolescence, but these early protective effects appear to have worn off, as they did for other symptom and functioning variables by the 8 year follow-up (Molina et al., 2009, JAACAP). We previously reported no significant associations between past year stimulant treatment and substance use in early adolescence (Molina et al., 2007, JAACAP). Our current findings provide a carefully statistically controlled extension of those results at the age when

substance use is ascending. Results suggest that prolonged stimulant treatment affords no visible protection from or predisposition toward adolescent substance use or SUD. A limitation of these findings is that the peak period of SUD risk in early adulthood has not occurred. If complete by December 2010, we will present the results of parallel analyses with the 12-year data.
Disclosure: B.S. Molina: None.

The Impact of Childhood Stimulant Medication on Later Substance Use
William E. Pelham Jr.*

Florida International University, Miami, FL

Background: Stimulant treatment of ADHD children has been commonplace for 40 years, and is currently recommended by influential professional organizations (e.g, American Academy of Child and Adolescent Psychiatry, 2007) as the first line and sufficient treatment for ADHD in childhood. At the same time, basic research on CNS drug effects in animals (e.g., behavioral sensitization) suggests that childhood stimulant therapy for ADHD could theoretically exacerbate later drug use in ADHD individuals. Given that ADHD is a risk factor for later substance abuse, this concern has great public health significance. Prior studies in ADHD children that address this question have revealed mixed findings with some suggesting that stimulants increase, decrease, and do not impact the later substance use of ADHD children. The present study reports on the longest and largest continual follow up of an ADHD sample that has evaluated this relationship. The Pittsburgh ADHD Longitudinal Study (PALS), funded by NIDA and NIAAA, follows 364 ADHD children and 240 controls into adolescence and young adulthood. This paper reports on stimulant treatment in the ADHD children and its relationship to later substance use in the PALS sample.
Methods: ADHD children were ascertained in childhood as a function of their referral to a university clinic. Information about lifetime stimulant treatment was gathered using childhood intake records, doctor's records, and parent report. Substance use outcomes (marijuana, alcohol, and tobacco) were measured with standardized instruments through self-report. Ordinal and logistic regressions examined the effects of length of time medicated with stimulants on the substance use outcome domains, controlling for a variety of childhood and concurrent measures (e.g., age, comorbid conduct problems, ongoing school functioning) that were thought to serve as possible confounds.
Results: Stimulants were used to treat 91% of the sample at some point in time, with the highest percentage medicated at grade 5 and a wide range of length of time medicated (0-16 years). For adolescents (age 11-17), longer stimulant use is not related to heavy use of alcohol, cigarette or marijuana when analyses control for current age. For young adults (age 18+), longer stimulant use predicts *higher* use of all three substances – that is longer stimulant pharmacotherapy is associated with higher frequent use of the substances.
Discussion: In contrast to earlier reports widely advertised by pharmaceutical companies, we can find no evidence that stimulant medication taken in childhood and adolescence has a beneficial impact on ADHD children's substance use in adolescence and young adulthood. In contrast, we find no association in adolescence and an *adverse* effect in young adulthood. The implications of these results in light of findings from basic research on drug effects on animals and humans as well as current treatment of ADHD and alternatives to pharmacotherapy are discussed.
Disclosure: W.E. Pelham, Janssen Pharmaceuticals–reimbursement and honoraria for a week of talks in Japan in 2008, Part 1; Johnson and Johnson–I am Co-PI on an NIMH-funded study for which J & J has agreed to provide Concerta at no cost., Part 4.

Co-Occurring Childhood Disruptive Disorders, ADHD and Psychophysiological Deficits in Adolescents and Young Adults
William G. Iacono*

University of Minnesota, Minneapolis, MN

Background: Attention-deficit/hyperactivity disorder (ADHD) is comorbid with other childhood disruptive disorders (conduct and oppositional disorder), occurs in the offspring of substance abusing and antisocial parents, and is associated with the development of substance use disorders (SUDs) and antisociality. Factors that underlie these associations are not well understood. We hypothesize that a genetic vulnerability to behavioral disinhibition underlies these relationships, and that gene-environment interplay over the course of development influences which of these combinations of externalizing psychopathology are likely to arise in any given individual.
Methods: Participants were drawn from the 1900 twin families that are part of the Minnesota Center for Twin and Family Research. Two cohorts were recruited, one older, consisting of 17-year-old twins and their biological mothers and fathers, and the other younger consisting of 11-year-old twins and their parents. Twins in both cohorts were followed up at regular intervals (3-4 years) spanning adolescence and young adulthood. In-person psychiatric interviews with parents and their twins covered childhood disruptive disorders when the twins were younger and nicotine, alcohol, illicit drug use, and antisocial personality disorder when the twins were older. Participants were also underwent a two-hour psychophysiological assessment designed to tap putative endophenotypes associated with the development of substance use and related disorders, including the P300 event related potential derived from the "rotated heads" task originally introduced by Begleiter and colleagues.
Results: Parents with substance use disorders tend to have offspring at elevated risk for ADHD and each of the other externalizing disorders. Each of these disorders is moderately to highly heritable. At age 17, the covariance among externalizing traits and disorders can be modeled as a highly heritable general liability for externalizing behavior. At age 11, the covariance among the three childhood disruptive disorders can be similarly modeled as a highly heritable externalizing liability. At both ages, parent-offspring transmission reflects the influence of a general externalizing liability captured by substance use and antisociality in parents and expressed in offspring as childhood disruptive disorders (at age 11) and substance use and antisocial disorders (at age 17). P300 amplitude also shows moderate heritability; is associated with ADHD, substance use and abuse, and antisocial behavior; and forecasts the subsequent development of SUDs. The association between P300 amplitude and externalizing disorders reflects shared genetic effects.
Discussion: A highly heritable general liability, transmitted from parent to child, accounts in part for the association of ADHD with other childhood disruptive disorders and the eventual development of SUDs. This general liability can be indexed as reduced amplitude of the P300 brain potential. The general liability manifest as the covariance among externalizing disorders and the P300 endophenotype provide an alternative avenue for research aimed at uncovering the etiology of externalizing disorders by focusing on underlying mechanisms that tie them together. Molecular genetic studies focused on these phenotypes may provide a particularly attractive supplement to studies focused on the etiology of each disorder alone.
Disclosure: W.G. Iacono, None.

Decreased Motivation in ADHD Is Associated with Deficit in Dopamine Reward Pathway
Nora Volkow*

National Institute on Drug Abuse, Bethesda, MD

Background: ADHD is typically characterized as a disorder of inattention and hyperactivity/impulsivity but there is increasing

evidence of deficits in motivation. Here we test the hypothesis that decreased function of the brain dopamine reward pathway is associated with decreased motivation in ADHD.
Methods: To evaluate this hypothesis we assessed the correlation between the measures of dopamine D2/D3 receptor and dopamine transporter availability (obtained using Positron Emission Tomography and the radioligands [^{11}C]raclopride and [^{11}C]cocaine, respectively) in the dopamine reward pathway (midbrain and nucleus accumbens), and measures of trait motivation (assessed using the Achievement scale on the Multidimensional Personality Questionnaire or MPQ) in 45 mever medicated ADHD participants and 41 matched controls.
Results: Both D2/D3 receptors and DAT in midbrain and nucleus accumbens were lower in ADHD participants than in controls. The trait measure of motivation was lower in ADHD participants than in controls (11 ± 5 vs 14 ± 3, $p < 0.001$) and correlated with D2/D3 receptors (accumbens: $r = 0.39$, $p < 0.008$; midbrain: $r = 0.41$, $p < 0.005$) and transporters (accumbens: $r = 0.35$, $p < 0.02$) in ADHD participants, but not in controls. ADHD participants also had lower values in the Constraint factor and higher values in the Negative Emotionality factor of the MPQ but did not differ in the Positive Emotionality factor - and none of these were correlated with the dopamine measures. In ADHD participants trait motivation was also negatively correlated with symptoms of inattention (CAARS A, E and SWAN-I).
Discussion: These findings provide evidence that disruption of the dopamine reward pathway is associated with motivation deficits in ADHD adults, which may contribute to attention deficits and supports the use of therapeutic interventions to enhance motivation in ADHD. It also supports the notion of a "motivation deficit" in ADHD.
Disclosure: N.D. Volkow, None.

Panel Session
Neuregulin Session 1: A Gene at the Crossroads of Synaptic Plasticity, Psychiatric Disorders, and the Self Medication Theory of Smoking

Neuregulin and Nicotine: Cholinergic Modulation of Synaptic Plasticity in Mono Allelic Disruptions of Nrg1
Lorna W. Role*

Stony Brook University, Stony Brook, NY

Neuregulin 1 plays critical roles in trophic and synaptic processes in the brain. Therefore, isoforms and variants of this gene, whose products signal through the ErbB family of receptor tyrosine kinases, could influence psychiatric disease risk by perturbing brain development and/or synaptic plasticity. Nrg1 is emerging as a nodal element at the confluence of key genetic and neurochemical networks; detailed dissections of which could offer important insights into the mechanisms of various psychiatric disorders and their comorbidities, and uncover promising new targets for the development of next generation pharmacotherapies. Like the CHRNA7 gene, encoding the $\alpha 7$ nicotinic acetylcholine receptors ($\alpha 7$ nAChRs), the NRG1 gene has been linked to schizophrenia and associated sensory-motor gating deficits. Interestingly, the prominence of nicotine addiction in schizophrenic patients may be related to evidence that gating deficits are normalized by nicotine self-administration. This observation is consistent with the evidence showing that presynaptic Type III Nrg1 is required for sustained enhancement of hippocampal transmission by nicotine and for axonal targeting of $\alpha 7$ nAChRs. This talk will present findings from *in vivo* recording of sensory motor gating circuits in the type II Nrg1 heterozygous mice and provide evidence for altered cholinergic modulation of cortico limbic circuits in slice electrophysiological studies.
Disclosure: L.W. Role: None.

NRG-ErbB4 Signaling Regulates Synaptic Plasticity and Neuronal Network Activity: Relevance for Schizophrenia and Self-Medication by Smoking
Andres Buonanno*

National Institute of Child Health and Human Development, NIH, Bethesda, MD

Genes encoding N*euregulin-1* (*NRG-1),* the NRG-1 receptor *ErbB4*, and the acetylcholine receptor alpha-7 (nAChR-7) have been genetically associated with schizophrenia. The heavy smoking frequently associated with schizophrenia is suggestive that patients smoke to automedicate. We have investigated how NRG-ErbB signaling modulates neurotransmission, synaptic plasticity and neuronal network activity, biological functions proposed to be compromised in schizophrenia and that may be corrected by smoking. More specifically, our studies show that NRG-ErbB4 signaling regulates glutamatergic and dopaminergic synaptic transmission and plasticity, as well as neuronal network activity associated with working memory (i.e. gamma oscillations). We have used neurochemical, immunohistochemical, electrophysiological and behavioral approaches to investigate how NRG-ErbB4 signaling reverses LTP and regulates gamma frequency oscillations in the hippocampus of wild-type mice and mice harboring mutations in the ErbB4 receptor. We found that NRG-1 rapidly and dramatically stimulates hippocampal dopamine release, and that subsequent activation of D4 receptors (D4Rs) reverses LTP by promoting AMPAR internalization. The depotentiating effects of NRG-1, D4R agonist and theta pulse stimuli on LTP are blocked by several D4R antagonists (including clozapine), and are absent in D4R knockout mice. NRG-1 also enhances by approximately 20-fold the power, but not frequency, of kainate-induced gamma oscillations in acute hippocampal slices. Consistent with these observations, ErbB4 receptors are restricted to GABAergic interneurons including parvalbumin-positive basket cells that regulate gamma oscillatory power, and accumulates at glutamatergic postsynaptic sites. ErbB4 mutant mice manifest behavioral deficits, as well as altered hippocampal synaptic plasticity and gamma oscillatory activity. The effects of NRG-1 on dopamine and glutamate neurotransmission/plasticity and gamma oscillatory power, and the selective expression of ErbB4 in GABAergic interneurons, could have important implications for understanding how imbalances in NRG-ErbB signaling contribute to the pathophysiology associated with schizophrenia. Interestingly, the nAChR-7 and ErbB4 receptors have overlapping cellular expression patterns and are reported to modular similar biological processes, suggesting that the function of both pathways may overlap significantly. Support for this work was from the Eunice Kennedy Shriver NICHD Institute.
Disclosure: A. Buonanno: None.

NRG1 Signaling and Risk for Schizophrenia: Epistasis with ERBB4 and AKT1
Daniel Weinberger*

National Institute of Mental Health, NIH, Bethesda, MD

Background: *NRG1* is a schizophrenia candidate gene and plays an important role in brain development and neural function. Schizophrenia is a complex disorder, with etiology likely due to epistasis between genes. We sought to examine epistasis between *NRG1* and selected NMDA-glutamate pathway partners implicated in its effects, including ERBB4, AKT1, DLG4, NOS1, NOS1AP and to validate any epistatic interactions using imaging techniques in individuals with the interacting genotypes.
Methods: We used machine learning algorithms (MLAs) to examine epistasis in a schizophrenia case-control sample ($N = 296/365$) and then performed logistic regression on the variable importance measures (VIM) to confirm the significance of the interactions. fMRI during the N back task was acquired in an independent sample of normal subjects ($n = 172$) to test for the effect of clinically interacting genotypes on an intermediate biologic phenotype associated with

increased risk for schizophrenia-cortical inefficiency (i.e. increased BOLD response for a fixed level of performance).
Results: None of the individual markers showed significant association with schizophrenia after correction for multiple testing. However, we observed interaction between *NRG1* 5′ and 3′ SNPs: rs4560751-rs3802160 (likelihood ratio test (LRT) p = 0.00020) and schizophrenia which was validated using fMRI of working memory in healthy controls; carriers of risk-associated genotypes showed inefficient processing in dorsolateral prefrontal cortex (DLPFC) (p = 0.015, FWE corrected). We observed epistasis between *NRG1* (rs10503929; Val1066Ile) and its receptor *ERBB4* (rs1026882; LRT p = 0.035); a three-way interaction with these two SNPs and *AKT1* (rs2494734) was also observed (OR = 27.13; 95% confidence interval 3.30, 223.03; LRT p = 0.042). These same two- and three-way interactions were biologically validated via fMRI: healthy individuals carrying risk genotypes for *NRG1* and *ERBB4*, or these two together with *AKT1*, were disproportionately less efficient in DLPFC processing. Lower-level interactions were not observed between *NRG1/ERBB4-AKT1* in association or neuroimaging, consistent with biological evidence that NRG1-ERBB4 interaction modulates downstream AKT1 signaling. We searched a schizophrenia genome-wide association study dataset (GAIN) and the dataset described by Need et al. (2009) for replication of epistasis. Imputation of our VIM SNPs in GAIN could not be performed as only one SNP participating in epistasis was found in HapMap. Using flanking SNPs for our 5′-3′ epistasis, we observed two interactions with LRT p-values < 0.05 (rs7812662-rs7830691 (0.024) and rs10098401-rs17631978 (0.042)). In the separate datasets from Need et al, individuals carrying all 3 risk genotypes did show marginally increased risk for schizophrenia (OR = 1.60, 95% CI 0.98, 2.61, p-value = 0.062) versus those carrying no risk genotypes in the German sample and the Aberdeen Sample (LRT p-value = 0.076) with individuals carrying all three risk genotypes showing increased risk (OR = 1.91, 95% CI 0.98, 3.69, p-value = 0.056).
Conclusions: Our data suggest complex epistatic effects implicating a *NRG1* molecular pathway in cognitive brain function and the pathogenesis of schizophrenia. These results represent the first demonstration of the application of MLAs for identifying epistatic effects in psychiatric genetics and the first use of neuroimaging as an approach to biological validation of clinical epistasis.
Disclosure: D. Weinberger: None.

Self-Medicating with Tobacco in Individuals with ADHD and Schizophrenia

Jean Gehricke*

Department of Pediatrics, University of California, Irvine, Irvine, CA

Smoking prevalence rates have declined in the United States over the past 40 years but the proportion of smokers with psychiatric comorbidity has risen. According to the self-medication hypothesis, smoking rates in individuals with mental disorders are high because of the symptom-reducing effects of nicotine and other tobacco constituents. Studies suggest that individuals with schizophrenia and attention-deficit/hyperactivity disorder (ADHD) are not only at a higher risk for smoking initiation and subsequent nicotine dependence, but also less successful with smoking cessation. In addition to reviewing evidence for the self-medication hypothesis of tobacco use in schizophrenia, findings will be presented from studies on the association between ADHD and smoking. These studies reveal the reinforcing effects of nicotine and tobacco smoke on clinical symptoms, behavior and brain activity in individuals with ADHD and schizophrenia. Potential associations between these findings and neuregulin 1 will be discussed.
Disclosure: J. Gehricke: None.

Panel Session
Neurodevelopmental Genomics: Opportunities for Advancing Developmental Neuropsychiatry

Translating Between Genes, Brain, and Behavior in Williams Syndrome: A Unique Neurogenetic, Neurodevelopmental Model

Karen Berman*

National Institute of Mental Health, Bethesda, MD

Background: Williams syndrome (WS), a rare neurogenetic, developmental disorder caused by hemizygous microdeletion of approximately 1.6 megabases on chromosomal band 7q11.23, has a unique profile of striking behavioral features: remarkable hypersociability combined with differential impact on cognitive functions – some mildly affected while others, particularly visuospatial construction, are severely impaired. Because the genes involved are known, WS affords a privileged setting for investigating how genes are translated in the brain to produce cognitive and behavioral features.
Methods and Results: Using multimodal imaging (PET, MRI, MRS, fMRI, DTI), we have identified several fundamental aspects of the brain phenotype in Williams syndrome: 1) underlying the syndrome's hallmark visuospatial construction impairment, is a neurostructural anomaly and adjacent neural hypofunction in the dorsal visual processing stream, as well as hippocampal involvement; 2) underlying the syndrome's hallmark social cognition and personality features, are structural and functional anomalies in the insula and in the orbitofrontal cortex, an important affect and social regulatory region that participates in a fronto-amygdalar circuit found to be dysfunctional in WS; and 3) underlying several of these gray matter structural and functional abnormalities are altered white matter axonal tracts (measured *in vivo* with MR diffusion tensor imaging). Identification of these brain phenotypes in WS has motivated two experiments aimed at linking specific genes in 7q11.23, such as *LIMK1,* to the neural and behavioral features of the syndrome. First, in extremely rare individuals with small deletions that include only a subset of the genes deleted in classic WS_*LIMK1* and genes extending telomerically, but not *GTF2IRD1 and GTF2I*_we found reduced gray matter volume and activation in several dorsal stream regions, as was seen in classic WS. Because these individuals did not have *GTF2IRD1* and *GTF2I* deleted, disruption of these genes does not appear to be necessary to produce the observed hypofunction and reduced gray matter volume. Moreover, when analyses were restricted to six individuals with **only** *LIMK1* and *ELN* deleted, we a found a similar pattern. Because *ELN* plays little part in neural organization, our findings strengthen the evidence for an important role of *LIMK1* in the dorsal stream abnormalities and associated visuospatial impairment in WS. Second, and further implicating *LIMK1*, we found that allelic variation in this gene is associated with reduced gray matter volume in the dorsal stream in a large cohort of healthy individuals (N = 244, P < .001).
Discussion: The incisive approach afforded by this known genetic abnormality and the specific cognitive/behavioral profile it produces in WS provides a unique opportunity for the study of neurogenetic, developmental mechanisms. The compelling evidence for *LIMK1* as a candidate mechanism for the visuospatial cognition problems in WS is supported by the fact that this gene is important for neural development, including axon guidance, synapse maturation, and dendrite formation, abnormalities of which appear to contribute to dorsal visual processing stream anomalies. Further work longitudinally documenting the developmental trajectory of these brain phenotypes in children with WS is underway.
Disclosure: K.F. Berman, None.

Fragile X Syndrome
Stephen T. Warren*

Emory University, Atlanta, GA

Background: Fragile X syndrome (FXS) is the paradigm of how use the lessons learned from a single gene disorder to approach a more complex disorder such as autism. FXS is due to the functional absence of the protein FMRP that regulates local protein synthesis, in an activity dependent manner, by binding to key transcripts within the postsynaptic space. The absence of FMRP results in excess translation of these messages that in turn enhances AMPA receptor trafficking thereby weakening of the synaptic strength, which presumably leads to the phenotype of intellectual disability and autism. To reduce the stimulatory signal to trigger translation, Group 1 mGluR receptor antagonists have been shown to "balance" the loss of the translational repressor FMRP and rescue FXS phenotypes in animal models.
Methods: In order to identify additional small molecules that may have potential therapeutic use in FXS, we developed a *Drosophila* chemical library screen exploiting the lethality of *dfmr1*-deficient flies on food containing excess glutamate. We screened 2,000 small molecules and drug in independent vials each containing 40 M of the test compound and 12 *dfmr1*-deficient embryos and scored for survival.
Results: Ten compounds were identified to reproducibly rescue glutamate lethality in the *dfmr1*-deficient flies. Among these were three GABA agonists and two muscarinic agonists.
Discussion: The loss of FMRP in FXS leads to activity-dependent over translation at the synapse. This results in the apparent outcome of excess glutamate excitatory activity, although the signals are downstream of the glutamate receptor. Thus group 1 mGluR receptor antagonists can dampen this enhanced excitatory signaling. Likewise, the data reported here point to another approach to dampen excitatory neurons; activation of the GABA inhibitory pathway. Although a well established interaction, the use of GABA agonists in FXS had not been widely considered previously. Clinical trials are now underway evaluating these compounds in patients with FXS.
Disclosure: S.T. Warren, Seaside Therapeutics, Inc (Chair, Scientific Advisory Board), Part 1; Seaside Therapeutics, Inc (Chair, Scientific Advisory Board), Part 2.

Genetic Underpinnings of Autism Spectrum Disorders
Hakon Hakonarson*

Children's Hospital of Philadelphia, Philadelphia, PA

Background: Autism spectrum disorders (ASDs) represent a group of childhood neurodevelopmental and neuropsychiatric disorders characterized by deficits in verbal communication, impairment of social interaction, and restricted and repetitive patterns of interests and behavior. Autism spectrum disorders affect as many as 1 in 150 children, making it more common than pediatric cancer, diabetes, and AIDS combined. It occurs in all racial, ethnic, and social groups and is four times more likely to strike boys than girls. ASD has debilitating consequences for the affected individual and entails great burden to the individual's family and to society as a whole. Although there have been concerted efforts to define the causes and best treatments for ASD, until very recently, progress has been quite limited.
Methods: We used Genome-wide association (GWA) approach to identify association to both common and rare variants in the human genome.
Results: We report the first confirmed genetic susceptibility locus for ASDs, as well as a group of individually rare copy number variants that belong to specific gene pathways, in particular, gene networks involving neuronal cell adhesion molecules and ubiquitin family of genes showing variations, both common and rare, that predispose to ASDs.
Discussion: These new discoveries, when coupled to high-throughput sequencing approaches of the human exome and ultimately the entire genome are likely to lead to new and improved diagnostic and therapeutic approaches to more effectively treat ASDs and related neurodevelopmental disorders.
Disclosure: H. Hakonarson, None.

Neurodevelopmental Genomics: Integration of Deep Phenotyping and Deep Sequencing
Raquel E. Gur*, Ruben Gur, Monica Calkins, Jan Richard, James Loughead, Hakon Hakonarson

University of Pennsylvania, Philadelphia, PA

Background: Mental illnesses emerge during childhood and adolescence and many persisting into adulthood. To prevent or intervene in this pathway it is essential to identify premorbid risk factors and early manifestations of these conditions. An integrative approach is required to elucidate genetic, epigenetic, and environmental factors, which shape neurodevelopmental trajectories. Linking disease phenotypes and intermediate variables, modulating disease manifestations in genetically susceptible individuals, will help articulate how these factors contribute to shaping the development of brain systems that underlie complex behavior. The ability to obtain quantitative phenotypic measures of brain and behavior affords bridging molecular biology with the phenomenology of disease. Large phenotypically and genomically characterized samples are required for reliable progress. A collaboration between the Center for Applied Genomics at Children's Hospital of Philadelphia and the Brain Behavior Laboratory at the University of Pennsylvania capitalizes on an unprecedented opportunity: an already genotyped large sample of children and adolescents, who have consented to being contacted for further research.
Methods: A cohort of 10,000 genotyped children and adolescents, age range 8-21, from the greater Philadelphia area are being characterized phenotypically and assessed along behavioral dimensions indicating vulnerability to major mental illnesses. The phenotypic dimensions include attention deficit, anxiety, mood, psychosis proneness and substance abuse. Neurobehavioral measures are obtained of cognitive and emotion processing related to neural systems vulnerable to neurodevelopmental aberrations. In addition, neuroimaging studies are conducted in a random subsample of 1000 to establish neural substrates of behavioral phenotypic trajectories. Neuroimaging modalities include structural imaging with deformation based morphometric characterization, diffusion weighted imaging examining white matter connectivity, arterial spin labeled perfusion imaging measuring resting cerebral blood flow, and blood oxygenation level dependent measures of cerebral activation for neurobehavioral probes of neural circuitries implicated in major mental illnesses.
Results: Computerized assessment procedures based on a modified K-SADS have been established with validation procedures. We started with the oldest group (18-21), who is departing for college or work. The table shows the composition of the current sample, about 4 months into data collection. The rate is now accelerating and we will be able to present phenotypic data on the clinical assessment, the neurobehavioral data that show the developmental effects of age and gender, and neuroimaging measures.

	Male 18 +	Female 18 +	Male <18	Female <18	Total
Participants	280	400	57	68	805

Discussion: Establishing a well-phenotyped sample with behavioral measures related to developmental disorders, neurobehavioral and neuroimaging measures, provides the growth-chart of typical development. Against it, children with vulnerability can be examined and gene networks underlying neuronal vulnerability leading to mental disorders can be established. Integrative analyses of the genomic, epigenetic, imaging and phenotypic datasets and measures will provide for optimal genotype-phenotype association.
Disclosure: R.E. Gur, Pfizer, Part 1; AstraZeneca, Part 1; Pfizer, Part 4; AstraZeneca, Part 4.

Panel Session
Novel Targets for the Development of Medicines for the Treatment of Tobacco Dependence

Translational Approaches for Nicotine Dependence: Utility of Genetically Modified Mice

Christie D. Fowler*, Jonathan Hollander, Paul Kenny

The Scripps Research Institute, Jupiter, FL

Background: Tobacco addiction imposes a significant negative impact on health worldwide; however, attempts to quit smoking are most often unsuccessful. Currently available smoking cessation therapeutics demonstrate limited effectiveness with reported adverse side effects being of concern. Thus, a pressing need exists to develop novel therapeutics for efficacious and safe smoking cessation.
Methods: Genetically modified mice are powerful tools for understanding the mechanics of drug addiction and are revealing important insights into the actions of nicotine in the brain. Recently, we developed a reliable mouse intravenous nicotine self-administration procedure to more directly examine addiction-related mechanisms through the use of genetically modified mice.
Results: Using the mouse self-administration procedure, we assessed volitional nicotine intake in mice with null mutation in the α5 nicotinic acetylcholine receptor (nAChR) subunit gene (*CHRNA5*). When given access to a range of nicotine doses, the α5 knockout mice exhibited dramatically increased intake compared with wildtype mice, particularly when higher unit doses of nicotine were available. Further, lentivirus-mediated re-expression of α5 nAChR subunits in the habenulo-interpeduncular pathway completely "rescued" this pro-addiction phenotype. These findings suggest that α5-containing (denoted α5*) nAChRs regulate nicotine intake through a novel mechanism independent of the midbrain dopamine systems classically implicated in reward and addiction, the major neuroanatomical target for available smoking cessation agents. Interestingly, the α5* nAChR partial agonist ABT-089 decreased nicotine self-administration in rats, suggesting that α5* nAChRs may be important targets for the development of novel smoking cessation pharmacotherapeutics. In the second series of experiments, we examined nicotine self-administration behavior in mice with null mutation in the hypocretin-1 receptor (Hcrt-1; also termed orexin-1) gene (*HCRTR1*). In contrast to the α5 knockout mice, Hcrt-1 knockout mice displayed significantly decreased nicotine intake compared to wildtype littermates across a range of doses. Further, the Hcrt-1 receptor antagonist SB-334867 also decreased nicotine intake and attenuated nicotine-induced lowering of brain-stimulation reward thresholds in rats. Thus, Hcrt-1 receptors may also be an important target for development of novel smoking cessation agents.
Discussion: Together, these findings reveal fundamental insights into the mechanisms of nicotine reinforcement and provide a foundation for further development of clinically efficacious therapeutics.
Disclosure: C.D. Fowler, None.

Fatty Acid Amide Hydrolase as a Novel Molecular Target for Tobacco Dependence

Danielle Piomelli*, Maria Scherma, Zuzana Justinova, Marco Pistis, Steven R. Goldberg

University of California, Irvine, Irvine, CA, Preclinical Pharmacology Section, Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD, B.B. Brodie Department of Neuroscience, University of Cagliari, Cagliari, Italy

Endogenous cannabinoid signaling is known to be involved in nicotine addiction, but the functions of specific endocannabinoid transmitters in the abuse-associated effects of nicotine have long remained unclear. This gap has recently been filled by the discovery of drugs that block the degradation of the major endocannabinoids operating in the brain - anandamide and 2-arachidonoylglycerol. It has been shown that pharmacological inhibitors of the anandamide-deactivating enzyme, fatty acid amide hydrolase (FAAH), selectively magnify the intrinsic modulatory actions of this transmitter on brain circuits that control pain and emotion. Indeed, several compounds in this class have been recently advanced to clinical testing for the treatment of pain. Experiments in rats have shown that one of these compounds, called URB597, markedly reduces both the rewarding effects of nicotine and the drug's ability to increase dopamine release in the shell of the nucleus accumbens, a core component of the reward system in the brain. Studies in squirrel monkeys have further demonstrated that URB597 reverses nicotine self-administration and prevents nicotine-induced reinstatement, a model of relapse into nicotine use. These findings suggest that FAAH inhibition counteracts the addictive properties of nicotine, and point to FAAH as a novel molecular target for tobacco dependence.
Disclosure: D. Piomelli, None.

Restoring Glutamate Homeostasis Inhibits Nicotine Relapse

Peter Kalivas*, Lori Knackstedt, Steven LaRowe, Robert Malcolm, Himanshu Upadhyaya, Athina Markou

Medical University of South Carolina, Charleston, SC, Med Univ So Carolina, Charleston, SC, UC San Diego, La Jolla, SC

Background: Addiction to drugs of abuse is characterized by relapse to drug taking. The regulation of synaptic plasticity by the elimination and release of glutamate from glia is disrupted by chronic use of addictive drugs, including cocaine and heroin. The physiological regulation of synaptic plasticity by glia has been termed glutamate homeostasis. In rats trained to self-administer cocaine or heroin, impaired glutamate homeostasis can be ameliorated by administering drugs that increase cystine-glutamate exchange and glial glutamate transport, including N-acetylcysteine and ceftriaxone, respectively. As well, N-acetylcysteine reduces both craving and relapse in cocaine addicted participants in double-blind clinical trials. However, it is unknown if nicotine addiction shares these characteristics with cocaine and heroin.
Methods: Two general experiments were performed. In the first, rats were trained to self-administer nicotine and following a 21-day self-adminstration protocol, the nucleus accumbens and ventral tegmental were dissected from the brain in order to measure the membrane expression of the catalytic subunit of the cystine-glutamate exchange, xCT, and the levels of the glial glutamate transporter, GLT-1. The second study was a double-blind pilot clinical study was then conducted to determine if restoring cystine-glutamate exchange with N-acetylcysteine inhibits cigarette use. Twenty-nine subjects were recruited and administered either 1200 mg of N-acetylcysteine bid or placebo over 4 weeks.
Results: The levels of xCT and GLT-1 were significantly reduced in the nucleus accumbens and ventral tegmental area, but not in the amygdala or prefrontal cortex, of rats trained to self-administer nicotine compared to yoked-saline controls. Also, after 4 weeks of daily treatments, participants in the N-acetylcysteine treatment group were smoking 25-30% fewer cigarettes than placebo-treated subjects.
Discussion: These data indicate that, akin to chronic cocaine administration, repeated use of nicotine impairs glutamate homeostasis. Also, by restoring glutamate homeostasis with N-acetylcysteine it was possible to reduce daily cigarette use in treatment-seeking smokers. Thus, nicotine use follows a similar pattern of neuropathology as cocaine and heroin use, and ameliorating this pathology reduces human nicotine abuse, as it does for cocaine abuse.
Disclosure: P. Kalivas, None.

Activation of the CRF-CRF1R System Mediates the Motivation for Nicotine During Abstinence in Nicotine Dependent Animals
Olivier George*

Scripps Research Institute, La Jolla, CA, CA

Background: The main psychoactive ingredient responsible for tobacco addiction has long been hypothesized to be nicotine. Nicotine acutely produces modest positive reinforcing effects by activating the reward systems, including the mesolimbic dopamine system. However this mechanism is not sufficient to explain the transition from nicotine use to nicotine dependence. Nicotine dependence has been hypothesized to result from neuroadaptative changes in the brain that produce a powerful need to continue tobacco use. Such neuroadaptation may involve the mechanisms responsible for the negative emotional states observed during abstinence and represent a powerful source of negative reinforcement leading to excessive drug intake. Recruitment of an anti-reward system, such as stress-regulatory extrahypothalamic corticotropin releasing factor (CRF) via activation of CRF1 receptors, may contribute significantly to the motivation for compulsive use of tobacco, defined as use driven by negative reinforcement. This talk highlights recent animal studies from our laboratory showing that activation of the extrahypothalamic CRF-CRF1 system during abstinence from chronic nicotine mediates the aversive response to nicotine withdrawal, and the excessive nicotine intake and anxiety-like symptoms observed after abstinence in rodents.
Methods: We used defensive burying to measure anxiety-like behavior, conditioned place preference to measure the motivational response to nicotine and withdrawal, and intravenous self-administration to measure nicotine intake in rats and mice that have been acutely or chronically exposed to nicotine.
Results: Blockade of the CRF1R prevents spontaneous nicotine withdrawal aversions and abstinence-induced increases in nicotine self-administration and in anxiety-like behavior. In contrast, blockade of the CRF1R does not prevent the rewarding response to nicotine in chronic nicotine treated mice or the aversive response to nicotine in acute nicotine treated mice.
Discussion: These results suggest that dependent rats may increase their nicotine intake after abstinence to obtain relief from the resulting CRF-CRF1 receptor-mediated negative emotional state. This CRF1 receptor-dependent mode of negative reinforcement may explain the chronic relapsing feature of nicotine addiction and represent the neurobiological equivalent of a subject "hooked" on tobacco. The recruitment of such anti-reward systems may critically explain the transition from drug use to drug dependence and suggest new targets for non-nicotine pharmacotherapy to aid smoking and smokeless tobacco cessation.
Disclosure: O. George, None.

Panel Session
Alzheimer's Revisited: Understanding Tomorrow to Remember Yesterday

Treating Alzheimer's: Where We Have Been and Where We Are Going
Pierre Tariot*

Banner Alzheimer's Institute, Phoenix, AZ

The major trials in AD have been or are based on clinical rationales and/or translatable biomarkers and have varied in the extent to which they have adequately tested hypotheses about the pathobiology of AD. The main therapeutic approaches in play will be summarized briefly, including neurotransmitter, glial modulation, neuroptrotective/neurotrophic, amyloid, and tau/tangle based therapies, and results from selected trials will be presented along with key elements of ongoing trials. A neuronal nicotinic receptor partial agonist that showed efficacy in animal models of cognition was studied in a novel adaptive randomization design showing absence of clinical benefit in AD. A RAGE antagonist with evidence of effects on A beta metabolism and glial function is currently under study. The putative mitochondrial stabilizer latreperdine (dimebon) showed positive effects in an earlier trial and negative effects in a recent phase 3 trial; ongoing phase 3 trials will be described. The antiamyloid monoclonal antibody bapinuezumab showed trends in clinical outcomes in a phase 2 trial, decreased fibrillar amyloid binding on PiB PET scans, and vasogenic edema in a minority of subjects; ongoing phase 3 trials will be described. IVIG showed encouraging clinical and biomarkers data in a phase 2 trial; the current phase 3 trial will be summarized. Phase 2 data for a gamma secretase inhibitor showed reduced production of beta amyloid in CSF, leading to a current phase 3 trial. An amyloid anti-aggregating agent is in phase 2. Valproate inhibits GSK3 *in vitro*, leading to the hope of clinically relevant antitangle effects in AD that were not seen in a phase 3 trial. Other agents of note will be summarized, including tarenflurbil, tramiprostae, omega 3 fatty acid, statins, glitazones, and lithium. It is possible that treating before symptoms and pathology are evident may be more effective. Initial prevention trials with NSAID's and ginkgo have not shown benefit, although late data from the largest NSAID trial offer intriguing hints. A particular challenge is that typical prevention trials require thousands of patients and many years to obtain an answer. The Alzheimer's Prevention Initiative (API) is a collaborative program that aims to implement proof of concept prevention and therapeutic surrogate development studies in non-demented individuals at increased genetic risk for developing Alzheimer's disease, including presenilin 1 (PS1) carriers in a remarkably large kindred in Antioquia, Colombia, in partnership with Francisco Lopera and his colleagues, and APOE4 homozygotes. Established biomarkers of AD progression and pathology will be characterized within these trials as to the extent to which a treatment's biomarker effects predict a clinical benefit before regulatory agencies will support the use of suitable biomarkers as "reasonably likely surrogate endpoints" in the accelerated approval of pre-symptomatic AD treatments.
Disclosure: P.N. Tariot: Part 1; Acadia, Eisai, Genentech, sanofi aventis, Abbott, AstraZeneca, Baxter, Bristol Myers Squibb, Pfizer, Forest, Lilly, Merck, Janssen, Elan, Wyeth. Part 2; sanofi aventis. Part 4; Abbott, Baxter, Elan, GlaxoSmithKline, Medivation, Pfizer, Merck, Toyama, AstraZeneca, AVID, BristolMyersSquibb, Lilly, Wyeth, Janssen, Medavante.

γ-Secretase Dysfunction in Sporadic Alzheimer's Disease and a Signaling Pathway for Therapeutic Modulation of γ-Secretase Function at the Synapse
Samuel E. Gandy*

Mount Sinai School of Medicine, New York, NY

Background: The pathogenesis of most familial Alzheimer's disease (FAD) involves dysfunction of γ-secretase, which, in turn, causes elevation of the Aβ42/40 ratio, favoring the assembly of neurotoxic Aβ oligomers. We investigated the possibility that multiple γ-secretase substrates might be misprocessed in subjects with sporadic AD. Further, we have investigated the regulation of Aβ speciation at the synapse. Together, these studies provide novel insights into the pathogenesis and potential therapy of sporadic AD.
Methods: Using immunoprecipitation-mass spectrometry, we analyzed in the cerebrospinal fluid (CSF) of various clinical populations the peptide products generated by processing of not only APP but also an unrelated γ-secretase substrate protein, alcadein (Alc). Alternatively, in our therapeutic studies, we have dissected the subtypes of metabotropic glutamate receptors (mGluRs) that modulate synaptic Aβ40 and 42 generation.
Results: In the diagnostic part of the study, the CSF covariance plots from elderly nondemented and other neurological disease control subjects were found to be negative in slope and indistinguishable from

each other, while the covariance plot for SAD patients was positive in slope and readily distinguishable the plots from either control subject group. In the therapeutic part of the study, stimulation of postsynaptic Group I mGluR with DHPG induced transient accumulation in the releasate of Aβ40 but not Aβ42. Following stimulation with the Group II mGluR agonist DCG-IV, we observed sustained accumulation of Aβ42 in the releasate, which could be blocked by a Group II mGluR antagonist. DAPT-sensitivity was employed to establish that this Aβ generation was likely to be due to the same γ-secretase activity that generates the peptide under normal physiological conditions.
Conclusions: The CSF results raise the possibility that the molecular pathogenesis of SAD might involve γ-secretase dysfunction despite the absence of a PS1 mutation. The therapeutic program points to a potential intervention that addresses precisely the γ-secretase dysfunction implicated by the CSF data and reveals a new strategy for development of selective Aβ42-lowering agents. We propose that suppressing Group II mGluR signaling might be a viable prophylactic or therapeutic strategy for AD by selectively reducing synaptic production and release of Aβ42. This is an especially attractive class of compounds because of their reported ability to enhance hippocampal cognitive function and neurogenesis while suppressing tau phosphorylation and microglial activation.
Disclosure: S.E. Gandy, Amicus Pharmaceuticals, Part 1; Elan/J&J, Part 1; Diagenic, Part 1; Amicus Pharmaceuticals, Part 4.

Predictive Performance of CSF Biomarkers for Conversion from Mild Cognitive Impairment to Alzheimer's Disease
Leslie M. Shaw*, John Q. Trojanowski

University of Pennsylvania Medical Center, Philadelphia, PA

Background: Qualification of imaging and biochemical biomarkers for use in detection of Alzheimer's Disease (AD) pathology is of increasing interest in academic, pharmaceutical, and regulatory disciplines. There are accumulating data that support the hypothesis that early detection (risk for AD) of disease pathology is possible at the pre-dementia stage of the disease when the clinical diagnosis is less certain. Here we summarize our experience in the qualification of CSF Amyloid beta42 (Abeta42), total tau (t-tau) and tau phosphorylated in the 181 threonine position (p-tau181).
Methods: We utilized an immunoassay platform and reagents (Luminex and Innogenetics xMAP research use only bead-based capture antibodies and reagents, AlzBio3, Ghent, Belgium) in this study. For analytical qualification we performed an interlaboratory quality assessment study and documented reproducibility during a total of 38 analytical runs: %CV values of 5.7%, 5.6% and 11.5% test-retest performance for ADNI subject CSF Abeta42, t-tau and p-tau181, respectively. For clinical qualification of this analytical system, we first established threshold CSF biomarker concentrations for AD detection using pre-mortem CSF samples collected from subjects diagnosed with AD at autopsy and an age-matched cognitively normal control (CN) group (all samples provided by the UPenn Alzheimer's Disease Clinical Core). We used Receiver Operating Characteristic curve analyses to determine threshold "cut-point" concentrations that effectively discriminated between AD and CN subjects and then applied the same analytical methodology for biomarker concentrations in CSF collected from 410 subjects enrolled in the Alzheimer's Disease Neuroimaging Initiative (ADNI) at baseline. For assessments of the predictive performance of CSF biomarkers, biomarker ratios and a logistic regression model that included Abeta42, t-tau and APOE genotype (LRTAA) we applied survival analysis techniques.
Results: Using the ADNI data and the non-ADNI subject-based disease detection cutpoints we confirmed the expected significantly decreased CSF Abeta and increased tau concentrations in 100 AD subjects compared to the 114 CN subjects and intermediate abnormalities of these biomarkers in the 196 ADNI subjects who provided CSF at entry into the study. For the 37 MCI subjects who converted to a clinical diagnosis of AD within 12 months of entry into the study the incidence of an AD-like biomarker profile was 86.5%, 89% and 86.5%, respectively, for Abeta42 alone, t-tau/Abeta42 and LRTAA, using the autopsy-confirmed cutpoints. Using Kaplan-Myer survival analyses each of these three biomarker measures provided conversion prediction based on the established cutpoints (respective p values of 0.0005, <0.0001 and 0.0001).
Discussion: These study data provide, in a large multicenter investigation, support for the hypothesis that CSF biomarkers can provide a basis for establishing in MCI subjects the risk for conversion to a clinical diagnosis of probable AD. Such information may be useful to establish risk-based cohorts in future treatment trials. Essential to the use of CSF biomarker measurements in this context is use of highly standardized analytical methodology and associated pre-analytical factors. Further characterization of the clinical utility of CSF biomarkers over longer periods of followup in the ADNI MCI subjects is underway including evaluations of combinations with imaging biomarkers.
Disclosure: L.M. Shaw, Pfizer, honorarium and travel expenses for symposium presentation, 2009, Part 1; Bristol Myers Squibb, technical review committee, honorarium, travel expenses, 2009, Part 1.

Imaging Biomarkers of Alzheimer's Disease: Current Concepts
Clifford Jack*

Mayo Clinic, Rochester, MN

Biomarkers of brain Aβ amyloidosis are reductions in CSF Ab42 and increased amyloid PET tracer retention. Biomarkers of neuronal injury and neurodegeneration are elevated CSF tau and structural Magnetic Resonance Imaging measures of cerebral atrophy. Neurodegeneration is accompanied by synaptic dysfunction, which is reflected by decreased Fluorodeoxyglucose uptake on Positron Emission Tomography. A recently proposed model relating disease stage to Alzheimer's disease (AD) biomarkers posits that (1) Aβ amyloid biomarkers become abnormal first, before neurodegenerative biomarkers and before cognitive symptoms, (2) neurodegenerative biomarkers become abnormal later and correlate with clinical symptom severity. This proposed sequential ordering of AD biomarkers determines the practical utility of different imaging measures at specific stages of the AD pathological cascade.
Disclosure: C.R. Jack, None.

Panel Session
Beyond Recreation: What Does Dopamine Have to Do with Addiction?

A Synaptic and Optogenetic Approach to Understanding the Dopamine System
Garret Stuber, Billy T. Chen, Antonello Bonci*

UCSF and Ernest Gallo Clinic and Research Center, San Francisco, CA, Ernest Gallo Clinic and research Center, Emeryville, CA

Dopamine neurons of the VTA and their widespread projections to areas such as the nucleus accumbens, prefrontal cortex and amygdala form the mesolimbic system. Results from many studies show that these neurons are centrally involved in both acute and chronic cellular and behavioral responses to cocaine as well as most other drugs of abuse. I will discuss the most recent discoveries from my laboratory that were generated by using combined optogenetic, behavioral and electrophysiological approaches. The discussion of these results will further highlight the role of dopamine in mediating addictive as well as reward-related behaviors.
Disclosure: A. Bonci, None.

Extended Access to Rapidly Delivered IV Cocaine Changes Brain and Behavior in Ways That May Increase Liability to Addiction

Terry Robinson*

University of Michigan, Ann Arbor, MI

Introduction: Many people use potentially addictive drugs but very few go on the develop addiction. A key question is addiction research, therefore, is what is responsible for the transition from recreational or circumstantial drug use to the compulsive patterns of drug-seeking and drug-taking behavior that characterizes addiction? There is increasing recognition that to study this question using animal models requires the use of procedures that result in the development of addiction-like symptoms. There are now a number of reports that one way to achieve this aim is to allow animals extended access to drugs, either by increasing the amount of time drug is available each day, or by allowing animals to self-administer drugs for months at a time. Rats allowed extended access to drugs like cocaine undergo a transition from the "controlled" pattern of intake seen when they have only limited access, to a pattern of intake associated with a number of symptoms of addiction. Another factor thought to increase liability to addiction is the rate that drug reaches the brain. Drugs, formulations and routes of administration that lead to the rapid uptake of drug to the brain are thought to increase liability to addiction. This presentation will review studies on how these factors interact to alter brain and behavior in ways that may contribute to addiction.
Methods: Behavioral (e.g., self-administration; measure of attention), molecular (gene expression) and anatomical (analysis of dendritic structure) methods are combined to study persistent effects of cocaine on brain and behavior.
Results: Only when rats were given extended access to cocaine did they develop a number of changes in brain and behavior that may contribute to addiction. For example, addicts show a number of persistent cognitive deficits that impair judgment and decision-making, but it has been difficult to demonstrate these in rats given limited access to cocaine. However, when given extended access rats show very persistent deficits on a sustained attention task characterized by a disruption in cognitive flexibility, and this is associated with persistent changes D2 receptors in the prefrontal cortex. Furthermore, extended access to cocaine produces especially robust behavioral sensitization and marked structural changes in dendrites on medium spiny neurons in the core of the accumbens. The rapid delivery of i.v. cocaine also produces especially robust behavioral sensitization and has a greater impact on the brain, relative to when it is delivered slower, but interestingly, under limited access conditions the rate of cocaine delivery has essentially no influence on self-administration behavior. However, when tested under extended access conditions we recently found that the rate of cocaine delivery has profound effects on self-administration behavior. Only animals in which cocaine was delivered rapidly greatly increased their intake when they were given the opportunity to take more cocaine, and these animals were also more prone to reinstate drug-seeking behavior after a long period of abstinence. The latter effect was also associated with persistent changes in the ability of cocaine to induce the immediate early gene, c-fos.
Discussion: The importance of these results for thinking about how the rate of drug delivery and extended access to drugs contribute to liability to addiction will be discussed.
Disclosure: T.E. Robinson, None.

Neural Mechanisms Underlying the Development of Compulsive Cocaine Seeking Habits

Barry J. Everitt*

University of Cambridge, Cambridge, United Kingdom

Drug addiction can be viewed as the endpoint of a series of transitions from initial, voluntary drug use through loss of control over this behavior such that it is ultimately instantiated as a compulsive habit. Based on functional imaging data in humans and the experimental analysis of drug seeking and taking behavior in animals, we have hypothesized that the switch from controlled to compulsive drug seeking represents a transition at the neural level from prefrontal cortical to striatal control over drug seeking and drug taking behavior, as well as a progression from ventral to more dorsal domains of the striatum, mediated by its serially interconnecting dopaminergic circuitry. Data will be presented that support the notion of a transition from ventral to dorsal striatal control over drug seeking and its dependence on the spiraling dopaminergic circuitry that links ventral with dorsal striatal areas. The dorsal striatal dominant control over drug seeking emerges as the behavior becomes progressively well established and habitual. However, a key issue for animal experimental studies of addictive behavior is the measurement of compulsive drug seeking. We have established a model of compulsive drug seeking, defined as persistence in the face of aversive outcomes (reminiscent of the DSMIV diagnostic criterion) using a cocaine seeking-taking chained schedule in which seeking responses are intermittently and unpredictably punished, but the chained taking response always delivers intravenous cocaine. We have shown that a consistent 20% or so of rats develop compulsive drug seeking behavior after an extended, but not a short, cocaine taking history. Highly impulsive rats, that we have earlier shown to have low D2/3 dopamine receptor binding potential in the ventral striatum compared to low impulsive rats and to more readily escalate their cocaine intake, are also highly represented in the group that compulsively seeks cocaine. These data suggest that impulsivity predisposes animals to compulsively seek and take cocaine after prolonged exposure to the drug. Rats that compulsively seek cocaine have, in addition to an expected reduction in indices of striatal dopamine transmission in the dorsal striatum *post mortem*, also have a marked reduction in prefrontal cortical and striatal serotonin transmission. Treatment of rats that compulsively seek cocaine with a 5-HT2 receptor agonist decreases this complusive cocaine seeking, while treatment of rats with a 5-HT2 receptor antagonist results in cocaine seeking despite punishment in animals even after a short cocaine taking history. These data have implications for understanding the transition from controlled to compulsive drug seeking in addiction.
Disclosure: B.J. Everitt, GSK, Part 1.

Understanding Dopamine's Role in Drug Abuse and Addiction: Insights from Imaging

Nora Volkow*

National Institute on Drug Abuse, Bethesda, MD

Background: Dopamine (DA) is associated with drug reward and is believed to be involved in addiction. However, Schulz and collaborators have shown that dopamine cells are not only involved with reward but also with prediction of reward so that while dopamine increases occur with initial exposure to a primary reward with repeated exposure the dopamine cells will no longer fire with the primary reward but to the conditioned stimuli that predict the reward. Here we test the hypothesis that cocaine addicted subjects will show an attenuation of the DA increases induced by the drug but an enhanced response to cocaine-cues.
Methods: Positron emission tomography (PET) and [11C]raclopride were used to assess change in DA induced by intravenous methylphenidate (stimulant that like cocaine increases DA by blocking DA transporters) in active and in detoxified cocaine abusers and compared these responses to those in controls. In parallel we have also used PET to assess the responses of active cocaine abusers to cocaine cues (cocaine-cues video).
Results: We showed that DA increases induced by methylphenidate were markedly attenuated in cocaine addicts when compared with controls and that these differences were most accentuated in the active cocaine abusers. Moreover in active cocaine abusers the effects of MP did not differ from those in placebo. In contrast in active cocaine abusers conditioned drug-related stimuli induced significant increases in DA that were associated with cocaine craving.
Discussion: These findings show that as predicted by preclinical studies, as drug abuse transitions to addiction the increases in DA once

induced by the reward (the drug) itself are transferred to stimuli that predict the reward and drive the compulsive drug seeking behavior characteristic of addiction.
Disclosure: N.D. Volkow, None.

Panel Session
Common Factors in the Progression and Treatment of Bipolar Disorder and Schizophrenia

The Relationship of *NRG1* to Initial and Progressive Neural Change in Schizophrenia

Nancy Andreasen*, Beng-Choon Ho, Thomas Wassink

University of Iowa Hospitals and Clinics, Iowa City, IA

Background: Brain changes (i.e., GM and WM loss, increase in CSF) occur in schizophrenia both prior to and after onset. *NRG1* has been replicated in multiple studies as a potential susceptibility gene for schizophrenia. It has functions that are potentially relevant for understanding the mechanisms that may drive brain changes in schizophrenia either prior to or after onset. It produces proteins that affect axon guidance, myelination, glial differentiation, synaptogenesis, and neurotransmission.
Methods: We used an Illumina candidate gene chip custom-designed for genetic studies of psychiatric disorders. We examined tag SNPs in patients from the Iowa Longitudinal Study of Schizophrenia, a prospective study of first episode patients, who have been followed using repeated structural Magnetic Resonance (sMR) scans for up to 15 years. Previous analyses of these data in 153 patients and 103 controls has demonstrated that 1) patients scanned at intake have evidence of brain tissue loss both globally and in specific regions (e.g., frontal lobes, thalamus), as compared with controls; and 2) patients also show evidence of additional brain tissue loss over time. To determine the potential role of *NRG1* polymorphisms in these brain changes, we examined them in relation to sMR measures obtained at intake, and to a percent change over time. sMR measures were obtained using a locally developed fully-automated analysis with BRAINS2. We analyzed the intake data using a GLM to examine between allele combinations and covaried for sex and age. We analyzed the follow-up data by calculating a percent change between intake and follow-up and then used a GLM to examine between-allele combinations, covarying for sex and number of years of surveillance.
Results: Multiple *NRG1* SNPs were associated with brain abnormalities in first episode patients at onset, suggesting that they may have affected developmental brain processes occurring prior to the occurrence of symptoms. The number of significant associations increased between intake and follow-up, suggesting that *NRG1* polymorphisms may also play a role in ongoing neuroprogressive changes after onset. Effects were greatest in WM and in frontal lobes and the thalamus at both intake and follow-up, a finding consistent with *NRG1*'s role in myelination, glial formation, synaptogenesis, and neuroplasticity. At follow-up an increased number of relationships were also found between *NRG1* polymorphisms and indicators of more diffuse neuropathy (e.g. overall decrease in cerebral size, increase of cortical CSF and VBR).
Discussion: These multiple genetic polymorphisms may affect *NRG1*'s role in mediating multiple brain developmental processes, such that brain morphology is altered in ways that lead to in increased susceptibility to develop schizophrenia and ongoing progression after onset. The initial and progressive loss of WM is of particular interest, given the various roles that *NRG1* plays in myelination and glial differentiation. *NRG1* has also been shown to regulate thalamocortical axon projection through the striatum to the frontal cortex. Significant SNPs are frequently located in introns; their effect may be exerted through producing abnormalities in splicing or by affecting gene expression, transcript stability, or aberrant isoform production. Future directions will include an examination of epistasis with other candidate genes such as *ERBB4*, *DISC1*, and *BDNF*.
Disclosure: N.C. Andreasen, Johnson & Johnson, Part 1; Johnson & Johnson, Part 4.

Overlapping Molecular Neuropathology in Post-Mortem Frontal Cortex from Bipolar Disorder and Schizophrenic Patients

Jagadeesh S. Rao*

National Institute on Aging, NIH, Bethesda, MD

Background: Bipolar disorder (BD) and schizophrenia (SZ) are severe, debilitating psychiatric disorders. Severity of symptoms over time, progression and cognitive decline are common in both illnesses. In such conditions, an increased arachidonic acid (AA) cascade signaling associated with upregulated neuroinflammation and excitotoxicity markers with reduced synaptic loss are widespread, similar to those reported in Alzheimer's pathology. On this basis, we tested the hypothesis that BD and SZ are associated with increased AA cascade and neuroinflammatory markers and excitatory markers, with synaptic loss.
Methods: To do this, we used Western blotting and RT-PCR to compare protein and mRNA levels of AA cascade markers, neuroinflammatory, glutamate transporters and synaptic markers in post-mortem frontal cortex from 10 BD, 10 SZ patients and 20 matched controls.
Results: Mean protein and mRNA levels of cytosolic and secretory phospholipase A_2 ($cPLA_2$ type IVA, $sPLA_2$ type IIA) and cyclo-oxygenase (COX)-2, were significantly elevated in postmortem from BD and SZ patients. Whereas, protein and mRNA levels of interleukin-1beta, glial protein acidic protein, Cd11b were significantly increased in both the illnesses. Excitatory amino acid transporter (EAAT)-2 protein and mRNA were reduced in postmortem frontal cortex from BD patients but not in SZ patients. Whereas, protein and mRNA levels of EAAT (3 and 4) were upregulated in SZ but not in BD patients. In addition, reduced synaptophysin and drebrin were found in postmortem frontal cortex from BD and SZ patients.
Discussion: The discrepancy in glutamate transporters levels in SZ patients explains SZ might be associated with hypoglutamatergic function, while BD is associated with hyperglutamatergic function. Increased neuroinflammation with upregulated AA cascade enzymes might lead to disease progression and cognitive defects in BD and SZ patients. Thus, down regulating this cascade might be considered for new therapy.
Disclosure: J.S. Rao, None.

Effects of Antipsychotic Medications on Cellular and Molecular Features of the Cerebral Cortex

David Lewis*

Western Psychiatric Institute & Clinic, Pittsburgh, PA

Background: Both *in vivo* and post-mortem investigations of brain morphology in schizophrenia have demonstrated smaller volumes of the whole brain and of certain brain regions. In addition, post-mortem studies have revealed evidence suggestive of oligodendrocyte and myelination alterations and consistently found disturbances in the expression of genes that regulate GABA neurotransmission in individuals with schizophrenia,. However, it is unclear whether prolonged treatment with antipsychotic medications contributes to these disturbances.
Methods: To investigate this question, we developed a non-human primate model of chronic antipsychotic exposure. Three groups of 6 macaque monkeys each were exposed to oral haloperidol, olanzapine or sham for $\sim$2 years. The resulting plasma drug levels were comparable to those seen in subjects with schizophrenia treated with

these medications. We used highly precise measures to determine regional brain volumes, unbiased stereological methods to determine total numbers of all classes of cortical neurons, including total oligodendrocyte and astrocyte numbers, and several methods to assess levels of gene expression. Some studies were also conducted in animals exposed chronically to higher serum levels of haloperidol decanoate and matched controls.
Results: After the exposure, we observed an 8-11% reduction in mean fresh brain weights as well as left cerebrum fresh weights and volumes in both drug-treated groups compared to sham animals. The differences were observed across all major brain regions, but appeared most robust in the frontal and parietal regions. The use of point counting and Cavalieri's principle on Nissl-stained sections confirmed a 14-15% reduction in the left parietal grey matter volumes of the antipsychotic-exposed monkeys. Use of the optical fractionator method to estimate the number of each type of cortical cell revealed no differences in total neuron number across subject groups, a concomitant 10.2% increase in neuron density in the antipsychotic-exposed monkeys and significant mean 14.2% reduction in glial cell number. Immunocytochemical studies revealed a significant 20.5% lower astrocyte number with a non-significant 12.9% lower oligodendrocyte number in the antipsychotic-exposed monkeys. Similar effects were seen in both the haloperidol and olanzapine groups. In contrast, no differences in GABA-related gene expression were seen across any of the conditions.
Discussion: Together, the findings of smaller cortical grey matter volume, lower glial cell number, and higher neuron density without a difference in total neuron number in the antipsychotic-exposed monkeys parallel the results of postmortem studies of schizophrenia, and raise the possibility that observations in human subjects might be due, at least in part, to medication effects. In contrast, these findings suggest that the GABA-related transcript abnormalities in the illness do not represent an antipsychotic medication effect.
Disclosure: D.A. Lewis, BMS Foundation, Bristol-Myers Squibb, Curridium Ltd, Pfizer, AstraZeneca, BioLine RX, Hoffman-Roche, Lilly, Merck, Neurogen and SK Life Science, Part 1; BMS Foundation, Bristol-Myers Squibb, Curridium Ltd, Pfizer, Part 4.

By Dampening Its Upregulated Brain Arachidonic Cascade, Mood Stabilizers and Atypical Antipsychotics May Slow Bipolar Disorder Progression
Stanley Rapoport*

National Institute on Aging, NIH, Bethesda, MD

Background: A common factor regarding the mechanism of action of anti-bipolar disorder (BD) drugs and neuropathology leading to BD progression may involve the brain arachidonic acid (AA, 20:4n-6) cascade. Studies described by J Rao in this panel indicate increased expression of neuroinflammation and excitotoxicity markers, and of AA-metabolizing enzymes (cytosolic phospholipase A2 (cPLA2), secretory sPLA2, cyclooxygenase (COX)-2), in postmortem BD brain. Neuroinflammation and excitotoxicity can release AA from membrane phospholipid via astrocytic cytokine receptors or synaptic glutamatergic NMDA receptors, and high AA concentrations are neurotoxic and can contribute to apoptosis and disease progression.
Methods: *In vivo* kinetics in rodent models, lipid analytical chemistry, molecular biology, enzyme activity analyses, post-mortem brain sampling.
Results: Approved BD therapy appears to target some of these BD changes, since lithium, valproate, carbamazepine and lamotrigine (unpublished), when given chronically to rats to produce therapeutically relevant blood concentrations, each downregulated parts of the brain AA cascade, including: cPLA2, sPLA2 and/or COX-2 expression, AA incorporation from plasma and AA turnover in brain phospholipid, AA-selective acyl-CoA synthetase activity, and concentrations of AA and its metabolite, pro-inflammatory prostaglandin E2 (PGE2). Each mood stabilizer also blocked the robust brain AA signal, imaged using quantitative autoradiography in unanesthetized rats, seen following intraperitoneal administration of a subconvulsive dose of NMDA (signal also was blocked by MK-801). Unpublished studies now indicate that two effective atypical antipsychotics, olanzapine and clozapine, reduced AA incorporation into brain secondary to lowering the unesterified plasma AA concentration in unanesthetized rats, brain COX mRNA and activity and PGE2 concentration. Expression of brain cPLA2 and sPLA2 was unaffected, while expression of COX-1 and calcium-independent iPLA2 (selective for anti-inflammatory docosahexaenoic acid, 22:6n-3) was upregulated.
Discussion: The ability of approved mood stabilizers and atypical antipsychotics to downregulate parts of the rat brain AA cascade provides a possible mechanism for their therapeutic efficacy in BD, in which an upregulated cascade associated with neuroinflammation and excitotoxicity likely contributes to disease progression.
Disclosure: S.I. Rapoport, None.

Panel Session
Negotiating the Path to Approval: Finding Solutions to Common IRB Issues

The IRB Experience: A Survey of the ACNP Membership
Stephan F. Taylor*, Katherine L. Wisner, Robert R. Conley, Lawrence S. Brown, Mark Rapaport, Thomas Kosten

University of Michigan, Ann Arbor, MI

Background: Protection of individuals who participate in research is overseen by Institutional Review Boards (IRBs), usually local, independent entities that review human research to ensure that participants are not harmed, exploited or subjected to unnecessary risks. Established by Federal regulations, IRBs are required for all federally-sponsored research, as well as studies that involve drugs and medical devices. In practice, IRBs exercise oversight over all research at institutions receiving federal support. In the last decade, investigators have seen a sharp increase in the amount of time spent assuring compliance with research regulations, and institutions have devoted increasingly larger budgets to operate IRBs. Because of concerns about the growing burden that IRBs place on investigators, the Council of the ACNP charged the Human Subjects Committee with surveying the membership to determine the extent of this burden, and to identify potential solutions.
Methods: From September through October of 2009, a web-based survey was made available to the membership of ACNP. 143 members responded to the survey, which represents 40.9 % of members self-identified as clinical researchers, out of a total membership of 887.
Results: Respondents reported that IRB review had placed significant burdens on research. 64% experienced approval delays of more than 3 months for protocols. 72% reported that the costs of conducting research had been increased by at least a moderate amount, and 42% reported at least a moderate, negative impact on research recruitment. IRB review increased the complexity of research, particularly when submitting to multiple IRBs in the same institution (35% of respondents). 78% of respondents had participated in multi-institutional studies with multiple IRBs, and 83% experienced delays due to differences in the standards applied by each IRB. Only 6% of respondents had participated in multi-site studies with a central IRB. In spite of these difficulties, 75% felt that IRBs enhanced human subject protection and 66% of respondents felt that IRBs strengthened the public trust, although many commented that the public has little understanding of IRBs and the process for reviewing human subjects research. 65% felt that neuropsychiatric studies are placed at a disadvantage in the review process, citing concerns about the capacity to consent and vulnerability of psychiatric subjects. Many comments spoke to the importance of representing the psychiatric point of view

in the review process. Comments to improve the processs included discussing protocols with IRB members and staff before submission, establishing ongoing lines of communication with IRBs, training workshops for study coordinators (sponsored by the IRB), appearing before the Board to answer questions, employing trained staff to negotiate the process, and becoming an IRB member. 62% of respondents had served on an IRB, and 16% as chairs. Lastly, the professionalism and efficiency of the commercial and NIH IRBs were repeatedly emphasized.
Discussion: While ACNP investigators identify clear burdens imposed by IRB oversight, there is also widespread appreciation of the important role that IRBs play. A more uniform process, such as the development and application of human subjects 'case law' may address some of the issues around inconsistency and inefficiency currently experienced.
Disclosure: S.F. Taylor, Organon/Schering Plough; St. Jude Medical; Neuronetics; St. Jude Medical; Neuronetics.

The Certificate of Confidentiality: Is the Hassle Worth It?
Bryon Adinoff*, Stephan F. Taylor, Robert R. Conley

UT Southwestern Medical Center at Dallas and VA North Texas Health Care System, Dallas, TX, University of Michigan, Ann Arbor, MI, Eli Lilly & Company, Indianapolis, IN

Background: The NIH Certificate of Confidentiality (CoC)promises to protect the identity of research participants and is often required in the study of vulnerable populations. Recent court cases, however, raised questions about whether the CoC can withstand legal challenge. Other potential problems include the anonymity demanded by the CoC conflicting with some institutions requiring the reporting of research participation in the clinical chart, the inability of the CoC to protect research data (as opposed to participant identity), the responsibility of the institution to support an investigator's defense of the CoC in the face of a legal challenge, and whether the difficulty of obtaining a CoC is worth its presumed protections. In response to these concerns, the ACNP Human Subjects Committee convened a group to make recommendations to the Executive Committee.
Methods: The subcommittee explored the pros and cons of the CoC through discussions with NIH Science Policy Advisors, CoC legal experts, and academic institutions/IRBs and an exploration of court cases challenging the CoC protections.
Results: (1) The CoC provides a unique and necessary protection for research data. (2) The courts have generally upheld the protections provided by the CoC, and most attorneys and courts have been responsive to the CoC concept and have abided by its protections. (3) The legal ramifications of having research information on the clinical chart, with respect to the CoC, are not clear. (4) The CoC offers protection of identifiers, not data. (5) Institutions are under legal obligation to defend the CoC against forced disclosure.
Discussion: The legal challenges to the CoC will be summarized and interpreted, urban myths surrounding the CoC clarified, and potential problems in CoC implementation identified. In general, the CoC is relatively easy to obtain yet offers powerful protections to research participants.
Disclosure: B. Adinoff, Shook, Hardy & Bacon LLP (medical malpractice for tobacco companies), Part 1.

Conflation of Risk Determinations for Protocols with Psychiatric Patients
Barbara Stanley*

Columbia University, New York, NY

Determination of research risk level is an important consideration in evaluating research protocols, not just to protect research subjects but also because intensity of the review and assignment of protections is dependent, in part, on risk level. To qualify for expedited IRB review, alteration or waiver of consent requirements, the research must be minimal risk *and* not involve a "vulnerable" population. Federal regulations call for additional protections for "vulnerable" subjects; these include full IRB review, surrogate consent and disallowing consent waivers. Vulnerability is analyzed within the framework of subjects' impaired decision-making capacity (e.g., cognitively disabled individuals, children) and/or a power differential resulting in potential coercion and undue influence (e.g., students, prisoners). Psychiatric patients are not, de facto, presumed to be vulnerable. IRBs may confound risk assessment with disorder and may determine that a minimal risk study (e.g. blood draw and questionnaire study) is greater risk simply because the study contains psychiatric patients. While some procedures may cause psychiatric patients more distress and, thereby, impact risk, this evaluation should be on a protocol by protocol basis not simply based on the study population. This presentation will discuss ways to clearly distinguish factors having an impact on risk determinations.
Disclosure: B. Stanley, None.

Inside the Black Box: What Really Happens in IRBs?
Paul S. Appelbaum*

Columbia University, New York, NY

Background: Institutional review boards (IRBs) are the gatekeepers that regulate access to human subjects for most neuropsychiatric researchers. Despite frequent expressions of dissatisfaction with IRBs_often focused on the time delay, cost, and focus on minutiae that appear to characterize the process_systematic data on how IRBs operate have not been available.
Methods: This presentation reports results from an NIH-funded study of IRBs at 10 of the 25 leading academic medical centers in the U.S. (by dollars of NIH funding). Meetings of two IRB panels at each participating center were audiotaped, transcribed, and coded, then subjected to quantitative and qualitative analysis.
Results: The efficiency of pre-review by IRB staff members influenced the extent to which IRB members could focus on substantive issues rather than technicalities or wording. In general, the review process for individual studies was confined to a small subset of IRB members, with those having specific expertise regarding the area of the study playing the greatest role. Many of the study risks could only be identified by those with a detailed knowledge of the conditions and interventions being studied. Community members generally limited their comments to small changes in consent forms. The administrative style of the IRB chair determined the degree of interaction among members and the thoroughness of discussions. The majority of areas that the federal regulations require to be reviewed were discussed for most protocols, but some issues appeared to have been systematically neglected. Protocols were almost never approved without requests for changes, however outright rejection was also rare. The special issues that arose in the review of psychiatric protocols will be described.
Discussion: For the most part, IRBs appear to be doing the job required of them by applicable regulations. However, IRB staff members play a critical but generally unrecognized role, and members vary greatly in their contributions. Greater attention to staff organization and training, and innovative approaches to applying specific expertise to protocol review could improve both the efficiency and effectiveness of IRB review.
Disclosure: P.S. Appelbaum, COVR, Inc., Part 1.

Panel Session
Neuregulin Session 2: Neuregulin in Neuropsychiatry 2010: Genetics, Neurobiology & Therapeutic Response

Neuregulin Signaling at Excitatory Synapses
Ulrich Mueller*

The Scripps Research Institute, La Jolla, CA

Neuregulin-1 (NRG1) and its ErbB2/B4 receptors are encoded by candidate susceptibility genes for schizophrenia, yet the essential functions of NRG1 signaling in the CNS are still unclear. Using CRE/LOX technology, we have inactivated ErbB2/B4-mediated NRG1 signaling specifically in the CNS. In contrast to expectations, cell layers in the cerebral cortex, hippocampus and cerebellum develop normally in the mutant mice. Instead, loss of ErbB2/B4 impairs dendritic spine maturation and perturbs interactions of postsynaptic scaffold proteins with glutamate receptors. Increased NRG1 levels promote spine maturation. ErbB2/B4-deficient mice show increased aggression and reduced prepulse inhibition. Treatment with the antipsychotic drug clozapine reverses the behavioral and spine defects. We conclude that ErbB2/B4-mediated NRG1 signaling modulates dendritic spine maturation, and that defects at glutamatergic synapses likely contribute to the behavioral abnormalities in *ErbB2/B4*-deficient mice.
Disclosure: U. Mueller, None.

Common Genetic Variation in Neuregulin 3 (NRG3) Regulates NRG3 Expression and Influences Risk for Schizophrenia
Amanda J. Law*

IRP, NIMH, NIH, Bethesda, MD

Background: The NRG-ErbB signaling pathway is critical to neurodevelopment and several genes in the network have been associated with schizophrenia. NRG3 is a specific ligand for ErbB4 and is the most recent signaling partner to be implicated in the disorder. Previous fine mapping of the 10q22-23 locus in schizophrenia has identified strong evidence of association between delusion severity and polymorphisms in intron 1 of NRG3 (Chen et al, 2009). The biological mechanisms remain unknown. We identified significant association of SNPs within intron 1 of NRG3 and increased risk for schizophrenia in 350 families with an affected offspring and confirm association to patient delusion- and positive symptom severity. Molecular cloning and cDNA sequencing in human brain revealed that NRG3 undergoes complex splicing giving rise to multiple structurally distinct isoforms. RNA expression profiling of these isoforms in the prefrontal cortex of 400 individuals revealed that NRG3 expression is developmentally regulated and pathologically increased in schizophrenia. Moreover, we demonstrate that a genome-wide significant risk SNP (rs10748842) resides within a DNA ultraconserved element and homedomain and strongly predicts brain expression of NRG3 isoforms that contain a unique developmentally regulated 5′ exon.
Methods: Family based samples were used for clinical genetic investigation of NRG3 polymorphisms. DNA was available from 350 families with an affected offspring. The samples were ascertained as part of the Clinical Brain Disorders/National Institute of Mental Health (NIMH) Sibling Study. Main effect analyses of single SNPs were conducted using the family-based association test (FBAT). To isolate, clone and sequence full length NRG3 cDNAs, adult and fetal human brain cDNA libraries were generated. Expression levels of families of NRG3 transcripts identified through PCR cloning were measured using quantitative real time RT-PCR in dorsal lateral prefrontal cortex (DLPFC) derived from 245 normal individuals, 42 fetal subjects and 113 individuals with schizophrenia. Effects of risk genetic variation on NRG3 eQTLs was examined.
Results: At the clinical genetic level, distorted transmission of alleles in intron 1 of NRG3 was observed in our family-based sample. Sequencing of NRG3 cDNA transcripts in human brain shows that the gene generates >15 novel alternatively spliced variants. Disease state and rs10748842 were associated with increased brain expression of novel NRG3 isoforms that contain a unique developmentally regulated 5′ exon (from $p = 1.097E^{-12}$ to $p = 1.445E^{-15}$).
Conclusion: Our observations strengthen the evidence that NRG3 is a schizophrenia susceptibility gene, provide quantitative insight into NRG3 transcription traits in the human brain and reveal a probable mechanistic basis for disease association.
Chen, P., et al. (2009). Fine mapping on chromosome 10q22-q23 implicates *Neuregulin 3* in schizophrenia. Am. J. Hum. Genet. *84(1)*, 21-34.
Disclosure: A.J. Law, None.

Analysis for Multiple Genetic Markers Associated with Antipsychotic Response
Roy Twyman*

Johnson & Jounson Pharmaceutical Research and Development, LLC, Titusville, NJ

Background: The neuregulin ligand family interact with four transmembrane tyrosine kinase receptors of the epidermal growth receptor (ErbB1-4) family. This NRG-ErbB signaling cascade regulates or modulates neuronal development and migration, synaptogenesis and maintenance, gliogenesis and myelination, and neuron-neuron/neuron-glial communication. Multiple functionally distinct isoforms enhances the complexity of the diverse neuregulin neurobiology suggesting a diffuse interplay across networks and the potential for a significant role in modulating behavior. Since associations have been found between schizophrenia and various non-coding polymorphisms and haplotypes of the *nrg1* gene, genetic variations in NRG-ErbB signaling cascade and interacting networks raises the possibility that gene-gene interactions might also play a role in drug response in treated schizophrenic patients.
Methods: A pharmacogenomic analysis for multiple markers associated with response to antipsychotic treatment in schizophrenics was conducted. Discovery and validation samples obtained from controlled clinical trials of paliperidone in acute exacerbations of schizophrenic patients.
Results: Genetic variation in the ErbB4 gene has been previously reported to potentially differentially affect treatment response to paliperidone. Two other genes whose allele frequency in combination with ErbB4 was present in a larger subset of subjects studied implicated an association of genetic load to variability in the treatment response to paliperidone.
Discussion: Since individualization by genetic profiling might maximize benefits for patients, candidate gene and GWAS approaches may be utilized to identify combinations of genetic factors that may contribute to variations in treatment response to antipsychotics.
Disclosure: R. Twyman, Johnson & Johnson, Part 1.

Novel Multiple Testing Methods: The Importance of NRG1 and ERBB4 in COGS Schizophrenia Families
David Braff*, Tiffany A. Greenwood, Laura Lazzeroni, COGs Investigators

University of California San Diego, La Jolla, CA

Objective: We created a custom 1,536-SNP chip to interrogate 94 functionally relevant candidate genes for schizophrenia and identify associations with 12 heritable neurophysiological and neurocognitive endophenotypes collected as part of the Consortium on the Genetics of Schizophrenia (COGS). Neuregulin and its ERBB4 receptor SNP's were selected for interrogation because of their *a priori* relevance in glutamate modulation in schizophrenia.
Methods: A subset of 540 subjects from 130 COGS families were genotyped. The association analyses were conducted using Merlin and a novel bootstrap total significance test (TST) was developed and is

first presented here. It robustly demonstrates the presence of significant associations in the context of complex family data and population stratification effects, thus resolving statistical analytical concerns regarding family-wise analyses. Novel Multiple Testing Methods: Traditional multiple testing methods assess whether individual test results are strong enough to remain significant in the context of multiple tests, but do not provide for a formal test of whether the total number of positive findings is statistically significant and exceeds what would occur due to chance alone. Therefore, we (LL) developed a novel strategy, the bootstrap total significance test (TST), to evaluate the overall association results in a statistically rigorous manner. The bootstrap simulates results that would be seen only by chance with 16,620 tests (1,385 SNPs and 12 endophenotypes), given the unique correlation structure of families, endophenotypes, and SNPs in this study. A formal hypothesis test and overall p value is then constructed by comparing the observed results to the bootstrap simulations.
Results: Associations were observed for 46 genes of potential functional significance with 3 SNPs at $p<10^{-4}$, 27 SNPs at $p<10^{-3}$, and 147 SNPs at $p<0.01$. Bootstrap TST analyses confirmed that 47 SNP-endophenotype combinations with the strongest evidence of association significantly exceeded that expected by chance alone ($p=0.001$). Many genes interact on a molecular level, and eight displayed evidence for pleiotropy, most notably NRG1 and ERBB4. The results of the Merlin single-marker analyses revealed associations between the 12 endophenotypes and 46 of the 94 genes. There were 3 SNPs with a $p<10^{-4}$, 27 SNPs with a $p<10^{-3}$, and 147 SNPs with a $p<0.01$, 23 genes significantly associated with at least one endophenotype with $p<10^{-3}$ as indicated. The most significant finding in these analyses was for a SNP in NRG1 which associated at $6.4x10^{-6}$ with a neurocognitive (SPA) endophenotype. NRG1 and ERBB4 associated with 5 and 8 endophenotypes, respectively, offering a compelling picture of the importance of Neuregulin-ErbB4 signaling in the neuropathology of schizophrenia and its associated endophenotypic heritable deficits. In concert, murine NRG1 hypomorphs show deficiencies in the PPI endophenotype.
Conclusions: This study confirms the importance of NRG1, ERBB4 and other glutamate related genes in the identification of genetic variation underlying the etiology of endophenotypes in schizophrenia. In addition, the observation of extensive pleiotropy for NRG1 and ERBB4 and singular associations for other genes in our data suggests alternative, independent pathways involving glutamate and other neurotransmitters (and neural circuits) mediating the pathogenesis in the "group of schizophrenias".
Disclosure: D.L. Braff, Allergan, Part 1; UCSD, Part 2; VAHCS, Part 2; Allergan, Part 2; NARSAD, Part 2.

Panel Session
New Approaches to Old Targets

Functional Selectivity at Dopamine Receptors as a Route to Novel Drugs

Richard B. Mailman*

Pennsylvania State University, Hershey, PA

Background: Functional selectivity is the property of many ligands/drugs to bind to a single receptor subtype, yet differentially affect the signaling pathways mediated by that receptor. In the extreme, a ligand may be a full agonist at one signaling pathway, and an antagonist at another pathway, even in the same cell. Although first reported nearly 20 years ago, this mechanism has been widely accepted only recently. Its relevance to psychiatry has been shown by the demonstration that aripiprazole is a functionally selective D_2 ligand, not simply a partial agonist. This demonstration of the clinical utility of functional selectivity has opened numerous scientific arenas that are the subject of this panel (ranging from the use of computational approaches to novel *in vivo* models). This presentation will focus on the discovery and mechanisms of new functional selective drugs targeting the dopamine D_1 and D_2 receptors.
Methods: These studies used a battery of D_1 and D_2-related signaling assays in different cell lines. In some cases, effects on the canonical cAMP signaling pathway has been studied in both different cellular milieus and using several different assay systems. In addition, a series of rationally designed mutations have been made in both receptors to determine the effects not only on ligand binding, but also functional signaling through multiple pathways. The ligands used in these studies are based on a series of rigid chemical backbones, of which the parent drug has already been used in clinical trials.
Results: 1) When a series of ligands was tested in different assay systems in which the common output was cAMP, some ligands showed striking differences in efficacy or potency across different systems. 2) Receptor mutations were found sometimes to cause profound effects on ligand binding, yet leave functional properties unchanged. Conversely, some mutations can have little effect on the signaling of one ligand, yet cause another ligand to become functionally selective. 3) We have suggested that non-selective (bi-directed) D_1 and D_2 dopamine ligands might present unique potential clinical candidates. We explored this by identifying existing compounds with structural features suggesting dual actions, and demonstrating that some of these ligands were functionally selective for both D_1 and D_2 receptors.
Discussion: These data indicate that some compounds may look similar in pharmacological properties regardless of the milieu of the target receptor, whereas other drugs are markedly affected by cell type and assay system. This suggests that the use of a single assay system for functional determinations may often be problematic unless the cell environment chosen has been shown directly relevant to the target cell *in situ*. In addition, subtle changes in drug structure (or alternatively, in receptor structure) were shown to cause pronounced differences in functional properties of a drug. This provides an opportunity to exploit new structural and computational approaches to discover new functionally selective ligands rationally. Finally, despite the advances made in understanding functional selectivity mechanisms, and in discovering novel functionally selective drugs, there remain many challenges in predicting which compounds should be considered as potential drug candidates. The ability to discover novel drugs (e.g., aripiprazole) that signal through "old" receptors make further studies of great interest.
Disclosure: R. Mailman, Biovalve Technolgies, Part 1; Effipharma Inc, Part 1; US Department of Justice, Part 2; Williams & Connolly, Part 2; The Chartwell Group, Part 2.

The Ability of the D2 Dopamine Receptor to Engage the Akt/GSK3 Signaling Cascade Through a Beta-Arrestin 2-Dependent Complex Reveals Behavioral and Antipsychotic Functional Selectivity

Marc G. Caron*, Nikhil Urs, Bernard Masri, Ramona Rodriguiz, William C. Wetsel

Duke University Medical Center, Durham, NC

Background: In the brain, dopamine (DA) is an important neuromodulator that mediates its actions through D1- and D2-like G protein-coupled receptors (GPCR-7TM). Similar to other GPCRs, D2Rs have recently been shown to signal not only through the activation of heteromeric G proteins but also through the ability of D2R/beta-arrestin 2 (Barr2) to scaffold intracellular signaling complexes. Antipsychotics display complex receptor pharmacological patterns but all of these clinically effective agents interact with D2Rs. Whether antipsychotics might show preferential activity at D2R/Barr2 and whether the D2R-mediated Akt/GSK3 signaling might selectively affect certain behaviors *in vivo* have been questions of interest.
Methods: We used *in vitro* monitors of G protein- and Barr2-dependent D2R signaling and vivo behavioral approaches in

genetically engineered mice to examine the ability of various antipsychotics to inhibit dopamine (DA)-associated behaviors and interfere with D2R signaling.
Results: We have used cellular assays based on bioluminescence resonance energy transfer (BRET) approaches to monitor G protein-dependent D2R coupling to the cAMP cascade or the interaction of D2R tagged with RLuciferase and β-arrestin 2-YFP as a way to follow Barr2-dependent coupling to profile a series of antipsychotics. Whereas at D2R G protein-dependent signaling most antipsychotics acted as inverse agonists with newer atypical agents being partial agonists, all antipsychotics uniformly and effectively antagonized the D2R/βarr2 interaction with a potency 3-150-fold greater than their ability to antagonize D2R G protein-dependent signaling. These observations of functional selectivity raise the possibility that blocking D2R/Barr2 interactions may be relevant to the clinical efficacy of these drugs. Moreover, in addition to the above apparent selectivity of antipsychotics, deletion of the GSK3b gene in a cell specific manner suggests that the D2R/βarr2/Akt may show behavioral selectivity. In mice lacking GSK3b in D2R expressing neurons, the locomotor enhancing effects of amphetamine and apomorphine are markedly attenuated whereas they are unchanged in mice lacking GSK3b in D1R expressing neurons. Conversely, the ability of mice lacking GSK3b in D2R expressing neurons to develop conditioned place preference to amphetamine was identical to wild type mice or to mice lacking GSK3b in D1R expressing neurons. Thus, these results suggest that the D2R/Barr2/Akt signaling pathway may display functional selectivity also at the behavioral level since not all behavioral responses depend on activation of this pathway. Lastly, we have shown that the mood stabilizer lithium can regulate Akt/GSK-3 signaling and related behaviors in mice by disrupting the D2R dependent signaling complex Barr2/Akt/PP2A presumably independent of its direct inhibitory effect on GSK3.
Conclusions: The Barr2-dependent Akt/GSK-3 signaling pathway plays an important role in the behavioral actions of dopamine and may represent a previous unappreciated target for selectively interfering with the action of DA.
Disclosure: M.G. Caron, Lundbeck A/G, Part 1; Forest Laboratories, Part 1; Roche, Part 1; Omeros, Part 1; Omeros, Part 2; Lundbeck A/G, Part 4.

Serotonin Activates the 5-HT2AR via a βarrestin2/PI3-K/Src/AKT Complex *in vivo* While Hallucinogenic N-Methyltryptamines Do Not
Laura M. Bohn*, Cullen L. Schmid

The Scripps Research Institute, Jupiter, FL

Background: Serotonergic hallucinogens, such as lysergic acid diethylamide (LSD) and dimethyltryptamines (DMT), produce their psychoactive effects through serotonin 2A receptor (5-HT2AR) activation; while serotonin itself is not considered to be hallucinogenic. Cellular evidence suggests that hallucinogenic and non-hallucinogenic agonists may preferentially utilize certain signaling cascades, although the implications of such signaling divergence have been difficult to demonstrate *in vivo*.
Methods: The mouse head twitch response was utilized as a behavioral measure of 5-HT2AR activation in WT and arrestin2 knockout mice. Biochemical assessments of receptor signaling involved western blotting of mouse cortical neuronal culture lysates and frontal cortex lysates as well as co-immunoprecipitation studies in frontal cortex.
Results: Serotonin activates a arrestin2/PI3-K/Src/AKT cascade in frontal cortex and in cortical cultures while N-methyltryptamines do not. Furthermore, disruption of this pathway in mice attenuates 5-hydroxytryptophan (5-HTP)-mediated head twitch responses but not those induced by N-methyltryptamines. Furthermore, inhibition of AKT and N-methyltransferase prevents 5-HTP induced head twitches in normal mice.
Discussion: This study shows that the signaling of two endogenous neurotransmitters may differ in their ability to activate the 5-HT2AR and that this difference hinges on the functional role of arrestin2 *in vivo*. While arrestin2 acts as a 5-HT2AR pro-signaling molecule when serotonin is the agonist, it also serves as a negative regulator of 5-HT2AR signaling when an N-methyltryptamine is the agonist. This work is funded by NIDA/NIH R01DA025158 to LMB.
Disclosure: L.M. Bohn, Trevena, Inc., Part 1; Sucampo Pharmaceuticals, Part 4.

Computational Methods to Aid Activation Pathway Specific Drug Design for G-Protein Coupled Receptors
Naragajan Vaidehi*

Beckman Research Institute of the City of Hope, Duarte, CA

Background: G-protein coupled receptors (GPCRs) are highly dynamic receptors with high plasticity to allow for versatility in their function. Extracellular ligands ranging from small molecules to large proteins activate multiple signaling pathways, depending on the nature of the receptor, ligand, the G-proteins (including the non G-protein dependent pathways) they couple to and the dynamic state of the cell. Modulation of cell signaling pathways by ligands of different efficacies depends intrinsically on how the chemical structure of the ligand modulates the potential energy surface of the receptor. Ligands with different efficacies can remodel the energy landscape of the receptors, thereby perturbing the conformational equilibrium among multiple conformational states thereby conferring functional specificity. Understanding activation dynamics and pathways is vital in designing functionally specific drugs for GPCRs.
Methods: We have developed computational method called "LITiCon" to predict the ligand selective receptor conformational state for a class A GPCR. LITiCon method allows systematic spanning of the receptor conformations that enables more comprehensive conformational sampling. Starting from the crystal structure of β1 and β2-adrenergic receptor (β2AR), we have used LITiCon computational method to predict the ligand stabilized receptor state with full (epinephrine and norepinephrine), partial (salbutamol and dopamine), and inverse agonists (carazolol) bound. We have also developed computational method to calculate the minimum energy pathway going from the inactive to the ligand selective state for each type of agonist.
Results and Discussion: We have validated the computational methods for predicting the activation pathways of several agonists for β2AR, and shown that they are in agreement with fluorescence lifetime measurements. The calculated pathways for the full agonists in the β2AR start with an energy downhill step leading to a stable intermediate following by a barrier crossing leading to the active state. We find that the polarization of the amino acid residues in the binding site of the agonist by water molecules facilitates the barrier crossing. We have demonstrated the use of these methods for designing functional selective ligands. We have also further used these methods to design mutant GPCRs that show greater thermal stability that should aid the expression and purification of thermal stable mutants for biophysical studies. Our computational method provides an unprecedented opportunity to understand activation mechanisms in GPCRs. The discussion will focus on the opportunities provided by the computational methods to understand and design functional selectivity drugs. The pitfalls of the current methods and opportunities of refining the computational methods in collaboration with experiments will be discussed.
Disclosure: N. Vaidehi, None.

Panel Session
Novel Findings on the Etiology, Pathogenesis, and Pathophysiology of Autism

Early Brain and Behavioral Development in Autism: Findings from Longitudinal Imaging Studies

Joseph Piven*

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

Background: Longitudinal studies of head circumference in autism, by our group and others, suggest that the onset of brain overgrowth may occur at the end of the first year of life. Coincidently this period coincides with recent data from high risk infant sib studies suggesting that the onset of social deficits in children, who are later found to be autistic at ages 2-4 years, also occurs during this period between 6 and 12-14 months of age (Zwaigenbaum et al., 2005).
Methods: Longitudinal brain MRI/DTI and behavioral measurements of infants and toddlers at risk for autism.
Results: Results from a longitudinal MRI study of toddlers with autism, followed up at 4-5 years of age, provide new evidence that cerebral cortical brain enlargement present by two years of age in autistic individuals is largely the result of increased cortical surface area occurring during this period of early development. Preliminary MRI, DTI and behavioral data from an ongoing multi-site, longitudinal neuroimaging study (IBIS Network) of infants at high risk for autism (i.e., siblings of older autistic children examined at 6, 12 and 24 months) will be presented to follow up on the above noted observations that suggest both brain overgrowth and autistic behavior have their onset during the latter part of the first year of life. We hypothesize that no differences in brain morphology or behavior will be noted at 6 months of life whereas onset of early symptoms of autism as well as increased cerebral cortical brain volumes and changes in fractional anisotropy in selected fiber tracts will be present by 12 months of age. These data will be contrasted with similar imaging data in young children with a related neurodevelopmental disorder, Fragile X Syndrome, to examine the specificity of these findings for autism.
Discussion: As the development of cortical surface area and cortical thickness have distinct genetic and neurobiological underpinnings, these new findings suggest novel candidate genes and neurobiologic mechanisms (e.g., over-proliferation of peri-ventricular progenitor cells) not previously hypothesized to be of importance in autism. Focus on very early MRI/DTI brain changes as are currently being studied in the IBIS Network, are likely to provide important clues for early detection of later developing autistic behavior as well as insights into critical windows for early intervention.
Disclosure: J. Piven, None.

Converging Evidence for Structural and Functional Brain Abnormalities in the Autism Spectrum Disorders

Robert T. Schultz*, Daniel Grupe, Christine Shin, Elinora Hyundi, Julie Wolf, Warren Jones

Children's Hospital of Philadelphia, Philadelphia, PA

Background: The autism spectrum disorders (ASD) are a highly heritable and heterogeneous group of disorders that share social-communicative deficits. Individuals with ASD have difficulties with social motivation, social perception and social cognition. Deficits in face perception, especially facial identity matching, are among the largest behavioral markers of ASD (Wolf et al., 2008), especially since these deficits have very little correlation with IQ. Hypoactivation of the fusiform gyrus (FG) during social perception tasks is perhaps the best replicated fMRI marker for ASD, but a few publications argue that the effects are a consequence of looking behavior during the fMRI tasks. New, unpublished data using fMRI and conventional anatomical MRI will be presented. Results converge on abnormalities of structure and functional the temporal lobe of males with ASD independent of looking behavior.
Methods: High resolution structural MRIs were collected on 113 males with an ASD and compared to 103 typically developing controls (TDC) males. In a second independent sample, fMRI data were collected during 5 separate face perception runs in 38 males, 18 with ASD and 20 TDCs. One run employed a task show requiring participants to focus on a cross hair between the eyes so as to force gaze to the eye region. All tasks utilized eye tracking measurement to characterize the impact of differential point of regard on regional brain activity.
Results: Overall brain size was enlarged in ASD, with greater enlargement in the white matter vs. gray matter, especially in the temporal lobes. White matter in anatomically defined FG was most enlarged. Voxel based analyses show that the ventral temporal pathway in the right hemisphere is specifically enlarged in ASD vs. TDC. fMRI results also converge on this brain region. Averaging across fMRI runs the experiment, the right amygdala and FG were significantly less active in ASD vs. TDCs. Receiver operator curves using peak signal in the right FG showed very good diagnostic sensitive and specificity. Analyses of individual runs employing face tasks that systematically varied depth of processing showed evidence of task by group interactions, as well as main effects of task and group. Analyses of the eye tracking data found no evidence that hypoactivation of the FG in ASD was mediated by looking behavior - group differences were significant when participants looked at the eye region of the face as well as when they looked at the mouth region.
Discussion: Structure and function of the FG is abnormal in ASD. Converging evidence across this other studies implicates temporal lobe regions, especially the FG in the pathobiology of ASD. Moreover, a recent genome wide association study (Wang et al, 2009) reported the first common variant with genome wide significance in the intergenic region between CDH9 and CDH10. Previously published expression data in adult brain tissue show that CDH10 is most strongly expressed in the amygdala and temporal lobe, further suggesting a key role of these brain regions in ASD.
Disclosure: R.T. Schultz, Pfizer, Part 1; Pfizer, Part 2; Pfizer, Part 3; Pfizer, Part 4.

Whole Genome and Targeted Approaches to Studying Neuronal Adhesion Molecules in Autism Spectrum Disorders

Matthew State*

Yale University, Department of Genetics, New Haven, CT

Background: Our laboratory has had a long-standing interest in the contribution of rare genetic mutations to Autism Spectrum Disorders which has in the identification of three Contactin family neuronal adhesion molecules (Contactin 4, CNTN4, and Contactin Associated Protein 2, CNTNAP2) as likely contributors to both social and cognitive disability. We have now expanded our studies of rare variation via a large-scale collaborative investigation of copy number variation (CNV) in 1500 ASD families with only one affected member as well as through targeted deep sequencing and functional analyses of Contactin and Contactin-associated molecules.
Methods: The Simons Simplex Collection (N = 6000) was studied using a combination of genotyping on the Illumina IM and IM duo platform and targeted mutation discovery via Raindance micro-emulsion PCR coupled with next generation massively parallel sequencing on the Illumina GAII instrument. CNV prediction was performed using the overlap of three algorithms, CNV confirmations were performed in both case and control groups using quantitative PCR. The sensitivity and specificity of these approaches as well as that for a pooling strategy for the discovery of individually rare mutations via next generation sequencing was evaluated prior to undertaking these studies.
Results: Combining three algorithms for the prediction of transmitted copy number variants provides >90% positive predictive value (PPV) over a wide range of CNV "sizes". *De novo* predictions of all but the

largest CNVs have a PPV of <50% due to a high rate of false negatives in one parent, and must be confirmed using alternative methods such as QPCR. Pooling of 8 samples prior to library preparation using the Raindance instrument to study 1152 genomic regions simultaneously provides 100% sensitivity and >99.9% specificity for the detection of a single rare mutation within the pool. New data will be presented regarding the findings of a large scale CNV study of Autism in a extremely well characterized simplex sample, with a focus on the identification and follow-up of rare *de novo* CNVs implicating neuronal adhesion molecules in the etiology of ASD. The results of a large-scale mutation discovery in Contactin and Contactin associated molecules will be presented.
Discussion: Autism genetics is undergoing a rapid transformation from gene hunting to gene finding, led by a combination of rapidly advancing technology and the widespread availability of large numbers of DNA samples from well-characterized individuals and families. Many of the recent discoveries have implicated individually rare sequence and structural mutations in neuronal adhesion molecules. Data from an unbiased CNV analysis and a targeted deep resequencing project will be presented and the implications of the findings for the understanding of some of the biological mechanisms underlying ASD will be considered.
Disclosure: M. State, None.

Modeling an Environmental Risk Factor for Autism in Mice Leads to Permanent Changes in the Immune System
Paul H. Patterson*

California Institute of Technology, Div. of Biology, Pasadena, CA

Background: Several types of maternal infection (influenza, rubella, genital and reproductive system viruses, and urinary tract bacteria) are associated with increased risk of schizophrenia or autism in the offspring. Modeling this risk factor in mice using influenza infection or activation of the dam's immune system by injection of the double stranded, synthetic RNA, poly(I:C) yields offspring with characteristic endophenotypes of these disorders. Features consistent with symptoms in autism include behavioral abnormalities (deficits in social interaction, prepulse inhibition, open field exploration, neonatal vocalizations) and neuropathology (spatially localized Purkinje cell deficit).
Methods: Pregnant C57BL/6J mice are injected with poly(I:C), saline, IL-6, or anti-IL-6 antibody (or a control antibody) plus poly(I:C) on E12.5, and the fetuses and postnatal offspring are examined at various ages.
Results: The cytokine IL-6 is a key mediator of the effects of maternal immune activation (MIA) on the fetus, as shown by blocking or genetically inactivating IL-6 in the MIA dam. Moreover, MIA activates downstream IL-6 signaling pathways in the placenta and in subpopulations of neurons in the fetal brain. In addition, IL-6 mRNA is induced in both of these tissues, which raises the possibility of a feed-forward mechanism that could lead to permanent changes in immune status as is seen in the brain and peripheral immune system of autistic subjects. There is also evidence for inflammation in the gastrointestinal (GI) tract in autism. Thus, it is of interest that we find that MIA leads to significant changes in lymphocytes from the spleen and the GI-associated, mesenteric lymph node of adult offspring. CD4+ T cells display elevated levels of the pro-inflammatory cytokines IL-6 and IL-17 in response to stimulation *in vitro*.
Discussion: These observations support the hypothesis that MIA can cause permanent changes in immune status of the offspring. Thus, the MIA model has face and construct validity for autism, and is proving useful for exploring the mechanism of how the maternal infection risk factor alters fetal brain and immune system development. Work by others has further shown that the MIA model is useful in evaluating potential therapeutic approaches.
Disclosure: P.H. Patterson, None.

Thursday, December 9, 2010

Panel Session
Cannabinoids Session 1: As the Smoke Clears: Clinical and Pre-Clinical Evaluations of Cannabis Dependence and Withdrawal

Rodent Diversity: Sensitivity to the Pharmacological Effects of Δ^9-Tetrahydrocannabinol Across Age and Sex
Jenny Wiley*

Research Triangle Institute, Research Triangle Park, NC

Background: Marijuana is among the most commonly used illicit drugs during adolescence; however, little is known about its effects during development. This study used a longitudinal approach to examine sensitivity to the effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) in a rodent model during adolescence [postnatal day (PN) 28-42] and into adulthood, with comparisons to effects in rats that began an identical dosing regimen as adults. Evaluation of sensitivity was achieved through use of a battery of tests in which cannabinoids produce the characteristic effects of hypothermia, catalepsy and suppression of spontaneous activity.
Methods: Male and female Long-Evans rats were periodically assessed in the test battery over the course of chronic dosing with vehicle or Δ^9-THC. In order to obtain an entire dose-effect curve in each rat, a cumulative dosing procedure was used for assessment at each time point. Prior to the start of the chronic dosing regimen, an initial assessment in the test battery was completed on PN30 for adolescent rats or during early adulthood (>PN65). Rats were then administered vehicle or 10 mg/kg Δ^9-THC twice daily for 30 days, with additional assessments in the test battery occurring on days 7, 14, and 31. Injections were terminated after day 31 and a 7-day wash-out period began. Rats were re-tested on day 38. In addition, a group of rats of each age and sex were chronically dosed with vehicle until day 38 when they were injected with cumulative doses of Δ^9-THC and tested. Studies were approved by the IACUC at Virginia Commonwealth University, Richmond, VA and were conducted in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*.
Results: In all groups of rats, Δ^9-THC produced significant dose-dependent suppression of spontaneous activity, hypothermia and catalepsy. Δ^9-THC-induced effects in this initial pre-treatment test were similar across doses in rats of the same age and sex, regardless of the assigned chronic treatment condition; however, significant differences between effects in adolescent and adult rats were apparent for some measures. For example, adolescents of both sexes exhibited greater sensitivity to Δ^9-THC-induced suppression of activity; however, adult males and females were more sensitive to Δ^9-THC effects in the bar test of catalepsy. Increased sensitivity to hypothermic effects was also found in adolescent (vs. adult) males, but not in female adolescents (vs. adults). Final cumulative dose-effect curves, conducted when adolescents had reached adulthood, revealed dose-dependent decreases in spontaneous activity regardless of age, sex, or treatment condition. Hypothermia occurred only in adolescent and adult rats of both sexes that received Δ^9-THC acutely or intermittently during testing over the course of the chronic dosing regimen. However, in both sexes, dose-dependent increases in catalepsy were observed in adolescent and adult rats in all treatment conditions, with adults attending the bar significantly longer than adolescents.
Discussion: In summary, sensitivity to the acute effects of Δ^9-THC differed across age and sex. Further, administration of Δ^9-THC during development altered this level of sensitivity, regardless of whether exposure was chronic or intermittent. Nevertheless, age and sex differences in sensitivity to Δ^9-THC's effects in the bar test persisted, suggesting that age and sex differences in Δ^9-THC's effects are task-specific. This work was supported by NIH grant DA-016644.
Disclosure: J. Wiley: None.

Effect of Long-Term Delta-9-THC Exposure on Rat Cognitive Performance

Loren Parsons*

The Scripps Research Institute, La Jolla, CA

Background: Heavy cannabis use by humans is associated with neurocognitive deficits including impairments of attention, inhibitory control, impulsive behavior, decision-making and memory. Neuroimaging studies have demonstrated cannabis-induced alterations in the function of the orbitofrontal cortex, hippocampus and components of the basal ganglia and it has been proposed that neural dysfunction in these areas contribute to a loss of inhibitory control that propels continued cannabis use. The purpose of this study was to characterize neurocognitive function in rats given long-term intermittent exposure to Δ9-THC doses that produce clinically relevant plasma Δ9-THC levels.
Methods: Δ9-THC Dosing: Male Wistar rats were treated with vehicle, 0.3 or 3.0 mg/kg Δ9-THC (i.p.) in repeating cycles of 14-day blocks in which Δ9-THC was administered 2x per day for 6 days, followed by an 8-day drug-free period during for behavioral testing. Behavioral Testing: Separate cohorts of rats were prepared for testing in five behavioral tasks. (1) 5-Choice Serial Reaction Time Task (5-CSRTT; n = 20/group); (2) Delay Discount (n = 7/group); (3) Differential Reinforcement of Low Rates of Responding (DRL-30; n = 7/group); (4) Novel Object Exploration, conducted in three stages to evaluate latency to exploration, recognition memory using substitution and recognition memory using displacement (n = 8/group). (5) Dependence Measures (precipitated withdrawal; 3 mg/kg SR141716A). Duration of dependence was evaluated with tests at 7-day intervals following cessation of Δ9-THC treatment. (n = 8/group).
Results: All tests were conducted over the course of at least 10 cycles of Δ9-THC treatment. 5-CSRTT: Significant Δ9-THC effects were evident in "challenge" tests employing variable inter-trial interval (ITI) times to increase task difficulty: a dose dependent decrease in accuracy at short ITIs and increased impulsive responding at long ITIs. Delay Discount: There was no significant effect of Δ9-THC on performance of this task. DRL-30: Enhanced performance (rewards/total responses) was evident in the 3 mg/kg group from 4 cycles of Δ9-THC treatment forward. Object Exploration: Δ9-THC treated animals displayed increased exploration latency (index of anxiety-like behavior) and decreased novel object recognition in substitution tests (index of recognition memory). Precipitated Withdrawal: SR14716A induced significant withdrawal signs in all measures, with the severity directly related to the Δ9-THC treatment dose. Significant antagonist-induced withdrawal persisted for up to 2 weeks following Δ9-THC treatment.
Discussion: Despite evidence of dependence, increased anxiety-like behavior and deficits in recognition memory, Δ9-THC treated rats displayed no alterations in attention, impulsive behavior or inhibitory control when tested under well-established, familiar conditions. However, 5-CSRTT trials with increased task difficulty revealed significantly increased impulsive behavior and decreased accuracy that correlated with the Δ9-THC treatment dose. These findings suggest that long-term Δ9-THC exposure results in diminished behavioral performance under conditions of increased cognitive load.
Supported by NIDA P20 DA024194.
Disclosure: L. Parsons: None.

What Is the Mechanism of Neurocognitive Impairment in Chronic Daily Cannabis Users?

Marilyn A. Huestis*

National Institute on Drug Abuse, Baltimore, MD

Background: Neurocognitive impairment has been observed after chronic daily cannabis smoking as compared to naïve cannabis users, abstinent former heavy cannabis smokers and current occasional cannabis smokers. Pope et al found that chronic daily cannabis smokers (greater than 5000 lifetime uses) were impaired at baseline and after seven days of sustained abstinence, but there were no significant differences in performance at 28 days of abstinence[1]. In contrast, Bolla et al. reported significant cognitive impairment at baseline that continued through 28 days of abstinence in a different cohort of heavy cannabis smokers[2]. We suggest that the mechanism of the observed cognitive impairment could be residual Δ9-tetrahydrocannabinol (THC) in the brain due to a high body burden of cannabinoids that occurs following extended daily cannabis smoking. THC is the primary psychoactive component in cannabis. THC concentrations in blood and plasma decrease rapidly after acute, occasional cannabis smoking[1], but what is the best approach for investigating chronic cannabis exposure?
Methods: For safety and ethical reasons, we would never dose cannabis smokers with the amount of cannabis and the length of time they self-administer the drug. We evaluated cannabinoid pharmacokinetics in whole blood, plasma and urine in chronic daily cannabis smokers following initiation of cannabis abstinence and residence on a closed research unit for up to 30 days. All urine voids throughout the study were analyzed by two-dimensional gas chromatography mass spectrometry with low limits of quantification of 0.25 ng/mL in whole blood, plasma and urine in order to quantify residual excretion of cannabinoids.
Results: We found THC 1 ng/mL in whole blood and plasma of some chronic daily cannabis smokers for up to 7 days and THC, not the inactive 11-nor-9-carboxy-Δ9-tetrahydrocannabinol, in urine of chronic smokers up to 24 days after the start of abstinence. Furthermore, females had significantly longer detection of THC in blood, plasma and urine than males, despite similar amounts of cannabis smoked per day and years of cannabis smoking; however, detection time was not significantly correlated to body mass index.
Discussion: These data suggest that residual THC in brain could be the mechanism of neurocognitive impairment in chronic cannabis smokers, and that with sustained cannabis abstinence, performance may return to baseline levels. Our current research further probes this hypothesis by evaluating CB1 cannabinoid receptor density during cannabis dependence and sustained cannabis abstinence.
[1]Huestis MA, Henningfield JE and Cone EJ. Blood cannabinoids: I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after marijuana smoking. *Journal of Analytical Toxicology*, 1992 Sep-Oct; 16(5):276-282.
[2]Pope HG, Jr., Gruber AJ, Hudson JI, Huestis MA and Yurgelun-Todd D. Neuropsychological performance in long-term cannabis users. *Archives of General Psychiatry*, 2001 Oct; 58(10):909-915.
[3]Bolla KI, Brown K, Eldreth D, Tate K, Cadet JL. Dose-related neurocognitive effects of marijuana use. Neurology. 2002 Nov 12;59(9):1337-43.
Disclosure: M.A. Huestis: None.

Precipitated Versus Natural Withdrawal in Outpatients with Cannabis Dependence Versus Healthy Controls

Barbara J. Mason*, Rebecca Crean, Vivian Goodell

The Scripps Research Institute, La Jolla, CA

Background: Cannabis withdrawal is not included in the DSM-IV because its "clinical significance is uncertain." However rats chronically treated with cannabinoids, but not drug naïve rats, showed marked withdrawal when administered the CB1 antagonist rimonabant. We aim to characterize precipitated (rimonabant) versus naturally-occurring (placebo) cannabis withdrawal across emotional, behavioral, and biochemical measures in paid volunteers with cannabis dependence and in relation to untreated non marijuana-using controls, to provide clear evidence of a cannabis withdrawal syndrome.
Methods: Subjects are male and female cannabis dependent volunteers and non marijuana-using controls, ages 21 to 30 years. This is a 28-day longitudinal study with two components: a single dose, double-blind, placebo-controlled study of precipitated cannabis withdrawal in the

lab using rimonabant 90 mg po, followed by outpatient assessments of key symptoms of cannabis withdrawal with financial compensation for monitored abstinence over the subsequent 28-day period.
Results: Cannabis subjects averaged >5 years of daily use of >1 gram of marijuana per day and had a baseline urinary THCCOOH/creatinine concentration of >400 ng/mL. Rimonabant-treated subjects showed significant elevations in plasma cortisol and ACTH and on the Marijuana Withdrawal Checklist and Spielberger State-Trait Anxiety Inventory relative to placebo following dosing on the challenge day, but then averaged lower scores on these scales, as well as on the Beck Depression Inventory, over the subsequent period of monitored abstinence. However untreated, non marijuana-using controls scored lower than cannabis groups on all scales at all time points. The THCCOOH/creatinine concentrations confirmed marijuana abstinence over the 28-day study.
Discussion: Rimonabant 90 mg precipitated a significant acute increase in cannabis withdrawal symptoms which then significantly decreased over the 28-day study, relative to placebo. Both cannabis groups showed elevated mood, anxiety, and cannabis withdrawal symptoms relative to non marijuana-using controls. These data suggest cannabis withdrawal has motivational components symptoms akin to other drugs of abuse which may be significantly altered by a single dose of the CB1 antagonist, rimonabant. Supported by P20DA024194 from NIDA.
Disclosure: B.J. Mason: Part 1; Addex Pharmaceuticals, Lilly, Forest Laboratories, Inc., GlaxoSmithKline, Johnson & Johnson Pharmaceutical Research & Development, LLC, Lohocla Research Corporation, Catalyst Pharmaceutical Partners, Inc.

Panel Session
Comparing Neural and Behavioral Characteristics in Schizophrenia and Psychotic Bipolar Disorder: Findings from the Bipolar and Schizophrenia Network on Parsing Intermediate Phenotypes (B-SNIP) Study

Neurocognitive Abnormalities in Schizophrenia and Bipolar Probands and Their Family Members

John Sweeney*, James Reilly, Scot Hill, Brett A. Clementz

University of Illinois Chicago, Chicago, IL, University of Illinois at Chicago, Chicago, IL, University of Illinois, Chicago, IL, University of Georgia, Athens, GA

Background: Neurocognitive abnormalities are well established in schizophrenia patients and their unaffected relatives. Paralleling multiple lines of evidence that now indicate phenotypic and genotypic overlap between schizophrenia (SZ) and bipolar disorder (BP), recent studies have documented prominent and persistent cognitive impairments in bipolar patients as well. Large scale studies investigating the relative prevalence and profile of these deficits in BP have not been conducted, nor has the expression of these deficits in unaffected relatives of BP patients been extensively investigated.
Method: In the BSNIP study, neuropsychological tests, cognitive neuroscience paradigms and cognitive oculomotor tasks were used to evaluate neurocognitive function in what now is a sample of 1200 subjects relatively equally divided among 5 groups: SZ and BP probands, unaffected relatives of these two groups, and healthy controls.
Results: Neuropsychological deficits on the BACS test were at 1.7 SD for Sz patients and 1.2 SD for BP probands. Deficits in Sz were greater than in BP in all BACS domains but verbal memory. Spatial span was notably affected in Sz relatives, while verbal memory impairment was more notable in BP relatives. Stop signal task performance was the only test where deficits were greater for BP than Sz probands. CPT, emotion identification and spatial span deficits were similar in Sz and BP probands; among these, only CPT deficits were greater in Sz than BP relatives. On an auditory oddball task, BP relative and healthy subjects had highly similar brain responses, while SZ and BP probands and SZ relatives showed abnormal P2 and P3 responses to target stimuli. Antisaccade deficits were greater in Sz than BP probands but were similar in the family member groups.
Discussion: Cognitive deficits are pronounced in BP patients. These were less pronounced than in Sz probands for several domains with the notable exception of the stop signal task. Deficits were present in unaffected Sz and BP family members, with these deficits being greater in Sz relatives on some but not all tests. The profile of deficits across BP and Sz suggests that different domains of function are relatively more affected in the two disorders. These observations clarify several similarities and differences across Sz and BP in neurocognitive function, and suggest that cognitive data may provide useful intermediate phenotypes for family genetic studies into the boundary of these disorders.
Disclosure: John Sweeney, Pfizer, Part 1; Janssen Research grant, Part 2.

Functional Network Connectivity Analysis of Resting State fMRI Data in Schizophrenia and Psychotic Bipolar Probands and Their Unaffected Relatives

Godfrey Pearlson*

Yale University/Institute of Living, Hartford, CT

Background: Functional network connectivity (FNC), measuring connections among brain circuits, (as opposed to between regions) can be used to characterize fMRI neural interactions during cognition or at rest.
Methods: We used FNC to examine differential interactions between independent resting state networks in 116 healthy controls, 63 bipolar psychotic (BP) and 51 schizophrenia (SZ) patients, 74 relatives of SZ & 27 relatives of BP, (age/sex/ethnicity matched) derived from the BSNIP study and imaged at 3T during a 5-minite scan during which subjects were instructed to lie still and focus on an asterisk. Resting state circuits were identified using Independent Component Analysis in the GIFT toolbox, which delineated 15 components, including the default mode network. FNC was implemented using the method of Jafri et al 2008.
Results: Both patient groups and both groups of unaffected relatives differed from healthy controls. Many of the abnormal functional network connections (both increased and decreased vs controls) were unique between patient groups ($p < 0.05$ FDR corrected). However, 3 connections (posterior cingulate-thalamus- cerebellum; posterior cingulate-thalamus-medial precuneus; left fronto-parietal-subgenual cingulate-sensorimotor) were commonly affected in both analyses and primarily appeared to be more tightly connected in relatives and probands compared to controls. In general, relatives displayed fewer differences from controls than probands, but they also displayed differences from controls not seen in their affected relatives.
Discussion: This large scale study suggests that specific anomalous functional connections are shared between SZ and BP probands. Also, unaffected relatives of SZ and BP subjects shared several connectivity abnormalities with their affected probands, but additionally differences that may represent compensatory mechanisms. Overall, we demonstrate the utility of FNC to identify both potential resting connectivity based "endophenotypic" and "disease" markers for mood disorders and psychosis.
Disclosure: G.D. Pearlson, None.

Are Brain Structural Alterations Similar in Schizophrenia and Psychotic Bipolar Disorder? Preliminary Structural MRI Data from the B-SNIP Study

Matcheri Keshavan*

Harvard University, Boston, MA

Background: Schizophrenia (SZ) and psychotic bipolar disorder (BP) overlap in clinical manifestations, pathophysiology, and etiology,

raising questions about the century-old Kraepelinian view that these are distinct diseases. Characterizing disease-related neurobiological alterations in these disorders is likely to have key implications to efforts addressing this question. The Bipolar and Schizophrenia Network for parsing Intermediate Phenotypes (B-SNIP) consortium seeks to examine a broad panel of endophenotypes (cognitive, electrophysiological, and imaging) in 500 SZ and 500 BP probands and their relatives (SZrel and BPrel). The B-SNIP project is currently ongoing in recruitment; we herein present findings from interim morphometric analyses of the structural MRI (sMRI) images.
Methods: MRI scan were obtained using 3.T magnets and analyzed using Freesurfer. Regions known in prior literature to be altered in SZ and BP were examined across groups using ANCOVAs with age, sex, intracranial volume and study site as covariates; significant tests were followed by pair-wise comparisons. A total 381 patients (SZ, n = 87; BP, n = 54; SZrel n = 85; BPrel n = 57; controls, n = 96) were processed.
Results: Both schizophrenia and bipolar probands show structural deficits compared to controls in orbitofrontal, heteromodal association cortices, medial temporal and and cingulate regions, with schizophrenia probands in general having more severe and widespread deficits compared to bipolar probands. Subcortical regions are larger in schizophrenia probands compared to both controls and bipolar probands. Structural measures in relatives of schizophrenia and bipolar probands showed deficits in these brain regions intermediate in severity between patient and control groups. However, the left medial orbitofrontal, superior frontal and pars orbital volumes were larger in bipolar relatives compared to controls.
Discussion: Our sMRI data support the view that there may be commonalities as well as unique distinctions in brain structural changes between schizophrenia and psychotic bipolar disorders. Further analyses are under way to investigate the heritability of these measures as well as relationships between sMRI alterations and other endophenotypic measures in psychopathological, cognitive, functional and physiological domains.
Disclosure: M.S. Keshavan, None.

Endophenotype/Genotype Relationships in Psychotic Disorders
Gunvant Thaker*, Ikwunga Wonodi, Nithin Krishna, Carol Tamminga, John Sweeney, Matcheri Keshavan, Godfrey Pearlson, James Gold, Elliot Hong

Maryland Psychiatric Research Center, Baltimore, MD

Background: Search for vulnerability genes of schizophrenia (SZ) and bipolar disorder (BD) has been complicated by their complex nature with high phenotypic and genetic heterogeneity. Furthermore, their boundaries are poorly demarcated resulting in substantial overlap in genetics and pathophysiology. The Bipolar and Schizophrenia Network on Parsing Intermediate Phenotypes (B-SNIP) study is examining endophenotypes to disentangle this etiologic and pathophysiologic overlap between the two. Endophenotype is defined as a specific, heritable, quantifiable brain deficit that marks psychosis risk. Research of the past three-four decades has identified several such endophenotypes. Studies suggest that many of these endophenotypes mark independent aspects of psychosis risk, however a large sample study that includes a comprehensive battery in the two disorders has not been carried out. Furthermore, it is unclear to what extent unique genes are associated with each of the endophenotypes.
Methods: Relationships across different endophenotypes were examined using Principal Component Analysis with varimax rotation in a sample of 156 subjects (schizophrenia probands, their relatives, and healthy control subjects). A total of 12 measures (P50 sensory gating, PPI, SPEM, antisaccade, oculomotor delayed response, and composite measures of memory, problem solving, processing speed, working memory, and attention) were included. Genetic associations were examined in a SZ sample (N = 451) and a large cohort (n = 971) that included both SZ and BD families (B-SNIP sample). Genetic studies either examined associations with candidate genes, or carried out a two-phase study that included a genome-wide screen in 100 subjects, followed by confirmation of top hits in a larger sample that used False Discovery Rate corrections. The latter set of studies used the predictive component of the smooth pursuit eye movement function as a phenotype.
Results: Cognitive measures, errors in antisaccade, and oculomotor delayed response task loaded on to the same factor. While, P50 sensory gating, PPI, smooth pursuit eye movement measures loaded on to three separate factors. Variation in NRG1 was associated with PPI in the first sample, while variation in a non-synonymous coding SNP in CHRNA5, rs16969968, was associated with sensory gating. Neuroxin 3 (NRXN3) was one of top genes screened by the genome wide screening in the discovery sample. The association was confirmed in African-American subjects in the confirmatory analysis. Variation in ALDH5a1 was associated with early motion processing and smooth pursuit response. ALDH5a1 gene codes for semialdehyde dehydrogenase enzyme, a GABA degradation enzyme. The phenotype associated with the deficiency in this enzyme is well described and include problems in early visual processing. In secondary study showed significant effects of variations in ALDH5a1 on visual evoked potential. Predictive pursuit response was significantly worse in SZ and their relatives compared with healthy control in the B-SNIP sample. BD probands and relatives performed at a intermediate level. We are in the process of genotyping NRXN3 and ALDH5a1 genes in this sample in attempt to replicate the findings.
Discussion: These findings show that cognitive measures, P50 sensory gating, PPI, and SPEM are independent suggesting that they mark independent aspects of psychosis risk. Variations in different genes are associated with different endophenotypes.
Disclosure: G.K. Thaker, Mitsubishi Tanabe Pharma, Part 4.

Panel Session
Drugs of Abuse and Postpartum Depression

Postpartum Depression: An Overview
Zachary Stowe*

Emory University, Atlanta, GA

Background: Maternal mental illness following childbirth has been described for centuries. These illnesses have been broadly grouped as the 'blues', postpartum depression (PPD), and postpartum psychosis (PPS). The debate about whether or not PPD and PPS represent a distinct set of illnesses has eased over the past decade with the increasing recognition that many of these women have a previous history of mood or anxiety disorders prior to childbirth. The postpartum period represents a unique neuroendocrine and psychosocial event that, fortunately, the majority of mothers traverse without significant mental illnesses.
Methods: Literature review and presentation of scientific data derived from the Emory Women's Mental Health Program and collaborators.
Results: There remain issues with respect to the optimal identification/detection of PPD utilizing rating scales. Despite the long term recognition, there remain sparse data on etiology, vulnerability factors, and treatment response that have incorporated assessment of 'recurrent' versus 'new onset' illness events. It seems intuitive, that sex steroids and/or their metabolism/interactions could account for the vulnerability to develop PPD. However, the extant data awaits replication. Recent data from our group and others have demonstrated that the 5-HTTLPR genotype is associated with depressive symptoms in women with a history of depression. Preliminary data suggested that response to antidepressants may be differ between 'recurrent' versus 'new onset' of depression in the postpartum period. The treatment of PPD is often complicated by a desire to breast feed. Remarkably, as a class, antidepressants now have more data in breast feeding than any

other class of medications. While adverse effects from medication exposure during breast feeding are limited, maternal tobacco and substance abuse have not been included in these reports. Across the vast majority of these epidemiological, etiological, treatment, and follow up studies - maternal tobacco and substance abuse is typically viewed as a consequence of maternal depression.
Conclusion: The role(s) of co-morbid substance abuse in the etiology, treatment response, and impact on infant development of maternal depression as well as its contribution to 'recurrent' versus 'new onset' episodes, if included, have typically relied on maternal self-report and therefore may be under-estimated.
Disclosure: Z.N. Stowe, Lifetime disclosure - Wyeth, GSK, Pfizer, Lilly, Bristol Myer Squibb, Forest Laboratories, Part 1; Lifetime disclosure - Wyeth, GSK, Pfizer, Part 4.

Association Between Drugs of Abuse and Postpartum Depression
Amy L. Salisbury*

Warren Alpert Medical School at Brown University, Providence, RI

Background: Current drug use by pregnant women in the United States is estimated at 5.2% for illicit drugs, 16.4% for nicotine and 11.6% for alcohol.[1] Major Depressive Disorder (MDD) occurs in over 11% of women at child-bearing age[1];and has been reported to occur in 10-25% of women during pregnancy. Although the prevalence of co-morbid MDD and substance use during pregnancy is often reported to be quite high, the rate is not clear. Moreover, the specific nature and timing of associations between substance use and MDD has not been previously described.
Methods: Data analyses (Chi-square) were performed on the 2008 National Survey on Drug Use and Health database[2] to determine co-morbidity statistics on pregnant women, depression, and substance use. Specific questions examined included: What is the prevalence of co-morbid substance abuse/dependence and depression in the past year for pregnant women? Do the associations vary by trimester? Do the associations vary by drug type?
Results: In the survey data, 978 respondents were pregnant. As reported in the NASDUH publication, 5.2% of the women reported current drug use. Further data analyses by trimester of pregnancy did not reveal differences based on trimester($X^2 = 1.776$, $p = .624$). There was also no difference between trimester on alcohol dependence ($X^2 = 4.47$, $p = .215$), however there was a trend for fewer women to report alcohol abuse in the third trimester (1.5%) than in the first (5.4%) or second trimester (4.19%; $X^2 = 8.86$, $p = .312$). In this sample, 7.67% reported a significant episode of MDD in the past year, with no difference between trimesters. pregnancy. All substances of abuse showed an association with prenatal and/or postpartum depression; the associations were often stronger when nicotine or alcohol was combined with other substances. There was a significant lack of data on the timing and onset of depression in relation to substance use during pregnancy. More than three times the number of women who reported MDD also reported illicit substance dependence in all trimesters of pregnancy (2.80% vs. 10.67%).
Discussion: The majority of published studies suggest that prenatal substance use is highly associated with incidence of major depression in pregnant and postpartum women. The data analyzed here suggest the same trend. However, timing of depression onset versus drug use onset was not able to be assessed. Specific associations by drug type and other psychotropic medications will be discussed. These data will also be discussed in relation to data from database samples and large national cohort studies. 1. Substance Abuse and Mental Health Services Administration. (2008). *Results from the 2007 National Survey on Drug Use and Health: National Findings* (Office of Applied Studies, NSDUH Series H-34, DHHS Publication No. SMA 08-4343). Rockville, MD. 2. United States Department of Health and Human Services. Substance Abuse and Mental Health Services Administration. Office of Applied Studies. National Survey on Drug Use and Health, 2008 [Computer file]. ICPSR26701-v2. Ann Arbor, MI: Inter-university Consortium for Political and Social Research [distributor], 2009-12-16. doi:10.3886/ICPSR26701.
Disclosure: A.L. Salisbury, None.

Animal Models of Postpartum Depression: Effects of Steroid Hormones
Liisa A.M. Galea*

University of British Columbia, Vancouver, BC, Canada

Background: Postpartum depression (PPD) affects 15% of women and very few women with PPD seek treatment. Women with untreated PPD have impaired cognitive ability, marital difficulties, and are more likely to abuse their children. Children of untreated PPD mothers have an increased risk to develop depression and have impaired cognitive, motor and social development. Adult neurogenesis in the hippocampus is associated with depression, is modulated by drugs of abuse and steroid hormones and may be a neural marker for depression. In the present experiments we examined the effects of animal models of postpartum depression on adult hippocampal neurogenesis and sucrose anhedonia. Further we tested stress reactivity and impulsivity in the offspring of PPD dams to understand effects of PPD on the offspring. Steroid hormones play a significant role in depression. During pregnancy and postpartum, levels of estrogens, progesterone and glucocorticoids fluctuate dramatically and may contribute to the etiology of PPD. We have created two animal models of PPD based on steroid hormones. In the ovarian hormone withdrawal model withdrawal from a hormone simulated pregnancy increases depressive-like behavior. In our corticosterone (CORT)-induced model, high chronic levels of CORT during the postpartum increased depressive-like behavior in the dams and increased anxiety-like behavior in the offspring.
Methods: In this study, a hormonally-simulated pregnancy was induced in ovariectomized female rats and the effect of a 'postpartum' drop in estradiol was examined on hippocampal cell proliferation and sucrose preference, a model of anhedonia Further we examined the effects of high corticosterone (40 mg/kg) given during gestation and/or the postpartum on hippocampal cell proliferation and sucrose preference. In Expt 1, 4 days after a hormone stimulated pregnancy rats were perfused to assess cell proliferation in the dentate gyrus. A separate group of rats was tested for consumption of, and preference for, sucrose at baseline, throughout the hormone simulated pregnancy and during the withdrawal. In Expt 2 female rats were given high CORT during gestation and/or the postpartum period for 10-21 days and the dams and offspring were examined for changes in hippocampal cell proliferation and in a variety of behavioural tests, including impulsivity.
Results: In Expt 1 hormone withdrawal decreased hippocampal cell proliferation which was rescued by commitment treatment with the tricyclic antidepressant, imipramine. Imipramine significantly increased hippocampal cell proliferation in intact females, but not in ovariectomized females suggesting that imipramine's effects on hippocampal cell proliferation in female rats is related to ovarian hormones. Further withdrawal from a hormone simulated pregnancy reduced sucrose preference. In Expt 2 high CORT during gestation resulted in increased depressive-like behavior and stress reactivity while high CORT during postpartum resulted in altered impulsivity in the offspring. High CORT during the gestation and the postpartum reduced cell proliferation but did high CORT during the postpartum did not alter sucrose preference in the dams.
Conclusions: Together these studies suggest an important role for steroid hormones on the dams and her offspring in these models of postpartum depression and may link impaired behavior in the offspring to maternal hormone environment.
Disclosure: L.A. Galea, None.

Alcohol Consumption in Mice Exhibiting Postpartum Depression-Like Behaviors

Jamie Maguire*

Tufts University, Boston, MA

Background: We recently characterized a mouse model, with deficits in GABAergic inhibition (*Gabrd*$^{-/-}$ mice), that exhibits depression-like behaviors during the postpartum period. Due to the well documented increase in drug and alcohol abuse associated with depression, we hypothesized that mice exhibiting depression-like behaviors in the postpartum period would exhibit increased drug and/or alcohol abuse. Given the well known actions of alcohol on $GABA_A$ receptors ($GABA_A$Rs), we sought to determine whether deficits in GABAergic inhibition may underlie the increased alcohol consumption associated with postpartum depression-like behaviors.
Methods: We utilized the two-bottle test to monitor voluntary consumption of either normal drinking water or 6% ethanol. To determine the influence of postpartum depression-like behavior on alcohol abuse, voluntary alcohol consumption was measured in virgin and postpartum wild type or *Gabrd*$^{-/-}$ mice. To determine the impact of stress on alcohol abuse, voluntary alcohol consumption was monitored in vehicle and Antalarmin treated wild type or *Gabrd*$^{-/-}$ mice.
Results: In a two-bottle test, mice exhibiting depression-like behaviors in the postpartum period demonstrated increased voluntary consumption of 6% ethanol compared to postpartum wild type mice. However, the mechanism underlying this increased alcohol consumption is unknown. In humans, major depression is associated with hyperexcitability of the hypothalamic-pituitary-adrenal (HPA) axis. Interestingly, we observe an exacerbated stress response in *Gabrd*$^{-/-}$ mice and changes in the activation of the HPA axis, evident by an increased basal firing rate of corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus (PVN) of the hypothalamus, which we hypothesized may play a role in these abnormal postpartum behaviors as well as the increased alcohol consumption. Abnormal postpartum behaviors can be mimicked in wild type mice with either physiological stress or administration of exogenous stress hormones. Due to the evidence of increased alcohol abuse associated with both depression and stress, we hypothesized that activation of the HPA axis may play a role in alcohol abuse. To test this hypothesis, we blocked HPA axis activation with a CRH antagonist, Antalarmin, and monitored alcohol consumption. Mice treated with Antalarmin exhibited a decrease in voluntary alcohol consumption compared to vehicle treated mice.
Discussion: Our data suggest that mice exhibiting postpartum depression-like behaviors exhibit a propensity for alcohol consumption in the postpartum period compared to pre-pregnancy and control postpartum mice. The increased alcohol consumption is associated with hyperactivity of the HPA axis in *Gabrd*$^{-/-}$ mice and is diminished by inhibiting the HPA axis with Antalarmin. This study demonstrates a correlation in postpartum depression-like behaviors and alcohol consumption in a novel mouse model and implicates the body's stress response in voluntary alcohol consumption associated with postpartum depression-like behaviors.
Disclosure: J. Maguire, None.

Panel Session
Kynurenine and Its Metabolites: Emerging Targets for Neuropsychiatric Disease

Kynurenic Acid: An Astrocyte-Derived Neuromodulatory Agent with Links to Cognitive Behaviors

Robert Schwarcz*, Michelle C. Potter, Richard Bergeron, Greg I. Elmer, Hui-Qiu Wu

University of Maryland School of Medicine, Baltimore, MD

Background: The kynurenine pathway of tryptophan metabolism accounts for over 95% of dietary tryptophan degradation in mammals and contains three neuroactive metabolites with purported links to neuropsychiatric diseases: kynurenic acid (KYNA), 3-hydroxykynurenine and quinolinic acid. At endogenous brain concentrations, the astrocyte-derived compound KYNA antagonizes the α7 nicotinic acetylcholine receptor (α7 nAChR) and, possibly, the glycine co-agonist site of the NMDA receptor. Several lines of evidence indicate that the functions of these two receptors, which are intimately involved in synaptic plasticity and cognitive processes, are modulated by fluctuations in brain KYNA levels. Thus, elevated KYNA reduces extracellular glutamate levels, disrupts prepulse inhibition and auditory sensory gating, and induces deficits in contextual learning and memory in experimental animals. These effects model aspects of cognitive deficits observed in schizophrenia and are therefore especially interesting in view of the fact that schizophrenia patients present with increased KYNA levels in brain and cerebrospinal fluid. Conversely, cognitive processes may be enhanced by reductions in brain KYNA levels. We tested this concept in mice with a targeted deletion of kynurenine aminotransferase II (KAT II), a major biosynthetic enzyme of KYNA in the mammalian brain.
Methods: Young (21-32 day-old) wild-type and KAT II knock-out mice, both on a FVB/N background, were used in all studies. Microdialysis was performed in the hippocampus of unanesthetized mice. Tissue and extracellular KYNA and glutamate levels were measured by HPLC with fluorimetric detection. Object exploration and recognition, contextual memory (passive avoidance) and spatial discrimination (T-maze) were tested according to conventional methods (described in detail by Potter et al., Neuropsychopharmacology 2010 Mar 24 - Epub ahead of print). Long-term potentiation was induced in CA1 pyramidal cells in hippocampal slices using a pairing protocol consisting of 3 brief high frequency tetani (50 pulses at 100 Hz, 4 s intervals), applied at the end of a 3 min depolarization at 0 mV.
Results: At 21 days of age, KAT II knock-out mice had reduced hippocampal KYNA levels (-71%) and showed significantly increased performance in three cognitive paradigms that rely in part on the integrity of hippocampal function, namely object exploration and recognition, passive avoidance and spatial discrimination. Moreover, compared to wild-type controls, hippocampal slices from KAT II-deficient mice showed a significant increase in the amplitude of long-term potentiation *in vitro*. These functional changes were accompanied by reduced extracellular KYNA (-66%) and increased extracellular glutamate (+51%) concentrations, measured by hippocampal microdialysis *in vivo*.
Discussion: Taken together, a picture emerges where a reduction in the astrocytic formation of KYNA increases glutamatergic tone in the hippocampus and enhances cognitive abilities and synaptic plasticity. These studies raise the prospect that interventions aimed specifically at reducing KYNA formation in the brain may constitute a promising molecular strategy for cognitive improvement in health and disease.
Acknowledgment: This work was supported by USPHS grant NS25296.
Disclosure: R. Schwarcz, Mitsubishi-Tanabe, Part 1; Mitsubishi-Tanabe, Part 4.

Inflammation-Associated Depression: A Role for Indoleamine 2,3 Dioxygenase-Dependent Kynurenine Metabolites

Robert Dantzer*, Keith W. Kelley, Jason C. O'Connor

University of Illinois at Urbana Champaign, Urbana, IL

Background: The concept of inflammation-associated depression initially developed in the context of psychiatric side effects of cytokine immunotherapy in patients with viral infections or chemotherapy and radiotherapy-resistant malignancies. It has now developed well beyond this early iatrogenic context and applies to the increased prevalence of depression that is observed in patients with chronic inflammatory disorders, to age-related depressive symptoms and even to treatment-resistant depression. The mechanisms of inflammation-associated depression represent potential targets for the development of new antidepressants. Clinically, reduced circulating tryptophan and increased kynurenine levels are correlated with increased depression scores of patients undergoing cytokine immunotherapy, and the same alterations in the metabolism of tryptophan are observed in patients with various chronic inflammatory disorders. These biochemical alterations point to a possible role of activation of the tryptophan degrading enzyme indoleamine 2,3 dioxygenase (IDO) in the pathophysiology of inflammation-associated depression.
Methods: To study the mechanisms of inflammation-associated depression, we have developed murine models of depressive-like behavior induced by acute or chronic activation of the peripheral innate immune system. In the first case, mice respond to intraperitoneal administration of lipopolysccharide by an episode of sickness which resolves in a few hours. This short duration permits an investigation of depressive-like behavior independently of sickness, based on increased duration of immobility in the forced swim and tail suspension test, and decreased consumption of sucrose in a two-bottle preference test. In the second case, mice are inoculated with *Bacillus Calmette-Guerin*. The subsequent sickness episode resolves within a few days and is followed by depressive-like behavior that can be evidenced for several weeks after inoculation.
Results: We have confirmed that inflammation-induced depression is associated with activation of peripheral and central IDO, which is the first and rate-limiting enzyme of the kynurenine metabolic pathway. Inhibition of proinflammatory cytokine production by administration of minocycline abrogates the increased expression of proinflammatory cytokines and the subsequent activation of IDO, resulting in inhibition of both inflammation-induced sickness and depressive-like behavior. Inhibition of IDO activation by pharmacological means (administration of 1-methyl tryptophan) or the use of IDO knock-out mice specifically abrogates inflammation-induced depression without altering expression of proinflammatory cytokines and inflammation-induced sickness behavior. In addition, administration of kynurenine restores depressive-like behavior in IDO-knockout mice previously administered lipopolysaccharide, which indicates that immune activation is inducing depression via the formation of neuroactive kynurenine metabolites.
Discussion: Because of the compartmentalization of kynurenine metabolism in the brain, the next step for this research is the determination of the cellular compartment in which degradation of kynurenine ultimately leads to development of depression. Supported by NIH (MH-51569, AG-029573, MH-71439, MH-079829).
Disclosure: R. Dantzer, Astra-Zeneca, Part 1; Lundbeck, Part 1; Bristol-Myers Squibb, Part 1.

Peripheral Activation of the Kynurenine Pathway by Interferon-Alpha Leads to Altered CSF Concentrations of Kynurenine and Its Metabolites Which Correlate with Depression

Andrew H. Miller*

Emory University School of Medicine, Atlanta, GA

Background: Converging evidence suggests that activation of the innate immune, inflammatory response including the release of cytokines of the innate immune system may contribute to the pathogenesis of major depression in certain depressed patients. One pathway that may be relatively unique to the mechanisms by which inflammatory stimuli contribute to the symptoms of depression is cytokine-induced activation of the enzyme, indoleamine 2,3-dioxygenase (IDO). IDO catabolizes L-tryptophan (TRP) into L-kynurenine (KYN), which is metabolized to quinolinic acid (QUIN) and kynurenic acid (KA). QUIN and KA are both neuroactive and may contribute to the behavioral changes experienced by patients during chronic exposure to inflammatory stimuli such as the innate immune cytokine, interferon (IFN)-alpha. Of note, activation of IDO appears to play a critical role in the development of depressive-like symptoms in laboratory animals following exposure to inflammatory stimuli such as lipopolysaccharide and bacille Calmette-Guérin, an attenuated form of Mycobacterium bovis. A relationship between depressive symptoms and peripheral blood TRP, KYN and KA during IFN-alpha treatment in humans has been described. However, whether peripheral blood changes in these IDO catabolites are manifest in the brain and whether they are related to central nervous system cytokine responses and/or behavior is unknown.
Methods: TRP, KYN, QUIN and KA were measured in cerebrospinal fluid (CSF) and blood along with CSF concentrations of relevant cytokines, chemokines and soluble cytokine receptors in 27 patients with hepatitis C after $\sim$12 weeks of either treatment with IFN-alpha ($n = 16$) or no treatment ($n = 11$). Depressive symptoms were assessed using the Montgomery Asberg Depression Rating Scale.
Results: IFN-alpha significantly increased peripheral blood KYN, which was accompanied by marked increases in CSF KYN (IFN-alpha-treated: 111.5 nM SD 32.6 versus controls: 76.6 nM SD 19.2, $t = 2.36$, $p = 0.03$). Increased CSF KYN was in turn associated with significant increases in CSF QUIN and KA (Spearman's rho $= 0.72$, $p < 0.001$, and Spearman's rh $= 0.48$, $p = 0.001$, respectively). Despite significant decreases in peripheral blood TRP, IFN-alpha had no effect on CSF TRP concentrations. Increases in CSF KYN and QUIN were correlated with increased CSF IFN-alpha, soluble tumor necrosis factor-alpha receptor 2 (sTNFR2) and monocyte chemoattractant protein (MCP)-1 as well as increased depressive symptoms (Spearman's rho $= 0.47$, $p = 0.019$ and Spearman's rho $= 0.39$, $p = 0.043$, respectively).
Discussion: These data demonstrate that peripheral administration of a cytokine of the innate immune system can activate IDO in concert with central nervous system cytokine responses, resulting in increased brain KYN, QUIN, KA, and ultimately depressive symptoms.
Disclosure: A.H. Miller, Shering Plough, Part 1; Roche, Part 1; Centocor, Part 1; Janssen, Part 1; Pfizer, Part 1; Lundbeck, Part 1; AstraZeneca, Part 1; GlaxoSmithKline, Part 1; Schering Plough, Part 4; Centocor, Part 4; GlaxoSmithKline, Part 4.

Involvement of the Kynurenine Pathway in Alzheimer's Disease

Gilles J. Guillemin*, A. Rahman, K. Ting, Kathryn Cullen, N. Braidy, R. S. Chung, W. Wu, B. J. Brew

University of New South Wales, Sydney, Australia, Kuwait University, Kuwait City, Kuwait, St. Vincent's Centre for Applied Medical Research, Sydney, Australia, University of Sydney, Sydney, Australia, University of New South Wales, Syndney, Australia, University of Tasmania, Hobart, Australia

Background: Some of the tryptophan catabolites produced through the kynurenine pathway (KP), and more particularly the excitotoxin quinolinic acid (QA), are likely to play a role in the pathogenesis of Alzheimer's disease (AD). We have previously shown that the KP is over activated in AD brain and that QA accumulates in amyloid plaques and within dystrophic neurons. We hypothesized that QA in pathophysiological concentrations affects tau phosphorylation.
Methods & Results: Using immunohistochemistry, we found that QA is co-localized with hyper phosphorylated tau (HPT) within cortical

neurons in AD brain. We then investigated *in vitro* the effects of QA at various pathophysiological concentrations on tau phosphorylation in primary cultures of human neurons. Using western blot, we found that QA treatment increased the phosphorylation of tau at serine 199/202, threonine 231 and serine 396/404 in a dose dependent manner. Increased accumulation of phosphorylated tau was also confirmed by immunocytochemistry. This increase in tau phosphorylation was paralleled by a substantial decrease in the total protein phosphatase activity. A substantial decrease in PP2A expression and modest decrease in PP1 expression were observed in neuronal cultures treated with QA. These data clearly demonstrate that QA can induce tau phosphorylation at residues present in the PHF in the AD brain. To induce tau phosphorylation, QA appears to act through NMDA receptor activation similar to other agonists, glutamate and NMDA. The QA effect was abrogated by the NMDA receptor antagonist memantine. Using PCR arrays, we found that QA significantly induces 10 genes in human neurons all known to be associated with AD pathology. Of these 10 genes, 6 belong to pathways involved in tau phosphorylation and 4 of them in neuroprotection. In 3xTg-AD mice ($n = 6$), we have detected an accumulation of kynurenine QA in a progressive and age-dependent manner. QUIN production is predominantly restricted to the hippocampal regions.
Conclusion: Altogether these results indicate a likely role of QA in the AD pathology through promotion of tau phosphorylation. Understanding the mechanism of the neurotoxic effects of QA is essential in developing novel therapeutic strategies for AD.
Disclosure: G.J. Guillemin, None.

Panel Session
New Perspectives on the Brain Regulation of Social Perception and Behavior

OPRM1 Variation, Reward Sensitivity and Social Behavior - Translation Across Species and Situation

Christina S. Barr*

NIH/NIAAA/LNG-Section of Comparative Behavioral Genomics, Bethesda, MD

Background: Mu-Opioid receptor gene polymorphisms that influence receptor affinity for β-Endorphin have arisen independently in humans and rhesus macaques and have been shown to moderate responses to alcohol in the respective species. We have previously demonstrated there to be effects of *OPRM1* C77G genotype on the development of infant attachment. Genetic variation that influences endogenous opioid response could also potentially impact the incentive-motivational aspects of other types of social interactions. We, therefore, wanted to investigate whether *OPRM1* genotype would influence social interactions during adolescence in rhesus macaques. Because alcohol-induced stimulation is higher as a function of this allele, we also examined whether it predicted individual differences in aggressive responses to provocation during periods of intoxication.
Methods: Behavioral responses to an unfamiliar intruder male were recorded in adolescent/young adult male rhesus macaques over 30 minutes in an Intruder Challenge Test. In a separate study, a 2.2 g/kg dose of alcohol (IV) was administered, and aggressive responses to a human intruder were measured over 5 minutes. Behavioral response factors were generated using factor analysis. Animals were genotyped for the *OPRM1* C77G polymorphism, and whether genotype predicted behavioral factor scores was assessed by ANOVA. The effects of the *OPRM1* G allele on individual behavioral responses to the presence of an intruder across time (10, 20, 30 min) were also assessed using repeated measures ANOVA.
Results: Factor analysis generated 4 factors relating to social interaction for the Intruder Challenge Test (Agonistic, Clingy, Curious/Bold, Threatening) and 3 aggression factors for the IV-Alcohol Aggression Test (Threatening, Distance Decreasing, Escalated). The *OPRM1* G allele predicted the Curious/Bold factor (positive loadings for locomotion and approach intruder, $P < 0.01$) for the Intruder Challenge Test and the Escalated Aggression factor (positive loadings for open-mouth threat and lunge, $P < 0.0005$) for the IV Alcohol Test. Repeated measures ANOVA of the behaviors loading onto the Curious/Bold factor demonstrated effects of genotype on the frequency with which the focal approached the intruder. G allele carriers approached the intruder more frequently ($F(1,25) = 14$, $p = 0.001$), especially during the first 10 min of testing (genotype x phase interaction, $F(2,50) = 4$, $p = 0.02$).
Discussion: We show that males carrying the *OPRM1* 77G allele, which predicts increased alcohol response and intake, are more rapid in their approach of an unfamiliar conspecific, a trait that could be adaptive in certain environmental contexts. We also show that this variant predicts aggressive responding to threat under conditions of intoxication. Our findings suggest that variation at the *OPRM1* locus may be associated with differences in sociality and alcohol-related violence in humans. The relevance to these findings to data recently obtained in human subjects will be also discussed. This research was carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.
Disclosure: C.S. Barr, None.

Why Rejection Hurts: New Evidence for Shared Mechanisms Underlying Physical and Social Pain

Naomi I. Eisenberger*

University of California Los Angeles (UCLA), Los Angeles, CA

Numerous languages characterize 'social pain,' the feelings resulting from social rejection or loss, with words typically reserved for describing physical pain (''broken hearts,'' ''hurt feelings'') and perhaps for good reason. It has been suggested that, in mammalian species, the social attachment system borrowed the computations of the physical pain system in order to prevent the potentially harmful consequences of social separation. In this talk, I will use a combination of behavioral and neuroimaging methodologies to explore the notion that physical and social pain rely on overlapping neural and experiential processes. Specifically, I will examine: 1) whether social pain activates pain-related neural circuitry, 2) whether individual differences in sensitivity to one kind of pain relate to individual differences in sensitivity to the other (e.g. Do individual differences in a pain-related mu opioid receptor gene (OPRM1) relate to individual differences in sensitivity to social pain?), and 3) whether factors that up- or down-regulate one type of pain affect the other in a similar manner (e.g., Can Tylenol reduce social pain?).
Disclosure: N.I. Eisenberger, None.

The Effects of Oxytocin on Trust, Prosocial Behavior and Recollections of Early Care: Social Panacea or Salience Enhancer?

Jennifer Bartz*, Eric Hollander, Jamil Zaki, Niall Bolger, Daphne Simeon, Kevin Ochsner

Mount Sinai School of Medicine, New York, NY

Background: Oxytocin (OXT) regulates social behavior, attachment and social memory in animals. While recent studies suggest OXT may have similar functions in humans, the role OXT plays in human social perception and behavior is neither simple nor obvious, e.g. OXT was shown to increase negative social emotions like envy. Rather than broadly facilitating positive social emotions, these data suggest OXT may increase the salience of social cues, thereby triggering the positive or negative emotions associated with those cues. We tested this alternative account of OXT function in humans. Study 1 tested the effects of OXT on trust and cooperation in healthy adults and adults

with borderline personality disorder (BPD), a disorder marked by interpersonal insecurity. Study 2 tested the effects of OXT on memories of maternal closeness and care in childhood. The popular view suggests OXT should facilitate trust and cooperation, and positively bias maternal memories in everyone. However, if OXT enhances the salience of social cues, baseline psychological set, and especially chronic beliefs about the reliability of close others (attachment anxiety), should moderate OXT's effects, with secure individuals benefitting from OXT but not more insecure, anxious individuals. This idea is consistent with broader interstionist views that individual differences uniquely shape our response to situations.
Methods: Study 1 used a randomized, between subject design in which 14 BPD and 13 healthy adults received intranasal OXT (Syntocinon, Novartis) or placebo (PL); trust and cooperative behavior were assessed while subjects played a social dilemma game. Study 2 used a randomized, mixed between-within subject design in which 31 healthy men received intranasal OXT/PL on two occasions; memories of maternal closeness and care in childhood were assessed with the Inclusion of Other in Self Scale (IOS) and Parental Bonding Instrument (PBI). In both studies, individual differences in attachment were measured at baseline with the Experience in Close Relationships Scale.
Results: Study 1 results showed a significant group x drug interaction for trust, $F = 4.83$, $p < .05$, and for response to partner hypothetical cooperation, $F = 5.06$, $p < .05$. OXT decreased trust and cooperative responses for BPD but not control subjects. Analyses focusing on individual differences in attachment across subjects showed that these effects were driven by the anxiously attached subjects. Study 2 results showed that, again, differences in attachment anxiety moderated OXT response. Specifically, attachment anxiety predicted change (OXT-PL) on the IOS, $b = -.45$, $t = -2.95$, $p < .01$, and PBI, $b = -.12$, $t = -2.16$, $p < .05$, such that securely attached individuals remembered being closer to their mother and remembered their mother as more caring following OXT (vs. PL) whereas anxiously attached subjects remembered being less close to their mother and remembered their mother as less caring following OXT (vs. PL).
Discussion: We found the effects of OXT on interpersonal perception and function were moderated by baseline psychological set, i.e. individual differences in attachment anxiety. These data argue against the popular notion that OXT has broad positive effects on social perception and function in humans and suggest a more nuanced role of OXT function, e.g. increasing the perceived salience of social cues. Although popularly dubbed "hormone of love" these data suggest a narrower answer to the question of who will benefit from OXT.
Disclosure: J.A. Bartz, None.

The Neural Substrate of Alexithymia in Borderline Personality Disorder

Antonia S. New*, Marianne Goodman, David Carpenter, Cheuk Tang, Erin A. Hazlett, Larry J. Siever

James J Peters VAMC/MIRECC, Mount Sinai School of Medicine, New York, NY, James J Peters VAMC, Bronx, NY, Mount Sinai School of Medicine, New York, NY, James J Peters VAMC/MIRECC, Mount Sinai School of Medicine, Bronx, NY

Background: Borderline personality disorder (BPD) is a common, serious and chronic psychiatric illness. Recently, investigators have come to view the abnormalities in social functioning as a core deficit in BPD. We have previously shown evidence of alexithymia from behavioral responses, psychophysiology and fMRI BOLD response to emotional pictures in adult BPD patient and controls. In the present study, we present white matter abnormalities using DTI tractography in adolescent BPD and their relationship to symptoms of BPD.
Methods: We conducted DTI (diffusion tensor imaging) tractography on 14 adolescent in patients with BPD and 13 age- and sex-matched controls. Subjects with BPD met DSM-IV (SCID-II) and Diagnostic Interview for BPD-revised (DIB-R) criteria for BPD. Controls had no current axis I or II diagnosis as assessed by the SCID-P and SCID-II, although BPD adolescents met criteria for major depressive disorder, as is characteristic of inpatients with BPD. DTI was acquired on a 3T Siemens Allegra and processed with both in-house software and FSL for pre-processing and DTI-tractography. A study-specific-template was created using the FSL-TBSS package and used to outline DTI-tractography (Carpenter et al 2008). Fractional anisotropy (FA) was calculated from the DTI-tractography of the following tracts: genu and splenium of the corpus callosum, inferior bilateral longitudinal fasciculus, and bilateral cingulum bundle (Figure 1).
Results: A tract specific decrease in FA was found in the ILF of BPD patients (left ILF $t = 3.13$ $p < 0.005$; right ILF $t = 2.92$ $p < 0.008$; $p = ns$ for other tracts). In addition to the bilateral decrease in FA in the ILF in BPD adolescents compared to controls, we also found significant inverse correlations between clinical measures of BPD symptoms of social dysfunction (Borderline Features Scale-Child) and FA in right ILF (BPD: $r = -0.66$, $p < .01$; HC: controls: $r = .08$, $p = ns$; r^2 difference $p < .03$). Correlations of FA in the ILF were not significant for measures of depression.
Conclusion: While the function of the ILF is poorly understood, individuals with brain lesions specifically in left ILF show poor ability to name objects (Mandonnet et al 2007) and in right ILF with difficulty in recognition of facial affect (Philippi et al 2009). This is very interesting because a profound deficit in the ability to name and describe feelings has been found in BPD. Moreover, we have shown high levels of alexithymia in adult BPD. Our finding of low FA in the ILF, supporting the notion of disruption in white matter efficient signal conduction, in the brain area related to object naming and emotion recognition provides a very interesting possible neural substrate for this difficulty in naming and describing affect in BPD. Future studies will need to examine whether this abnormality persists into adulthood, is predictive of BPD in at risk adolescents and relates to symptoms of social dysfunction in BPD. References: Carpenter DM, Tang CY, Friedman JI, Hof PR, Stewart DG, Buchsbaum MS, et al (2008): Temporal characteristics of tract-specific anisotropy abnormalities in schizophrenia. *Neuroreport* 19:1369-1372. Mandonnet E, Nouet A, Gatignol P, Capelle L, Duffau H (2007): Does the left inferior longitudinal fasciculus play a role in language? A brain stimulation study. *Brain* 130:623-629. Philippi CL, Mehta S, Grabowski T, Adolphs R, Rudrauf D (2009): Damage to association fiber tracts impairs recognition of the facial expression of emotion. *J Neurosci* 29:15089-15099.
Disclosure: A.S. New, None.

Panel Session
Novel Treatment Targets from Preclinical Models of Neurodevelopmental Disorders

Behavioral Assays in Genetic Mouse Models to Discover Treatments for Autism

Jacqueline Crawley*

National Institute of Mental Health, Bethesda, MD

Introduction: Autism is a major mental illness with a strong genetic component. As candidate genes linked to autism are identified, mice with targeted mutations of these genes are becoming available. Genetic mouse models offer useful translational tools to test hypotheses about genetic and environmental causes of autism, and to discover effective treatments for the diagnostic and associated symptoms of autism spectrum disorders.
Methods: The key to translational applications is robust, highly replicable functional assays. Our laboratory has been developing novel mouse behavioral paradigms with high face validity to the three diagnostic symptoms of autism. This presentation will focus on behavioral tests for mice that are proving useful in treatment discovery. The core deficit in reciprocal social interactions is being

modeled longitudinally across developmental stages with juvenile and adult reciprocal social interactions, and in our automated 3-chambered social approach task. Communication in mice is being approached with measures of the emission, detection, and responses to social olfactory cues and auditory ultrasonic vocalizations. The third diagnostic category of motor stereotypies, repetitive behaviors, insistence on sameness, and narrow restricted interests is being analyzed in mice by quantitating stereotyped motor behaviors, repetitive self-grooming, perseveration during the reversal phase of T-maze and Morris water maze spatial tasks, and restricted exploration of complex environments. Comprehensive control parameters are scored to detect physical deficits that will result in artifacts which confound the interpretation of specific autism-relevant phenotypes.
Drug treatments are administered before the behavioral test, using dose-response methods standard in behavioral neuropsychopharmacology. Behavioral interventions are administered at specific time points during early development.
Results: Examples will be presented of treatments in the BTBR T + tf/J (BTBR) inbred strain mouse model of autism, which displays low sociability and high repetitive behaviors. The control group is the standard inbred strain C57BL/6J (B6) which displays high sociability and low repetitive behaviors. The prototypic mGluR5 antagonist MPEP reduced repetitive self-grooming in BTBR, while having no effects in the standard C57BL/6J inbred strain (B6), and no effect on general open field exploratory locomotion. Acute treatment with risperidone, the antipsychotic approved for treating "irritability" associated with autism, reduced repetitive self-grooming in BTBR only at doses that were sedating in the open field. Early behavioral intervention with B6 juvenile peers rescued the social deficits in adult BTBR.
Discussion: We have developed an experimental design that will be useful for evaluating a range of treatments for the diagnostic symptoms of autism. Early success with an mGluR5 antagonist in ameliorating repetitive self-grooming in BTBR provides a proof of principle for the translational value of mouse models of autism.
Disclosure: J.N. Crawley, Pfizer, Inc., Part 4.

Pathogenesis and Treatment of Fragile X Syndrome
Mark Bear*

MIT, Cambridge, MA

Background: Fragile X syndrome is caused by transcriptional silencing of the FMR1 gene encoding the fragile X mental retardation protein (FMRP). One consequence of the loss of FMRP in the Fmr1 knockout mouse model is excessive basal hippocampal protein synthesis. Excessive protein synthesis has been shown to alter in synaptic plasticity triggered by activation of metabotropic glutamate receptor 5 (mGluR5). The mGluR theory of fragile X posits that diverse symptoms of the disease are caused by hypersensitivity to mGluR5 activation. We have investigated how mGluR5 regulates protein synthesis and whether manipulations of this pathway can correct the excess in fragile X. In addition, we have investigated the Tsc2 + /- mouse model of tuberous sclerosis complex (TSC), another disease with a high prevalence of autism which has been hypothesized to have pathophysiology that overlaps with fragile X.
Methods: Hippocampal slices were prepared from WT and mutant mice, and assays of protein synthesis and mGluR5-dependent synaptic plasticity were performed. Treatments that corrected these phenotypes *in vitro* were tested on behavioral phenotypes *in vivo*.
Results: We find that the excess protein synthesis in fragile X is completely corrected by treatment with an mGluR5 negative allosteric modulator (NAM). Stimulation of slices with an mGluR5 agonist reliably activates the ERK1/2 MAP kinase pathway in all genotypes. Interestingly, an inhibitor of ERK could also correct excessive protein synthesis in the Fmr1 KO, but an inhibitor of mTOR could not. Consistent with this finding, we do not observe a phenocopy of fragile X in the Tsc2 + /- mice in which mTOR signaling is enhanced. Treatment of fragile X mice with an inhibitor of ERK completely suppressed audiogenic seizures, similar to what has been observed previously with mGluR5 NAMs.
Discussion: We conclude that excessive protein synthesis in fragile X is downstream of glutamate-mGluR5-ERK signaling, suggesting additional therapeutic targets. Our data are not consistent with the hypothesis of excessive mTOR activation as a basis for altered protein synthesis or mGluR-dependent synaptic plasticity in fragile X.
Disclosure: M. Bear, Pfizer, Part 1; Consultant, Part 1; Seaside Therapeutics, Part 2.

Evidence from Mutant Mice and Human Studies That Tuberous Sclerosis Mutations in Combination with Gestational Immune Activation Trigger Phenotypes Associated with Autism
Alcino Silva*, Dan Ehninger, Yoshitake Sano, Petrus De Vries, David Franz, Manpreet Kaur, Yong-Seok Lee, Daniel H. Geschwind, Jennifer K. Lowe, Jo Anne Nagawa, Mustafa Sahin, Vicky Whittemore

UCLA, Los Angeles, CA

Background: An emerging theme in the biology of autism spectrum disorders (ASDs) is the involvement of signaling mechanisms that regulate protein synthesis, such as those involving mammalian target of rapamycin (mTOR) signaling. For example, heterozygous mutations in the TSC1 or TSC2 genes, key regulators of mTOR signaling, cause tuberous sclerosis and elevate the risk for autism 50-fold compared to the general population. Prenatal viral infections also constitute a risk factor for neuropsychiatric disorders, including schizophrenia and ASDs, and they may also modulate mTOR signaling.
Methods: To test for an interaction between the Tsc2 heterozygous mutation in mice and gestational Poly I:C (a model of gestational immune activation), we performed timed matings between Tsc2 heterozygotes males in the C57BL/6Ncrl genetic background and C57BL/6J wild-type (WT) females and injected pregnant females at E12.5 with either Poly I:C (20 mg/kg) or saline control. To test if seasonal influenza infections interact with TSC gene mutations in the pathogenesis of TSC-related ASD, we obtained birth date and clinical information from human TSC populations (clinical samples from 4 sources in the US and the UK) and estimated gestational age during peak seasonal flu activity for TSC-ASD individuals and, as a reference group, for TSC individuals without major neuropediatric phenotypes.
Results: We will report interactive effects of these two autism risk factors Tsc2 heterozygous mutation and gestational immune activation) in a mouse model for TSC: a Tsc2 heterozygous mutation combined with gestational immune activation, but not each these factors individually, had adverse effects on intrauterine survival and adult social approach behavior, a model of social problems in autism. Moreover, our studies in human TSC populations are also consistent with an interaction between prenatal viral infections in late gestation and TSC mutations in the generation of ASDs phenotypes in these populations.
Discussion: Taken together, the results described above show that a heterozygous Tsc2 mutation and the Poly I:C model of gestational viral infections display interactive effects on intrauterine survival and social behavior in adult mice. Studies in human TSC populations indicated an association between high seasonal flu activity in late gestation and autism. Collectively, our data raise the possibility that TSC gene mutations interact with gestational viral infections in the pathogenesis of TSC-related autism.
Disclosure: A. Silva: Part 4; Roche.

Rescue of the Cognitive Defects in the Adult Angelman Syndrome Mouse Model
Edwin Weeber*

University of South Florida, Tampa, FL

Background: Angelman syndrome (AS) is a neurological disorder characterized by severe developmental delay, cognitive disruption,

propensity for seizure with an associated characteristic electroencephalogram, sleep disturbances and an ataxic gait. AS is estimated to occur in 1 of every 15,000-20,000 births and is caused by various disruption of the imprinted maternal UBE3A allele. UBE3A encodes for an E3 ubiquitin ligase and is expressed throughout neurons of the CNS. The mouse model for AS was developed through the null mutation of exon 2 of the UBE3A gene and mice inheriting the maternal copy of the mutated UBE3A show all of the major phenotypes established in the human condition. Biochemical investigation revealed a change in the phosphorylation state of Calcium Calmodulin protein kinase II (CaMKII) at both the autonomously active threonine 286 and autoinhibitory threonine 305/306 sites of the kinase. Point mutation of the threonine 305/306 site to prevent phosphorylation was sufficient to rescue the major AS phenotypes when bred with the maternal deficient UBE3A mouse model. The genetic rescue of the overt AS phenotype suggested that therapeutic intervention could be possible.
Methods: Two separate strategies were used to test the hypothesis that the cognitive disruption in the AS mouse model was reversible in the adult. AS mice were aged to 16-18 weeks and bilaterally injected: 1) intrahippocampally with sero-type-9 adeno-associated viral (AAV-9) particles containing a UBE3A-GFP fusion protein. 2) in the ventricles with ~280 nM Reelin protein. Reelin is a naturally occurring extracellular matrix protein in the CNS shown to modulate synaptic function in the hippocampus, enhance LTP, modulate ligand-gated receptors and increase spine density *in vivo*. Five weeks post injection mice were tested for associative and spatial learning and memory using contextual fear conditioning and hidden platform water maze respectively. Behavioral controls included measurements of activity, anxiety, motor coordination and pain perception.
Results: AAV-9 mice showed UBE3A expression throughout the hippocampal formation with greater expression in the dentate granule cell layer. Neither experimental group showed significant differences in any of the subsequent control behavioral tests and experimental and treatment groups revealed the characteristic motor coordination defect. However, both AAV-9 and Reelin injected AS mice showed significant increases in freezing behavior 24 hours and 1 week following fear conditioned training. The increase in freezing was comparable to that of wild type mice. All groups performed comparable during hidden platform training. A probe test was given on day 7 following 6 days of spaced training. Both Reelin and AAV-9 injected mice showed significant increases in target platform crossings and both revealed improved search strategies.
Disscussion: The present studies suggest that the major measurable cognitive disruption in the AS mouse model can be rescued in the adult. The strategies of a future human therapeutic acting as a cognitive enhancer, gene therapy for UBE3A, or a biochemically-induced increase in paternal expression from the silenced paternal allele may be efficacious treatments for the severe cognitive disruption seen in human AS patients.
Disclosure: E. Weeber, None.

Panel Session
The Stressed Synapse. The Impact of Stress and Glucocorticoids on Glutamate Transmission and Brain Function

Dual Regulation of Glutamatergic Transmission and Working Memory by Stress in Prefrontal Cortex

Zhen Yan*

State University of New York at Buffalo, Buffalo, NY

Background: The prefrontal cortex (PFC), a key brain region controlling cognition and emotion, is strongly influenced by stress. Through the action of corticosteroid stress hormones, acute stress has been shown to enhance learning and memory, while chronic stress often produces detrimental effects on these measures. In this study, we have identified the molecular and cellular mechanisms that may underlie the complex actions of stress in PFC.
Methods: A combination of electrophysiological, biochemical, immunocytochemical and behavioral approaches has been used to examine the impact of stress *in vivo* and *in vitro* in male rats at the prepubescent or early adolescent period. Cellular knockdown and pharmacological methods have been used to identify key molecules involved.
Results: Acute behavioral stressor or short-term corticosterone treatment *in vitro* induces a long-lasting potentiation of NMDAR- and AMPAR-mediated synaptic currents and a marked increase of NMDAR and AMPAR surface expression in PFC neurons. These effects of acute stress are mediated by glucocorticoid receptors (GRs), and require the induction of serum- and glucocorticoid-inducible kinase (SGK) and the activation of Rab4, which controls receptor recycling between early endosomes and the plasma membrane. Furthermore, behavioral tests indicate that working memory, a key function relying on the recurrent excitation within networks of PFC neurons, is enhanced by acute stress via a GR-dependent mechanism. Inhibiting SGK, which blocks stress-induced enhancement of glutamatergic transmission, also blocks stress-induced facilitation of working memory, suggesting that the GR/SGK-induced glutamate receptor membrane trafficking in PFC may underlie the working memory improvement by acute stress. In contrast to the enhancing effect of acute stress on glutamatergic transmission, *in vivo* chronic stress or *in vitro* long-term corticosterone treatment induces a long-lasting depression of NMDAR and AMPAR synaptic responses. Moreover, in chronically stressed animals, the expression of NR1 and GluR1 subunits is selectively decreased, which is accompanied by their increased ubiquitination. These effects of chronic stress are blocked by proteasome inhibitors that reduce the degradation of ubiquitin-conjugated proteins. Furthermore, behavioral tests indicate that the object recognition memory is impaired by chronic stress.
Discussion: Corticosterone, the major stress hormone, serves as a key controller for neuronal responses that underlie behavioral adaptation, as well as maladaptive changes that lead to cognitive and emotional disturbances in stress-related mental disorders. Here we demonstrate that acute or chronic stress induces a significant potentiation or depression of glutamatergic transmission in PFC, respectively, which is likely caused by the altered levels of NMDAR and AMPAR subunits. These results have identified a form of long-term potentiation (LTP) and long-term depression (LTD) of synaptic transmission induced by natural stimuli *in vivo*, providing a potential mechanism for the beneficial effects of acute stress and detrimental effects of chronic stress on cognitive processes subserved by PFC. In addition to providing a basis for the biphasic effects of stress and glucocorticoids on synaptic plasticity and memory, key molecules revealed by this study should also provide valuable targets for designing novel therapies that modify the neuronal stress response.
Disclosure: Z. Yan, None.

Mechanisms of Stress-Induced Release of Glutamate and the Dampening Action of Antidepressant Agents

Maurizio Popoli*

University of Milan, Milano, Italy

Background: Behavioral stress is a main risk factor for neuropsychiatric diseases. Acute stress induces rapid changes in the release of neurotransmitters, hormones and cytokines that may become damaging if the stress response is inadequate or excessive. Inappropriate stress response acts as a trigger, producing a vulnerable phenotype in genetically predisposed individuals and increasing the risk for mental ilness. Converging evidence has suggested that acute stress is associated with increase of excitatory transmission in certain forebrain areas. In this work we investigated the mechanism whereby acute stress increases glutamate (GLU) release, and if psychiatric drugs prevent the effect of stress on GLU release.

Methods: Rats were chronically (2 wks.) treated with vehicle or different drugs employed for therapy of mood/anxiety disorders (fluoxetine, desipramine, venlafaxine, escitalopram, agomelatine) and then subjected to inescapable footshock (FS)-stress (40-min FS; 0.8 mA, 20 min total of actual shock with random intershock length between 2-8 sec). The action of stress on GLU release and synaptic transmission were analyzed by complementary methodologies, including superfusion of freshly prepared synaptosomes (SPT), patch-clamp recordings of acute slices, molecular analysis of presynaptic machinery, electron microscopy (EM) ultrastructural analysis of synapses and confocal microscopy (CM).
Results: Acute stress induced marked increase in (15 mM K^+) depolarization-evoked release of GLU from SPT of prefrontal/frontal cortex (P/FC) in superfusion, and the chronic drug treatments mostly or completely prevented the GLU release increase [1]. Stress rapidly increased circulating levels of corticosterone (CORT) in both vehicle- and drug-treated rats, and GLU release increase was blocked by previous administration of selective antagonist of glucocorticoid receptor (GR; RU 486). Experiments with protein synthesis inhibitors showed that this is a non-genomic effect of CORT. On the molecular level, stress induced accumulation of presynaptic SNARE complexes in synaptic membranes (both in vehicle- and drug-treated rats), and changes in synaptic signaling regulating SNARE complex. Patch-clamp recordings of pyramidal neurons in prefrontal cortex layer III revealed that stress increases GLU transmission through both pre- and postsynaptic mechanisms, altering both N and P quantal parameters. Antidepressants normalize release by reducing release probability, but do not normalize postsynaptic changes. Superfusion of SPT with sucrose or ionomycin, as well as EM and CM confirmed that the readily releasable pool of vesicles was increased by stress.
Discussion: Acute FS stress rapidlly up-regulates GLU release in P/FC (both in isolated SPT and whole tissue), by rising CORT level, stimulation of GR by CORT and rapid accumulation of presynaptic SNARE complexes. Chronic treatment with different antidepressants prevents Glu release up-regulation, by a mechanism downstream of SNARE complex assembly. This novel effect of antidepressants on the response to stress, shown here for the first time, is likely related to the antidepressant/anxiolytic action of these drugs.
[1] Musazzi et al. (2010) Acute stress increases depolarization-evoked glutamate release and presynaptic SNARE complex accumulation in prefrontal/frontal cortex. The dampening action of antidepressants. *PloS ONE* 5(1): e8566. doi:10.1371/journal.pone.0008566.
Disclosure: M. Popoli, Servier Pharmaceuticals, Part 4.

The Effects of Stress on Glutamate Uptake and Metabolism
Gerard Sanacora*, Mounira Banasr, Golam Chowdhury, Kevin Behar

Yale, New Haven, CT

Background: Growing evidence implicates glial dysfunction and abnormal glutamatergic neurotransmission in the neuropathology of stress-related illnesses including major depressive disorder. Postmortem studies have repeatedly found a decreased density and number of glial cells in cortical regions, including the prefrontal and cingulate areas from depressed patients. However, it is unclear whether these changes are related to the pathophysiology of the disorders. The following studies sought to determine the relationship between stress, glial mediated glutamate transporter activity and behavior.
Methods: The rodent chronic unpredictable stress (CUS) model was used to examine the effects of stress on glial cell function and glutamate metabolism employing carbon magnetic resonance spectroscopy and molecular biology methods. The behavioral effects of specific pharmacologic agents with actions on glutamate transporter activity were examined to explore the relationship between glutamate uptake, stress and antidepressant-like activity. Lastly, heterozygous GLT1 (EAAT2) knockout mice, expressing markedly reduced levels of the primary glial glutamate transporter, were examined in several behavioral models to explore the role of the transporter in mediating stress responsivity.
Results: We demonstrated that chronic unpredictable stress (CUS), a rodent model of depression, resulted in a reduced level of glial fibrillary acidic protein (GFAP) expression and glutamate/glutamine cycling glial function in the prefrontal cortex (PFC), as well as behavioral changes on measures of sucrose preference, active avoidance and forced swim. Drugs such as riluzole and ceftriaxone that have effects on GLT1 expression and function were demonstrated to prevent the effects of CUS on glial metabolism, glutamate cycling, and behavior. We further demonstrated that reduced function of the GLT1 transporter can modify stress related behavioral effects in the PFC. Using both heterozygous GLT1 knockout mice and injections of dihydrokainate (DHK), a selective GLT1 inhibitor, into the PFC we are able to demonstrate that decreased glutamate transporter function leads to an increased sensitivity to stress, and a decreased antidepressant-like efficacy for riluzole and ceftriaxone.
Discussion: These data suggest that glial mediated glutamate uptake in the PFC may contribute to the behavioral and physiological response to stress. These studies support the hypothesis that glial dysfunction observed in the cortex tissue samples from patients with major depressive disorder may not only be a consequence of the illness but also be a susceptibility factor for depression. Together, these results suggest modulation of glial mediated glutamate clearance may be a viable target for future antidepressant drug development.
Disclosure: G. Sanacora, AstraZeneca, Part 1; Bristol-Myers Squibb, Part 1; Eli Lilly & Co, Part 1; Evotec, Part 1; Novartis, Part 1; Roche, Part 1; Transform Pharmaceuticals, Part 1; Sepracor, Part 1; Eli Lilly & Co, Part 2; AstraZeneca, Part 4; Bristol-Myers Squibb, Part 4; Merck & Co., Part 4; Roche, Part 4; Sepracor Inc, Part 4.

Circadian Disruption as a Stressor: Effects on Prefrontal Cortex and Relevance to Cognitive and Mental Health
Ilia Karatsoreos*, Sarah M. Bhagat, Erik Bloss, John H. Morrison, Bruce McEwen

The Rockefeller University, New York, NY

Background: The prefrontal cortex (PFC) plays an important role in control of mood, impulsivity, decision-making and cognitive flexibility. Importantly, it is clearly affected by both acute and chronic stress. Chronic stress results in increased anxiety, learned helplessness and impaired cognitive flexibility, with the medial PFC showing shrinkage and loss of synaptic connections. Current paradigms show that stress can alter glutamatergic signaling within the PFC, suggesting putative mechanisms by which the effects of stress can alter cognition and emotionality. Disruption of circadian (daily) rhythms is pervasive in modern industrialized society, with our endogenous circadian clock being out of synch with environmental light-dark cycles. This is especially obvious in certain occupations, many of which have high cognitive demands, such as aircrews and medical residents. Disruption of sleep and circadian rhythms is also a hallmark of numerous psychiatric syndromes, and the role that such disruption plays in the exacerbation, or etiology, of such disorders is still not known. New data from our lab indicate that chronic circadian disruption causes remodeling of circuitry in medial PFC (mPFC) and reduced cognitive flexibility and dysregulation of metabolic endocrine function. This pattern of results suggests that circadian disruption may serve as a chronic stressor that could perturb the normal functioning of the mPFC, and lead to behavioral abnormalities.
Methods: C57Bl6 mice were housed in a 20hr light dark cycle, in opposition to the endogenous, approximately 24hr period of the circadian clock. Mice were weighed weekly throughout the course of the experiment, and body temperature recorded throughout. After 8-10wks of circadian disruption (CD), mice were tested in the open field task (OFT), the light-dark emergence task (LDE), and a modified version of the Morris water maze task (MWM) that tested cognitive flexibility. At the end of the study period, blood was collected from the animals and assayed for the important metabolic hormones leptin and

insulin. In a separate group of animals, brains were removed and cells of layer II/III of the prelimbic PFC (PL) were filled with Lucifer yellow, reconstructed using computer assisted morphometry, and analyzed.
Results: CD animals had reduced apical dendritic length and decreased dendritic complexity in the PL. Basal dendrites were unaffected, paralleling changes observed in chronic stress. Using the MWM, we found impaired ability to shift learning to a new quadrant, although acquisition of the initial platform location was normal, indicative of a reduction in cognitive flexibility. In the OFT, CD animals entered the center of the field more rapidly than controls, and were faster to emerge into the light in the LDE. CD mice also showed increased body weight and elevated levels of plasma insulin and leptin.
Discussion: Our data provide a novel model of circadian disruption induced changes in PFC morphology and mediated behaviors, with ecological and clinical relevance in the context of the disrupted sleep cycles experienced by many individuals in modern society. We propose that this model will provide an excellent jumping off point to further understand how environmental stressors can alter the structure and function of the PFC, and provide insight into potential therapeutic targets to reduce the effects of stress on the brain.
Disclosure: I.N. Karatsoreos, Sepracor Inc., Part 4.

Panel Session
Alzheimer's Disease: Beyond the Amyloid Hypothesis

Viewing the Pathogenic Roles of Alzheimer's-Related Genes Through a Different Lens

Ralph A. Nixon*

NYU Langone Medical Center/Nathan Kline Institute, New York, NY

Background: Links between genes causing Alzheimer's Disease (AD) and processes promoting Aβ overproduction or aggregation are persuasive, though still indirect, support for an Aβ toxicity model of AD pathogenesis. Guiding most AD drug development, the amyloid-β cascade hypothesis has yielded effective amyloid-lowering strategies but so far with less-than-hoped-for therapeutic efficacy. These considerations and other emerging data suggest that pathogenic factors besides Aβ may be essential for AD development and may need to be considered in designing effective therapies. Although amyloid and neurofibrillary pathologies have been the principal sources for clues to AD pathogenesis, the origin of hallmark "neuritic dystrophy" characteristic of affected neurons in AD, is providing further mechanistic insights. The grossly swollen dystrophic neurites in AD brain are filled mainly with autophagic and lysosomal compartments containing incompletely degraded substrates, including Aβ. This massive "storage" of waste proteins in neurons, reminiscent of lysosomal storage diseases, reflects a marked impairment of autophagy - the major lysosomal mechanism for protein clearance. This pathology is part of a continuum of lysosomal system functional deficits in AD, which begin to develop before amyloid is deposited. The deficits are also linked to actions of the genes driving AD, which increasingly have been recognized to modulate autophagy and endocytosis, the main pathways of the lysosomal system. I will discuss how the same AD genetics that implicate Aβ in AD also implicate *primary* lysosomal system dysfunction as a mechanism that both cripples neuronal functions critical for synaptic plasticity and neuron survival and promotes accumulation of toxic proteins, including Aβ and tau.
Methods and Results: Our studies (Lee et al. Cell, *in press*) show that the AD-related gene presenilin1 (PS1) is essential for lysosomal proteolysis and autophagy and plays a novel role in lysosome acidification required for protease activation. In cells lacking PS1, including neurons in mice conditionally depleted of PS1, a failure to deliver the proton pump vATPase to lysosomes results in autophagy failure and build-up of autophagosomes and autolysosomes containing waste proteins. PS1 mutations causing familial AD (FAD) confer partial loss of these same functions in fibroblasts from PS-FAD patients and in neurons of PS1/APP mutant mice. Lysosomal dysfunction also develops in sporadic AD and in AD mouse models driven in part by other AD-related genes. Supporting the pathogenic significance of this dysfunction, we find that partially restoring deficient autophagy in the CRND8 mouse model of AD by genetically manipulating lysosomal protease activities substantially ameliorates lysosomal pathology, amyloid burden, neuritic dystrophy, and memory deficits.
Discussion: Autophagy is essential for neuron survival and loss of PS1 support for autophagy explains the accelerated neuritic dystrophy and neuronal loss seen in PS1-FAD. Because Aβ generated during autophagy is normally degraded in lysosomes, impaired lysosomal Aβ clearance may also accelerate amyloidogenesis. Emerging evidence also links genes causing inherited forms of other neurodegenerative disease to the lysosomal system, reinforcing the concept that primary lysosomal system dysfunction is a pathway to neurodegeneration in AD and possibly other aging-related neurological disorders. Supported by NIA.
Disclosure: R. Nixon, None.

How Amyloid Precursor Protein Causes Alzheimer's Disease-Related Pathologies via Non-Amyloid Mechanisms

Sanjay W. Pimplikar*

Cleveland Clinic, Cleveland OH

Background: Alzheimer's disease (AD), the most common form of dementia, afflicts about 5.3 million Americans. The cause of AD is not conclusively known and there is no cure for or effective treatment against the disease. Two membrane proteins, amyloid precursor protein (APP) and presenilin, play a major role in AD pathology. A prevailing hypothesis of AD pathogenesis posits that increased accumulation of amyloid-β (Aβ) peptide, produced when presenilin cleaves APP, is the primary cause of the disease. Although this premise has received significant support, it is becoming increasingly clear that non-Aβ factors also play a significant role in disease causation. The cleavage of APP by presenilin, which results in the secretion of Aβ in the extracellular space, also causes release of APP IntraCellular Domain (AICD) in the cytoplasm. AICD is a small peptide of 50-59 residues, and when released from the membrane it has been shown to alter signaling pathways, modulate gene transcription and cause apoptosis *in vitro* in tissue culture cells.
Methods: We generated a transgenic mouse model to study the *in vivo* effects of AICD. We used CaMKIIα promoter to drive the expression of AICD transgene in postnatal forebrain and hippocampal neurons. Mice were aged and were tested at different time points for behavioral deficits. The brains were analyzed by biochemical and histochemical procedures for the presence of AD-related pathological features.
Results: We found that the AICD transgenic mice recapitulated a number AD-related pathological feature in a time-dependent manner. Chronologically, the AICD transgenic mice developed neuroinflammation, tau hyperphosphorylation and aggregation, memory impairments and neurodegeneration. In addition, they also showed aberrant neural activity and reduced adult neurogenesis. These pathologies occurred without any increase in the amyloid load or the deposition of amyloid plaques. Also, a number of these features were rescued by treating mice with the non-steroidal anti-inflammatory drug ibuprofen.
Discussion: Our findings show that AICD alone is able to cause AD-related pathologies in pre-clinical settings. Since the brains from AD patients show higher levels of AICD compared to non-demented individuals, these data suggest that AICD can also contribute to clinical AD. We will summarize the pathological effects of increased levels of APP or APP metabolites and discuss how our studies add to the knowledge base. These findings, together with the observations that other non-amyloid mechanisms can be operative in AD, provide one

possible explanation for the lack of success of Aβ-directed therapeutic strategies in clinical trials. We will present data that suggest the mechanism underlying the effects of AICD and discuss how this new insight into disease pathogenesis provides potential novel ways for therapeutic intervention.
Disclosure: S.W. Pimplikar, None.

Neurodegenerative Mitochondrial Mechanisms, Involving the Interaction of apoE Isoforms with tom40 [Outer Mitochondrial Membrane Channel Protein], with Apoptosis and Altered Mitochondrial Dynamics Including Decreased Neurite Regeneration and Amyloid Cascade
Allen D. Roses*

Deane Drug Discovery Institute, Duke University Medical Center, Durham, NC

Background: The association of apolipoprotein E (APOE) genotypes, particularly the carriage of the APOE ε4 allele, with the risk and age of onset of late-onset Alzheimer's disease (LOAD) remains the most confirmed example of a genetic association for a complex disease. However, it lacks sufficient specificity and sensitivity for application in clinical practice. Recent published data supports the hypothesis that age of onset distribution of Alzheimer's Disease can be predicted by a variable polyT polymorphism in the TOMM40 gene, inherited over evolution in different ethnic groups.
Methods: 10-fold Sanger sequencing of the APOE-TOMM40 linkage disequilibrium region and analyzed all polymorphisms [SNPs and CNVs] using phylogenetic methodology in several independent series of AD patients and controls matched for APOE genotypes.
Results: We have identified a polymorphic poly-T variant locus, rs10524523, in the TOMM40 gene that provides greatly increased precision in the estimation of AD risk and disease onset for APOE ε3 carriers. In two independent clinical cohorts, longer lengths of rs10524523 were associated with a higher risk for LOAD. For APOE ε3/ε4 patients who developed LOAD after age 60, individuals with long poly-T repeats linked to APOE ε3 develop LOAD an average of 7 years earlier than individuals with shorter poly-T repeats linked to APOE ε3 (70.5 years ± 1.2 versus 77.6 years ± 2.1, $P = 0.02$, $n = 34$). The age of onset distribution data have been confirmed in a prospectively followed "normal" cohort from Mayo Clinic, Scottsdale, Arizona. Additional psychological and imaging biomarkers have been related [University of Wisconsin] and may be predicted, by the size of the polyT repeats on each chromosome.
Discussion: The genetic data focus the pathogenic process on the variable intraneuronal interactions of apoE3 (1-272) and/or apoE4 (1-272) with the mitochondrial outer molecular membrane channel protein. The TOMM40 gene contains this intronic polyT polymorphism. The role of the sizes of each of the polyTs on each inherited DNA strand are under investigation but include creating differences in exon splicing and quantitative expression. The cascade of amyloid is a variable consequence over time of the rate of mitochondrial apoptosis, with the apoE molecules functioning as "pro-apoptotic death signals." Cytochrome c is released and catalyzes the caspase pathways leading to a subsequent aggregation of amyloid and other cellular proteins. The prospective validation of the AD age-specific risk estimates will be tested in a combination diagnostic validation, and delay of onset clinical trial. The design of this study has been accepted by the FDA and negotiations with pharmaceutical partners are underway.
Disclosure: A.D. Roses, Eli Lilly and Company, Pharmacogenetic consulting and project management, Part 2; Cabernet Pharmaceuticals, Inc., self-owned PGX consulting company, Part 2; Zinfandel Pharmaceuticals, Inc., self-owned drug development organization, Part 2; Shiraz Pharmaceuticals, Ins, self-owned companion diagnostic company, Part 2.

Neuroimaging Biomarker Options for Monitoring Alzheimer's Disease Treatments
Gary Small*, Vladimir Kepe, Graham B. Cole, Markus Donix, Alison Burggren, Susan Y. Bookheimer, Jorge Barrio

UCLA Semel Institute for Neuroscience & Human Behavior, Los Angeles, CA, UCLA, Los Angeles, CA

Background: Neurodegeneration and age-related cognitive decline afflict millions of people: 20% of those aged 65 or older have mild cognitive impairment, and 10% have Alzheimer's disease or related dementias. Recent research has focused on developing brain imaging to track neuropathological changes associated with these conditions, particularly amyloid senile plaques. However, several available techniques label alternative structures and mechanisms, such as tau accumulation, synaptic activity, and inflammation - all useful targets for monitoring novel treatments.
Methods: A review of recent structural and functional imaging studies from UCLA and other centers will be used to demonstrate how several methods provide varied data that assist in detecting neurodegeneration and monitoring interventions.
Results: Voxel-based MRI morphometry indicates the predictive value of medial temporal atrophy and its potential utility in clinical trials. Although brain volume measures may lack target specificity, high-resolution MRI and cortical unfolding methods have identified medial temporal subregions that may improve MRI utility for clinical trial monitoring. [F-18]FDG-PET provides measures of synaptic activity through regional glucose metabolism, and findings from the Alzheimer's Disease Neuroimaging Initiative indicate that its use can reduce the number of subjects needed for a randomized clinical trial. Cholinesterase inhibitor and anti-inflammatory drug trials have demonstrated significant effects on both [F-18]FDG-PET signals and cognitive measures. A randomized clinical trial of the anti-amyloid, humanized monoclonal antibody bapineuzumab resulted in a modest decrease in [C-11]PIB-PET signals but only limited treatment efficacy on cognitive measures. [F-18]FDDNP-PET, which measures tau neurofibrillary tangles as well as amyloid senile plaques, predicts future cognitive decline in normal aging and mild cognitive impairment. [F-18]FDDNP-PET signals are associated with cerebrospinal fluid tau levels, and studies of supranuclear palsy (a pure tauopathy) demonstrate high signals in brain regions with high concentrations of tauopathy. Although [F-18]FDDNP-PET has not yet been studied in a clinical treatment trial, it may prove particularly useful in monitoring anti-Alzheimer's treatments since tau tangles are closely associated with disease progression.
Discussion: A variety of imaging techniques provides opportunities to track neurodegeneration and cognitive decline in treatment trials. Choice of a particular method should depend upon the known mechanism of action of the treatment. Thus, [F-18]FDDNP-PET would be a reasonable choice for monitoring anti-tau treatments, while [18]FDG-PET would be more appropriate for tracking drugs that directly affect synaptic function. Combining multiple neuroimaging methods (e.g., structural MRI plus PET) may improve specificity and sensitivity and offer added value to using only one imaging technique.
Disclosure: G.W. Small, Eisai, Part 1; Forest, Part 1; Medivation, Part 1; Novartis, Part 1; Pfizer, Part 1; Eisai, Part 2; Forest, Part 2; Novartis, Part 2.

Panel Session
Cannabinoids Session 2: Cannabinoids: Is There a Future?

Radioligands for PET Imaging of Cannabinoid Receptors Type 1 and Type 2

Andrew Horti*, Yongjun Gao, Hayden Ravert, Paige Finley, Heather Valentine, Dean Wong, Alena Savonenko, Robert Dannals

Johns Hopkins University, School of Medicine, Baltimore, MD

Background: Cannabinoid receptors subtype 1 (CB_1) and subtype 2 (CB_2) and cannabinoid drugs constitute a vibrant field in modern medicine and pharmacology. However, the physiological and pharmacological roles played by the cannabinoids are still not fully understood. Positron-emission tomography (PET) is the most advanced imaging technique for non-invasive research of receptors. Quantitative PET imaging of CB_1 and CB_2 in animal and humans has been limited by the drawbacks of the available radioligands. The latest research has revealed several CB_1 PET radioligands with improved imaging properties. Molecular imaging of the CB_1 receptor in human brain with these radioligands has now become possible and their application is underway. The CB_2 receptor is found in the immune system and is also expressed in small quantities in normal brain tissue. There are multiple lines of evidence that CB_2 is upregulated under many pathological conditions including neuroinflammation (NI), Alzheimer's disease (AD), multiple sclerosis and cancer. PET CB_2 radioligands for human use are not available. A836339 (2,2,3,3-tetramethylcyclopropanecarboxylic acid [3-(2-methoxy-ethyl)-4,5-dimethyl-3H-thiazol-(2*Z*)-ylidene]amide) was recently developed by Abbott as a selective CB_2 agonist ($K_i = 0.64$ nM). The radiosynthesis of [^{11}C]A836339, [^{11}C]-**1** and its evaluation in mouse models of NI and AD is presented as a novel approach to image NI and AD.
Methods: [^{11}C]-**1** was prepared by ^{11}C-methylation of the corresponding alcohol derivative. Whole body and regional brain distribution of [^{11}C]-**1** were studied in control mice and after blockade with selective CB_2 ligands. The mouse model of NI (mice treated with lipopolysaccharide (LPS)), and mouse model of AD (APPswe/PS1dE9) were used in these studies.
Results: [^{11}C]-**1** was prepared with a radiochemical yield of 26%, radiochemical purity $>99\%$ and specific radioactivity of 300 TBq/mmol. [^{11}C]-**1** exhibited low uptake in the control brain (0.4%ID/g tissue) with little specific binding. The whole body distribution of [^{11}C]-**1** in control mice demonstrated high specific uptake in the CB_2 rich spleen. Uptake of [^{11}C]-**1** in the LPS (NI) mouse brain regions was significantly greater than that in controls (ratio up to 3.3). Blocking with various CB_2 ligands (60% blockade) demonstrated that binding of [^{11}C]-**1** in the LPS mouse brain is mediated by CB2 receptors. A regional brain distribution study (baseline and blockade) of [^{11}C]-**1** in AD mice demonstrated 36% specific CB_2 binding in the cortex and lower binding in all other brain regions. The CB_2 specific binding is consistent with distribution of the Aβ amyloid plaques in this model of AD.
Discussion: [^{11}C]-**1**, a new selective CB_2 radioligand with high binding affinity, has been synthesized. [^{11}C]-**1** manifests low uptake in the normal mouse brain, but it specifically radiolabels the cerebral CB_2 receptors *in vivo* in mouse models of NI and AD. These results suggest that [^{11}C]-**1** is a candidate for PET imaging of CB_2 receptors in neuroinflammation and Alzheimer's disease.
Disclosure: A.G. Horti, None.

Quantification of Cerebral Cannabinoid Receptors and Occupancy of Subtype 1 (CB1) in Healthy Subjects and Schizophrenia by the Novel PET Radioligand [^{11}C]OMAR

Dean F. Wong*, Hiroto Kuwabara, Andrew Horti, Vanessa Raymont, James Brasic, Maria Guevara, Weiguo Ye, Robert Dannals, Hayden Ravert, Ayon Nandi, Arman Rahmim, Jeffrey E. Ming, Igor Grachev, Christine Roy, Nicola Cascella

Johns Hopkins University, School of Medicine, Baltimore, MD, Sanofi-Aventis Research and Development, Bridgewater, NJ

Background: Several studies have examined the link between the cannabinoid CB1 receptor and several neuropsychiatric illnesses including schizophrenia. Recently several attempts at *in vivo* human PET CB1 imaging have occurred and will be briefly reviewed. We will emphasize our first human studies in both healthy control patients (HC) and patients with schizophrenia (SZ) using the novel PET tracer, [^{11}C]OMAR (JHU 75528). We have shown its utility as a tracer for imaging human CB1 receptors and to investigate normal aging and the differences in the cannabinoid system of HC vs. SZ in binding and clinical controls. We will also review unpublished studies of CB1 receptor occupancy with an antagonist drug.
Methods: A total of 10 HC and 10 SZ were studied with high specific activity [^{11}C]OMAR. Binding was obtained by compartmental modeling with radial arterial input measured and expressed as the distribution volume (V_T). The control cohort consisted of ten healthy males, with a mean age of 33 ± 11 years, ranging from ages 21-51 and comprised 8 African-Americans (AA), 1 Asian (A) and 1 Caucasian (C). The SCZ cohort consisted of 9 males and 1 female, with a mean age of 42 ± 9 years, ranging from ages 30-54 including 7 AA, 1 A and 2 C. The occupancy study was with 7 HC and 4 SCZ with AVE1625, an antagonist for CB1.
Results: Imaging with baseline (^{11}C)OMAR followed by a second PET following 5 h post dosing with a single oral 5-60 mg (HC) and 5-70 mg (SZ). V_T was highest in the globus pallidus and the cortex in both controls and patients with schizophrenia. Controls showed a correlation with the known distribution of CB1 and decline of [^{11}C]OMAR binding with age, most significantly in the globus pallidus. Overall, we observed elevated mean binding in patients with schizophrenia across all regions studied, and this increase was statistically significant in the pons ($p < 0.05$), by the students t-test. When we ran a regression of the control subjects V_T values with age and then compared the patient data to 95% prediction limits of the linear regression, three patients fell completely outside for the globus pallidus, and in all other regions there were at least 1-3 patients outside of the prediction intervals. There were no statistically significant correlations between PET measures and the BPRS subscores, but there was a significant correlation ($p < 0.05$) between V_T and the ratio of the BPRS psychosis to withdrawal score in the frontal lobe ($r = 0.49$), parietal lobe($r = 0.60$), and middle and posterior cingulate regions ($r = 0.71$ and $r = 0.79$ respectively). For the occupancy studies, after a single dose of AVE1625, CB1 brain occupancy (36-90%) was demonstrated for HC and 25-69% for SZ.
Discussion: In conclusion, [^{11}C]OMAR can image human CB1 receptors in normal aging and schizophrenia and shows strong evidence of an association of elevated binding specific brain regions and symptoms of the disease. The occupancy studies demonstrated feasibility of measuring human CB1 occupancy (25-92%) with a candidate antagonist under investigation.
Disclosure: D.F. Wong, Sanofi-Aventis, Part 4; Merck, Part 4; Otsuka, Part 4; Sepracor, Part 4; Lundbeck, Part 4; GE Healthcare, Part 4; AVID, Part 4; DANA, Part 4; Lilly, Part 4; Roche, Part 4.

Some Pharmacological Actions and Potential Therapeutic Applications for the Plant Cannabinoid Δ^9-Tetrahydrocannabivarin
Roger G. Pertwee*, Daniele Bolognini, Maria Grazia Cascio, Francesca Comelli, Barbara Costa, Lisa A. Gauson

Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom, Department of Biotechnology & Bioscience, University of Milano-Bicocca, Milano, Italy

There is growing evidence that plant cannabinoids in addition to Δ^9-tetrahydrocannabinol and cannabidiol possess pharmacological properties that could possibly be exploited in the clinic, one of these phytocannabinoids being Δ^9-tetrahydrocannabivarin (THCV). We have reported previously that THCV displays significant potency as a CB_1 receptor antagonist, both *in vitro* and *in vivo*. The present investigation has yielded evidence that THCV can also behave as a CB_2 receptor agonist, again both *in vitro* and *in vivo*. The measured *in vitro* response was inhibition of cyclic AMP production stimulated by 10 μM forskolin in Chinese hamster ovary (CHO) cells either transfected with human CB_2 receptors or untransfected ($n = 4$). Cannabinoids were administered in dimethyl sulphoxide and EC_{50} and E_{max} values calculated using GraphPad Prism 5. For *in vivo* experiments, acute inflammation was induced in the right hind paws of adult male C57BL/6J mice by intraplantar injection of carrageenan. Mice were injected intraperitoneally 30 min before carrageenan with THCV or its vehicle, ethanol:cremophorEL:saline 1:1:18, and paw oedema was assessed 2 h after carrageenan. Data are expressed as means and variability as SEM or 95% confidence limits (CLs). *In vivo* values were compared by Student's t test or, for multiple comparisons, by one-way analysis of variance followed by Tukey's *post-hoc* test ($n = 8$ to 10). Both THCV and the CB_1/CB_2 receptor agonist, CP55940, inhibited cyclic AMP production by CB_2 CHO cells. Their EC_{50} values with 95% CLs shown in parentheses were 38 nM (12 & 124 nM) and 6.9 nM (3.5 & 13 nM), respectively, their corresponding E_{max} values being 40% (32 & 48%) and 55% (50 & 60%). THCV and CP55940 were antagonized in this assay with equal potency by the CB_2-selective antagonist/inverse agonist, AM630. Importantly, the cells used in these experiments were preincubated with 10 μM AM630 for up to 24 h and then subjected to intense washing as we found this procedure causes subsequently administered AM630 to behave as an apparent neutral CB_2 receptor antagonist rather than as a CB_2 receptor antagonist/inverse agonist. Neither THCV nor CP55940 inhibited cyclic AMP production in untransfected CHO cells. In contrast to its vehicle, which did not significantly affect paw oedema, THCV (0.3 mg/kg) decreased the mean volume of carrageenan-injected hind paws from 114 ± 9.2 μl to 80 ± 0.8 μl ($P < 0.05$). This ability of THCV to reduce oedema was attenuated significantly ($P < 0.001$) by the CB_2-selective antagonist/inverse agonist, SR144528 (1 mg/kg i.p.) but not by the CB_1-selective antagonist/inverse agonist, rimonabant (0.5 mg/kg i.p.), injected 15 min before THCV. Paw volumes were 80 ± 1.9 μl after THCV (0.3 mg/kg), 74.4 ± 3.5 μl after rimonabant plus THCV and 117.5 ± 7.5 μl after SR144528 plus THCV. Hence, THCV behaves as a reasonably potent CB_2 receptor agonist *in vitro* and induces reductions in oedema that seem to be CB_2 receptor-mediated in an *in vivo* model of acute inflammation. Accordingly, THCV may have therapeutic potential as an anti-inflammatory agent. Our discovery that THCV activates CB_2 receptors but blocks CB_1 receptors, raises the possibility that it also has therapeutic potential for the management of Parkinson's disease (PD), chronic liver diseases and stroke. Indeed, evidence has recently emerged from experiments using a rat model of PD that THCV may be effective both at delaying disease progression in PD and at ameliorating parkinsonian symptoms. Funded by GW Pharmaceuticals and NIH (DA-03672).

Disclosure: R.G. Pertwee, Funding from GW Pharmaceuticals, Part 1.

Cannabinoids: Is There a Therapeutic Future?
Christian C. Felder*

Neuroscience Division, Eli Lilly and Company, Indianapolis, IN

Both phyto and small molecule cannabinoid therapeutics have entered the clinical arena with both successes and failures. Medical marijuana and mimetics such as levonantrodol, nabilone, and marinol progressed to late stage clinical testing and beyond, but were deemed suboptimal due to dysphoric side effects. Direct acting cannabinoid agonists, particularly at the CB-1 receptor, result in undesirable central effects including dysphoria, dizziness, thought disturbance, and somnolence. A new focus on blocking the endocannabinoid inactivation mechanism to provide a more physiologic cannabinoid receptor stimulation mechanism suggests that separation of the therapeutic benefit from psychotropic side effects may be possible. Inhibition of the fatty acid amido hydrolase (FAAH) and/or monoacyl glycerol lipase (MAGL) enzymes provides a physiologic elevation of endocannabinoid tone and desirable preclinical efficacy without concomitant motor or behavior disturbances in rodents and non-human primates. Several FAAH inhibitors are in phase 2 clinical testing for neuropsychiatric indications. A CB-1 cannabinoid receptor inverse agonists has progressed to the market for obesity, but was associated with undesirable increase in mood disorders and subsequently terminated. Peripherally restricted CB-1 receptor antagonists or negative allosteric modulators may offer a potential resurrection of this therapeutic opportunity. This presentation will offer an overview of the current therapeutic landscape for cannabinoid based therapeutics with detailed discussion of the pharmacological mechanisms of each approach. Cannabinoid receptors, endocannabinoid release and inactivation mechanisms, and both orthosteric and allosteric ligand pharmacology will be described and reviewed. An overview of the current knowledge of relevant bioactive endocannabinoids and additional candidates will be discussed. This background will serve to provide sufficient context to enhance the learning from the presentations of the other three speakers.

Disclosure: C.C. Felder, Eli Lilly & Co., Part 5.

Panel Session
Emerging Nanotechnology-Based Drug Delivery Methods and Their Applications to Addiction Research

Programmed Transdermal Delivery of Addictive Substances Through Voltage Gated Carbon Nanotube Membranes
Bruce J. Hinds*

University of Kentucky, Lexington, KY

Addiction treatment is one of the most difficult health care challenges due to the mixture of complex changing neurochemical pathways and psychological behavior. Generally the most effective treatments require psychological monitoring/counseling and adaption of therapeutic techniques. For large population addiction, such as nicotine, it is cost and time prohibitive to have face to face meetings. A promising system is where a dosing regiment (within a doctors' prescription limit) can be *remotely* programmed to account for daily environmental factors, patient input, and counselor feedback from phone interviews or internet-based surveys. Needed for this system is an ultra low power, compact, and programmable delivery device not currently available with electroporation or mechanical pumps. Carbon nanotubes (CNTs) have three key attributes that make them of great interest for novel applications such as programmed drug delivery; 1) atomically flat graphite surface allows for ideal fluid slip boundary conditions and thousand fold faster fluid flow 2) the cutting process to open CNTs inherently places 'gate keeper' valve chemistry at CNT core entrance and 3) CNT are electrically conductive allowing for electrochemical reactions and application of electric fields gradients at CNT tips.

Towards this goal, a composite membrane structure containing vertically aligned carbon nanotubes passing across a polystyrene matrix film have been fabricated [Science 2004]. Pressure driven flux of a variety of solvents (H2O, hexane, decane ethanol, methanol) are 4-5 orders of magnitude faster than conventional Newtonian flow due to atomically flat graphite planes inducing nearly ideal slip conditions [Nature 2005]. These properties are nearly ideal for introducing efficient electro-phoretic and electro-osmotic flow to be used as the basis of a programmed transdermal delivery device. CNT tips are functionalized with a high density of negative charge allowing only the unidirectional flow of positive cations under small bias, thus inducing an efficient flux of neutral molecules. Efficiencies as high as 1 neutral molecule per ion are seen in the small CNT pores, allowing standard watch batteries to operate for 2 weeks. These CNT membranes are 400 fold more energy efficient than conventional nanoporous materials. An *in-vitro* cell, composed of a reference electrode, reservoir solution, CNT membrane electrode, gel contact and human skin sample were assembled in a Franz cell. A differential mass-transport model of diffusion in series (reservoir/CNT/gel/skin) explained observed dosage profile. Therapeutically useful fluxes for Nicotine treatment were controllably switched between, with 0.56 and 2.0 micromole/cm2-hr at 0 mV and -600 mV respectively. Support was provided by NIH NIDA (R01DA018822) and NSF CAREER (0348544).

Disclosure: B.J. Hinds, None.

Development of Novel Delivery Systems and Manufacturing Methods Based on Nanotechnology

James Talton*

Nanotherapeutics, Alachua, FL

The absorption of nanoparticles from the gastrointestinal tract to improve the absorption rate and/or bioavailability has great potential for almost all classes of pharmaceuticals. While many currently available pharmaceuticals are delivered without absorption difficulties, such as orally, there is an increasing need to develop delivery systems for drugs with low solubility and/or permeability to obtain the desired pharmacological effect. Nanoparticle delivery involves several crucial parameters that influence uptake, such as particle diameter, the nature of the particle and surface characeristics that effect targeting to and uptake into cells. Post-absorptive events, including translocation processes, are suggested to be as important as initial uptake into the epithelial cells or M-cells of the gut associated lymphoid tissue. Many insoluble compounds are dry or wet milled in order to increase the dissolution properties, which results in an increased absorption after oral administration for compounds with substantial permeability of the free drug in solution. Unfortunately, many insoluble compounds as well as water-soluble and protein drugs also have low permeability across membranes limiting therapies to injectable routes. Administration of drug nanoparticles, nanocarriers, and microencapsulated particles have also been described that provide protection from enzymatic degradation and slow-release from diffusion of drug from hydrophobic bioerodible / biodegradable polymers. Particle interactions on the nanometer or micrometer scale have been shown to promote particle growth and agglomeration, and are inherently unstable without surface modifiers. The described invention describes multi-component compositions and methods to manufacture stable drug PDSs into patient-friendly FDFs that retain significantly improved biological properties with high shelf-life stability. Particulate delivery systems, or PDSs, in the form of nanoparticles, microparticles, and microencapsulated particles, are usually formed either by size reduction (dry or wet milling), spray-drying, precipitation upon addition of a non-solvent, gelling the drug / polymer upon changing the pH or addition of a precipitating ions (salts), or complexing a drug with a polymer of an opposite charge. These systems, however, tend to be characterized by poor reproducibility and scalability in manufacturing, low encapsulation efficiencies, damage/denaturation of the drug when it is a macromolecule due to the use of organic solvents and / or spray-drying, and poor shelf-life. This presentation will review current manufacturing methods and products on the market and in development.

Disclosure: J. Talton, None.

Targeted Nanoparticles for Gene Silencing

Jessica L. Reynolds*, Supriya D. Mahajan, Ravikumar Aalinkeel, Bindukumar B. Nair, Donald E. Sykes, Adela Bonoiu, Hong Ding, Roy Law, Paras N. Prasad, Stanley A. Schwartz

SUNY at Buffalo, Buffalo, NY

Background: Gold nanoparticles (GNP), specifically gold nanorods (GNR), are useful for a range of biomedical applications due to their biocompatibility and their ability to bind and deliver many biomolecules. Their surfaces can be modified to incorporate cationic charges which can form stable electrostatic complexes with anionic nucleic acids such as small interfering RNA (siRNA), for the purpose of targeted gene silencing. The parallel epidemics of drug addiction and human immunodeficiency virus (HIV-1) infections are major public health problems worldwide. Addictive drugs are risk factors for acquiring HIV-1 infections and may facilitate the pathogenesis of HIV encephalopathy. The 32-kDa dopamine and adenosine 3′,5′-monophosphate-regulated phosphoprotein (DARPP-32), associated with dopaminergic neurons in the brain, is involved in the pathogenesis of drug addiction. Previous studies demonstrated that drugs of abuse enhance HIV-1 infection. Thus we investigated the effects of silencing DARPP-32 expression using nanotechnology on the pathogenesis of HIV-1 infections.

Methods: Monocyte-derived macrophages (MDM) and primary normal human astrocytes (NHA) were treated *in vitro* with either methamphetamine or heroin. Further, GNR were complexed with DARPP-32 siRNA to form "nanoplexes" which also were used to treat cultures of MDM and NHA. Uptake of nanoplexes into the cells was determined using dark-field imaging of GNR. Gene and protein expression were analyzed by quantitative PCR and western blot analysis. MDM were infected *in vitro* using HIV-1 and p24 antigen expression was determined using ELISA.

Results: We note for the first time that DARPP-32 is expressed by MDM. Methamphetamine or heroin significantly upregulated DARPP-32 expression by MDM and NHA. Uptake of nanoplexes within the cytoplasm of cells was observed by dark-field imaging. Effective gene silencing by the nanoplexes was evidenced by a reduction in the expression of DARPP-32 in MDM and NHA, with no observed cell cytotoxicity. DARPP-32 silencing resulted in significant modulation of the activity of downstream effector molecules such as ERK and CREB. DARPP-32 silencing decreased p24 antigen production in MDM infected with HIV-1 *in vitro*. DARPP-32 silencing prevented an increase in p24 antigen production induced by drugs of abuse in MDM. Moreover gene expression of DARPP-32 was increased in MDM isolated from HIV-1 subjects.

Discussion: While DARPP-32 plays a significant role in signal transduction within the CNS and is intimately involved in the pathogenesis of addictive behaviors, we have demonstrated that it also is expressed by immunocompetent cells and is associated with immunoregulatory activities. As drugs of abuse have been shown to increase susceptibility to and progression of HIV-1 infections, nanoparticle-mediated silencing of DARPP-32 expression within the immune system of drug using, HIV-1 infected patients may be useful in managing the progression of their infections.

Disclosure: J.L. Reynolds, None.

Clathrin Triskelia as Potential High-Relaxivity Magnetic Resonance Nanoprobes for Molecular Imaging of Dopamine Receptors

Gordana D. Vitaliano*, David J. Rios, Franco Vitaliano, Lu Yang, David Y.W. Lee, Susan Andersen, Perry Renshaw, Martin H. Teicher

McLean Hospital, Boston, MA

Background: A rapidly developing area in drug addiction research is molecular-level imaging, encompassing the study of receptors, transporters, enzymes, genes and intracellular processes. Magnetic Resonance Imaging (MRI) is a noninvasive visualization technique with high spatial resolution, but low sensitivity for visualization of molecular targets. In order to improve MRI sensitivity for molecular brain imaging, our goal was to develop small (<50 nm) clathrin triskelia-based nanoplatforms with high molecular relaxivity that incorporate high payloads of Gadolinium (Gd) contrast agents, which can be delivered non-invasively and target specific dopamine receptors in the rat brain.
Methods: Gadolinium-2-(4-Isothiocyanatobenzyl) diethylene-triamine-pentaacetic acid (Gd-DTPA-ITC) was conjugated to clathrin triskelia through reactive lysine residues. We determined the chelate to protein molar ratio by using a standard Arsenazo III-based spectrophotometric method. We calculated relaxivity for each sample by using T1 data and Gd concentrations as determined by NMR and spectrophotometric analyses. We then conjugated Maleimide-PEG-Dopamine-3 Antibody (D3Ab) and Maleimide-PEG-rhodamine to clathrin triskelia through cysteine residues, and delivered nanoprobes intranasally. Animals were sacrificed 2 hours after intranasal administration and immunohistochemistry and fluorescent analyses were performed. For autoradiography we PEGylated Clathrin-triskelia with Maleimide-PEG-Dopamine-3 ligands (BP 897 or SB-277011A), radiolabeled with C14, and delivered intranasally. Brains were removed after 2 hours and autoradiography was performed. Autoradiograms were developed, quantified and compared with immunohistochemistry and fluorescent images.
Results: Electron Microscopy has shown a large proportion of Gd-DTPA-clathrin triskelia with a mean hydrodynamic radius of 20 nm. The mean Chelate/Protein molar ratio was 27 ± 2.4. At 0.47T, Gd-DTPA-ITC-Clathrin-Triskelia displayed relaxivity of $1{,}604\ mM^{-1}s^{-1}$ per particle, and $22\ mM^{-1}s^{-1}$ per Gd ion. Two hours after intranasal administration D3-Triskelia nanoprobes were found only in D3 related brain regions in rats. Autoradiography, fluorescent and light microscopic examination of the D3 brain regions confirmed specific targeting of D3 receptors with different D3-nanoprobes. Confocal laser microscopy confirmed integrity of the nanoparticles in the rat brain. Clathrin and D3Ab fluorescence co-localized in the D3 brain regions.
Discussion: We demonstrated that Clathrin triskelia can serve as robust MRI platforms onto which multiple functional motifs can be added through chemical modifications; and that small and stiff molecular structures with large rotational correlation times can exhibit over 400-fold greater molecular relaxivity than any currently approved Gd-MRI contrast agent. Triskelia nanoprobes were successfully nasally delivered non-invasively into the rat brain, were able to target specific dopamine receptors, and remained stable in the rat brain. These preliminary results should encourage further investigations into the use of clathrin triskelia as a new nanoplatform for MR contrast enhanced molecular brain imaging and drug delivery. Targeted, high precision dosing can be developed for genes, RNA interference and antisense gene therapeutics, and neurotrophic, neuroprotective and psychotropic drugs for treating addiction and other brain disorders.
Disclosure: G.D. Vitaliano, ExQor Technologies Inc., Stockholder, Part 1.

Panel Session
From Mouse to Man: Modeling Obsessive Compulsive Disorder in Mice, and Relevance to the Human Disorder

Synaptic and Circuitry Mechanisms of Compulsive/Repetitive Behavior

Guoping Feng*

McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA

Background: Obsessive-compulsive disorder (OCD) is characterized by persistent intrusive thoughts (obsessions), repetitive actions (compulsions) and excessive anxiety. Currently, the causes of OCD are largely unknown. At the neuroanatomical level, surgical lesions and neuropsychological studies have indicated that the dysfunction of cortico-striatal-thalamic-cortical circuitry may play a key role in the pathogenesis of OCD. This observation is further supported by functional brain imaging studies which show increased functional activity in orbitofrontal cortex, anterior cingulate cortex, the caudate nucleus and the thalamus of OCD patients. Despite these important findings little is known about the mechanisms underlying the dysfunction of cortico-striatal-thalamic-cortical circuitry in OCD.
Methods: We deleted SAPA3 gene in mice using homologous recombination in ES cells. A Cre-inducible BAC transgenic mouse was generated by placing floxed STOP cassette in front of ATG codon of the SAPAP3 gene in a BAC clone. D1R-Cre and D2R-Cre were obtained from GENSAT. Grooming behavior was recorded with 24-hour video taping in home cage and scored by investigators blinded to genotypes. Elevated zero test and dark-light emergence test were used to measure anxiety-like behavior.
Results: We hypothesize that dysfunction of the cortico-striatal glutamatergic synapses plays a key role in compulsive and repetitive behavior. Using genetic approaches in mice we show that deletion of SAPAP3, a postsynaptic scaffolding protein highly expressed in the striatum and critical for synaptic function, leads to increased anxiety and compulsive-like grooming behavior resulting in facial skin lesions; both were alleviated by treatment with a selective serotonin reuptake inhibitor. Electrophysiological, structural, and biochemical studies of SAPAP3 mutant mice revealed defects in cortico-striatal synapses. Furthermore, lentivirus-mediated selective expression of SAPAP3 in the striatum rescued the synaptic and behavioral defects of SAPAP3 mutant mice. To directly dissect the relative contribution of the direct and indirect pathways in compulsive/repetitive behavior in SAPPA3 mutant mice, we generated Cre-inducible SAPAP3 transgenic mice. We selectively induced SAPAP3 transgene expression in medium spiny neurons (MSNs) of the direct or indirect pathway in SAPAP3 mutant mice, by crossing the inducible SAPAP3 transgenic mice to D1R-Cre and D2R-Cre mice, respectively. We found that selective expression of SAPAP3 transgene in MSNs of the directly pathway, but not MSNs of the indirectly pathway, rescued the compulsive/repetitive grooming behavior in SAPAP3 mutant mice.
Discussion: Our results provide direct evidence for a critical role of cortico-striatal glutamatergic synapses in compulsive/repetitive behavior, and illustrate the importance of synaptic dysfunction of the direct pathway of the basal ganglia in such behavior.
Disclosure: G. Feng, None.

5-HT1B-Induced Mouse Model of OCD-Like Behavior

Nancy A. Shanahan, Lady Velez, Virginia A. Masten, Stephanie C. Dulawa*

University of Chicago, Chicago, IL, University of California at San Diego, San Diego, CA

Background: Obsessive-compulsive disorder (OCD) is characterized by intrusive thoughts, images, or impulses and/or repetitive behavior. OCD patients exhibit reduced prepulse inhibition (PPI), and symptom

exacerbation following challenge with 5-HT1B receptor agonists. In mice, 5-HT1B agonists induce PPI deficits and perseverative hyperlocomotion. Long-term treatment with serotonin reuptake inhibitors (SRIs) provides the only pharmacological monotherapy for OCD. We tested the specificity and time-course for SRI antidepressants to block 5-HT1B-induced OCD-like behavior in mice. We also examined the effect of effective vs. ineffective antidepressant treatments for OCD on 5-HT1B receptor expression and functional coupling in brain regions implicated in OCD.

Methods: Mice were treated chronically with clomipramine, fluoxetine, or desipramine. Mice then received acute 5-HT1B agonist challenge and were assessed for PPI and perseverative hyperlocomotion. Additionally, separate groups of mice were treated with fluoxetine for 4, 14, 21, 28, or 56 days and assessed for OCD-like behavior or depression-like behavior for comparison. Finally, 5-HT1B receptor expression and G-protein coupling were assessed in the orbitofrontal cortex (OFC), dorsofrontalcortex (dFC), caudate/putamen and nucleus accumbens following chronic antidepressant treatment.

Results: Only chronic treatment with SRIs attenuated 5-HT1B-induced OCD-like behavior. SRI reversal of 5-HT1B-induced OCD-like behavior required 3-4 weeks to emerge, while reversal of depression-like behavior required 2 weeks. Chronic SRI treatment reduced 5-HT1B receptor expression in the OFC.

Conclusions: Our results show that 5-HT1B-induced OCD-like behaviors are reduced by chronic administration of effective, but not ineffective, treatments for OCD. Furthermore, SRIs reverse OCD-like behavior weeks after they reduce depression-like behavior, as observed in clinical populations. Our findings also suggest that downregulation of 5-HT1B receptors in the OFC may underlie the therapeutic effects of SRIs in OCD. This novel mouse model provides a tool for identifying the neural substrates underlying aspects of OCD, and the therapeutic mechanisms of SRIs in this disorder.

Disclosure: S.C. Dulawa: None.

Role of Slitrk5 in Obsessive Compulsive-Like Behaviors

Francis S.Y. Lee*, Sergey V. Shmelkov Catia C. Proenca, Deqiang Jing, Adília Hormigo, Ipe Ninan, Shahin Rafii

Weill Cornell Medical College, New York, NY

Background: There are several disorders that have OCD-like clinical manifestations, such as Gilles de la Tourette's syndrome. Recent human genetic analyses have linked the SLITRK1 gene to these pathological conditions, though the underlying mechanisms are not well understood. The Slitrk1 gene belongs to a new family of six members (Slitrk1-6) encoding one-pass transmembrane proteins, which contain two extracellular leucine-rich repeat (LRR) domains, similar to Slit proteins, and a carboxy-terminal domain that is similar to Trk neurotrophin receptors. These proteins have been shown to affect neuronal process outgrowth. Slitrk1 knockout mice exhibit elevated anxiety-like behaviors, but do not show any other behavioral abnormalities. Though little is known about Slitrk1, the function of other members of Slitrk family remains obscure. We hypothesized that abnormal expression of Slitrk5 may lead to behavioral phenotypes similar to the involvement of SLITRK1 in Tourette's syndrome. In order to investigate the function of this protein and to delineate the expression pattern of the Slitrk5 gene in mouse tissues, we generated a knockout/knockin mouse by replacing Slitrk5 gene with a reporter gene.

Methods: Slitrk5-/- and WT littermate mice were tested at different postnatal ages in obsessive-compulsive and anxiety related behavioral, with subsequent evaluation of neuronal morphology in relevant brain regions.

Results: We have observed that targeted inactivation of Slitrk5 in mice leads to OCD-like behavioral phenotypes, including overgrooming with elements of self-mutilation, and is alleviated by the selective serotonin reuptake inhibitor, fluoxetine. Slitrk5-/- mice display selective overactivation of orbitofrontal cortex, abnormalities in striatal anatomy and cell morphology, as well as alterations in glutamate receptor composition, which contribute to deficient corticostriatal neurotransmission.

Discussion: Overall, our data suggest that Slitrk5 may play a central role in the development of OCD-like behaviors. While human genetic studies have implicated another Slitrk family member, SLITRK1, in Tourette's syndrome, these associations have not been consistently replicated. In this context, our studies link Slitrk5 to core symptoms of OCD: self-injurious repetitive behavior and increased anxiety. In all, we provide a novel animal model of OCD-like behaviors, involving a novel neuronal transmembrane protein, which modulates region-specific glutamatergic neurotransmission. This model can be used to further dissect the role of Slitrk5 in molecular pathways underlying the pathogenesis of obsessive-compulsive behaviors.

Disclosure: F.S. Lee, None.

Using Translatable Human Biomarkers to Assess Clinical Relevance of Mouse Models of Obsessive Compulsive Disorder

Susanne E. Ahmari*, Bina Santoro, Lauren Leotti, Daniel Flicker, Rebecca A. Piskorowski, Cara Malapani, Victoria Risbrough, Steven A. Siegelbaum, Edward E. Smith, Mark Geyer, René Hen, Helen Blair Simpson

Columbia University/New York State Psychiatric Institute, New York, NY, Columbia University, New York, NY, University Of California, San Diego, CA

Background: Though Obsessive Compulsive Disorder (OCD) is one of the most disabling and chronic psychiatric disorders, with 2-3% lifetime prevalence, the pathophysiology underlying OCD remains unclear. This is partly because it is difficult to make OCD mouse models that recapitulate symptoms in multiple cognitive, behavioral, and emotional domains: obsessive thoughts, compulsive behaviors, and anxiety. In addition, individual patients display widely variable obsessions, compulsions, and anxiety levels, rendering it difficult to establish a unified group of symptoms to model in animals. To address this problem, we have created a framework for performing parallel studies in humans and mice to help determine whether animal models have relevance to human OCD symptoms. As an example, we apply this framework to HCN1 [hyperpolarization-activated cyclic nucleotide-gated channel 1] knockout mice, which demonstrate abnormal repetitive arching behavior.

Methods: Humans: 21 unmedicated OCD subjects and matched healthy controls were tested in prepulse inhibition (PPI) and a Stop Signal Reaction Time (SSRT) task. Clinical measures were obtained at time of testing (YBOCS, OCI-R, HAM-D, YGTSS, STA-I, ASI). Mice: $HCN1^{-/-}$ and wild-type (WT) littermates were treated with 18 mg/kg fluoxetine vs vehicle for 4 weeks. Groups were tested in a behavioral battery of obsessive-compulsive and anxiety-related tasks before and after fluoxetine treatment.

Results: Humans: We found impaired PPI in unmedicated OCD subjects (prepulse intensities: 78 dB and 86 dB). We also found an improvement in the ability of OCD subjects to inhibit behavioral responses compared to matched controls, represented by a smaller Stop Signal Reaction Time (SSRT = an estimate of the time taken to stop a response). This difference was linked to an overall slower reaction time in OCD patients, and increased accuracy on the task. Mice: Mice with constitutive knockout of HCN1 pacemaker channels exhibited an abnormal repetitive arching behavior that was absent in WT littermates. Arching was alleviated by chronic treatment with high-dose fluoxetine, a selective serotonin reuptake inhibitor used in our OCD treatment studies. We also noted an increase in PPI in $HCN1^{-/-}$ mice following fluoxetine treatment. Studies of SSRT are ongoing.

Discussion: We have demonstrated abnormalities in two translatable neurocognitive tasks (PPI, SSRT) in OCD patients vs matched healthy

controls. This is the first study to examine these candidate endophenotypes in unmedicated OCD subjects.
1) Our human results confirm that PPI abnormalities are seen in OCD subjects, and support use of PPI to help validate OCD mouse models.
2) Though we also see abnormalities in the SSRT, our findings run counter to the published literature (i.e. we see improved inhibition in OCD subjects); this result can be reconciled by our finding that OCD patients sacrificed speed on the task in favor of improving overall accuracy. This suggests caution in using this task as a reliable translatable biomarker.
3) We illustrate the process of applying translatable biomarkers using the $HCN1^{-/-}$ mouse model, which demonstrates repetitive behavior and PPI increases after fluoxetine treatment.
4) Further work is needed in the field to identify additional reliable translatable biomarkers of OCD; these in turn will increase our ability to validate mouse models, which can be used to dissect the molecular and cellular substrates underlying OCD pathophysiology.
Disclosure: S.E. Ahmari, None.

Panel Session
Glutamatergic Anomalies in Alcoholism: Exciting Advances from Studies of Mice, Rats and Man

In Vivo Translational Study of Brain Glutamate in Aging and Alcoholism

Natalie May Zahr*, Dirk Mayer, Meng Gu, Edith V. Sullivan, Adolf Pfefferbaum

Stanford University, Stanford, CA, SRI International, Menlo Park, CA

Background: *In vivo* magnetic resonance spectroscopy (MRS) permits translational study through noninvasive longitudinal tracking of brain chemistry changes caused by human aging and substance abuse and experimental manipulations in models. A metabolite of particular interest to mechanisms of alcoholism neurotoxicity and age-related neurodegeneration is glutamate (Glu). Advances in MRS acquisition parameters now permit the direct *in vivo* detection and quantification of Glu and glutamine (Gln) at high magnetic field-strengths. As Glu is predominately neuronal and Gln a glial metabolite, these advances enable *in vivo* investigation of neuron-glial metabolic interactions and alterations in glutamatergic synaptic regulation by conditions affecting these constituents.
Methods: MRS detection of Glu overcame challenges from spectral overlap with signals from other metabolites and a complicated multiplet structure due to strong J-coupling through the application of Constant Time PRESS (CT-PRESS), which enabled separation and quantification of Glu and Gln. In humans, CT-PRESS examined the effects of age in 3 brain regions targeted by cortical glutamatergic efferents-basal ganglia, cerebellum, and pons-and to test whether performance on frontally based cognitive tests would correlate with regional Glu levels. In rats, CT-PRESS examined the effects of 16 and 24 wks of vaporized EtOH exposure on basal ganglia Glu and Gln levels and associated liver condition.
Results: Healthy elderly individuals (77 ± 5yo) had lower Glu in basal ganglia but not pons or cerebellum than young adults (26 ± 4yo). Levels of basal ganglia Glu correlated selectively with cognitive tests showing age-related decline. In rats, 16 wks of EtOH exposure (blood alcohol levels (BAL) = 293 mg/dL) resulted in higher Gln levels in the EtOH than control group. After 24 wks, when Gln levels no longer distinguished the groups, Glu levels were higher in the EtOH (BAL = 445 mg/dL) than Con group.
Discussion: In humans, selective relations between performance and basal ganglia Glu provide *in vivo* support for age-related modification of Glu levels as contributing to cognitive decline in normal aging. In rats, mild liver damage in the EtOH group suggests a mechanism for brain changes in Gln and Glu: liver failure impairs the major organ for ammonia (NH_3) elimination via the urea cycle and can lead to elevated brain NH_3. The mechanism of brain NH_3 detoxification is the formation of Gln from Glu by the enzyme Gln synthetase. With prolonged EtOH exposure, Gln synthetase levels may be compromised, leading to a build-up of Glu. These findings demonstrate the potential of *in vivo* MRS to contribute to a mechanistic understanding of glutamatergic system modification in the development of alcoholism and the age-related cognitive decline. (Support: AA005965, AA012388, AA017168, AA013259-INIA, AG017919.)
Disclosure: N.M. Zahr, None.

Magnetic Resonance Spectroscopy of Human Glutamate-Glutamine System Disruption in Alcohol Use Disorders

Robert J. Thoma*, Charles Gasparovic, Paul Mullins, Ronald A. Yeo, Arvind Caprihan, David Ruhl

University of New Mexico, Albuquerque, NM, University of New Mexico, Albuquerque, CA, MIND Research Network, Albuquerque, NM, Mind Research Network, Albuquerque, NM

Background: Acute ethanol exposure inhibits NMDA glutamate (Glu) receptors and sudden withdrawl from chronic alcohol use may lead to an increased activation of these receptors with excitotoxic effects. In the longer term, brain levels of Glu and its metabolites, such as glutamine (Gln), are likely to be altered, providing a measure of overall glutamatergic dysfunction. However, few studies have assessed concentrations of these metabolites in clinical populations of individuals either actively abusing alcohol or currently in prolonged remission.
Methods*:* Twenty-two healthy controls were compared to a group of 17 participants with alcohol dependence, ten with active dependence (AD) and seven in remission for at least one year (AD-R). Neurometabolite concentrations were measured with proton magnetic resonance spectroscopy (^{1}H-MRS) in a predominantly gray matter voxel including the bilateral anterior cingulate gyri. Tissue segmentation provided an assessment of the proportion of gray matter in the ^{1}H-MRS voxel. The Drinker Inventory of Consequences Scale (DrInC) exam was administered to all participants.
Results: Glu was significantly lower and Gln was significantly higher in the AD and AD-R groups relative to the control group; no other neurometabolite concentrations differed across groups. These results were not confounded by age, proportion of gray matter in the ^{1}H-MRS voxel, or smoking history. Neurometabolite concentrations did not differ across AD and AD-R groups. Subsequent regressions in the combined clinical group, treating voxel gray matter proportion as a covariate, revealed that both the absolute concentration of Gln, and the Gln/Glu ratio, were positively correlated with total score on the DrInC, while gray matter proportion was negatively correlated with the DrinC.
Conclusions: The current findings of higher Gln and lower Glu in the combined AD-A and AD-R groups indicate a perturbation of the Gln-Glu cycle. The absence of any difference between AD and AD-R groups suggests that glutamatergic dysfunction either predates the onset of abuse or persists long after prolonged alcohol abstinence.
Disclosure: R.J. Thoma, None.

Ethanol and Nicotine Co-Abuse Is Regulated by, and Produces Long Term Neuroadaptations in, the Glutamatergic System in the Corticolimbic Pathway

Zachary Rodd*, Zheng-Ming Ding, Gerald A. Deehan, William Truitt, William McBride

Indiana University: School of Medicine, Indianapolis, IN

Background: The transition from controlled drug consumption to uncontrollable intake has been hypothesized to involve alterations in the glutamate (Glu) system in the corticolimbic (CL) pathway. While past research has focused on alterations in the Glu system produced by administration of a single drug of abuse (i.e., cocaine), the vast

majority of human drug use is characterized by polydrug abuse. In alcoholics the rate of concurrent nicotine dependence can exceed 92%. Therefore, elucidating the effects of co-abuse of ethanol and nicotine on the Glu system in the CL pathway could provide insights into alterations observed in the human condition.
Methods: Ethanol (EtOH) can alter the Glu system in the CL. Briefly, Wistar rats were implanted with guide cannuale aimed at the VTA. One week later, rats received injections of 0, 0.5, 1.0, or 2.0 g/kg EtOH during conventional microdialysis procedures. Recently, we have developed an EtOH-Nicotine (Nic) co-abuse model. Alcohol-preferring (P) rats will readily consume EtOH-Nic solutions. P rats given 1 h operant access to EtOH-Nic solutions will orally consume the two reinforcers at a level which is intoxicating (BECs $>$ 120 mg%) and results in high levels of Nic self-administration ($>$ 8 mg/kg). Following a 10 day abstinence period, a No-Net Flux microdialysis experiment was conducted to assess basal Glu levels and clearance in the medial prefrontal cortex (mPFC) in P rats that were drug naïve, EtOH-exposed, or with a past history of EtOH-Nic co-abuse. Similar to the period of initiation of EtOH-Nic co-abuse in humans, P rats will readily consume EtOH-Nicotine solutions during adolescence. P rats were allowed to self-administer water, EtOH, Nic, or EtOH-Nic during periadolescence (PND 30-60), but were drug abstinence for 40 days prior to sample collection (PND 100). The gene expression of G-couple receptors in the nucleus accumbens shell and anterior cingulate in these rats were determined through the use of a focused microarray.
Results: In the initial microdialysis study, extracellular Glu levels in the VTA were increased in rats administered a low dose (0.5 g/kg; 35% increase) of EtOH, but reduced at a higher dose (2.0 g/kg; 20% decrease). The No-Net Flux data indicated that Glu clearance in the mPFC was significantly reduced in P rats with a past history of EtOH or EtOH-Nic self-administration. The focused microarray analysis revealed that EtOH and EtOH-Nic usage during periadolescence produced numerous alterations in G-coupled receptors during adulthood, including the expression of Glu receptors in the nucleus accumbens shell (some unique to the EtOH-Nic group).
Discussion: The initial results indicated that low dose ethanol stimulates glutamatergic projections to the VTA, suggesting that VTA glutamate may be involved in the activating and reinforcing effects of EtOH. The reduction in Glu clearance following chronic EtOH and EtOH-Nic consumption (No-Net Flux data) would theoretically translate into Glu lasting longer in the synaptic cleft, possibly having enhanced post-synaptic actions. The findings of the focused microarray data set indicated that consumption of EtOH or EtOH-Nic can produce long term alterations in the Glu system in the CL. Overall, the data indicate that consumption of EtOH or EtOH-Nic results in acute and chronic alterations of the Glu system in the CL pathway, and that the development of pharmacotherapeutics for the treatment of alcoholism and/or alcohol-nicotine co-abuse should include the Glu system.
Disclosure: Z.A. Rodd, None.

The Importance of Glutamate Signaling Through Homer in Regulating Binge Alcohol Drinking Behavior

Karen Szumlinski*, Debra K. Cozzoli

University of California at Santa Barbara, Santa Barbara, CA

Background: Alcohol is a drug of abuse well-characterized to affect both pre- and postsynaptic aspects of glutamate neurotransmission and in recent years, converging proteonomic, behavioral genetic and pharmacological evidence supports a potential role for the scaffolding/signaling molecule Homer in regulating alcohol-induced glutamate plasticity of relevance to addiction and alcoholism.
Methods: A series of neurochemical and immunoblotting studies were performed on groups of mice trained to binge drink alcohol, as well as mice exhibiting high vs. low binge alcohol drinking phenotypes to examine for relations between mesocorticolimbic levels of extracellular glutamate, as well as glutamate receptor and Homer, and binge alcohol drinking. Behavioral pharmacological and genetic approaches were then used to examine the role for mGluR5/Homer2/PI3 K and PKCepsilon signaling within the nucleus accumbens (NAC) and central nucleus of the amygdala (CeA) in the propensity to binge alcohol drink.
Results: A history of binge alcohol drinking sensitizes the capacity of alcohol to stimulate glutamate release within the NAC. Using 2 distinct models, a history of binge alcohol drinking elevated the expression and/or activational state of members of the mGluR5/Homer2/PI3K/PKCepsilon signaling pathways within the NAC and CeA, but not in other mesocorticolimbic structures examined. A high alcohol drinking phenotype was also found to be positively associated with elevated PI3 K and/or PKC signaling within the NAC and inhibition of signaling through mGluR5/Homer2 to PI3K and PKCepsilon reduced binge alcohol intake in 2 distinct binge drinking models.
Discussion: This collection of data supports the notion that idiopathic or alcohol-induced increases in extended amygdala mGluR5/Homer2 signaling may be critical for the manifestation of binge alcohol drinking behavior and the development of this prevalent form of alcoholism. This work was funded by NIAAA grant R01AA016650.
Disclosure: K.K. Szumlinski, None.

Panel Session
Pediatric Bipolar Disorder Is a Valid and Prevalent Diagnosis Whose Expression in Children May Include Chronic and Severe Irritability in Place of "Classical" Manic Symptoms

Supporting the Validity of Pediatric Bipolar Disorder

Joseph Biederman*

Massachusetts General Hospital, Boston, MA

Background: To evaluate the validity of an early-onset form of bipolar disorder (BPD) in light of ongoing debate and proposed changes in DSM-IV.
Methods: A growing number of empirical studies and extensive reviews of the literature[1] are amassing evidence for this diagnosis in children and adolescents.
Results: The literature indicates that pediatric BPD is a disabling condition characterized by extreme affective and behavioral dysregulation, aggression, severe irritability, and a chronic course. Epidemiological studies estimate that at least 1% of youth may be affected[2], and clinical studies document that up to 20% of psychiatrically referred children and adolescents satisfy criteria for bipolar spectrum disorders with many requiring repeated inpatient hospitalizations[3]. Irritability, which is one of DSM-IV's mood criteria for mania, is the most common abnormal mood associated with pediatric BPD and the one that commonly drives the clinical referral. Studies have noted that the type of irritability observed in children with mania is extremely severe and arguably distinct from other forms of irritability seen in other psychiatric conditions[4]. Studies of children and adolescents show high rates of ADHD in pediatric patients with mania and adults with early onset BPD. Since juvenile mania is commonly associated with extreme violence and severe behavioral dysregulation, many children with this diagnosis will also meet diagnostic criteria for conduct disorder (CD). Taken together, these findings suggest that early onset mania represents a highly virulent form of the disorder that is heavily comorbid with disruptive behavior disorders. While the literature has paid little attention to subsyndromal cases, it is very clear that the onset of this disorder in insidious and children with incipient disorders are likely to be seen in the clinic. Since intervention with incipient cases may be critical to avoid progression and morbidity, there is a critical need for clinician to be able to diagnose those subsyndormal cases.
Conclusions: Research on pediatric BPD has begun to shift from debating the validity of a diagnosis to understanding its neuro-

biological underpinnings and clinical correlates. Despite debate, there is an increasing recognition that a substantial minority of children suffer from an extraordinarily severe form of psychopathology associated with extreme irritability, violence, and incapacitation that is highly suggestive of mania. Advancements in neurosciences, neurobiology, psychopharmacology, genetics and neuroimaging will undoubtedly help advance the understanding of this complex and crippling disorder. Such advances will shed light on the brain circuits that may underlie the spectrum of conditions that comprise pediatric bipolar disorder.

References: 1. Geller B, et al. Child and adolescent bipolar disorder: A review of the past 10 years. *JAACAP* 1997;36(Sept.):1168-1176.
2. Lewinsohn P, et al. Bipolar disorders in a community sample of older adolescents: Prevalence, phenomenology, comorbidity, and course. *JAACAP* 1995;34(4):454-463.
3. Biederman J, et al. Further evidence of unique developmental phenotypic correlates of pediatric bipolar disorder: Findings from a large sample of clinically referred preadolescent children assessed over the last 7 years. *J Affect Disord* 2004;82(Suppl 1):S45-S58.
4. Mick E, et al. Heterogeneity of Irritability in ADHD Subjects with and without Mood Disorders. *Biol Psychiatry* 2005;58(7):576-582.

Disclosure: J. Biederman, Alza, Part 1; AstraZeneca, Part 1; Bristol Myers Squibb, Part 1; Eli Lilly and Co., Part 1; Janssen Pharmaceuticals Inc., Part 1; McNeil, Part 1; Merck, Part 1; Organon, Part 1; Otsuka, Part 1; Shire, Part 1; NIMH, Part 1; NICHD, Part 1; Fundacion Areces, Part 1; Medice Pharmaceuticals, Part 1; Spanish Child Psychiatry Association, Part 1; Novartis, Part 1; UCB Pharma Inc., Part 1; Lilly, Part 1; Janssen and McNeil, Part 1; Lilly, Part 2; Janssen and McNeil, Part 2; Alza, Part 4; AstraZeneca, Part 4; Bristol Myers Squibb, Part 4; Eli Lilly and Co., Part 4; Janssen Pharmaceuticals Inc., Part 4; McNeil, Part 4; Merck, Part 4; Organon, Part 4; Otsuka, Part 4; Shire, Part 4; NIMH, Part 4; NICHD, Part 4.

Arguments Against Including Temperamental Dysregulation Disorder with Dysphoria in DSM-5

Robert Kowatch*

Cincinnati Childrens Hospital, Cincinnati, OH

Background: There are several strong arguments against including Temper Dysregulation Disorder with Dysphoria (TDD) as an official diagnosis in the DSM-5.

Methods: The TDD diagnosis, as currently conceived, does not have symptom criteria that are specific to TDD as a syndrome. The TDD diagnosis rests on two primary criteria: recurrent severe temper outbursts and chronically irritable and/or sad mood. Temper outbursts are a behavioral manifestation of irritable mood, TDD as it is currently proposed, can be fulfilled with the presence of a single symptom. However the symptom of irritability is a DSM-IV diagnostic criterion for a range of psychiatric disorders in children and adolescents that span the Mood, Anxiety and Disruptive Behavior Disorder categories including Bipolar Disorders, Major Depressive Disorder, Dysthymic Disorder, Cyclothymic Disorder, Generalized Anxiety Disorder, Post-Traumatic Stress Disorder, Acute Stress Disorder and Oppositional Defiant Disorder. As noted in the DSM-5 Task Force document "Justification for Temper Dysregulation Disorder with Dysphoria", the limited scientific support for the TDD diagnosis emerges primarily from one research group. This fact in itself is problematic, as replication by independent research teams is a requirement for establishing the scientific validity of research findings. Recently in psychiatry we have repeatedly seen the lack of replication of genetic and neuroimaging findings across different research groups. In addition the studies that do have bearing on TDD do not examine it directly, but instead focus on an overlapping but not identical population of youth with Severe Mood Dysregulation (SMD). The proposed TDD criteria will likely identify a broader range of patients when applied in clinical settings. Irritability and temper outbursts are among the most common presenting complaints in child and adolescent psychiatry. Since TDD has these as its primary diagnostic criteria without any other accompanying symptoms, it could readily become the default diagnosis for the vast majority of children presenting with these symptoms. The rationale that TDD will reduce the inappropriate use of medication in children and adolescents with temper outbursts also seems at odds with perceptions of how the pharmaceutical industry approaches the DSM. Official diagnostic status in DSM-5 will allow TDD to become a target for pharmaceutical companies to obtain an FDA indication for the treatment of TDD.

Discussion: In summary, the inclusion of Temper Dysregulation Disorder with Dysphoria as a diagnosis in the DSM-5 is not warranted for many reasons. The level of scientific evidence to support TDD is too limited to justify a new diagnostic entity. Application of the TDD criteria in clinical practice will most likely label a highly heterogeneous group of children and adolescents who will have divergent developmental trajectories of psychopathology. Youth with a broad range of symptomatology are lumped together into the TDD diagnostic category, research into the pathophysiology and treatment of youth with severe irritability will be adversely affected - greater heterogeneity reduces the signal to noise ratio. Inclusion of TDD will compromise the already precarious public perception of child and adolescent psychiatry.

Disclosure: R.A. Kowatch, AstraZeneca, Part 1; Forest, Part 1; Merck, Part 1; Medscape, Part 1; NIMH, Part 4; NICHD, Part 4; Oxley Foundation, Part 4; CCHMC, Part 5.

Non-Episodic Irritability Is Not a Developmental Presentation of Bipolar Disorder

Ellen Leibenluft*, Kenneth Towbin, Daniel Pine

NIMH, Bethesda, MD

Background: Over the last decade, researchers have advanced the idea that bipolar disorder (BD) presents in youth, not with the episodes of mania characteristic of "classic" BD, but instead with severe, non-episodic irritability.

Methods: To test the hypothesis that mania presents in youth as severe, non-episodic irritability, we embarked on a program of research centered on recruiting youth with unequivocal, episodic BD, as well as youth with "severe mood dysregulation" (SMD). The criteria for SMD were designed to ascertain reliably youth with severe, non-episodic irritability and the "hyperarousal" symptoms common to ADHD and the "B" criteria of mania. Approximately 150 youth in each group have been compared on longitudinal course and family history, as well as clinical neuroscience measures. The latter included response reversal, face emotion processing, and frustrating paradigms, used both behaviorally and in concert with ERP's and/or fMRI. This work in clinical samples was supplemented by post-hoc analyses of community-based data sets to examine longitudinal outcomes of irritability.

Results: *Outcome:* Over a median follow-up period of 28.4 months, 1/84 SMD (mean 11.6 ± 2.3y) developed a (hypo)manic episode, whereas 58/93 BD (mean 12.9 ± 2.8y) developed such episodes ($p < .001$). In a post-hoc analysis of the Great Smoky Mountain Study ($N = 1420$), the lifetime prevalence of a proxy form of SMD was 3.3%. At 18.3 ± 2.1 years, youth with SMD at age 10.6 ± 1.4 years were significantly more likely to be diagnosed with a unipolar depressive disorder ($p < .02$) than youth who never met criteria for SMD. Similarly, a 20-year follow-up of the Children in the Community sample ($N = 631$) found that irritability at age 13.8 ± 2.6 was associated with dysthymia, generalized anxiety disorder, and major depression, but not BD, at age 33.2 ± 2.9. *Family history:* Parents of BD youth (proband $N = 33$, parent $N = 42$) and SMD youth (proband $N = 30$, parent $N = 37$) were interviewed by clinicians blind to the child's diagnostic status. Compared to parents of SMD youth, those of BD youth were more likely to themselves meet criteria for BD ($p < .01$). *Clinical neuroscience:* Compared to controls, both youth with SMD and those with BD have deficits in face emotion labeling and response reversal, and experience more frustration when playing a rigged game. However, in

each instance the mediating brain circuitry differs between patient groups. For example, BD show executive attention deficits during frustration, whereas SMD show deficits in early attentional processing regardless of emotional condition. During both explicit and implicit face emotion processing, amygdala activity differs between SMD and BD.
Discussion: Longitudinal work in both clinical and epidemiological samples indicates that irritable youth are not at high risk to "grow up" to develop classic BD. Preliminary family data indicate that youth with BD are more likely than those with SMD to have a parent with BD. While the two patient groups share several behavioral deficits, the mediating neural circuitry differs between groups. Thus, to the extent that one conceptualizes BD categorically, youth with the SMD phenotype (i.e., severe non-episodic irritability) should not be diagnosed with BD. Ultimately, however, SMD and BD may be found to have some overlapping pathophysiological mechanisms.
Disclosure: E. Leibenluft, None.

Developmental & Nosological Issues in Defining Pediatric Bipolar Disorder
David Shaffer*

Columbia Univ and NY State Psychiatric Institute, New York, NY

Dr. Shaffer will review in detail evidence from a nosological, developmental perspective bearing on the diagnosis of pediatric bipolar disorder. He will first emphasize the problems inherent in adopting uncritically a set of adult diagnostic labels and appropriating them for classification systems in childhood. This has inherent risks when psychopathological phenomena in children show significant differences from those in adults, before a reasonable body of research demonstrates strong similarities between the childhood and adult condition. Such research ideally should collect data on the relationships, in prospective studies, manifesting among children, adolescents, and adults with the disorder. In the second part of his talk, Dr. Shaffer then will also discuss a similar set of problems that arise when problems in children are given different labels for syndromes that do appear to resemble syndromes that commonly manifest adults. Again, evidence of such similarity emerges from studies examining external validators, particularly the longitudinal outcome of the pediatric condition, and Dr. Shaffer will review the nature of such evidence. For both sets of problems, Dr. Shaffer will use examples from the psychiatric literature to illustrate the nature of problems that can arise.
Disclosure: D. Shaffer, None.

Panel Session
Social Cognition and Social Neuroscience in Schizophrenia

Social Cognition in Schizophrenia: Stability of Impairments Across Phases of Illness and 12-Month Prediction of Functional Outcome in First-Episode Patients
William P. Horan*

UCLA Semel Institute, Los Angeles, CA

Background: Social cognitive impairments in chronic schizophrenia are large in magnitude and are associated with poor outcome. However, little is known about the scope, stability, and functional correlates of social cognitive impairments during the early course of schizophrenia. In the current study, three different aspects of social cognition required for meaningful social interaction (emotion processing, Theory of Mind, and social/relationship perception) were evaluated in patients during early to late phases of illness.
Methods: Participants included patients in three distinct phases of illness: prodromal (n = 50), first-episode (n = 81), and chronic (n = 54), and three corresponding demographically matched healthy control samples. Measures included the Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT), the Awareness of Social Inference Test (TASIT), and the Relationships Across Domains (RAD) test. Cross-sectional analyses compared the matched patient and control groups on these three tests. Longitudinal analyses within the first-episode patient sample evaluated whether performance on these tasks was temporally stable and predicted functional outcome across a 12-month follow-up period.
Results: Each social cognitive measure revealed clear cross-sectional impairments in prodromal, first-episode, and chronic patients compared to their corresponding control groups. The magnitude of impairments on the social cognitive tasks was medium to large and there was no evidence of progression or improvement across the three patient cohorts. Among first-episode patients, each social cognitive test demonstrated good 12-month longitudinal stability (test-retest r's:.70 -.86). Higher baseline and 12-month social cognition scores were both significantly associated with better real-world outcomes in work functioning, level of independence, and social functioning at follow-up (r's:.34 -.59). Furthermore, cross-lagged panel analyses were consistent with a causal model in which baseline social cognition drove later functional outcome in the domain of work, above and beyond the contribution of symptoms.
Discussion: Wide-ranging social cognitive impairments are present in early schizophrenia and are consistent in magnitude across early to late phases of illness. This fits the pattern of a stable vulnerability marker as opposed to an indicator of severity or chronicity. Social cognitive impairments also robustly predict real-world functional outcome during the early course of schizophrenia. These impairments therefore appear to be useful targets for early psychosocial and pharmacological interventions to promote functional recovery.
Disclosure: W.P. Horan, None.

Social Appraisal, Negative Emotion, and Medial Frontal Connectivity in Chronic Psychosis
Stephan F. Taylor*

University of Michigan, Ann Arbor, MI

Background: Social information processing is impaired in schizophrenia, in a variety of domains, including the tendency to appraise individuals as threatening or persecutory. Social information processing entails complex emotional operations, and neurocircuits of the brain involved in emotion overlap considerably with those involved in social function, such as the medial prefrontal cortex (mPFC), which is implicated in schizophrenia. In previous work, we have demonstrated aberrant activity in the mPFC to negatively-valenced stimuli. Here, we used a social appraisal task to elucidate network dysfunction during social appraisal, focusing on negative emotional expressions, which we predicted would engage aberrant mPFC activity and poor connectivity.
Methods: Twenty-one stable outpatients with chronic psychotic disorders (16 schizophrenic, 5 schizoaffective) and 21 matched, healthy subjects underwent functional magnetic resonance imaging. Subjects performed an explicit social appraisal task, in which subjects judged whether or not they liked face stimuli (negative, neutral and positive expressions), contrasted with a gender identification task. A psychophysical interaction (PPI) analysis was conducted to evaluate the distributed networks that carry out this social cognitive function.
Results: For social appraisal, patients were slower to respond, but particularly slow when they judged negatively-valenced faces, compared with the control subjects. This slowness correlated positively with the amount of negative emotion reported by the patients. Appraisal activated the mPFC across all face valences. For negative faces, the patients exhibited greater activation of the dorsal anterior cingulate cortex (dACC; -9, 30, 24, Z = 5.07), as well as increased activity in the precuneus. For positive faces, the controls exhibited greater activity in the cerebellum. PPI analysis of the dACC revealed co-modulation of the mPFC in controls, significantly less in patients

(0, 54, 21, Z = 3.96). In controls, coupling of the dACC occurred with fusiform face areas, whereas more diffuse regions of the visual cortex exhibited coupling in the patients. Activation in visual cortex was impaired during the preference task for negative faces, which correlated with poor social adjustment and impaired social cognition.
Discussion: Patients with chronic psychosis exhibit aberrant processing of negatively-valenced social stimuli during social appraisal, here involving dysfunction of the mPFC and dACC, such that the normal coupling of these adjacent regions is disrupted. The social appraisal task also showed coupling between dACC and visual processing areas, and abnormal activity in early processing in schizophrenia may reflect cortical deficits, correlated with broad functional measures, in line with data showing that early visual processing is associated with impaired social functioning in schizophrenia.
Disclosure: S.F. Taylor, None.

Emotion Processing in Schizophrenia: Face and Prosody
Ruben Gur*, Raquel E. Gur, David I. Leitman, Theodore Satterthwaite, Christian Kohler, Amy Pinkham, James Loughead

University of Pennsylvania, Philadelphia, PA

Background: Emotion processing deficits in schizophrenia have been extensively documented and linked to abnormal activation of limbic and frontal regions, other deficits in social cognition and to clinical features. However, most studies to date have focused on either facial or vocal emotion identification, and few have examined the interaction between these modalities or on quantifying expression deficits. We present recently completed and ongoing work on identification and expression of affect on the face and through prosody in patients with schizophrenia and healthy comparison subjects.
Methods: Facial and vocal affect stimuli have been generated using standardized published procedures. The stimuli have been validated and then used in field studies and in fMRI experiments examining affect identification. Analysis of fMRI results have incorporated both evaluation of signal change and psychophysiological interactions (PPI) analysis to examine inter-regional communication. Computerized semi-automated methods for quantifying facial and vocal change have been developed more recently, and demonstrated sensitivity to individual differences in healthy people. We also developed procedures for eliciting emotional expressions in a standardized yet effective fashion. These methods have been applied in tandem to quantify emotional expression deficits in patients with schizophrenia.
Results: Both behavioral deficits in affect identification and abnormal activation to affective stimuli, as well as abnormal connectivity between limbic and frontal and striatal regions are evident in schizophrenia. They appear more severe for threat-related emotions (anger, fear) than for affiliative emotions (happy, sad) and seem to reflect deficits in early stages of facial and vocal stimulus processing, which lead to greater reliance on deficient frontal executive functions. The deficits in facial affect processing seem to parallel those in vocal affect identification, although the latter are relatively more severe. Both deficits are associated with more severe neurocognitive impairment, but their correlation with clinical symptomatology appears more specific and modulated by whether the deficit is primarily for threat related or for affiliative emotions. Deficits in expression of affect in face and voice are also evident in schizophrenia, and the application of classifiers based on support vector machines (SVM) yields reliable diagnostic assignment. The expression deficits correlate well with clinical ratings of flat and inappropriate affect.
Discussion: Advanced behavioral and neuroimaging methodology can help delineate the extent and potential causes of deficits in social cognition that are core features of schizophrenia. Large samples are needed to ferret out the links between affect identification deficits and clinical features, and these should incorporate paradigms where both facial and vocal affect are studied simultaneously. Implementing quantitative methods for analysis of facial and vocal affective expressions could eventually lead to more objective methods of assessing flat affect that could supplement and ultimately replace clinical ratings as diagnostic tools and as objective yardsticks for gauging treatment effects.
Disclosure: R.C. Gur, Brain Resource Center, Sydney, Part 1; Pfizer, Part 4; AstraZeneca, Part 4; Merck, Part 4.

Self vs. Other in Schizophrenia: What Behavioral, Neural, and Cognitive Training Experiments Tell Us About Future Treatment Directions
Sophia Vinogradov*

UC San Francisco, San Francisco, CA

Background: Behavioral evidence indicates that, for both schizophrenia patients and healthy subjects, memory for self-generated information shows unique associations with social cognition, compared with memory for externally presented information. In other words, the ability to remember that "self was source" on an earlier sentence completion task strongly relates to basic social cognitive performance on face recognition and emotion identification tasks. However, in schizophrenia, this relationship is attenuated and is also influenced by attention and executive functions. Moreover, in fMRI experiments, schizophrenia patients show relatively decreased activation within dorsal medial prefrontal cortex compared to healthy subjects when engaged in this process.
Methods: Randomized controlled trial of neuroscience-based computerized cognitive training and sequential behavioral and fMRI assessments.
Results: Eighty hours (sixteen weeks) of intensive computerized cognitive training of general and social cognitive functions results in significant behavioral improvement in self-referential source memory in schizophrenia, as well as "normalization" of the neural correlates of this process observed in fMRI experiments.
Discussion: We will discuss the implications of these findings for the design of future behavioral treatments that target social cognition functions in schizophrenia.
Disclosure: S. Vinogradov, None.