Neuropsychopharmacology (2011) 36, S1–S74

Monday, December 5, 2011

Panel Session
Memory Erasure: Mechanisms and Potential Utility in Psychiatry

Memory as a New Therapeutic Target
Karim Nader*

McGill University, Montreal, Canada

Background: Traditionally, new memories were thought to be stabilized (consolidated) once in the brain. We tested whether recall/reactivation of a consolidated memory causes it to become unstable again and require another round of re-stabilization. I will discuss some of the evidence for the existence of this memory process and its clinical implications.
Methods: We use a number of behavioral tasks that have been relatively well described for their consolidation mechanisms. We then test whether the representations stored there, when recalled, can return them to a state that is susceptible to amnesic treatments again. In addition, we examine how molecular correlates of long-term memory change when a memory impaired.
Results: Reconsolidation has been reported across appetitive and aversive paradigms, in species as simple as c. elegans to humans, and using a wide range of amnesic treatments. The behavioral impairments in reconsolidation can be relatively specific to the reactivated memory, generalizes across contexts and can be very long lasting making. Reconsolidation is not ubiquitous for all memories. There are experimental parameters that determine when a memory will and will not undergo reconsolidation. Understanding of how these constraints are manifested in the brain will be critical for guiding us to optimize the chances of successful translational application of these findings.
Conclusions: The clinical implications of reconsolidation are that it may be possible to treat psychopathologies such as post-traumatic stress disorder (PTSD), addiction, chronic pain, kindling, and obsessive compulsive disorder. For example, in the case of PTSD if we could impair reconsolidation of their traumatic memory this should in turn weaken the traumatic memory. In the first study to test the results of challenging reconsolidation of traumatic memories in long-standing PTSD, the strength of the traumatic memory was indeed reduced. This demonstrates the feasibility of targeting reconsolidation therapeutically.
Disclosure: K. Nader, None.

Temporary, but not Permanent, Disruption of Fear Potentiated Startle Following PKMζ Inhibition in the Amygdala
Michael Davis*

Emory University, Atlanta, USA

Background: Maintenance of late-phase LTP can be blocked by disruption of protein kinase Mζ (PKMζ), implying that memories may be maintained by this persistently active kinase (Sacktor, 1993; Ling, 2002). Inhibition of PKMζ can also disrupt memory for various tasks (Shema, 2007; Serrano, 2008; Migues, 2010). However, many of these experiments assessed memory shortly after infusion, raising the possibility that apparent memory erasure may instead reflect a disruption in retrieval of the memory or an interruption in the behavioral response from which memory is inferred. Some studies that assessed memory at longer times after infusion used repeated testing (Migues, 2010) which can have a profound impact on the stability of memory over time. The current study tested whether PKMζ inhibition in the amygdala permanently disrupts olfactory mediated fear memory by testing the retention of fear memory at various intervals after PKMζ blockade.
Methods: Rats were trained to fear an odor by pairing it with a footshock. 7 days later they were infused with a PKMζ antagonist into the amygdala and tested for fear-potentiated startle to the odor after either 2 hrs, 48 hrs or 15 later. In another experiment they were tested 1 day and then again 10 days later.
Results: Fear memory was disrupted when the injection-to-interval was 2 hr, 48 hr but not 15 days. It was also blocked when the same rats were tested 1 day and then 10 days later, perhaps due to a facilitation of extinction produced by the 1 day test that involved tests trials with the odor not followed by shock.
Conclusions: These results do not support the suggestion that PKMæ activity underlies permanent memory storage of fear memories in the amygdala. However, PKMζ inhibition does have long lasting effects when infused into a cortical area (Shema *et al.*, 2007) and we are currently testing whether infusion of the inhibitor into the piriform cortex might have a long-lasting effect.
Disclosure: M. Davis, None.

Disrupting Fear Memories: Retrieval, Reconsolidation and the Passage of Time
Cristina Alberini*

Mount Sinai School of Medicine, New York, USA

Background: It is becoming increasingly clear that the processes of memory formation and storage are exquisitely dynamic. Elucidating the nature and temporal evolution of the biological changes that accompany encoding, storage and retrieval is key for identifying means that can be used to disrupt the storage or retention of memories associated to pathological states. For example, traumatic experiences may lead to debilitating psychiatric disorders including acute stress disorder and posttraumatic stress disorder, which are strongly associated with the reexperiencing of the trauma through intrusive memories and nightmares. Retrieval or reactivation of an apparently consolidated memory can render the memory labile again, and reconsolidation is the process that mediated its restabilization.
Methods: We use the fear-based task inhibitory avoidance and a variety of molecular or pharmacological interferences in rats to study the mechanisms and functions of fear memory reconsolidation.
Results: We found that inhibitory avoidance reconsolidation evolves with the age of the memory: Young memories (one week old) are sensitive to post-retrieval disruption, but older memories (2 weeks old) are resistant. Furthermore, we found that retrievals of older memories lead preferentially to extinction rather than reconsolidation. Furthermore, an effective reconsolidation disruption of inhibitory avoidance was found with the glucocorticoid antagonist RU38486 but not with the b-adrenergic antagonist propranolol.
Conclusions: We conclude that the main function of reconsolidation is to contribute to a lingering consolidation process and, in fact, mediate memory strengthening. These results have important implications for framing the optimal parameters to be used in therapeutic settings.
Disclosure: C. Alberini, None.

Selectively Erasing a Fear Memory in Mice
Sheena Josselyn*

Hospital for Sick Children, Toronto, Canada

Background: Over 40 million adult Americans suffer from some sort of anxiety disorder, making these disorders amongst the most prevalent mental illnesses. As inappropriate or excessive fear and/or anxiety is a core feature of these disorders, a more thorough understanding of how the brain encodes and stores fear memories will likely facilitate the development of more targeted treatments.
Methods: The primary goal of our research is to understand how fear memories are encoded and stored in the brain. Identifying the physical basis of a memory within the brain (the memory trace) has been a long-standing challenge for scientists since Lashley's "search for the engram" in the 1950's. My lab applied more modern molecular techniques in our search for the elusive engram. Instead of deleting an entire brain region, we used a more targeted network approach in which we specifically ablated only those neurons in a structure thought to be involved in a fear memory. Specifically, we used an inducible diphtheria-toxin system to ablate a defined population of neurons in the lateral amygdala (LA, a critical fear center in the brain) of mice.
Results: Selectively inducing cell death in neurons in a small population of LA neurons (those with increased levels of the transcription factor CREB) after learning blocked subsequent expression of that fear memory. Importantly, ablating a similar portion of random neurons had no effect and mice could re-acquire a memory. The resulting memory loss produced by ablating LA neurons with high levels of CREB was robust and persistent, suggesting that the fear memory had been erased.
Conclusions: Our findings show that certain ablating a portion of LA neurons erased a fear memory. We are now using more selective methods of "silencing" just these neurons and, furthermore, asking whether a similar approach "erases" the rewarding memories of cocaine exposure in mice.
Disclosure: S. Josselyn, None.

Panel Session
Striving for the Correct Diagnosis of Mental Health Disorders

What Will the New DSM-5 Provide for Us?
David Kupfer*

University of Pittsburgh School of Medicine, Pittsburgh, USA

Background: The Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) will represent the first time in more than 20 years that the field of psychiatry has formally reviewed and updated the diagnostic criteria for all psychiatric disorders recognized by the U.S. healthcare system. In addition, DSM-5 will contain a wealth of information intended to advance the study and practice of psychiatry.
Methods: Our understanding of how DSM-5 may impact the future of research and clinical care will be informed largely by outcomes from two sources: literature reviews conducted amongst the DSM-5 Work Groups and the DSM-5 Field Trials. These field tests are being conducted both in large, academic-medical settings and in routine clinical practice settings. Field trials are taking place in a variety of geographic locations, amongst a wide range of patients, with a variety of clinical experts (e.g., psychiatrists, psychologists).
Results: Findings from the field trials will help us assess whether DSM-5 generates diagnoses that are reliable, useful to clinicians, and informative for treatment planning and monitoring course of illness. Regarding outcomes from literature reviews, DSM-5 experts have provided numerous recommendations on how DSM-5 can address current gaps in our understanding of psychiatric disorders. This includes providing a revised chapter organization that integrates findings from neuroscience and better reflects our current knowledge of underlying interrelationships between similar disorders; calling greater attention to meta-issues that cross all diagnostic categories, like those related to developmental lifespan, gender, and culture; consideration of categorical versus dimensional approaches to diagnosis; and integration of diagnostic severity measures with disorder criteria.
Conclusions: The goal of DSM-5 is to provide a representation of psychiatric disorders consistent with the state of the science, while ensuring that patients receive the best care possible.
Disclosure: D. Kupfer, Part 1: N/A, Part 2: American Psychiatric Association - Consultant.

New Approaches to Psychiatric Diagnosis: The NIMH Research Domain Criteria Project
Bruce Cuthbert*

NIMH, Bethesda, USA

Background: The purpose of this presentation is to give a brief overview of the RDoC project, explain its major features, and outline the goals for the project in contributing to NIMH's future research portfolio in the neurobiology of mental disorders.
Methods: N/A
Results: While the DSM and ICD diagnostic frameworks represent current consensus thinking regarding psychiatric nosology, these diagnostic systems were created before current advances in genetics, neuroscience, and behavioral science. Diagnostic categories are highly heterogeneous, each reflecting the influence of many different neurobiological dimensions. As a result, diagnoses fail to inform treatment with sufficient precision, and trials for new interventions frequently fail because they target only one particular mechanism. To address these problems, the National Institute of Mental Health (NIMH) is developing the Research Domain Criteria project. This effort represents the implementation of goal 1.4 of the NIMH Strategic Plan, and is intended to result in a classification scheme for research that is dimensional and informed by neurobiological findings. Dimensions are construed as functional constructs (such as fear or reward-related behavior) that are relevant to symptoms of mental disorders, that can be related to particular neural or hormonal systems, and that are grouped into a small number of higher-level domains. The initial draft Research Domain Criteria (RDoC) matrix comprises five domains: Negative Valence Systems, Positive Valence Systems, Cognitive Systems, Systems for Social Processes, and Arousal/Regulatory Systems. The NIMH plans a series of five consensus workshops, one for each of the major domains, to define these constructs and specify their defining features.
Conclusions: This presentation will conclude with some remarks regarding the future of the RDoC project in shaping HIMH's funding portfolio regarding the neurobiology of mental disorders.
Disclosure: B. Cuthbert, None.

Using Biological and Cognitive Measures to Discriminate Among Depressive Subtypes
Alan Schatzburg*

Stanford University School of Medicine, Stanford, USA

Background: Biologic and cognitive measures were assessed to discriminate among patients with major depression with and without psychotic features (PMD and NPMD) who were otherwise matched for endogenous depressive symptom severity and healthy controls.

Methods: 115 PMD and NPMD patients and 50 healthy controls (HC's) were compared on hourly serum cortisol levels collected from 6PM to 9AM, responses to challenge with a mineralocorticoid agonist, cognitive testing, structural MRI and functional MRI using working and verbal memory tasks.
Results: Patients with MDD with psychotic features (PMD) demonstrated statistically significant elevations in serum cortisol measured from 6 PM to 1 AM in comparison to the other 2 groups. Both PMD and NPMD patients demonstrated blunting of response to challenge with a mineralocorticoid agonist. In addition, patients with PMD demonstrated significantly poorer performance on tests of executive function, working memory, response inhibition, and verbal memory. Poorer verbal memory was associated with higher serum cortisol measures. PMD patients demonstrated significantly reduced amygdalar but not hippocampal volumes. Using f-MRI, on working memory, PMD patients demonstrated significantly greater activation of para-hippocampal regions even on simple tasks. On verbal memory, the poorer performance was associated with significant alterations in prefrontal activation during encoding suggesting that the verbal memory deficits in PMD involve encoding alterations. These data when taken together suggest that PMD can be separated from NPMD on a variety of tests. An approach to the simultaneous application of these various dimensions for subtype discrimination is presented.
Conclusions: Data presented suggest that meaningful discrimination among groups of patients with specific subtypes can be achieved; however, there are limitations in achieving high levels of sensitivity or specificity on an individual patient and test basis. Implications of these data for taxonomy will be discussed.
Disclosure: A. Schatzberg, Part 1: BrainCells CeNeRx CNS Response Corcept Eli Lilly Forest Labs GSK Jazz Lundbeck Merck Neuronetics Novadel Novartis PharmaNeuroBoost Sanofi-Aventis Sunovian Takeda Xytis, Part 2: Amnestix BrainCells CeNeRx Corcept (co-founder) Forest Labs Merck Neurocrine Novadel Pfizer PharmaNeuroBoost Somaxon Synosia, Part 3: PharmaNeuroBoost American Psychiatric Association, Part 4: None, Part 5: Stanford University.

International Study to Predict Optimized Treatment for Depression (iSPOT-D), a Randomized Clinical Trial: Rationale and Protocol

Leanne Williams*

University of Sydney Medical School, Sydney, Australia

Background: Clinically useful treatment moderators of Major Depressive Disorder (MDD) have not yet been identified, though some baseline predictors of treatment outcome have been proposed. The iSPOT-D study includes clinical, genetic, cognitive and emotional task, psychophysiological and brain imaging measures to identify biologically-based predictors and moderators of treatment outcome. Aims are to identify: i) which of these pretreatment measures predicts treatment response. ii) which pretreatment measures moderates MDD treatment response to escitalopram, sertraline or venlafaxine. iii) A model to incorporate the effects of multiple predictors or moderators iv) Test i) and ii) in the first half of he sample, and use the second half of the sample to replicate and confirm findings.
Methods: iSPOT-D is a multi-centre, international, randomized, prospective, open-label trial. Target enrolment is 2016 MDD outpatients (672 per treatment arm). Study-eligible patients are antidepressant medication (ADM) naïve or willing to undergo a one-week wash-out of any non-protocol ADM. Assessments include clinical interview and symptom ratings, functional status, cognitive and emotional performance and associated brain activation on psychophysiological measures (EEG, ERPs, heart rate). Bloods are taken for genotyping. A matched subsample also have brain imaging with structural MRI, fMRI and DTI. Outcomes include the 17-item Hamilton Rating Scale for Depression (primary) and self-reported depressive symptoms, social functioning, quality of life, emotional regulation, and side-effect burden (secondary).
Results: The first 50% of the sample ($n = 1008$) completed pretreatment and week 8 assessments as of March 18 2011. To test aim i), we are using regression models to assess the predictive effect of each measure on outcome. Independent variables in the model will include main fixed effects for treatment and the possible predictive variable. For aim ii), regression models will be used to assess the moderating effect of each characteristic on outcome. Pretreatment measures will be assessed separately for each pairwise comparison of treatment (escitalopram vs. sertraline, escitalopram vs. venlafaxine-XR, sertraline vs. venlafaxine-XR). Independent variables will include main fixed effects for treatment, the possible moderator variable and the twoway interaction between the characteristic and treatment. For aim iii), recursive partitioning methods will be used to identify how baseline characteristics interact with treatment and with each other in their association with treatment response.
Conclusions: Candidate moderators or predictors include measures of depressive symptoms, functional status, side-effects, cognitive and emotional task performance, genetics, EEG and brain imaging. iSPOT-D includes an innovation in study design with the use of these multiple measures for the identification of objective markers that may moderate or predict response to antidepressants. Identifying these markers will be an important first step in a "personalized medicine" approach to the management of MDD.
Disclosure: L. Williams, Part 1: Consulting fees from Brain Resource Ltd. Advisory Board fees from Pfizer, Part 2: Stockholder and stock options in Brain Resource Ltd.

Panel Session
Genetic and Molecular Mechanisms of Normal Cognitive Aging

Aging-Associated Changes in the Human Brain Transcriptome

Vahram Haroutunian*

Mount Sinai School of Medicine and JJ Peters VAMC, Bronx, USA

Background: Most studies of gene expression in the aged brain have focused on disease related changes including those associated with Alzheimer's disease. However, the study of global gene expression in multiple regions of the normal human brain and comparison of gene expression in young-old (<85 years) and oldest-old persons (>85 years) can provide insights into mechanisms that contribute to successful cognitive aging.
Methods: Gene and protein expression changes in multiple (17) brain regions of non-demented young-old persons (N = 10–15, mean age 75 yr.) were compared to oldest-old persons (N = 7–10, mean age 94 yr.) using two microarray platforms, qPCR and Western blotting. In secondary analyses, the relationships of these age-associated gene expression changes to cognitive impairment were ascertained.
Results: 332 probe sets showed significantly altered expression in oldest-old cognitively intact subjects relative to cognitively intact young-old persons. 65–75 of these 332 "age-associated" probe sets were also differentially expressed in cognitively impaired persons. Strikingly, every single transcript among these common genes associated with dementia was upregulated in cognitively intact oldest-old persons relative to cognitively intact young-old persons. Gene ontology classification of these common probe sets from the

two age categories showed them to be linked to canonical immune function pathways. These pathways included antigen presentation, IL-4 signaling, natural killer cells; complement, acute phase response and glucocorticoid receptors.
Conclusions: The results suggest that successful aging to advanced old-age is associated with a robust and preserved CNS immune response system. It is possible that persons who survive to advanced old age with intact cognition are those individuals whose CNS immune system is able to respond to and defend against physiological insults (e.g., amyloidogenic, cardiovascular) effectively.
Disclosure: V. Haroutunian, None.

Protection against Cognitive Decline and Dementia by Longevity Genes
Yousin Suh*

Albert Einstein College of Medicine, Bronx, USA

Background: Despite evidence of a substantial genetic component, the inherited biological factor(s) that underlie human life span (longevity) remain unknown. In an innovative approach, we have assembled and characterized families with exceptional longevity from a selected homogenous background of Ashkenazi Jews, and have identified several biological markers that may account for their longevity. Our main hypothesis is unique genotypes and phenotypes protect against age-related diseases to assure exceptional healthy longevity. In particular, we are focusing on "longevity" genes and pathways that protect against cognitive decline and dementia, a hallmark of aging in humans.
Methods: We are conducting a systematic genetic study in two well-established human cohorts available at Einstein; i) the Longenity Study (directed by Nir Barzilai) established to discover genotypes and phenotypes associated with human exceptional longevity, and ii) the Einstein Aging Study (directed by Richard Lipton) focused on longitudinally-followed cognitive function using a battery of neuropsychological tests.
Results: We have discovered several such longevity genes including cholesteryl ester transfer protein (*CETP*) gene. We found that a hypomorphic variant in *CETP* (I405V) is significantly enriched in centenarians as compared to younger controls and carriers have significantly higher cognitive function than non-carriers among centenarians. Moreover, the CETP variant is longitudinally associated with slower memory decline and lower risk for dementia and Alzheimer's disease (AD).
Conclusions: Our results suggest that longevity-related genotypes may modulate or independently alter risk for dementia and cognitive decline and that common biological pathways underlie longevity and age-related neurodegenerative disease phenotypes.
Disclosure: Y. Suh, None.

Neuroimaging Genetic Influence in Normal Cognitive Aging: The Role of Memory and Cognition Related Genes
Venkata Mattay*

GCAP, NIMH, NIH, Bethesda, USA

Background: Identifying genes and proteins involved in cognitive aging could facilitate the development of novel treatments to combat cognitive impairment. Neuroimaging is proving to be a valuable tool to not only unravel the neurobiology underlying normal cognitive aging but to also explore the impact of genes on vulnerability to cognitive aging and age-related neurodegenerative diseases. As illustrations of the utility of this approach, we will show data related to the effects of memory and cognition related genes like CACNA1C, SERT, BDNF and WWC1 on normal aging related changes in memory circuits.
Methods: BOLD fMRI data was collected on a 3T MRI scanner during a simple declarative memory task in healthy subjects ranging from 19–85 years of age (N = 125 to 258 depending on gene). Effect of CACNA1C, SERT, BDNF and WWC1 genes on age-related change in hippocampal activation was assessed using a general linear model in SPM5.
Results: 1) A significant age x gene interaction was found in hippocampal activity for CACNA1C (N = 201; $p<0.005$, FWE-WB) and WWC1 genes (N = 258; $p<0.05$, FWE-SVC) - A allele homozygotes of CACNA1C and C allele homozygotes of WWC1 genes who had increased hippocampal activity at younger ages showed a relatively exaggerated decline in activity with age compared to G allele carriers of CACNA1C and T allele carriers of WWC1 genes respectively. 2) SERT and CACNA1c genes showed an epistatic effect - SERT L allele homozygotes carrying CACNA1c A allele (increased calcium and low serotonergic signaling) showed significantly greater age-related decline in hippocampal activity when compared to all other allele groups (N = 192; $p<0.01$, FDR-SVC). 3) BDNF met carriers showed a signficantly steeper slope in age-related decline in hippocampal activity than val homozygotes (N = 125; $p<0.05$, FDR-SVC).
Conclusions: Taken together, these results demonstrate a modulatory role for genes associated with individual variability in cognition on age-related decline in hippocampal function. Further, they suggest that some of these genes may potentially influence cognitive reserve or resilience of individuals to withstand the effects of aging and associated neurodegenerative disorders.
Disclosure: V. Mattay, None.

Novel APOE4 Findings in Cognitively Healthy and Compromised Aging Individuals
Terry Goldberg*

Litwin Zucker Center/Feinstein Institute/AECOM, Manhasset, USA

Background: APOE4 is the major risk variant for Alzheimer's disease with an OR = 3.8 for a single copy and 15 for two copies. Its population-attributable risk is about 30%. In this series of studies we examined several understudied features of APOE4 in a series of molecular, imaging, and biomarker experiments.
Methods: In studies of post mortem human cortical tissue we used RT-qPCR to measure expression and Western blots using polyclonal and monoclonal antibodies to measure protein level. In our imaging paradigm we measured H1-MRS metabolites acquired in a GE 3T magnet in cognitively healthy middle aged and older subjects and subjected data to a series of age × genotype interaction analyses to test whether genotypic differences were amplified with age. Finally we conducted atheoretical cluster analyses of biomarker data (not including APOE genotype) from large ADNI cohorts of controls and MCI subjects, and then assessed if a disproportionate number of E4 cases were observed in a cluster, as such suggesting an E4 signature.
Results: Using Western blot analysis with a polyclonal antibody for APOE in human post mortem cortical tissue from individuals who did not have AD histopathology, we found that APOE protein levels were significantly lower in E4 carriers than in E3 homozygotes. In 1H-MRS metabolite measures acquired at 3T in a posterior cingulate voxel in 67 cognitively healthy middle aged and older individuals, we found that APOE genotype interacted with age such that older E4 individuals had lower rations of NAA/Cr (a measure of neuronal integrity) and higher ratios of Cho/Cr (a measure of membrane turnover) than did E3 carriers, a pattern similar to that observed in AD itself. Last, in a large ADNI cohort of control and MCI subjects we conducted atheoretical cluster analyses that included morphometric, CSF, and cognitive biomarkers, but did not include APOE genotype. Multiple clusters were derived and one in each population was found to contain a

disproportionate number of E4 carriers, suggesting that an E4 signature is present before the onset of AD.
Conclusions: Expression level and protein level (reduced in E4 cortex) are consistent with tg mouse models and suggest that compromises in lipid based repair function may have a role in pathogenesis of AD. In vivo data from MRS and biomarker and cognitive data suggest that an E4 signature may be present well before AD and MCI.
Disclosure: T. Goldberg, Part 1: GSK (consultant) Merck (consultant), Part 2: None, Part 3: Neurocog Trials, Part 4: Pfizer Esai, Part 5: No.

Panel Session
Adolescent Brains: The Constancy of Change

Clinical Studies during Adolescence: Autism and Bipolar Disorder

John Sweeney*

University of Illinois, Chicago, USA

Background: The pattern of neurocognitive and brain dysfunction in major psychiatric disorders during adolescence is not well characterized. The focus in autism has been on early childhood, and pediatric bipolar disorder has only recently become a target for systematic study.
Methods: Behavioral and fMRI studies of sensorimotor systems, working memory, behavioral flexibility, behavioral inhibition and affective processing during late childhood and adolescence will be emphasized. Patients with autism were seen cross-sectionally. Bipolar patients were seen during an acute episode close to illness onset, after stabilization, and at 3 year followup. Matched controls were studied in parallel. Data from at least 50 subjects per group will be presented.
Results: In autism, variable patterns of differences from healthy subjects were seen through adolescence. In some sensorimotor tasks, performance that improved during early to mid-adolescence in healthy controls did not do so in autism. In contrast, on some executive function tests, performance in individuals with autism improved at a near normal rate during adolescence but from a lower baseline and hence achieved a lower final developmental level. In pediatric bipolar patients, cognitive deficits were moderate after stabilization from the index episode, but became significantly more pronounced over the following three years even in the context of state-of-the-art psychosocial and pharmacological treatment.
Conclusions: A growing body of neurobiological and cognitive neuroscience studies highlights the major changes in brain function and organization that occur during adolescence. The clinical data collected in our studies over the past 10–15 years with adolescent patients highlight the atypical developmental trajectories that are evident during this period, and help place these disorders in a broader neurodevelopmental context.
Disclosure: J. Sweeney, Part 1: Takeda and Pfizer consultant Janssen grant, Part 2: NA, Part 3: NA, Part 4: investigator grant from Janssen, Part 5: NA.

Anatomic MRI of the Developing Brain: Ages 3 to 30

Jay Giedd*

NIMH, Bethesda, USA

Background: In this presentation I will summarize results from our 20 year longitudinal MRI/genetics/neuropsych study of typical and atypical brain development from ages 3 to 30 years.
Methods: Subjects come to the NIH clinical center at approximately 2 year intervals for brain imaging (anatomic MRI, functional MRI, DTI, MTI, MEG); DNA (blood or saliva); and an evolving battery of cognititive/behavioral/emotional assessments. One fourth of the subjects are healthy singletons, one fourth are healthy monozygotic or dizygotic twins, and one half are from various clinical populations including ADHD, Autism, and Childhood onset Schizophrenia.
Results: White matter increases across this age span. Gray matter follows an inverted U shaped developmental trajectory with peak size occurring at different ages in different regions.
Conclusions: Developmental trajectories (i.e. size by age) are more predictive than static measures for discerning young from old, male from female, and health from pathology. Graph theory approaches are beginning differences in connectivity between populations.
Disclosure: J. Giedd, None.

The Imagen Gene X Neuroimaging Study on Reinforcement-Related Behaviour in Adolescents: GWAS and Epigenetic Results

Gunter Schumann*

Institute of Psychiatry, London, United Kingdom

Background: In the IMAGEN study we aim to identify the genetic and neurobiological basis of individual variability in impulsivity, reinforcer sensitivity and emotional reactivity, and to determine their predictive value for the development of frequent psychiatric disorders (Schumann *et al.*, 2010).
Methods: Comprehensive behavioural and neuropsychological characterization, functional and structural neuroimaging and genome-wide association analyses of 2000 14-year-old adolescents are combined with functional genetics in animal and human models.
Results: Here we will present exemplary data of the ongoing IMAGEN analysis: (1) We will show the results of a genome-wide association study of the stop-signal reaction task, a functional neuroimaging measure of impulsivity and explore its relevance for psychopathology in adolescents. (2) In an attempt to further identify mechanisms of gene x environment interactions in the circadian rhythme gene Period1 (PER1) (see Dong *et al.*, 2011), we will report the results of an ongoing study exploring the association of epigenetic methylation patterns of PER1 with emotional reactivity, as measured by the fMRI-BOLD response to angry faces, and its moderation by psychosocial stress. We will also relate epigenetic findings to genotype effects of Per1. In preliminary analyses in the IMAGEN sample we have identified a main effect of a PER1 genotype in the left prefrontal cortex, providing first evidence for a role of PER1 in modulating cognitive/attentional control during the processing of threat-related social stimuli.
Conclusions: In this presentation we will be exploring the possibilities of genome-wide genetic as well as epigenetic association analyses in a large functional neuroimaging sample. In addition to presenting our results, we will discuss strengths and limitations, and indicate how these approaches will help elucidate the neural basis of mental disorders and will lay the groundwork for development of treatments that target specific pathological processes across mental disorders rather than heterogeneous categories of mental illness.
References:
Dong L *et al.* Genotype-specific expression of human Period1 influences stress-induced alcohol consumption. *American Journal Psychiatry* 2011; in press
Schumann G *et al.* The IMAGEN study: reinforcement-related behaviour in normal brain function and psychopathology. *Mol Psychiatry* 2010 15(12): 1128–39;
Disclosure: G. Schumann, None.

Sex Differences in Normative Developmental Trajectories of Brain and Behavior

Ruben Gur*

University of Pennsylvania, Philadelphia, USA

Background: The marked changes in behavior and brain structure and function during adolescence may help understand neural substrates of behavior. Integration of behavioral domains with multimodal neuroimaging parameters requires large datasets, especially if dimensions of individual differences are examined. The purpose of the presentation is to report data on a large genotyped community sample that was characterized clinically, neurocognitively and with multimodal neuroimaging.
Methods: A community cohort of 8000 adolescents (age 10–20) was clinically assessed and tested with a computerized neurocognitive battery. A sample of 800 was studied with multimodal neuroimaging using structural and functional MRI at 3T. The neurocognitive battery measured accuracy and speed for executive, memory, intellectual and social cognition domains. The sMRI used MPRAGE for volumetric measures and DTI for FA and diffusivity, fMRI examined resting BOLD and activation for working memory and emotion identification tasks.
Results: Sex differences in neurocognitive measures were prominent and accentuated with age. Females performed better on attention, memory and social cognition tests while males performed better on spatial processing and motor speed tests. Sex differences in ICV-corrected brain volumes and WM integrity were more subtle but consistent with the behavioral differences, and systematically related to performance. At this age range WM and FA increased with age, reflecting myelination, while GM volume and diffusivity decreased with age, reflecting pruning.
Conclusions: Integration of multimodal neuroimaging with neurocognitive data is needed to understand changes in adolescence, and such integration requires large samples. With a community-based sample we documented normative characteristics of adolescents and established links between behavioral measures and underlying neuroanatomy and neurophysiology. Sex difference and age effects can thus help illuminate mechanistic models of brain behavior relations.
Disclosure: R. Gur, Part 4: Investigator initiated grants from Pfizer and AstraZeneca.

Panel Session
The Noradrenergic System as a Therapeutic Target for Drug Dependence

Functional Neuroanatomy of Norepinephrine-Dopamine Interactions within the Mesocorticolimbic Reward System

David Weinshenker*

Emory University, Atlanta, USA

Background: Norepinephrine signaling via the α1-adrenergic receptor (α1AR) is critical for several neurochemical and behavioral responses to drugs of abuse. However, the constellation of neural substrates underlying the contribution of α1ARs to mesocorticolimbic function have not been well defined.
Methods: We assessed cocaine- and morphine-induced locomotor activity following infusion of the α1AR antagonist terazosin into either the nucleus accumbens (NAc) or the ventral tegmental area (VTA). Next, we mapped α1AR distribution throughout the mesocorticolimbic system using double label immunocytochemistry at the electron microscopic level. Finally, we performed co-immunoprecipitation experiments in vitro to test potential physical interactions between α1ARs and D1 DA receptors (D1R).
Results: Terazosin attenuated cocaine- and morphine-induced locomotor activity when infused into the NAc, but not the VTA. α1ARs were enriched on presynaptic elements (unmyelinated axons and axon terminals) in all brain regions. α1AR-expressing terminals were co-localized with markers for glutamate and DA in the NAc, glutamate in the PFC, and GABA in the VTA. α1ARs were co-expressed in ~ 70% of D1R-positive dendrites in the PFC, and the presence of D1Rs was associated with a decrease in the ratio of plasma membrane bound/intracellular α1ARs. α1bARs and D1Rs were capable of forming heterodimers in transfected HEK 293 cells.
Conclusions: These results form α1AR activation in the NAc and PFC, but not the VTA, is necessary for psychostimulant- and opiate-induced locomotor activity, and these effects are likely mediated by modulation of drug-induced DA and/or glutamate release. α1ARs and D1Rs display a remarkable degree of co-localization in PFC dendrites, and the presence of D1Rs is associated with altered trafficking of α1ARs. α1ARs and D1Rs are capable of forming heterodimers, which may represent a novel catecholamine signaling complex in the PFC that contributes to addiction processes.
Disclosure: D. Weinshenker, Part 1: I am co-inventor on a patent concerning the use of selective dopamine beta-hydroxylase inhibitors for the treatment of cocaine dependence (US-2010-0105748-A1; "Methods and Compositions for Treatment of Drug Addiction"). Some of the data supporting this patent were generated using the selective DBH inhibitor nepicastat, which is a proprietary compound owned by Biotie Therapies. At this time, the patent has not been licensed, and I have received no royalties from it.

Noradrenergic Alpha1 Receptors as a Novel Target for the Treatment of Nicotine Addiction

Bernard Le Foll*

Centre for Addiction and Mental Health, Toronto, Canada

Background: Nicotine is the main psychoactive ingredient in tobacco and its rewarding effects are considered primarily responsible for persistent tobacco smoking and relapse. Although dopamine has been extensively implicated in the rewarding effects of nicotine, noradrenergic systems may have a larger role than previously suspected. Our aim was to investigate the effect of prazosin a noradrenergic alpha(1) receptor antagonist in an animal model of nicotine addiction.
Methods: We have investigated the effect of prazosin 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 prazosin on food self-administration as a control. We used the drug discrimination paradigm to assess motor performance and subjective effects induced by nicotine. Microdialysis was used to measure impact of prazosin on dopamine release in the nucleus accumbens induced by nicotine.
Results: We found that the noradrenergic alpha(1) receptor antagonist prazosin (0.25–1 mg/kg) dose dependently reduced the self-administration of nicotine (0.03 mg/kg), an effect that was maintained over consecutive daily sessions; but did not reduce food self-administration. Prazosin also decreased reinstatement of extinguished nicotine seeking induced by either a nicotine prime (0.15 mg/kg) or nicotine-associated cues, but not food-induced reinstatement of food-seeking, and decreased nicotine-induced (0.15 mg/kg) dopamine release in the nucleus accumbens shell. However, prazosin did not have nicotine-like discriminative effects and did not alter the dose-response curve for nicotine discrimination.
Conclusions: These findings suggest that stimulation of noradrenergic alpha(1) receptors is involved in nicotine self-administration

and relapse, possibly via facilitation of nicotine-induced activation of the mesolimbic dopaminergic system. The findings point to alpha(1) adrenoceptor blockade as a potential new approach to the treatment of tobacco dependence in humans.
Disclosure: B. Le Foll, Part 1: Pfizer Gideon Richter, Part 2: Pfizer, Part 3: Pfizer, Part 4: Pfizer.

Preclinical Evidence for a Role of Noradrenergic Systems in Addiction

George Koob*

The Scripps Research Institute, La Jolla, USA

Background: Norepinephrine has widespread distribution in the brain and has hypothesized functions in arousal, attention, stress, anxiety, and affective disorders, all of which may interface with different stages of the addiction cycle.
Methods: Addiction has been conceptualized as a chronic relapsing disorder with compulsive drug seeking that is composed of three stages: binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation (craving). Animal models with excellent face and construct validity for the compulsivity associated with the different stages of the addiction cycle have been used to explore the role of noradrenergic systems in addiction.
Results: Such studies have shown a role for noradrenergic systems in motivational components of opioid, stimulant, and alcohol withdrawal. Perhaps even more compelling, the alpha-1 noradrenergic antagonist prazosin decreases compulsive drug taking associated with extended access to cocaine, heroin, and alcohol. The neurocircuitry implicated in these effects is hypothesized to involve projections from the ventral noradrenergic bundle originating in the brainstem and projecting to the extended amygdala with a focus on the bed nucleus of the stria terminalis. Hypothesized key interactions of noradrenergic systems with the brain and hormonal stress systems mediated by corticotropin-releasing factor in the hypothalamus and extended amygdala, respectively, provide a mechanism by which dysregulated noradrenergic systems can contribute to the motivation for drug seeking in dependence.
Conclusions: Altogether, these results suggest that noradrenergic systems, when activated during the course of addiction, contribute to the dysregulation associated with brain stress systems, and, as such, may be a viable target for the pharmacotherapy of addiction.
Disclosure: G. Koob, Part 1: Addex Pharmaceuticals, Alkermes, Arkeo Pharmaceuticals, Casa Palmera, Embera Neurotherapeutics, GlaxoSmithKline, Lilly, Psychogenics, Part 2: None, Part 3: None, Part 4: None, Part 5: N/A.

Results of A Pilot Trial of the Alpha-1 Adrenergic Antagonist, Prazosin, for Alcohol Dependence

Tracy Simpson*

VA Puget Sound Health Care System, Seattle, USA

Background: Current medications for alcohol dependence (AD) show only modest efficacy. None target brain noradrenergic pathways. Theory and pre-clinical evidence suggest that noradrenergic circuits may be involved in alcohol reinforcement and relapse. We therefore tested the a-1 adrenergic receptor antagonist, prazosin, as a pharmacotherapy for AD.
Methods: We randomized 24 participants with AD but without posttraumatic stress disorder to receive either prazosin or placebo in a 6-week, double-blind pilot study. Medication was titrated to a target dose of 4 mg QAM, 4 mg Q3PM and 8 mg QHS by the end of week 2. Participants received 5 medical management treatment sessions. Participants were reminded three times each day via a text pager to take medications and to call a telephone monitoring system once daily to provide self-reports of alcohol consumption and craving, the primary outcome measures. Results were analyzed using mixed linear regression adjusted for drinking days per week at baseline and week number.
Results: Twenty of the 24 (83%) subjects completed. Among the completers, the prazosin group reported fewer drinking days per week than the placebo group during the final 3 weeks of the study. Since only 1 woman was randomized to placebo and only three women completed the trial, the following results focus on the 17 male completers. The prazosin group reported fewer drinking days per week and fewer drinks per week during the final 3 weeks of the study; average total number of drinking days for the placebo group 5.7 (SEM 1.9) versus 0.9 (SEM 0.5) for the prazosin group, and average total number of drinks 20.8 (SEM 6.5) for the placebo group versus 2.6 (SEM 1.3) for the prazosin group. Rates of adverse events were equivalent across conditions.
Conclusions: Prazosin holds promise as a pharmacologic treatment for AD and deserves further evaluation in a larger controlled trial.
Disclosure: T. Simpson, None.

Panel Session
NMDA Receptor Complexes: A Point of Convergence for Schizophrenia Candidate Pathways

Neuregulin1-ErbB4 Signaling suppresses the Src Upregulation of NMDA Receptors

Michael Salter*

Hospital for Sick Children, Toronto, Canada

Background: A commonly held view is that the primary causal mechanism underlying the cognitive dysfunction and other core behavioral manifestations in schizophrenia is "hypofunction of the NMDA receptor". In the present study, we investigated this possibility by taking advantage of the candidate schizophrenia genes, *Nrg1* and *Erbb4*. We examined the effects of NRG1-ErbB4 signaling on basal NMDA receptor (NMDAR) function and on the upreglation of NMDAR function, and subsequent synaptic potentiation, by the non-receptor tyrosine kinase Src.
Methods: We made whole cell-recordings from neurons in acute brain slices from adult wild-type and mutant rodents. We studied excitatory post-synaptic responses evoked by stimulating inputs to neurons in CA1 hippocampus or in prefrontal cortex. For the hippocampus we also studied long-term potentiation (LTP) evoked by electrical stimulation patterned on theta rhythm.
Results: We found, contrary to prediction of the NMDAR hypofunction theory, that activating the NRG1-ErbB4 pathway had no effect on NMDAR function. And yet, NRG1-ErbB4 signaling blocked LTP in CA1, the dominant form a synaptic plasticity arising from NMDAR activation. We resolved this apparent paradox by discovering that NRG1-ErbB4 signaling blocks the enhancement of NMDAR function by the non-receptor tyrosine kinase Src. NRG1-ErbB4 signaling blocked Src enhancement of NMDAR function in the hippocampus and in the prefrontal cortex. In addition, we discovered that NRG1-ErbB4 signaling, by inhibiting Src, dramatically suppressed neuronal membrane response to brief theta rhythm-patterned stimulation.
Conclusions: Our findings suggest that suppression of the Src-mediated enhancement of NMDARs may be a common neural mechanism in schizophrenia. That NRG1-ErbB4 signaling suppresses responses during theta rhythm but is without effect on responses to individual, isolated stimuli demonstrates a previously unknown critical timing period during which neuronal activity is exquisitely vulnerable.
Disclosure: M. Salter, None.

Rac1-PAK Cascade: A Promising Drug Target for Synaptic Deterioration in Mental Illnesses

Akira Sawa*

Johns Hopkins School of Medicine, Baltimore, USA

Background: Synaptic dysfunction associated with morphological deterioration, such as a decrease in spine density, is seen in schizophrenia. Downstream cascade(s) of NMDA receptor complexes is expected to be a good drug target to ameliorate such pathology. Then, we have tested whether PAK inhibitors may ameliorate such disturbance mainly by using DISC1 knockdown elicited spine deterioration. We previously reported that DISC1 is involved in proper spine maintenance by regulating Kalirin-7-Rac1 cascade in response to NMDA receptor activation.
Methods: In primary neuron culture, we knocked down DISC1 by the RNAi method. Three different PAK inhibitors, originally provided by Afraxis, are applied. We examined how these inhibitors can ameliorate decreases in spine size and density that are elicited by DISC1 knockdown.
Results: We observed promising effects of PAK inhibitors against DISC1 knockdown-elicited spine deterioration when the knockdown and administration of inhibitors were simultaneously taken place. We also observed some levels of recovery from the spine deterioration by applying these inhibitors several days after DISC1 knockdown.
Conclusions: We now provide evidence that inhibition of PAK may be a promising strategy against spine deterioration elicited by DISC1 knockdown.
Disclosure: A. Sawa, Part 4: Astellas Tanabe-Mitsubishi Takeda.

Dysbindin-1 Reductions in Schizophrenia may affect Cognition via Multiple Effects on NMDA Receptor Biology, including Induction of Arc Expression

Konrad Talbot*

University of Pennsylvania, Philadelphia, USA

Background: Two of the three major isoforms of dysbindin-1 are concentrated in postsynaptic densities (PSDs). These isoforms (dysbindin-1A and 1C) are reduced in auditory cortices and hippocampal formation of schizophrenia cases. Their postsynaptic roles may be related to NMDA receptor function. A candidate dysbindin-1 binding partner, sec8, also binds the PSD and regulates NMDAR delivery to the cell membrane. In homozygous sandy mice (dys -/-), which lack all major dysbindin-1 isoforms, cell surface levels of hippocampal NMDAR subunits 2A and 2B are altered. With Wei-Yi Ong, Bailey Glen, and Antonieta Lavin, we thus explored the potential causes and consequences of dysbindin-1 effects on NMDAR, specifically in the hippocampus of sandy mice.
Methods: The hippocampus of male dys-/- and wild-type littermates on a C57BL6/J background were studied. mRNA expression was quantified on Affymetrix mouse gene arrays with significant results of interest tested with RT PCR and Western blotting. NMDAR currents and long-term potentiation (LTP) were tested in hippocampal slice preparations.
Results: While microarray data indicated decreased gene expression of NR2A and NR2B (but not NR1) in dys-/- mice, this was not confirmed by RT-PCR. The microarray data did indicate, however, significantly decreased gene expression of NMDAR-inducible Arc (activity-regulated cytoskeleton-associated protein), which was confirmed by both RT-PCR and Western blotting. Since Arc plays a pivotal NMDAR-dependent role in LTP, we tested it in hippocampal field CA1 of dys -/- mice. The animals showed reductions in evoked NMDA pyramidal cell currents and in LTP. The latter abnormality was rescued by 10 uM of the NMDAR co-agonist glycine.
Conclusions: These findings indicate that dysbindin-1 can affect NMDAR functions not only via its trafficking functions, but via effects on NMDAR function itself and on gene and protein expression of the NMDAR effector Arc. All of these effects may contribute to cognitive deficits in schizophrenia.
Disclosure: K. Talbot, None.

N-methyl D-aspartate Receptor Complexes in Brains of Schizophrenia Patients

Chang-Gyu Hahn*

University of Pennsylvania, Philadelphia, USA

Background: N-methyl-d-aspartate (NMDA) receptor (NR) hypofunction is currently a leading postulate for the pathophysiology of schizophrenia. This project aims to delineate molecular underpinnings of NR hypofunction in brains of patients with schizophrenia.
Methods: The postmortem dorsolateral prefrontal cortex (DLPFC) of 15 matched pairs of schizophrenia and control subjects were separated for synaptic membranes, para-synaptic (PPF) and postsynaptic density (PSD) fractions. PSD fractions were immunoprecipitated for NR complexes which were analyzed by the AQUA LC-SRM/MS and were analyzed for MK-801 binding and Src activity assays.
Results: Proteomic analyses revealed several alterations in NR complexes derived from the patient group. We found a significant decrease in NR1: NR2B associations and an increase in NR: PSD-95 interactions in the patient group, which suggests altered NR assembly in schizophrenia. Previously, we have reported attenuated tyrosine phosphorylation of NR2 subunits in schizophrenia. NR complexes of the patient group exhibited decreases in NR's association with PLCg and PKCg, which can be mediated by NR2 phosphorylation. NR2 phosphorylation in turn is governed by Src, a non-receptor tyrosine kinase. We measured Src activity in membrane fractions and found a significant decrease in Src activity in the patient group ($p < 0.05$). NR functionality can be influenced by binding capacity of the receptors. MK-801 binding assays showed that the Bmax was higher, while Kd is lower in the patient group ($p < 0.01$).
Conclusions: The subunit assembly, protein interactions and signaling activity of NR complexes are altered in schizophrenia. These changes overall are consistent with attenuated NR function, which can be at least in part mediated by altered Src activity. Given that overall ligand binding capacity appears to be increased, we suggest that the post-receptor level dysregulation plays an important role for NR hypofunction in schizophrenia.
Disclosure: C. Hahn, Part 1: PI of the investigator initiated grants from Asztrazeneca, GSK and Phizer, Part 2: None, Part 3: None, Part 4: PI of the investigator initiated grants from Asztrazeneca, GSK and Phizer, Part 5: None.

Panel Session
Enteric Hormone Modulation of Cerebral Neurotransmission and Eating Behaviors in Obesity

Enteric Hormone Modulation of Cerebral Neurotransmission and Eating Behaviors in Obesity

Dianne Lattemann*

VA Puget Sound Health Care System, Seattle, USA

Background: This presentation will summarize the influence of metabolic hormones and diet on aspects of CNS dopaminergic functioning and food reward behaviors. It will provide brief historic, behavioral, and anatomical overview for the field.

Methods: In addition to multiple behavioral paradigms, brain activation indexed by cFos, cellular markers of dopamine activity in multiple CNS sites, and metabolic assessments are used to simultaneously evaluate behavior, CNS function, and metabolic status in rats.
Results: We have found that the metabolic hormones insulin and leptin decrease food reward, and the GI hormone ghrelin increases food reward. This is modified by the background diet of the rat independent of obesity status (pre-obese). Insulin increases dopamine uptake, thus decreasing dopamine signaling. These actions are mediated at multiple sites of CNS reward and energy-regulatory circuitry, including the ventral tegmental area, striatum, and hypothalamus.
Conclusions: Both metabolic/enteric hormones and diet composition significantly modify food reward, acting at molecular, synaptic, and behavioral levels. Since insulin and leptin are markers of caloric abundance with slightly different secretory patterns and time course of action, we speculate that insulin may act rapidly to modulate food reward in a meal-to-meal context whereas both insulin and leptin may act long-term to reflect stable caloric abundance and decrease motivation for feeding. Consistent with this, ghrelin–a hormone that signals hunger–increases motivation for sucrose without changing the sucrose preference profile of rats. Together these findings emphasize a coordinated action of enteric hormones, connecting CNS energy regulatory and reward circuitries.
Disclosure: D. Lattemann, None.

Impaired Striatal Akt Signaling Disrupts Dopamine Homeostasis and Increases Feeding

Aurelio Galli*

Vanderbilt, Nashville, USA

Background: The prevalence of obesity and related disorders has increased dramatically worldwide despite efforts to understand and target homeostatic mechanisms of feeding. Dopamine (DA) rich midbrain structures, such as striatum, provide motivation for feeding. In these central circuitries, DA dysfunction is posited to contribute to obesity pathogenesis. Here, we sought to identify a mechanistic link between metabolic dysregulation and the maladaptive behaviors that potentiate weight gain. Insulin acts centrally to regulate both homeostatic and reward-based high-fat (HF) feeding. We have shown that insulin regulates DA homeostasis, in part, by controlling a key element in DA clearance, the DA transporter (DAT). We show that upon HF feeding, striatal neurons rapidly become insensitive to insulin stimulation and that midbrain insulin signaling deficiency is a link between DA dysregulation and increased HF calorie intake.
Methods: Using a model of HF feeding diet-induced obesity (DIO) in rats, we show that consumption of fat-rich food impairs striatal activation of the insulin-activated intracellular signaling kinase, Akt. HF-induced Akt impairment, in turn, reduces DAT cell surface expression and function, thereby impairing DA homeostasis and amphetamine (AMPH)-induced DA efflux. In addition, HF-mediated dysregulation of Akt signaling impairs DA-related behaviors such as increased caloric intake. We restored nigrostriatal Akt phosphorylation using recombinant viral vector expression technology. We observed a rescue of DAT expression in HF fed rats. This restoration of DAT surface expression was associated with rescue of locomotor responses to AMPH and normalization of HF diet-induced hyperphagia.
Results: Acquired disruption of brain insulin action by consuming a HF diet may confer risk for and/or underlie "food-abuse" disorders and the recalcitrance of obesity to standard therapies.
Conclusions: Our molecular model explains how even short-term exposure to "the fast food lifestyle" creates a vicious cycle of disordered eating that cements pathological changes in DA signaling leading to weight gain and obesity.
Disclosure: A. Galli, None.

Reward Mechanisms in Feeding and Addiction: Paradoxical Roles for Hypocretin (Orexin) Transmission

Jonathan Hollander*

Background: Evidence suggests that compulsive drug use in addiction and compulsive eating in obesity may share common underlying neurobiological mechanisms. Consistent with this hypothesis, I will present recent evidence from our laboratory showing that dopamine D2 receptors (D2Rs) in striatum, known to play an important role in drug addiction, also regulate the development of compulsive eating. I will also data from very recent studies seeking to between understand the roe for feeding-related neuropeptides in regulating consumption of food and addictive drugs. Specifically, I will present studies investigating food intake and intravenous nicotine and cocaine self-administration behavior in mice with null mutation in the hypocretin-1 receptor (Hcrt-1-R), also known as the orexin-1 receptor.
Methods: We trained hungry Hcrt-1-R KO mice to respond for food rewards (25 g pellets) under a fixed-ratio 5 time-out 20 sec (FR5TO20) schedule of reinforcement. We also permitted the Hcrt-1-R KO mice to respond for intravenous nicotine (0.01–0.4 mg/kg per/infusion) or cocaine (0.03–3 mg/kg/infusions) rewards under the FR5TO20 reinforcement schedule.
Results: We found that Hcrt-1-R KO mice consumed dramatically less nicotine or cocaine than wildtype mice. Similarly, treatment of wildtype mice with the selective Hcrt-1-R antagonist SB-334867 also decreased nicotine and cocaine intake in these mice. These findings demonstrate that Hcrt-1-R transmission plays a critical role in regulating consumption of addictive drugs. In contrast to this diminished motivation to consume addictive drugs, we found that the Hcrt-1-R KO mice consumed significantly greater motivation to seek and consume food. This enhanced food intake was detected most reliably when animals were tested under conditions of negative energy balance. Ongoing studies are seeking to clarify the underlying neurobiological substrates that regulate these dissociable roles for Hcrt-1-R transmission in drug and food seeking behaviors.
Conclusions: Taken together, these findings demonstrate that Hcrt-1-R transmission is central to regulating drug intake, but that these receptors may actually protect against overconsumption of food in hungry animals.
Disclosure: Jonathan Hollander, None.

PET Studies of Dopaminergic Neurotransmission in Obesity

Robert Kessler*

Vanderbilt University School of Medicine, Nashville, USA

Background: Obesity in humans leads to insulin resistance with increased insulin levels as well as increased leptin and decreased ghrelin/acyl ghrelin levels. Previous and new studies of dopaminergic neurogransmission in obesity are reviewed and the relationships in humans of BMI and enteric hormones with dopaminergic neurotransmission in the brain are examined.
Methods: Previous literature on obesity and dopaminergic neurotransmission in humans was reviewed and analyzed. Obese and lean women were studied using PET [18F]fallypride studies to estimate regional DA D2 receptor levels, and insulin levels, insulin sensitivity, leptin levels, ghrelin and acyl ghrelin levels measured.

In a small subset (N = 5) obese subjects were studied 7 weeks after bariatric surgery. In addition, the relationship of polymorphisms of the letpin gene to DA release in lean and overweight subjects is currently underway.
Results: Previous studies of dopaminergic neurotransmission in obesity suggest a progression of changes in dopaminergic neurotransmission with the devlopment of obestiy. Studies performed at baseline and at 7 weeks after bariatric surgery demonstrated significant decreases in BMI (43 to 38), insulin, and leptin levels; DA D2 receptor levels significantly decreased in hypothalamus, striatum, and substantia nigra ($\approx$ 10%) consistent with increased extracellular DA levels which may be related to changes in insulin and leptin signaling. Studies in obese and lean women at baseline demonstrated significant relationships between insulin sensitivity, leptin, and acyl ghrelin levels with striatal, hypothalamic, and thalamic DA D2 receptor levels.. As results become available, effects of genetic polymorphisms on DA release will be discussed.
Conclusions: Progressive changes in enteric hormone signaling with the development of obesity appear to be an important mediator of obesity induced changes in CNS DA neurotransmission which in turn may affect eating behaviors.
Disclosure: R. Kessler, Part 4: Novo Nordisc.

Study Group

Crisis in Psychiatric Drug Discovery: Solutions from Academia, Government and the Advocacy Community

Mark Rasenick*, William Potter, John Greden, Anand Pandya, Beth Hoffman, Jeffrey Nye, Patrick Kennedy

University Illinois Chicago, Chicago, IL, United States

The panel will present evolution of the current abandonment of psychiatry research by industry and discuss strategies, including partnerships between mental health advocates and the researchers, to address the problem. An example of a pairing between an advocacy organization (Cystic Fibrosis Foundation) and a pharmaceutical company (Vertex Laboratories) will be presented. There is a crisis in CNS drug development and it is hoped that novel approaches will arise from this panel and the subsequent discussion.
Disclosure: M. Rasenick, Pax Neuroscience, Eli Lilly, Lundbeck, Sepracor, Part 1, Eli Lilly, Lundbeck, Part 4.

Study Group

Assessing Brain Developmental Trajectories from Infancy to Adulthood

James Swanson*, John Gilmore, Claudia Buss, Damien Fair, Jay Giedd, Xavier Castellanos, Raquel Gur, Linda Chang, Anders Dale, Thomas Insel

UC Irvine, Irvine, CA, United States

James Swanson will introduce the Study Group topic and participants. **John Gilmore** will lead the discussion of methods for assessment of brain developmental trajectories in normal and high-risk children from birth to 6 yrs. Studies of cohorts of typically developing children, twins, and children at risk for schizophrenia have revealed novel findings about early postnatal brain development, including regional variation in cortical growth trajectories, rapid maturation of white matter pathways associated with development of language and working memory, and genetic polymorphisms related to neonatal brain structure. Challenges and opportunities for future studies in this important age range will be discussed. **Claudia Buss** will lead the discussion of effects of maternal stress during pregnancy on developmental trajectories of the brain and body composition. Brain circuits involved in regulating energy balance are programmed during fetal development, increasing susceptibility for becoming obese. To combine assessment of brain development and body composition, whole body MRI scanning has been added to a neonatal brain MRI protocol and methods are being developed for quantification of subcutaneous and visceral fat deposition as well as for differentiation between white and brown adipose tissue. Challenges and advances in methods development will be discussed. **Damien Fair** will lead the discussion of developmental trajectories of functional connectivity MRI (fcMRI) from childhood through adulthood. Recent experiments have combined fcMRI, graph theory, and pattern classification techniques to discover organizing principles that guide maturation of functional networks in both typical and atypical development, including a trend toward "segregation" between regions close in anatomical space and "integration" between selected regions distant in space. Implications of this developmental characterization of brain growth in children with and without developmental neuropsychiatric disorders will be discussed. **Jay Giedd** will lead the discussion of developmental trajectories of brain anatomy from childhood to early adulthood (from 3–30 yrs of age). Longitudinal studies of typically developing children and adolescents demonstrate increases in white matter volume and regionally specific inverted U shaped trajectories of gray matter volume and suggest the path of development rather than size is more predictive of male/female differences, cognitive (e.g. IQ) or behavioral/emotional traits, or clinical status (e.g. Autism, ADHD, Schizophrenia). **Xavier Castellanos** will lead discussion of discovery science and use of large R-fMRI datasets aggregated across institutions. **Raquel Gur** will lead discussion of longitudinal studies of brain development and trajectories of complex phenotypes. **Linda Chang** will lead discussion of use of DTI and MRS in neonates, adolescents and adults. **Anders Dale** will lead discussion of development and utilization of multimodality imaging technologies for describing maturational trajectories. Thomas Insel will integrate the 8 topics and lead the general discussion.**Disclosure:** J. Swanson, Noven, Johnson and Johnson, Part 1.

Study Group

Can Vulnerability Markers Identify Informative Neurodevelopmental Abnormalities Across the Spectrum of Early Psychosis?

Kristin Cadenhead*, Jean Addington, Barbara Cornblatt, Elaine Walker, Daniel Mathalon, Diana Perkins, Larry Seidman, Tyrone Cannon, Matcheri Keshavan

UCSD, La Jolla, CA, United States

The 8 Universities comprising the North American Prodromal Longitudinal Studies (NAPLS) consortium aim to advance knowledge regarding the onset of psychosis by means of extensive clinical and biomarker (neurocognitive, electrophysiological, neuroimaging, neuroendocrine and genetic) assessment of subjects at clinical high risk (CHR) for psychosis as part of the NAPLS study. In addition to the important information generated by the NAPLS study, each site has collected longitudinal biomarker information across a range of early psychosis spectrum groups

including first psychotic episode (FE), adolescent first degree relatives (REL) of patients with schizophrenia, and adolescents with schizotypy (SPD) as part of ongoing studies bearing upon neurodevelopmental antecedents of schizophrenia. Adolescence and early adulthood are critical periods of neurological, psychological and social development. Increasing evidence suggests that individuals destined to become psychotic show evidence of dysmaturational trajectories of development. To introduce discussion, the study group will present individual site vulnerability marker data across domains that show evidence of neurobiological differences and neurodevelopmental abnormalities in FE, SPD, REL and CHR subjects compared to healthy adolescents. The findings across the 8 sites in the "early psychosis spectrum" are consistent with the mid-study baseline data from the NAPLS 2 project which now includes 360 CHR subjects and healthy controls. By examining vulnerability markers across the full spectrum of early psychosis, and comparing it to the NAPLS 2 findings, the study group will address the following questions: 1) What converging neurodevelopmental information have we gained across genetic high risk, clinical high risk and first episode psychosis populations? 2) Can neurodevelopmental markers help to improve prediction of psychosis? 3) How can this work be translated so that we can ultimately develop better treatment interventions that target specific deficit areas and dymaturational processes?
Disclosure: K. Cadenhead, None.

Study Group Session

Ethical, Legal, and Social Challenges in Research on Psychiatric Genetics

Paul Appelbaum*, Jennifer McCormick, Barbara Koenig, Laura Roberts, Hank Greely

Columbia University, New York, NY, United States

The explosive growth in research on the genetics of psychiatric and related disorders has generated a series of ethical, legal, and social challenges for researchers that will be explored in this panel. Given substantial public investment in large-scale genomic research, pressure has grown for data to be broadly available. **Dr. McCormick** will address the consequences of NIH's requirement for broad sharing of data from genome-wide association (GWA) studies for privacy and informed consent, in light of research suggesting that genetic data may never be fully identified. Qualitative data from participants in studies of the genomics of addiction will be used to explore these questions. In addition, a consensus is developing that at least some genetic data should be revealed to research subjects, especially as whole genome sequencing becomes more common. **Dr. Appelbaum** will explore the theoretical bases for researchers' duties to return data, the practical issues that are likely to arise, subjects' desire for information, possible legal concerns, and results from studies on the consequences of returning data. The need for large, well-characterized subject pools in genomic research has stimulated the development of biobanks. **Dr. Koenig** will draw on experience with the creation of the Mayo biobank and two biobanks for substance use and bipolar disorders to explore mechanisms for ongoing oversight and whether biorepositories focused on behavioral disorders raise unique concerns. In resolving these issues, the views of stakeholders are likely to be crucial. **Dr. Roberts** will characterize stakeholder perspectives from two large studies involving patients, family members, non-patients, investigators, and IRB members, including views regarding consent, confidentiality, genetic counseling, conflict of interest, sample retention, and community consent. **Prof. Greely**, one of the nation's leading experts on neuroethics and genetic ethics, will lead the discussion.
Disclosure: P. Appelbaum, None.

Study Group Session

PSTD Biomarkers Study Group

Alexander Neumeister*, Victoria Risbrough, Charles Nemeroff, Thomas Neylan, Charles Marmar, Dewleen Baker, Scott Orr, Murray Stein

Mount Sinai School of Medicine, New York, NY, United States

The purpose of this discussion group is to discuss the present state of the art for identification and validation of biomarkers for PTSD risk and resilience and treatment outcome. Experts in the field will present short examples of different biomarkers and their validity as well as limitations. The floor will then be opened for a frank discussion of the current approaches to identifying biomarkers for PTSD and surrogate markers of treatment outcome. This panel will highlight major findings of PTSD biomarkers from a number of different markers (sleep, psychophysiological, plasma and CSF neuropeptides and hormones, cognitive performance, and imaging) and experimental designs (prospective, longitudinal, cross-sectional, twin studies). Discussants will describe the pros and cons of these measures for specific purposes as well as the future of biomarker identification in PTSD. Three broad questions pertaining to biomarker research in PTSD will be addressed by this discussion group: (1) Biomarkers of risk: A number of the participants have experience with powerful prospective study designs across a wide range of measures (psychosocial, psychophysiological, sleep, cognitive, peripheral blood markers and imaging). What are we trying to achieve with these studies in terms of identifying biomarkers of risk (psychometric, neuropsychological, imaging, physiological, genetic, etc.)? For example, development of prevention tools (especially relevant to military and high trauma civilian professionals like police/fire fighters), targeting for potential prophylactic treatments, targeting for appropriate treatment after diagnosis etc. What is the sensitivity and selectivity that we need for a biomarker of risk for PTSD (likely depends on how we want to use the biomarker, see above)? What are markers that are feasible to screen for in small targeted populations vs. larger populations? Should we be focusing on biomarkers before or immediately after trauma? What are the design challenges that occur with prospective studies (e.g. appropriate control groups for repeated testing effects like practice effects) and how can they be overcome? (2) Biomarkers of treatment efficacy. What are the challenges and opportunities for developing biomarkers of treatment response? (3) How can we improve the power and reliability of biomarker studies? Studies use a variety of clinical symptom measures; there is variability in measures/scales/interviews used for PTSD clinical symptoms/diagnoses. However, biomarker research in many cases will require pooling of cohorts to extract enough power to detect effects (e.g. genetics). Is there a way to "normalize" across different symptom measures (e.g. principle component analysis) to allow combination of different studies? When conducting biomarker research, what are the critical factors that we must measure in tandem to avoid confounds (e.g. co-morbid disorders, trauma severity, time post trauma, child hood trauma, neuropsychological function etc.). Others argue that biomarkers of broader phenotypes (e.g. fear learning, extinction) is another approach of value. If so, what are the best relevant phenotypes, and what is the best approach for their use in biomarker research? PTSD biomarker research is in its relatively early stages however there are exciting opportunities for

new approaches and new technologies to advance the field rapidly. This discussion group is meant to be a place for panel members and attendants to "take stock" of the current biomarkers, their limitations and utility. Through this discussion we hope to achieve a better understanding of the critical gaps in the field, the pitfalls to be avoided and the best approaches for biomarker identification and use in treating and preventing PTSD.
Disclosure: A. Neumeister, None.

Study Group Session

The ALCOHOL CLINICAL TRIALS INITIATIVE (ACTIVE): Progress Report and Feedback

Raymond Anton*, Henry Kranzler, Daniel Falk, Roger Meyer, Stephanie O'Malley, Bernard Silverman

Medical University of South Carolina, Charleston, SC, United States

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 refine clinical trials methodology for medications to treat alcohol dependence. This ongoing process aims 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. Novel analyses of data from well-conducted and published clinical trials that can inform and impact the future planning and performance of new clinical trials of alcohol dependence are being performed. These data analyses will be presented and discussed in relation to their potential utility to advance the field of alcoholism pharmacotherapy in an interactive forum during this study group. **Dr. Raymond Anton** (Chair of ACTIVE) will present a brief overview of the ACTIVE process and key questions to be addressed. This will be followed by two presentations of about 15–20 minutes each. These presentations are intended to describe the approach and results of theses important new analyses. The formal presentations will be followed by a panel discussion with audience participation.
The first presentation will be by **Dr. Henry Kranzler** and will cover the length of clinical trials, the role of "continuous response analyses" of drinking measures in defining medication efficacy, and the effects of placebo response effects on outcome differences (effect sizes). **Dr. Daniel Falk** (NIAAA) will present data on the triangulation of drinking data (including changes from pre-study drinking) onto other alcohol- related outcomes (e.g., alcohol social/ health problems, blood pressure, quality of life etc.) as a first attempt to examine "harm reduction goals" as endpoints for alcoholism clinical trials. **Dr. Henry Kranzler** will coordinate a panel of ACTIVE experts from academia and industry including **Drs. Roger Meyer, Stephanie O'Malley, and Bernard Silverman** who will briefly comment on the work and engage in interactive questions with the audience. The goal of this study group is to update the ACNP membership on the work that has been done to date by the ACTIVE group and to solicit input from the membership and other participants to guide subsequent efforts. These efforts will include the ongoing publication of papers on key methodological issues in the conduct of clinical trials for alcohol dependence. Recommendations from the audience participants as to other key issues to consider for future analyses and consensus development will be an important contribution to the ACTIVE effort.
Disclosure: R. Anton, The following are supporter of the ACTIVE workgroup though grants to ACNP: Eli Lilly, Johnson & Johnson, Alkermes, Schering, Lundbeck, Glaxo Smith Kline, Abbott Labs, Part 1, Dr. Anton has received $10,000 per year from the ACNP supported by grants from companies in part one, for his administrative duties as Chair and coordinator of the ACTIVE workgroup, Part 2, Eli Lilly, Merck, Hythiam Inc., Part 4.

Study Group Session

Utilizing the NIH's CTSA Network to Advance Neuropsychopharmacology Research

Anantha Shekhar*, William Potter, Kathleen Brady, John March, Srijan Sen, Linda Brady

Indiana University School of Medicine, Indianapolis, IN, United States

The Clinical and Translational Science Awards (CTSAs) program of the NIH supports a national consortium of medical research institutions designed to transform how biomedical research is conducted. Its goals are to speed the translation of laboratory discoveries into treatments for patients and to train a new generation of clinical and translational researchers. Through the CTSA consortium, researchers across the country are working together in new ways to advance medical research across many disease areas and conditions, including cancer, cardiovascular disease, diabetes and obesity. However, this large resource is currently not well leveraged to develop new treatments for neuropsychiatric disorders. The aim of this panel is to provide information about the CTSA resources, demonstrate its potential through some illustrative projects and invite discussion concerning mechanisms to enhance the role of the CTSA's in facilitating neuropsychiatric research. The first speaker, **Dr. Kathleen Brady** from the Medical University of South Carolina will present an overview of the CTSA consortium and describe some of the unique resources available within the consortium to advance new discoveries into novel therapies and biomarkers. The next two presentations will describe illustrative case studies of how the CTSA consortium can be leveraged to facilitate neuropsychiatric research. **Dr. John March** from Duke University will present how select centers from the CTSA consortium were leveraged to rapidly develop the design and implementation plan for a multisite study of a novel device based therapy with magnetic seizure therapy for treatment resistant depression. Next, **Dr. Srijan Sen** from University of Michigan will describe how he was able to leverage the pilot programs from another group of interested CTSA sites to design and fund a large, multi-site biomarker study of genetic vulnerability to stress and depression. Following these case studies, **Dr. Linda Brady** from the national Institute of Mental health will discuss how the individual Institutes and Centers (ICs) within the NIH can leverage the CTSA consortium to advance their novel therapeutics and biomarker programs. Finally, **Dr. Anantha Shekhar** from Indiana University will act as the discussant to summarize the potential of the CTSAs to advance neuropsychiatric research challenges. Through illustrative case studies, this panel will demonstrate that: a) The CTSA consortium provides a readymade network of select academic research centers with the capability to conduct phase I and II studies in neuropsychiatric subjects in a highly complex regulatory environment. b) By leveraging the CTSA pilot grant mechanism and its mission to assist young investigators, junior faculty can successfully design, get funded and implement large multi-site biomarker and biological therapy studies rapidly. c) The national infrastructure of translational research within the CTSAs is an excellent opportunity for ICs to support large studies in a cost effective manner. In summary, the CTSA consortium provides the foundation with a ready-made network of like-minded academic medical centers with the clinical research resources, regulatory expertise, subject recruitment capabilities and pilot funding resources to take novel discoveries in neuropsychopharmacology

and rapidly move them into first in human in terms of drug, device or behavioral therapy investigation, as well as advance the exploration of biomarkers for neuropsychiatric disease states.
Disclosure: A. Shekhar, Research grants from Johnson & Johnson and Eli Lilly, Part 4.

Four Rodent Models of Psychosis: (Not) Lost in Translation
Herbert Meltzer*, Anthony Grace, Akira Sawa, Maria Karayiorgou, Bryan Roth

Vanderbilt University Medical Center, Nashville, USA

To evaluate the ability of four rodent models to guide development of effective treatments for psychosis and cognitive impairment. Comparing phenotypes and prevention/treatment data following transgenic DISC 1, 22q11.2 and other microdeletion mouse models, subchronic PCP treatment and neonatal MAM, to clinical and biological phenotypes of schizophrenia, including prevention and treatment data.
Each of these models produces enhanced locomotor activity, a model of psychosis, as well as deficits in cognition, electrophysiological alterations and neurochemical changes. Some of these phenotypes can be prevented from occurring, or transiently or permanently reversed, by various treatments, including typical or atypical antipsychotic drugs, specific ligands for monoamine or amino acid receptors or transporters, or selective GABAergic or glutamatergic modulators. These treatment-related effects will be compared with available clinical and treatment data, leading to critical evaluation of the models for construct validity, especially development of novel treatments, or novel models with better predictive power. Each model has intriguing similarities with positive, negative and cognitive deficits in schizophrenia, but also clear differences with respect to the domains of pathophysiology and treatment. Preclinical data from the the MAM and PCP models can be compared with clinical data. The transgenic mouse models can also be used to test correspondence with clinical measures and effects of current treatments. The models suggest novel and sometimes different treatment approaches. The speakers and discussant will endeavor to provide guidance on the utility and validity of these and other models of schizophrenia.
Disclosure: H. Meltzer, Part 4: ACADIA, Amgen, BioLIne Rs, Dainippon Sumitomo, Cypress, Janssen, EnVivo, Novartis, Otsuka, Roche, Sunovion, Part 1; Dainippon, EnVivo, Forest, Roche, Otska.

Tuesday, December 6, 2011

Mini-Panel Session
Medication Discovery for Addiction: Translating the Dopamine D3 Receptor Hypothesis

Translational Approach to Dopamine D3 Receptor: From Mechanism of Action to Clinical Studies
Emilio Merlo Pich*

GlaxoSmithKline, King of Prussia, USA

Background: Dopamine D3 receptors (D3R) are G protein-coupled 7-TM receptors expressed in the nucleus accumbens and in the dopaminergic neurons of substantia nigra-VTA, structures involved in mediating the effects of addictive drugs. In rodents D3R are upregulated by chronic amphetamine or cocaine, and the null mutation produces a phenotype partially resistant to psychostimulant via an unclear mechanism of action. In late '90ies a series of antagonists discovered within GSK, among them SB-277011A, GW598809 and GSK618334, were investigated in preclinical studies for nicotine dependence. Based on an acceptable safety profile GW598809 and GSK618334 were progressed in humans. In parallel, the mechanism of action at the cellular levels was investigted at the University of Brescia using in vitro primary cultures of dopaminergic neurons. Since predictive validity of animal models in neuropharmacology was questioned, we engaged in validating the approach and understanding the mechanism of action.
Methods: Preclinical studies in rats were performed using ex-vivo autoradiography to measure receptor occupancy and conditioned place preference (CPP) as pharmacodynamic endpoint. A translational PKPD model was developed. Human studies were performed using the D3R-preferential [^{11}C]PHNO PET ligand to estimate D3R displacement in substantia nigra-VTA, while pharmacodynamic signals were obtained in smokers using craving scores and fMRI BOLD signals. Primary mesencephalic neurons from mouse embryo where incubated with nicotine and D3R antagonists to measure neurotrophic changes.
Results: Based on preclinical data the PKPD-receptor occupancy model appropriately estimated the concentrations that produced significant pharmacologic effects in humans. Accordingly, GW598809 decreased craving score and GSK618334 reduced amygdala fMRI activation to a gambling task. The mechanims of action studies showed that the neurotrophic effects of nicotine were blocked by D3R antagonists.
Conclusions: The preclinical model was predictive of exposures that delivered pharmacologic human effects. The in vitro data suggested that D3 antagonist can attenuate some neuroadaptive changes of addictive drugs. These data support a possible use of D3R antagonists as therapy for relapse to smoking or cocaine use. Acknowledgment. This presentation summarizes the excellent work of several colleagues: F. Micheli, M. Mugnaini, P. Cavallini, M. Bani, A. Andorn, L. Iavarone, I. Rabiner, J. Beaver, G. Ghibellini, R. Gunn, C. Heitbreder and Ginetta Collo.
Disclosure: E. Merlo Pich, Part 1: I am a full time employee of GSK, Part 2: I am a full time employee of GSK, Part 3: I am a full time employee of GSK, Part 5: GlaxoSmithKline.

Buspirone: New Look at an Old Drug
Phil Skolnick*

NIDA, NIH, Bethesda, USA

Background: This presentation is focused on the demonstration that buspirone (Buspar) potently blunts the reinforcing effects of i.v. cocaine in primates without consistently affecting food maintained responding. Data will be presented demonstrating that buspirone binds with high affinity to D3 and D4 receptors.
Methods: Rhesus monkeys were trained to respond for food pellets and IV injections of cocaine under a FR 30 schedule of reinforcement. The potency and efficacy of buspirone at D1-D5 receptors was determined using standard methodologies.
Results: Cocaine self-administration was characterized by a bell-shaped dose response curve. Buspirone (0.1–0.32 mg/kg) suppressed cocaine (3–100 mcg/kg/inj.) self administration but did not consistently affect food maintained responding. Buspirone binds to recombinant dopamine receptors with Ki values (using [3H]methylspipirone) of: D4 (9 nM) > D3 (93 nM) > D2 (232 nm) > > > D1 = D5.
Conclusions: Among the hundreds of compounds assayed in this primate model, buspirone is among a handful to produce a profound suppression of responding for cocaine at doses that do not remarkably alter responding for food. While the mechanism(s) responsible for these behavioral effects are unknown, an antagonist action at D3 and/or D4 receptors is a viable hypothesis.
Disclosure: P. Skolnick, Part 1: DOV Pharmaceutical, Inc. Sepracor, Part 2: DOV Pharmaceutical, Inc., Part 3: DOV Pharmaceutical, Inc.

Monkey Models of Stimulant Abuse: Effects of Dopamine D_3-Selective Agonists, Partial Agonists and Buspirone

Michael Nader*

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

Background: The research to be described involves several nonhuman primate models of dopamine D_3 receptor function, with the goal of identifying pharmacotherapeutic targets for cocaine and methamphetamine abuse.
Methods: Unconditioned behaviors involving drug-elicted yawning and conditioned behaviors using drug-food choice and drug discrimination are employed.
Results: PG 619, a low efficacy D_3 agonist which binds to D_3 receptors with ~100-fold selectivity over D_2 receptors, elicited yawning similar to the D_3 agonist quinpirole only in monkeys with a cocaine self-administration history. Furthermore, quinpirole, but not PG 619, reinstated cocaine seeking in the same monkeys and PG 619 attenuated cocaine-primed reinstatement. In studies designed to determine the effects of acute and chronic drug administration on stimulant reinforcement, PG 619 was examined in adult male rhesus monkeys self-administering cocaine (0.01–0.3 mg/kg/inj) or methamphetamine (0.01–0.3 mg/kg/inj) under a concurrent schedule with food as the alternative reinforcer. In a study involving cynomolgus monkeys, the effects of acute buspirone were examined on cocaine-food choice. PG 619 resulted in rightward shifts in the cocaine and methamphetamine choice dose-response curves and reduced overall drug intake without affecting the number of total reinforcers; tolerance did not develop to these effects after 5 days of treatment. In contrast, buspirone decreased cocaine choice, but only at doses that decreased total reinforcers in the session. In preliminary studies in one monkey, neither quinpirole nor PG 619 substituted for the discriminative stimulus effects of cocaine in drug discrimination. Quinpirole but not PG 619 functioned as a reinforcer.
Conclusions: These findings add to the growing data supporting the use of D_3 compounds as treatments for cocaine and methamphetamine addiction.
Disclosure: M. Nader, None.

Mini-Panel Session
Vaccines, Viral Vectors, and Cocaine Addiction: Neutralizing Cocaine before it gets to the Brain

Cocaine Vaccine: Promises vs. Reality

Thomas Kosten*

Baylor, Houston, USA

Background: This presentation will present phase 2 studies of a human cocaine vaccine (TA-CD) and animal studies of new cocaine vaccines with improved immunogenicity as an alternative pharmacotherapy for cocaine addiction.
Methods: 1. Human laboratory cocaine administration studies; 2. Randomized placebo controlled, clinical trials; 3. Rodent studies of new vaccine carriers with antibody levels presented.
Results: New innovations in technology have facilitated the development of drug-protein conjugate vaccines, which elicit antibodies of high affinity that are specifically capable of neutralizing the drug in the body and attenuating its pharmacological effects. 1. TA-CD vaccine in humans produces dose-dependent blockade of up to 50 mg of smoked cocaine. 2. A randomized placebo controlled, clinical trial of TA-CD in outpatient cocaine addicts showed significant reductions in cocaine use among those 40% of vaccinated subjects who attained antibody levels above 43 ug/ml, which is the calculated antibody level needed to block 0.5 mg/ml of intravenous cocaine. 3. A new vaccine carrier increased peak antibody levels 3 fold and raised antibodies to these levels in half the time required with TA-CD in rodents.
Conclusions: Recent advances in biotechnology make vaccines feasible as potential pharmacotherapies for drug addiction. Unlike small molecules targeting the neural pathways and receptors involved in drug addiction, these protein therapies target the drug itself, providing alternative strategies for medications development. A cocaine vaccine has shown preliminary success by acting as a buffer to slow the pharmacokinetics of drug entry into the brain and thereby markedly reducing euphoria.
Disclosure: T. Kosten, Part 1: none, Part 2: NIH, Baylor, VAMC, Reckitt Benckizer, Catalyst Pharma.

Steps Toward Cocaine Hydrolase Gene Therapy

Stephen Brimijoin*

Mayo Clinic, Rochester, USA

Background: We are conducting animal studies to determine whether sustained delivery of a powerful cocaine hydrolase might be developed as a treatment to reduce risk of relapse in recovering cocaine abusers. Our studies are based on the concept that gene transfer of modified human plasma butyrylcholinesterase (BChE) is the most practical route to this goal. In the past 10 years the natural cocaine-hydrolyzing action of BChE has been enhanced up to 2000-fold by structure-based mutagenesis. Direct injection of the modified BChE rescues rats from lethal overdose and blocks cocaine-primed reinstatement of drug-seeking behavior in animals that formerly self-administered the drug (Brimijoin *et al.*, 2008). Our objective is now to establish safety and efficacy of BChE gene transfer in multiple models.
Methods: Mutagenesis of human BChE was carried out with standard methods. Most rats received a quadruple mutant (A199S/s287g/a328w/y332g) designated "CocH" (Pancook *et al.*, 2003). Experiments began with CocH cDNA in standard E-1 deleted type-5 adenoviral vectors (T5-AD) using a cytomegalovirus promoter. Later studies used an ApoE promoter in helper-dependent adenoviral vector (hdAD) lacking most viral genes and able to persist as a non-replicating episome without arousing host immune responses. Vectors (10^{11} viral particles) were given through the tail vein to Wistar rats (200–300g). Cocaine hydrolase activity and plasma / tissue levels of ^{3}H-cocaine were determined by radiometric assays.
Results: By 4 days after T5-AD vector injection, rat plasma cocaine hydrolase activity increased about 50,000-fold (Gao *et al.*, 2005). High levels only lasted one week, but the treatment blunted or prevented acute pressor effects of iv cocaine, as well as the slow rise in brain FosB levels during repeated daily ip cocaine administration. For longer enzyme expression, rats were given hdAD-CocH vector, which generated high plasma cocaine hydrolase activity for up to a year. In a reinstatement model treated rats showed reduced cocaine-primed drug-seeking behavior for at least six months (see Carroll abstract, this mini-panel). Vector treatments in themselves caused no rise in serum liver enzymes (markers of hepatotoxicity) or hepatic infiltrates and they acted to protect rats and mice against cocaine-induced liver damage.
Conclusions: Cocaine hydrolase gene transfer may merit clinical trial if it proves to have a good margin of safety. To address questions of toxicity and prepare for human studies, more tests are needed in multiple species. In addition it is important to explore additional BChE mutants (higher catalytic efficiency implying greater therapeutic effect for a given dosage) and other transduction platforms. Finally we will pursue recent indications that hydrolase vectors and cocaine vaccine may be even more effective as a combined treatment.
Disclosure: S. Brimijoin, None.

Long Term Reduction of Cocaine-Seeking Behavior in Rats Treated with Cocaine Hydrolase delivered by a Viral Vector

Marilyn Carroll*

University of Minnesota, Minneapolis, USA

Background: Cocaine abuse continues to result in serious health and societal consequences. We previously found that an iv injection of cocaine hydrolase (CocH), based on human butyrylcholinesterase, acutely blocked cocaine-primed reinstatement and abolished lethal cocaine-induced seizures in rats (Brimijoin *et al.*, 2008). The enzyme also reduced short-access cocaine self-administration but not escalation and bingeing during long access (Carroll *et al.*, 2010). We have now transduced CocH in vivo by gene transfer with a helper-dependent adenoviral vector (CocH vector) in order to determine if a single treatment would chronically attenuate the escalation and reinstatement of cocaine-seeking behavior.
Methods: In an escalation study rats trained to self-administer cocaine (0.4 mg/kg in daily 2-h sessions) were given CocH vector and moved directly into 6-h sessions for up to 90 days. In our reinstatement study, rats self-administered 0.4 mg/kg iv cocaine during daily 2-h sessions for 10 days. In a reinstatement study other rats were allowed daily short access to cocaine (2 h) for 14 days, then they received one tail vein injection of CocH vector, empty vector, or saline and were given 14 days of extinction, with stimulus lights and drug pumps deactivated. No group or sex differences in cocaine infusions were noted during these two phases. The rats were then tested with no cocaine access on an 8-day reinstatement procedure whereby each 2-h session began with an ip priming injection of saline (S), cocaine (C), 5, 10, and 15 mg/kg, or amphetamine (A) in the following order: S, C, S, C, S, C, S, A. A cycle of S, C, injections continued weekly for 4 mo, then monthly for up to 6 mo.
Results: Escalation of cocaine intake during long-access was somewhat reduced in the animals that developed the highest cocaine-metabolizing activity, and high CocH plasma enzyme activity correlated with reduced cocaine self-administration. During the initial 8-day reinstatement period the CocH vector-treated group showed no reinstatement of cocaine seeking after cocaine-priming injections, while empty vector and saline groups showed substantial reinstatement. All groups reinstated robustly after amphetamine priming, indicating that vector treatments produced no general decreases in responding. This pattern continued for the next 6 mo, with the CocH vector-treated group maintaining high CocH plasma levels and failing to show cocaine-primed reinstatement, while controls showed robust reinstatement.
Conclusions: CocH vector reduced long-access escalation of cocaine intake when serum CocH was high (compared with empty vector, saline controls, or rats with poor vector response), and it selectively suppressed reinstatement of cocaine seeking for up to 6 mo. Taken together, our results suggest that viral transfer of CocH interrupted but did not abolish an established pattern of ongoing drug seeking, and it may be highly useful for preventing relapse to cocaine addiction over extended periods of time.
Disclosure: M. Carroll, None.

Panel Session
Circadian Rhythms, Sleep Deprivation and Mood Disorders

Rhythms and Blues: How Circadian Genes Regulate Mood

Colleen McClung*

UT Southwestern Medical Center, Dallas, USA

Background: Circadian rhythm disruptions are prominent in patients with bipolar disorder, seasonal affective disorder and major depression. Moreover, several human genetic studies have linked specific circadian gene variants with these disorders. However, the mechanism by which the genes that control circadian rhythms regulate mood states remains unclear.
Methods: We have utilized genetic mouse models and viral mediated gene transfer to determine the importance of specific circadian genes in various limbic regions of the brain. Furthermore, we have employed molecular, cellular, optogenetic and physiological analysis to understand how these genes regulate mood-related circuitry.
Results: We find that one of the central circadian regulators, CLOCK, is keenly involved in the modulation of dopaminergic transmission in the ventral tegmental area (VTA). Manipulations of CLOCK specifically in this region lead to increased dopamine cell firing and changes in mood and anxiety-related behavior. Moreover, CLOCK is crucially involved in the synchronization of neuronal networks within limbic circuitry as mice explore novel environments. As a transcription factor, CLOCK directly regulates some of the important proteins involved in dopaminergic transmission. Furthermore, rhythms in dopamine signaling are necessary for proper behavioral responses to anxiety-related paradigms.
Conclusions: These results show that CLOCK and other circadian genes have an important role in limbic regions of the brain in the regulation of mood and anxiety-related behavior. They are centrally involved in both the regulation of daily rhythms in these circuits, and the ability of neurons within and between regions to synchronize activity. These studies not only underline the importance of rhythmicity in mood, but identify the circadian genes as novel targets for new therapeutic interventions.
Disclosure: C. McClung, Part 1: GlaxoSmithKline-honorarium Pfizer-honorarium Servier-honorarium, Part 2: None, Part 3: None, Part 4: GlaxoSmithKline-research funding Pfizer-research funding, Part 5: No.

Circadian Gene and Sleep Modulation of Reward Circuitry: Implications for Vulnerability to Bipolar Disorder

Mary Phillips*

University of Pittsburgh, Pittsburgh, USA

Background: Altered reward function, unstable circadian rhythms, and tendency to eveningness, characterize bipolar disorder. Animal studies indicate that circadian-associated genes, influence response to reward. To determine relationships among genetic variations in these genes, abnormal reward circuitry and circadian function that may mediate vulnerability to bipolar disorder, we examined: 1. relationships between functional variation in reward circuitry and sleep in healthy adolescents; 2. the impact of circadian-associated gene-mediated variations upon reward circuitry function associated with eveningness in these adolescents; and 3. reward circuitry function in adults with bipolar disorder.
Methods: Neuroimaging data were collected in 92 healthy adolescents (11–13 years); and in 20 depressed bipolar I, and 20 healthy, adults during a card-guessing paradigm designed to examine reward-related brain function to anticipation and receipt of monetary reward and loss. Data were collected using a 3T Siemens Trio scanner.DNA was obtained from all adolescent participants. In 65 adolescents, actigraphy was conducted over 2 weekday, and 2 weekend, nights in participants' homes.
Results: In adolescents, greater eveningness - later sleep mid-point - was associated with reduced activity in a key component of reward circuitry, mediodorsal prefrontal cortex (MdPFC) to reward ($p < 0.05$, corrected), Genetic variation in the circadian-associated gene *Period 2 (PER2) rs2304672* SNP also significantly impacted activity in this region to reward: G carriers showed significantly reduced activity vs. CC homozygotes in this region to reward. Relative to healthy adults, euthymic bipolar adults showed significantly greater ventral striatal activity during reward anticipation ($p < 0.05$, corrected).

Conclusions: To our knowledge, these findings are the first to show that genetic variation in *PER2* modulates nocturnal-related activity in reward circuitry in adolescents that, in turn, may mediate vulnerability to bipolar disorder.
Disclosure: M. Phillips, Part 2: I receive a consultant fee of more than $10,000 per year from Cardiff University, UK.

Neurobiological Consequences of Disrupted Sleep: Implications for Depression

Peter Meerlo*

University of Groningen, Center for Behavior and Neurosciences, Groningen, Netherlands

Background: Chronically disrupted sleep may have serious repercussions for health and perhaps sensitizes individuals to psychiatric disorders. Indeed, short sleep and insomnia often precede and predict the onset of depression. However, the neurobiological mechanisms through which insufficient sleep may contribute to the development of depression are unknown. We therefore developed an animal model of chronic sleep restriction to study effects of sleep loss on neurobiological and neuroendocrine systems that have been implied in the pathophysiology of depression.
Methods: Rats were exposed to a schedule of chronically restricted sleep allowing them about 4 h of sleep per day. We assessed the consequences of restricted sleep after 1 day or several weeks, with special emphasis on hypothalamic-pituitary-adrenal (HPA) axis regulation, serotonergic function and hippocampal neurogenesis.
Results: While one day of sleep restriction had no major effects on the systems we examined, a week of restricted sleep altered HPA axis regulation and reduced hippocampal cell proliferation. In addition, after a month of sleep restriction we found a significant reduction in hippocampal volume. These changes may in part be related to alterations in serotonergic signalling since sleep restricted rats displayed blunted physiological responses to direct serotonin-1A receptor stimulation. This desensitization of the serotonin-1A system persisted for many days even with unlimited recovery sleep. Importantly, control experiments indicate that the reduction in serotonin-1A sensitivity is not a by-product of stress but a result of sleep loss per se.
Conclusions: The gradually developing changes in neurotransmitter receptor systems and neuroendocrine reactivity in our model are remarkably similar to what is seen in depressed patients. These experimental studies thus provide support for the hypothesis that chronically disrupted sleep may contribute to the symptomatology of psychiatric diseases.
Disclosure: P. Meerlo, None.

Glutamatergic Neurotransmission and Synaptic Homeostasis in the Rapid Antidepressant Effect of Sleep Deprivation

Francesco Benedetti*

Scientific Institute and University Vita-Salute San Raffaele, Department of Clinical Neuroscience, Milano, Italy

Background: Recent studies in humans and animals showed net synaptic potentiation in wake and depression in sleep. Sleep deprivation (SD) causes immediate antidepressant effects, similar to NMDA antagonists. In bipolar depression repeated SD can lead to sustained euthymia, and SD-induced changes in glutamate turnover in prefrontal cortex, as detected by MR spectroscopy, are proportional to mood amelioration.
Methods: In patients affected by non-psychotic bipolar depression we used a combined TMS/EEG method to record TMS-evoked potentials which correlate with the strength of cortical synapses. We studied these physiological correlates of cortical effective connectivity in seven sessions across 3 consecutive cycles of SD alternated to undisturbed sleep. In C57BL/6 mice we investigated, with the same repeated SD protocol (1) up- and down-regulation (micro arrays, real time PCR) of genes in hyppocampus and anterior cingulate cortex, and (2) behavioral changes at tail suspension and forced swimming.
Results: In humans, the severity of depression in bipolar patients correlated with indices of synaptic strength across the repeated sleep-deprivation/recovery-sleep cycles. Self- and observer-rated mood was proportional to TMS-evoked global mean field power (GMFP). Responders eventually increased, and non responders decreased, GMFP before/after treatment. In mice, behavioral antidepressant-like effects of SD were paralleled by up-regulation of several components of the Wnt canonical/non-canonical pathway and of other pathways involved in axonal remodelling, synaptic homeostasis and glutamatergic neurotransmission (Homer, scaffolding proteins, etc.).
Conclusions: The behavioral antidepressant effects of SD in patients affected by bipolar depression and in mice models correlate with changes in indices of synaptic plasticity.
Disclosure: F. Benedetti, None.

Panel Session
Synaptic Plasticity: From Adaptive Molecular Mechanisms to Dysregulation in Psychiatric Disorders

Mechanisms of LTP and LTD: Recent Advances

Robert Malenka*

Stanford University, Stanford, USA

Background: The most well characterized forms of synaptic plasticity, NMDA receptor-dependent long-term potentiation (LTP) and long-term depression (LTD), have been implicated in playing important roles in many forms of adaptive and pathological experience-dependent plasticity. An update of the mechanisms underlying these phenomena will be provided with a focus on new approaches that are allowing the detailed molecular mechanisms of LTP and LTD to be elucidated in unprecedented detail.
Methods: Viral mediated expression of shRNAs combined with the ability to express wildtype or mutant versions of the "knocked down" protein was used to manipulate a number of different postsynaptic proteins. The effects of these molecular manipulations on cell culture models of LTP and LTD as well as LTP and LTD in acute hippocampal slices was examined.
Results: Knockdown of PSD95, AKAP79/150 and Pick1 all blocked LTD in hippocampal slices as well as the endocytosis of AMPA receptors triggered by NMDA receptor activation in cultures. These effects were rescued by simultaneous expression of wiltdype versions of the proteins but not by specific mutants with known effects on protein-protein interactions. The postsynaptic knockdown of complexin, a protein that is required for neurotransmitter release blocked LTP both in culture and in slices. These effects were rescued by expression of wildtype complexin but not by mutants that interfere with its interactions with SNARE proteins.
Conclusions: These results expand our understanding of the detailed molecular mechanisms underlying the classic forms of NMDA receptor-dependent LTP and LTD. For LTD, they support the hypothesis that the PSD95/AKAP interaction is critical for positioning calcineurin in the appropriated subsynaptic domaine. For LTP, they demonstrate that complexin-dependent membrane fusion is critical for the activity-dependent delivery of AMPA receptors to synapses.
Disclosure: R. Malenka, Part 1: Pfizer, Inc. (scientific advisory board), Part 2: Pfizer, Inc.

Regulation of AMPA Receptor Function During Fear Memory and Erasure
Richard Huganir*

Johns Hopkins University School of Medicine, Baltimore, USA

Background: The primary focus of this presentation is to discuss the role of the regulation of AMPA receptors in fear conditioning and the erasure of fear memories. We have been studying the regulation of AMPA receptor function by protein phosphorylation and the regulation of the subcellular targeting of AMPA receptors to synapses. Studies in our lab have provided evidence that the regulation of AMPA receptor function mediates several cellular models of learning and memory such as long-term potentiation (LTP) and long-term depression (LTD).
Methods: In these current studies we have used electrophysiological and behavioral techniques in wildtype and mutant knockin mice. We have found that fear conditioning potentiates synaptic transmission at the thalamic inputs into the basolateral amygdala during training by the addition of AMPA receptors to these synapses. Moreover, we have shown that a central component of extinction-induced erasure of fear memories is the synaptic removal of calcium-permeable AMPA receptors from these synapses resulting in the depotentiation of synaptic transmission. Interestingly, a transient up-regulation of this form of plasticity, which involves phosphorylation of the GluA1 subunit of the AMPA receptor, defines a temporal window in which fear memory can be degraded by behavioral experience. These results reveal a molecular mechanism for fear erasure and the relative instability of recent memory.
Results: In summary, studies from our laboratory indicate that the regulation of receptor function is a major mechanism for the modulation of synaptic transmission and is a critical determinant of animal behavior including fear. Importantly, recent evidence has implicated the regulation of AMPA receptor function in several neurological and psychiatric disorders including Alzheimer's disease, schizophrenia and autism as well as in chronic pain and drug addiction.
Conclusions: In conclusion, our results demonstrate that fear conditioning transiently increases the presence of calcium permeable AMPA receptors at synapses in the amygdala are selectively sensitive to mGluR1 LTD. This sensitivity provides a window for depotentiation of synapses potentiation by fear conditioning resulting in erasure of traumatic memories. These findings identify the mGluR1 as a new pathway for potentially developing therapeutics for treating traumatic memories and posttraumatic stress syndrome (PTSD).
Disclosure: R. Huganir, Part 1: Under a licensing agreement between Millipore Corporation and The Johns Hopkins University, R.L.H. is entitled to a share of royalties received by the University on sales of products described in this article. R.L.H. is a paid consultant to Millipore Corporation. The terms of this arrangement are being managed by The Johns Hopkins University in accordance with its conflict-of-interest policies, Part 2: Under a licensing agreement between Millipore Corporation and The Johns Hopkins University, R.L.H. is entitled to a share of royalties received by the University on sales of products described in this article. R.L.H. is a paid consultant to Millipore Corporation. The terms of this arrangement are being managed by The Johns Hopkins University in accordance with its conflict-of-interest policies.

The Gene Silencing Factor REST and Maternal Deprivation Epigenetically Regulate the Switch in NMDA Receptor Phenotype During Brain Development
R. Suzanne Zukin*

Albert Einstein College of Medicine, Bronx, USA

Background: NMDARs are critical to synaptogenesis, formation of neural circuitry and higher cognitive functions such as learning and memory. Early in postnatal development, synaptic NMDARs undergo a hallmark switch in subunit composition and function from primarily NR2B- to NR2A-containing receptors. This is significant in that NR2B-containing NMDARs exhibit slower decay times, carry more Ca^{2+} current per unit charge, and preferentially tether to the plasticity protein CaMKII. Although the switch in NMDAR phenotype has been an area of intense interest for nearly two decades, mechanisms that regulate the switch are, as yet, unclear. REST is a gene silencing factor that actively represses neuron-specific genes.
Methods: Experiments to assess protein abundance were performed via Western blot analysis and coimmunoprecipitation. Experiments to assess NMDAR phenotype were performed by whole cell patch recording from slices of hippocampus. Experiments to assess the imact of REST on the developmental switch in NR2 phenotype were performed by REST overexpression and/or expression of siRNA to REST and directly in the dentate gyrus of live animals by means of the lentivirus expression system.
Results: Here we show that REST is transiently upregulated during the second postnatal week and is enriched at the NR2B promoter. Upon binding, REST recruits its co-repressors CoREST, G9a and MeCP2 to effect epigenetic remodeling and silence GluN2B at hippocampal synapses. At the same time, the epigenetic landscape at the NR2B promoter changes from transcriptionally active to repressive. Acute knockdown of REST by direct delivery of siRNA into the hippocampus of intact rats *via* the lentivirus expression system prevents the decline in NR2B and change in synaptic NMDAR subunit composition and function. These findings document a causal role for REST-dependent epigenetic remodeling in the switch in NMDAR phenotype at hippocampal synapses. We further show that the REST-dependent decline in NR2B is regulated by adverse experience early in life. Brief periods of maternal deprivation during the first postnatal week prevent enrichment of REST and epigenetic marks of repression at the NR2B promoter, silencing of NR2B and the switch in synaptic NMDAR phenotype.
Conclusions: Thus we identify a novel experience-dependent epigenetic mechanism that regulates synaptic NMDAR phenotype during hippocampal development with far reaching implications for stress-induced alterations in brain function.
Disclosure: S. Zukin, None.

Alterations in Hippocampal Learning and Memory Mechanisms in Schizophrenia
Carol Tamminga*

University of Texas Southwestern Medical Center, Dallas, USA

Background: Alterations in normal brain processes generate the behavioral and cognitive symptoms of psychiatric diseases. We observe alterations from normal in learning and memory functions in schizophrenia (behavioral and molecular) and have developed a model of these as a putative basis for psychosis.
Methods: To test this model we have undertaken three complementary approaches. First, we contrasted cerebral perfusion *in vivo* in normal humans and schizophrenia using high resolution MR and the VASO technique, measuring rCBV in individual hippocampal subfields. Second, we examined markers of glutamate transmission and synaptic strength in DG and CA3 tissue from control and schizophrenia postmortem brain. Third we are examining a DG-selective NR1 knockout mouse model to test the plausibility of the overall model and to generate an addressable animal paradigm.
Results: The perfusion data support the previous reports of increased neuronal activity in schizophrenia hippocampus, but with evidence of subfield-specific changes, namely a rCBV increase in CA3 along with a decrease in DG (DG: SZ = 2.9[1.6], NL = 4.9[1.6]; CA3: SZ = 6.2[2.2], NL = 4.3[1.8]). In human hippocampal tissue, micro-dissected into subfields, the CA3 subfield shows a trend increase in NR2B protein (SZ = .32[.13], NL = .25[.11]) and in P(831)-GluR1 (SZ = .87[.57], NL = .60[.27]), both markers of increased synaptic strength. These observations are in the context of reduced NR1 mRNA and protein in DG (protein: SZ = 2.3[.79],

NL = 3.3[1.2]), suggesting reduced glutamate signaling in DG and in the mossy fiber innervations to CA3.
Conclusions: As a whole, we have interpreted these observations in schizophrenia to support a model of hippocampal pathology in psychosis where CA3 is hyper-associational, sometimes highly so, and where mistakes of association get laid down in memory with psychotic content.
Disclosure: C. Tamminga, Part 1: Intracellular Therapies; PureTech Ventures; Eli Lilly Pharmaceutical; Sunovion; Astellas; Cypress Bioscience; Merck; International congress on Schizophrenia Research; NAMI; APA; Finnegan Henderson Farabow Garrett & Dunner, LLP, Part 2: UTSW; APA; Intracellular Therpies Finnigan Henderson, Part 4: None.

Panel Session
Emerging Methods to Examine Fear Regulation

Development and Expression of Fear Memories during Adolescence
Francis Lee*

Weill Cornell Medical College, New York, USA

Background: Highly conserved neural circuitry between rodents and humans has allowed for in-depth characterization of behavioral and molecular processes associated with emotional learning and memory. Despite increased prevalence of affective disorders in adolescent humans, few studies have characterized how associative-emotional learning changes during the transition through adolescence or identified mechanisms underlying such changes.
Methods: Behavioral, biochemical, and electrophysiological studies were performed in wild-type, and BDNF mutant mice as they transitioned from childhood to adolescence.
Results: By examining fear conditioning in these mice, as they transitioned into and out of adolescence, we found that a suppression of contextual fear occurs during adolescence. Although contextual fear memories were not expressed during early adolescence, they could be retrieved and expressed as the mice transitioned out of adolescence. This temporary suppression of contextual fear was associated with blunted synaptic activity in the basal amygdala and decreased BDNF-dependent signaling in the hippocampus. Gain of function, and loss of function mutant BDNF mice demonstrate that this form of plasticity is modulated by the level of BDNF signaling during this "sensitive period."
Conclusions: These findings reveal a unique form of brain plasticity in fear learning during early adolescence and may prove informative for understanding endogenous mechanisms to suppress unwanted fear memories.
Disclosure: F. Lee, None.

Epigenetic Regulation of Gene Expression to Examine Mechanisms of Amygdala Plasticity and Fear Learning *in Vivo* and in Amygdala Primary Cultures
Kerry Ressler*

Emory University/HHMI, Atlanta, USA

Background: Understanding mechanisms of genetic modulation may lead to a greater understanding of fear behavior and psychopathology. This talk will examine data related to methylation and histone acetylation within the amygdala to study epigenetic regulation of gene expression to examine mechanisms of BDNF- and Homer1a-dependent plasticity and fear learning *in vivo* and in amygdala primary cultures.
Methods: Epigenetic signatures, including gene-specific regulation of DNA methylation and Histone acetylation, are examined *in vivo*, in the mouse amygdala after fear conditioning. In parallel, these approaches are used to examine primary amygdala cell cultures following pharmacological treatment and transfection for genetic manipulations.
Results: BDNF-dependent epigenetic regulation of the TrkB-MAPK-CREB-Homer1 pathway is associated with fear learning and amygdala plasticity.
Conclusions: New mechanistic approaches are rapidly allowing the molecular, cellular, and circuit dissection of fear in mammalian model systems which may lead to novel insights into the diagnostic, prevention and treatment approaches to fear-related disorders.
Disclosure: K. Ressler, None.

Optogenetic Investigation of Circuit Mechanisms of Anxiety and Anxiolysis
Karl Deisseroth*

Stanford University, Stanford, USA

Background: We describe the use of optogenetic methods to achieve gain or loss of function of targeted circuit elements within the amygdala, to probe the circuit mechanisms of anxiety and anxiolysis.
Methods: Microbial opsin genes are delivered by viral vectors to the CaMKIIa-positive cells of the BLA, and light for optical control is delivered either to BLA itself or to candidate downstream structures to control the projections selectively. Behavioral measures include open-field and elevated-plus maze, and circuit physiology measures include two-photon imaging and patch clamp physiology in acute slices.
Results: We demonstrate bidirectional control of acute anxiety responses via basolateral amygdala dependent regulation of central nucleus activation.
Conclusions: Optogenetic gain and loss of function experiments in freely moving mice now allow precise causal circuit interrogation in the setting of symptom-related behaviors.
Disclosure: K. Deisseroth, None.

Using Multi-Electrode Recording in Freely Moving Rats to Probe the Regulation of Fear Memory Formation and Extinction
Donald Rainnie*

Emory University, Atlanta, USA

Background: Synchronized communication between neurons of the medial prefrontal cortex (mPFC) and the basolateral amygdala (BLA) is thought to play an essential role in evaluating and adapting ongoing behavior as required by environmental demands. Moreover, abnormal communication between these two regions is thought to contribute to the etiology of psychopathologies such as PTSD and major depression. Significantly, Mayberg and colleagues have shown that deep brain stimulation (DBS) of the PFC can alleviate otherwise treatment-resistant depression. However, little is known about how these areas communicate in awake behaving animals or how neurons in the BLA may respond to DBS.
Methods: We have used chronically implanted multi-electrode arrays to simultaneously record neural activity in the mPFC and BLA of awake behaving animals during fear conditioning and extinction training, and also to record neural activity in the BLA in response to DBS of the mPFC.
Results: Tone presentations prior to fear conditioning caused no significant changes in the power spectra of the local field potentials (LFPs) recorded in either the mPFC or BLA. During fear conditioning, and on fear recall, tone presentation elicited coherent gamma bursts ($\sim$55 Hz) in the BLA and mPFC LFPs, and elicited coherent activity in a delta band ($\sim$2–4 Hz) that far outlasted tone presentation. Extinction training significantly diminished coherent activity in both of these frequency domains. In contrast, DBS (130 Hz) stimulation of the mPFC decreased the

power of BLA LFPs at frequencies below 20 Hz and appears to increase power in a high gamma band (60–90 Hz).
Conclusions: Acquisition and recall of fear memory is associated with coherent neural activity in the BLA and mPFC in two distinct frequency bands, which dissipate during extinction. DBS of the mPFC may alleviate depression by disrupting coherent activity in frequencies below 20 Hz.
Disclosure: D. Rainnie, None.

Panel Session
Feast or Famine: Is Disordered Eating Related to Disordered Reward?

Analysis of Brain Reward Circuits following Food-Restriction reveals Common Glucocorticoid-Initiated Gene Expression Changes
Ralph DiLeone*

Yale University School of Medicine, New Haven, USA

Background: While it has been known that food restriction enhances learning, motivation, and drug intake, the neural mechanisms underlying these behavioral changes are not well defined. Furthermore, unlike with drug addiction, the molecular basis of neural response to specific experiences (e.g. restriction) has not been identified and studied. The presentation will include the characterization and experimental analysis of molecular responses to food restriction in mesocorticolimbic brain regions.
Methods: To identify changes in gene expression that may mediate relevant behavioral plasticity, microarray analysis was completed after five days of mild food restriction. Gene expression was assessed within the hypothalamus, as well as three brain regions within the mesocorticolimbic circuitry, the ventral tegmental area, nucleus accumbens and the medial prefrontal cortex. Expression was confirmed and the role of glucocorticoid receptors in mediating both molecular and behavioral effects was evaluated. Chromatin immunoprecipitation was used to study direct regulation by glucocorticoid receptors.
Results: Validated genes were shown to be up-regulated across multiple brain regions, and time course studies suggest rapid and persistent induction following restriction. Experimental manipulations of corticosterone demonstrate that it is a key signal leading to changes in expression and that it can potentiate the motivation to seek food in a restricted state. Among regulated genes, NF-kB components are of particular interest since this pathway is known to regulate inflammatory processes in cells, and can influence responses to drugs of abuse and stress.
Conclusions: These data suggest stress-hormone mediated transcription as one mechanism by which the food restricted state leads to changes in behavior, including enhanced learning and motivation. The changes described here are likely relevant to eating disorders where food restriction is frequently experienced. Many of these pathways are also known to influence drug addiction and mood in animal models.
Disclosure: R. DiLeone, None.

Nucleus Accumbens Serotonin (5-HT) 5-HT_{2C} Receptor is involved in Sensitivity to Obesogenic Food
Noelle Anastasio*

University of Texas Medical Branch, Galveston, USA

Background: The neurobehavioral processes underlying the vulnerability to eating disorders may involve dysregulation of brain serotonin (5-HT) pathways that are well-characterized to regulate feeding, satiety, and mood. The etiology of eating disorders may involve aberrant 5-HT_{2C}R function in hypothalamic circuits known to control eating, and/or within limbic-corticoaccumbens circuits that drive reward and motivation. Serotonin actions at 5-HT_{2C}R within the nucleus accumbens (NAc) may modulate the processing of the motivational significance of food rewards as intra-NAc shell infusion of a 5-HT_{2C}R agonist decreased fat and sucrose intake while a 5-HT_{2C}R antagonist stimulated brown chow intake. The purpose of this study was to assess the role of nucleus accumbens shell (NAcSh) 5-HT_{2C}R in sensitivity to obesogenic food.
Methods: Recombinant AAV vectors were constructed with a separate expression cassette for eGFP and shRNA directed at the 3′ untranslated region of the rat 5-HT_{2C}R to decrease expression of all endogenous 5-HT_{2C}R isoforms. The 5-HT_{2C}R shRNA-AAV-eGFP viral vector was bilaterally infused into the NAcSh of male rats, while control animals received bilateral intra-NAcSh infusions of AAV-eGFP. All animals were subjected to the sucrose two-bottle choice and a novel binge-eating paradigm.
Results: Knockdown of NAcSh 5-HT_{2C}R increased preference for 1% sucrose solution vs control. Exposure to a sub-preference concentration of sucrose (0.05%) following withdrawal resulted in sucrose preference in rats with a loss of NAcSh 5-HT_{2C}R. Knockdown of the NAc 5-HT_{2C}R increased bingeing on an obesogenic food vs. controls.
Conclusions: Sensitivity to palatable/obesogenic food is enhanced following knockdown of the 5-HT_{2C}R in the NAcSh. 5-HT_{2C}R in the NAcSh may play a role in regulation of responsiveness to rewarding stimuli.
Disclosure: N. Anastasio, None.

Imaging of Brain Dopamine in Binge Eating Disorder
Gene-Jack Wang*

Brookhaven National Laboratory, Upton, USA

Background: Binge eating disorder is characterized by episodes of eating an objectively large amount of food in a short time period with a feeling of loss of control. The neurobiology of binge eating disorder is poorly understood. Brain dopamine, which regulates motivation for food intake, is likely to be involved. We assessed the involvement of brain dopamine in the motivation for food consumption in binge eaters.
Methods: PET scans with [^{11}C]raclopride were done in obese binge eaters and obese subjects without binge eating disorder. Changes in extracellular dopamine in the striatum in response to food stimulation in food-deprived subjects were evaluated after placebo and after oral methylphenidate, a drug that blocks the dopamine reuptake transporter and thus amplifies dopamine signals.
Results: Neither the neutral stimuli (with or without methylphenidate) nor the food stimuli when given with placebo increased extracellular dopamine. The food stimuli when given with methylphenidate significantly increased dopamine in the caudate and putamen in the binge eaters but not in the non-binge eaters. Dopamine increases in the caudate were significantly correlated with the binge eating scores but not with body mass index.
Conclusions: The lack of correlation between body mass index and dopamine changes suggests that dopamine release per se does not predict body mass index within a group of obese individuals but that it predicts binge eating. These results provide evidence of involvement of the caudate nucleus in the pathophysiology of binge eating disorder. Inasmuch as binge eating is not exclusively found in obese individuals, further studies are warranted to assess the neurobiological factors that may differentiate obese and non-obese binge eaters.
Disclosure: G. Wang, Part 1: No, Part 2: No, Part 3: No, Part 4: Orexigen Therapeutic Inc, Part 5: No.

Individual Differences in Cue Reactivity: Food and Drugs
Harriet de Wit*

University of Chicago, Chicago, USA

Background: Drug-related cues are known to elicit drug craving and possibly precipitate relapse. Recent evidence suggests that individuals differ markedly in their propensity to respond to cues, suggesting that they may also differ in risk for relapse. Little is known about sources of variation in responses to cues in humans. We have studied inter-individual variation in cue reactivity in cigarette smokers. In one recent analysis, we sought to determine whether individuals' ratings of craving for food after a food-related cue was related to craving elicited by a cue related to another reinforcer, cigarette smoking.
Methods: Regular cigarette smokers participated in a 4-session study, in which they abstained for 18 hours from either smoking or eating before the sessions (smoking only, eating only, both eating and smoking, neither). They rated their desire for food or cigarettes after presentations of food-related or smoking-related cues.
Results: Certain individuals reported strong cue-induced craving after both smoking and food cues. That is, subjects who reported strong smoking cue-induced craving after abstaining from smoking also rated stronger food cue-induced craving for food after fasting.
Conclusions: These correlations suggest that certain individuals may be susceptible to responding to cues, regardless of their modality. This finding fits well with recent reports from non-human studies, in which individuals differ in their tendency to approach stimuli associated with a reward.
Disclosure: H. de Wit, Part 4: Research study supported by Unilever.

Panel Session
Cortical Dopamine in Schizophrenia: Quantifying Levels, Understanding Function

Validation of [C-11]FLB457 as a Tool to Measure Cortical Dopamine Release
Raj Narendran*

University of Pittsburgh, Pittsburgh, USA

Background: The use of PET and SPECT endogenous competition binding techniques has contributed to the understanding of the role of dopamine (DA) in schizophrenia. An important limitation of these imaging studies that utilized [^{123}I]IBZM and [^{11}C]raclopride is the fact that measurements of acute changes in synaptic DA were restricted to the striatum. Given the broad interest to characterize the role of cortical DA function in cognition and schizophrenia, we conducted a series of experiments in healthy humans to validate the use of the high affinity DA $D_{2/3}$ receptor radioligand [^{11}C]FLB457 to measure amphetamine-induced dopamine release in the cortex.
Methods: 1. To compare the ability of two high affinity DA $D_{2/3}$ radioligands [11C]FLB457 and [^{11}C]fallypride to measure amphetamine-induced changes in DA transmission in the human cortex (i.e., [^{11}C]FLB457 binding potential, BP_{ND}). 2. To examine the effects of dopamine depletion with alpha-methyl-para-tyrosine (AMPT) on [^{11}C]FLB457 BP_{ND}. 3. To evaluate the reproducibility and reliability of [^{11}C]FLB457 BP_{ND} in the same imaging paradigm that was used to measure amphetamine-induced DA transmission. 4. To evaluate the fractional contribution of specific binding to $D_{2/3}$ receptors in the human cerebellum, which is typically used as a measure of non specific binding for the derivation of [^{11}C]FLB457 BP_{ND}. 5. To evaluate the relationship between decrease in [^{11}C]FLB457 BP_{ND} as measured with PET and peak increase in extracellular fluid (ECF) dopamine as measured with microdialysis in the primate cortex after different doses of amphetamine (0.15, 0.3, 0.5, 1.0 mg/kg).
Results: 1. Amphetamine induced DA release led to a significant decrease in [^{11}C]FLB457 BP_{ND} in most of the cortical regions (ROIs) evaluated. In contrast, no significant decrease in [^{11}C]fallypride BP_{ND} was detected in cortex following amphetamine. 2. AMPT induced DA depletion led to no significant changes in [^{11}C]FLB457 binding in the cortical ROIs. 3. The test-retest variability of [^{11}C]FLB457 BP_{ND} was 15% in all the cortical ROIs. 4. The contribution of specific binding to $D_{2/3}$ receptors in the cerebellum was lower than that in the cortical ROIs and mostly representative of nonspecific binding. 5. Preliminary results suggest a linear relationship between peak increase in ECF DA release and decrease in [^{11}C]FLB457 BP_{ND} in the cortex.
Conclusions: Results of the experimental data acquired to date support the use of [^{11}C]FLB457 as a tool to measure amphetamine-induced dopamine release in the human cortex.
Disclosure: R. Narendran, None.

Decreased Cortical Dopamine Release in Schizophrenia: Evidence from in Vivo Imaging
Anissa Abi-Dargham*

Columbia University, New York, USA

Background: Despite a long-standing interest in assessing cortical dopamine transmission to understand the pathophysiology of cortical dysfunction and its relationship to cognitive deficits and negative symptoms in schizophrenia (SCZ), these studies have not been possible until very recently. Recent evidence has shown that a high affinity radiotracer for D2/3 receptors, [^{11}C]FLB457, can be used to study amphetamine induced dopamine release in extrastriatal regions, including the cortex. Here, we used this paradigm to probe cortical amphetamine induced dopamine release and compared patients with schizophrenia to matched healthy controls.
Methods: Eleven patients with schizophrenia and 12 controls, matched for age, sex, ethnicity and family socio-economic status, as well as smoking, underwent two positron Emission Tomography (PET) scans after bolus injection of [^{11}C]FLB457 before and after iv administration of 0.3 mg/kg of D-amphetamine. The [^{11}C]FLB457 specific to nonspecific equilibrium partition coefficient, BPND was measured. The main outcome measure was the relative change in [^{11}C]FLB457 BPND induced by amphetamine, denoted ÄBPND.
Results: There were no statistically significant differences between baseline and amphetamine condition scan parameters related to injected activity, free fraction in arterial plasma or brain non-displaceable component (V_{ND}, cerebellum distribution volume) of [^{11}C]FLB457 for both groups. Patients with SCZ showed significantly less displacement compared to controls in many regions of interest (ROIs), including the medial temporal lobe, the anterior cingulate and the prefrontal cortex, as well as midbrain and thalamus. The effect size of the decrease ranged from 0.5 to 1. Linear mixed modeling with ROI as repeated measure showed a significant effect of diagnosis $F(1,20.87) = 7.01$, $p = 0.015$. Furthermore, dopamine release was correlated in most ROIs with performance on 3 back at baseline, prior to the scan, with low release associated with low performance in patients but not in controls.
Conclusions: This is the first in vivo demonstration of impaired cortical dopamine release and its functional significance in schizophrenia, in vivo, with a novel imaging paradigm. These results suggest that dopamine-enhancing strategies would be therapeutic in some patients.
Disclosure: A. Abi-Dargham, Part 1: BMS-Otsuka, Sunovion, GSK, Merck, Boehringer-Ingelheim, Lundbeck, Part 2: BMS-Otsuka, Part 3: BMS-Otsuka, Part 4: GSK.

Dysregulation of the Norepinephrine Transporter sustains Cortical Hypodopaminergia and Schizophrenia-Like Behaviors in Neuronal Rictor Null Mice
Aurelio Galli*

Vanderbilt, Nashville, USA

Background: The mammalian target of rapamycin (mTOR) complex 2 (mTORC2) is one of two highly-conserved protein kinases that are critical regulators of cell growth and metabolism. mTOR complex 1 (mTORC1) and mTORC2 are functionally distinct multiprotein complexes that are defined by their subunit composition, rapamycin sensitivity, and substrate selectivity. mTORC2 is the kinase responsible for phosphorylation of Akt at Ser residue 473. Defects in its Ser473 phosphorylation are linked to schizophrenia. Using genetic and pharmacological approaches, we corroborate the relationship between dysregulation in Akt signaling and disruptions in dopamine (DA)-associated behaviors linked to schizophrenia.
Methods: We have generated an animal model in which mTORC2/Akt signaling down-regulation is achieved by neuronal deletion of a key mTORC2 regulatory subunit, rictor. We used a Cre-lox strategy to restrict the genetic deletion to neurons.
Results: A compelling hypothesis in schizophrenia is that impaired mTORC2/Akt signaling triggers aberrant regulation of DA homeostasis. Termination of DA signaling at prefrontal synapses involves two mechanisms: degradation *via* enzymes including COMT, and clearance *via* the norepinephrine (NE) transporter (NET), which takes up both major brain catecholamines, DA and NE. We show that the insulin receptor, which controls mTORC2/Akt signaling, regulates NET cell surface expression and function at the level of the single bouton. We hypothesized that dysregulation of mTORC2 and inhibition of Akt signaling may provide a mechanistic link to cortical hypodopaminergia through NET trafficking. Deletion of the neuronal mTORC2 eliminates Akt Ser473 phosphorylation. Rictor-null mice show prepulse inhibition deficits, and enhanced expression of cortical NET, leading to reduced prefrontal DA and elevated cortical NE content. Strikingly, NET inhibition reversed cortical hypodopaminergia as well as prepulse inhibition deficits.
Conclusions: Akt phosphorylation is associated with cortical hypodopaminergia by altering NET function/trafficking and the resulting phenotype can be reversed by its pharmacoblockade.
Disclosure: A. Galli, None.

Developmental Disruption of Prefrontal Cortex Interneurons by Altered Dopamine Transmission during Adolescence
Kuei Tseng*

RFUMS/The Chicago Medical School, North Chicago, USA

Background: Adolescence is a vulnerable period for onset of some major psychiatric disorders such as schizophrenia. However, we know very little about the neurodevelopmental processes that may contribute to this vulnerability. While many brain regions are fully mature by the time of adolescence, the prefrontal cortex (PFC) continues to undergo many structural and functional changes during this developmental period. At the cellular level, changes in PFC function are dependent on local dopamine transmission and the activity of parvalbumin (PV)-positive GABAergic fast-spiking (FS) interneurons, which exert inhibition over pyramidal output cells and enable synchronous firing in the PFC. We therefore hypothesized that the increased susceptibility for the onset of schizophrenia could be due to an interference of the normal maturation of PFC interneurons by altered dopamine transmission during the periadolescent period.
Methods: Using histochemical, biochemical, electrophysiological and behavioral measures, we first examined how PV-interneuron activity changes during postnatal development. Next, we examined the impact of abnormal facilitation of the mesocortical dopamine transmission (non-contingent repeated exposure to cocaine; 15–25 mg/day/5 days, i.p.) during adolescence (postnatal days 35–40) on PFC PV-interneuron development.
Results: We found that PV-interneuron function in the normal PFC increases during the periadolescent transition to adulthood. Electrophysiological data expand upon these findings, by showing that the increased PV level is associated with an augmentation of glutamatergic drive onto FS/PV-interneurons. Periadolescent exposure to cocaine arrests the characteristic developmental facilitation of PV-positive interneuron function in the adult PFC. Histochemical and electrophysiological analyses of cortical metabolic and synaptic activity indicate that a developmental dysregulation of interneuronal circuits can elicit a sustained disinhibited PFC state. Such PFC disinhibition is associated with increased impulsive behavior as revealed by deficits in the delay-discounting working memory task.
Conclusions: Together, our data indicate that a developmental dysregulation of interneuronal circuits in the prefrontal cortex could trigger and sustain a disinhibited frontal cortical state and contribute to the late adolescent onset of prefrontal deficits observed in schizophrenia.
Disclosure: K. Tseng, Part 4: Supported by Rosalind Franklin University and NIH R01-MH086507.

Panel Session
Neuroimaging Genomics: Discovering a Signal in the Complexity of Genes, Brain and Behavior

Genome-Wide Association Implicates *FGF14* in Amygdala Volume and Fear Processing
David Glahn*

Yale & Olin Neuropsychiatry Research Center, Institute of Living, Hartford, USA

Background: The amygdala has a preferential role in processing emotional stimuli and fear conditioning. Amygdala dysfunction has been documented in a number of mental illnesses, including mood disorders, schizophrenia, anxiety disorders, post-traumatic stress disorder and addiction. Determining the genetic factors that influence amygdala function and structure should provide empirically defined candidate genes for these mental illnesses.
Methods: To that end, we genotyped more than one million genetic variants throughout the genome of 605 Mexican American individuals from randomly selected extended pedigrees, for which we have extensive neuroanatomical and neurocognitive data.
Results: We undertook a genome-wide association analysis of amygdala volume and identified a single nucleotide polymorphism (SNP), rs1336722, which was significantly associated with amygdala volume ($p = 6.7 \times 10^{-8}$). Located within intron 1 of the *FGF14* gene, the C allele (frequency = 0.44) accounts for nearly 4% of the phenotypic variation seen in amygdala volume and is associated with a decline in volume. Three other SNPs (rs1415060, rs1336709, and rs9518703) within intron 1 of *FGF14*, which were in linkage disequilibrium with rs1336722, also showed genome wide significance. To determine if rs1336722 influences fearful facial perception in healthy individuals, subjects were asked to choose which of five words (e.g. happy, sad, angry, fear, or neutral) best represents the emotion portrayed on a series of 40 faces. While the identification of fearful facial expressions, relative to neutral

faces, was significantly influenced by rs13366722 ($p = 0.002$), task performance in general was not influenced by this SNP ($p = 0.766$).
Conclusions: *FGF14* codes for a fibroblast growth factor that is expressed in the developing and adult central nervous system. Mutations within this gene have been associated with dyskinesia, cerebellar ataxia and mild mental retardation. Further, animal models have suggested a function for *FGF14* in neuronal signaling, axonal trafficking and synaptosomal function and indicate that reduced *FGF14* may result in decreased response to dopamine agonists. We have identified a novel polymorphism that is associated with amygdala volume and are currently re-sequencing and genotyping the *FGF14* gene more comprehensively to identify potential functional variation that may contribute to amygdala volume and the development of mental illnesses, particularly mood disorders.
Disclosure: D. Glahn, None.

Imaging Genetics Validation of Molecular Interactions in Psychiatric Risk Pathways
Daniel Weinberger*

NIMH/NIH, Bethesda, USA

Background: Psychiatric genes are involved in networks and pathways that subserve risk relevant cell biology, and the risk predicatibility of a particular gene likely depends on the integrity of other related proteins. Studies in cell and animal models systems indicate that gene interactions are critical for determining biological read out, but this has seen little confirmation in human studies. Imaging genetics is an approach to validation in living human brain of interactions predicted from basic cell and animal studies. This presentation will focus on two examples of interacting genes that have been implicated in risk for and pathophysiology of psychiatric illness: 1) the link between DTNBP1 (dysbindin) and DA synaptic activity via D2 receptor trafficking after ligand induced internalization, which has been demonstrated in cell culture (Iizuki *et al*., 2008) and in mice (Yuanyuan *et al*., 2009); and 2) the interaction of DISC1 and NKCC1. Downregulation of DISC1 in new hippocampal neurons leads to altered synaptic architecture (Kim *et al*., 2009) and this effect appears to be mediated by the activity of the chloride cotransporter, NKCC1 (encoded by SLC12A2) (Kim *et al*., *Cell* in press).
Methods: We tested the cognitive effects of the DTNBP1-DA interaction in mice and the cortical physiologic effects in humans with fMRI by genetically varying dysbindin expression and COMT enzyme activity. Based on the D2 trafficking effects, it was predicted that decreased dysbind expression and increased synaptic dopamine would translate into abnormal D2 signaling which would be disadvantageous in both the mouse and human paradigms. Combination of single (COMT or DTNBP1) and dual C57 knock out mice were created (total N = 71 animals) and tested in the forced choice alternating T maze working memory task. For the human fMRI experiments, normal subjects (N = 115 for DTNBP1 x COMT and N = 229 for DISC1 x NKCC1) performed a working memory and episodic memory task, respectively. Based on the data in cell culture and mice, we predicted that there would be nonlinear, epistatic effects of COMT and DTNBP1 on physiological efficiency during the N back working memory task in normal subjects, associated with exaggerated D2 signaling. The effect of a DTNBP1 haplotype associated with decreased expression of dysbindin in human prefrontal cortex (Bray *et al*., 2005) was tested on the background of COMT val/met genotypes. During the episodic memory task, we tested only one genetic hypothesis, namely, whether normal subjects who are both minor allele carriers of rs1000731 in DISC1 and minor allele carriers of rs10089 in SLC12A2 (NKCC1) would have altered physiological engagement of the hippocampal formation during memory encoding compared with all other genotype combination.
Results: Compared with wild type animals, each single KO mouse showed fewer days to criteria, but the combined KO, either combined heterozygotes or combined homozygotes, showed dramatic failure of learning the task ($p < 0.001$). In the human fMRI data, as predicted by the basic cell culture experiments and the mice, met alleles associated with relatively greater synaptic DA, and more physiologically efficient in general, were significantly less efficient on the background of the Bray risk associated haplotype in DTNBP1. On the background of nonrisk haplotypes, the met allele was more efficient (all P values < 0.05 corrected). In the imaging analysis of the interaction of DISC1 and NCKK1, as predicted, individuals with minor alleles at these two SNP loci had significantly reduced engagement of the parahippocampal cortex during encoding compared with all other genotypes, which did not differ from each other ($p < 0.01$ corrected).
Conclusions: These data demonstrate that nonlinear, epistatic interactions of genes subserving functional molecular pathways implicated in psychiatric disorders at the cellular and animal model level can be validated biologically in the living human brain with fMRI. The results also illustrate a critical principal of the biological impact of interacting genes in pathways, that the effects can be nonlinear and not predicted simply by adding the individual gene effects. These results also have implications for understanding variation in genetic association of individual genes across different populations, because genetic background in relevant pathways is not generally considered.
Disclosure: D. Weinberger, None.

Epistasis and Epigenetic DNA Methylation are Involved in Risk for Schizophrenia Phenotypes
Alessandro Bertolino*

University of Bari, Bari, Italy

Background: The function of genes is modulated by other genes and by epigenetic factors, including DNA methylation. Dopamine modulates prefrontal and striatal dysfunction in schizophrenia and is inactivated by COMT, but the effect of methylation of its SNPs (e.g, $Val^{158}Met$) is not known. Moreover, genetic interactions within the dopamine D2 c-AMP independent pathway, which includes AKT1 and GSK3b, are not known.
Methods: A large sample of subjects was genotyped (> 300 healthy subjects, 66 patients with schizophrenia). DNA methylation, mRNA and protein expression were assessed with standard methods from PBMCs and post-mortem DLPFC (NIMH brain bank). Stress was assessed with questionnaires and cognition was studied with several tasks, including the N-Back working memory (WM) and the Variable Attentional Control (VAC) tasks which also served for BOLD fMRI at 3T.
Results: Because it creates a CpG dinucleotide, only the COMT Val^{158} allele can be methylated. Methylation of the Val^{158} allele of Val/Val humans: is associated with lifetime stress and with WM performance; it interacts with stress to modulate prefrontal activity during WM (greater stress and lower methylation are related to reduced cortical efficiency); is inversely related to mRNA expression and protein levels. Finally, methylation of *COMT* in prefrontal cortex and in PBMCs of rats are correlated. The two risk alleles within *DRD2* (rs1076560) and *AKT1* (rs1130233) are associated with reduced AKT1 protein expression and GSK3b phosphorylation, with deteriorated cognitive performance and brain activity during the VAC, and with greater response to treatment with olanzapine in patients with schizophrenia. Further downstream in this pathway, an intronic SNP within GSK3b is associated with mRNA expression in DLPFC, with behavioral

performance and related prefrontal activity during attention and WM.
Conclusions: These results demonstrate that predictable genetic interactions and epigenetic effects modify risk for different phenotypes of relevance to schizophrenia.
Disclosure: A. Bertolino, Part 1: Eli Lilly, Janssen Pharmaceuticals, Astra Zeneca, Bristol Myers Squibb, Part 2: None, Part 3: None, Part 4: Unrestricted grants from Janssen Pharmaceuticals, Astra Zeneca, Eli Lilly, Part 5: No.

A Developmental Study integrating Neuroimaging and Genomics

Raquel Gur*

University of Pennsylvania, Philadelphia, USA

Background: Elucidation of normal developmental trajectories of brain structure and function is required for understanding processes underlying mental disorders. Abnormalities in brain structure and function are already evident in vulnerable populations, e.g. psychosis-prone. To integrate neuroimaging and genomics, large well-characterized samples are required. We present a study where genotyped youth were clinically and neurobehaviorally assessed and a subsample participated in MRI.
Methods: Imaging was conducted in 1000 randomly selected from a cohort of 10,000 genotyped and phenotypically characterized youths age 8–21. Computerized phenotypic assessments included a modified K-SADS and neurobehavioral measures of cognitive and emotion processing. MRI was performed on a Siemens 3T scanner. Structural imaging used T1-weighted 3D MPRAGE and DTI. Functional imaging used BOLD contrast acquired with GE- EPI sequence while performing emotion identification and working memory tasks.
Results: The sMRI showed increased WM and reduced GM volume for this age range. DTI showed developmental increase in FA and reduced diffusivity across the brain. fMRI showed increase in effective connectivity associated with improved performance on both tasks and improved system connectivity for resting BOLD in reference to working memory. Sex differences modulated developmental trajectories and brain-behavior relations. GWAS identified genes linking neuroimaging parameters to performance.
Conclusions: Because brain and behavior change during development it is important to establish their trajectories in reference to gene action. Correlations can be established for brain-behavior parameters and this can permit identification of vulnerable youths and gene networks underlying neuronal vulnerability leading to mental disorders. Integrative analyses of the genomic, epigenetic, imaging and phenotypic datasets offers optimal genotype-phenotype association methodology.
Disclosure: R. Gur, Part 4: Pfizer investigator initiated grant AstraZeneca investigator initiated grant.

Panel Session
Molecular Mechanisms Informing PTSD Risk, Treatment and Prophylaxis

Molecular Mediators of Stress differentiate Resilience and Risk for PTSD in a Highly Traumatized Population

Kerry Ressler*

Emory University/HHMI, Atlanta, USA

Background: We have previously found that the FKBP5 chaperone protein, which modulates glucocorticoid receptor sensitivity, appears to interact with history of childhood trauma to differentially associate with risk vs. resilience in adult risk for post-traumatic stress disorder (PTSD). Utilizing convergent genomic approaches, we now show that another molecular mediator of stress response, the pituitary adenylate cyclase-activating polypeptide (PACAP), is also apparently involved in disorders of fear regulation. Specifically, PACAP is known to broadly regulate the cellular stress response. In contrast, it was previously unclear if the PACAP-PAC1 receptor pathway has a role in human psychological stress responses, such as PTSD.
Methods: We utilized a large epidemiological sample in parallel with animal studies to examine genetic, epigenetic, gene expression and endocrine markers to predict PTSD. Here we find, in heavily traumatized subjects, a sex-specific association of PACAP blood levels with fear physiology, PTSD diagnosis and symptoms in females. We examined 44 single nucleotide polymorphisms (SNPs) spanning the PACAP (encoded by ADCYAP1) and PAC1 (encoded by ADCYAP1R1) genes, demonstrating a sex-specific association with PTSD. Additionally we utilize animal studies of fear conditioning and estrogen replacement to examine the fear- and stress-dependency of PAC1 mRNA expression.
Results: PACAP blood levels associate with PTSD symptoms in a cohort of highly traumatized females. Further, a single SNP in a putative estrogen response element within ADCYAP1R1, rs2267735, predicts PTSD diagnosis and symptoms in females only. This SNP also associates with fear discrimination and with ADCYAP1R1 messenger RNA expression in human brain. Methylation of ADCYAP1R1 in peripheral blood is also associated with PTSD. Complementing these human data, ADCYAP1R1 mRNA is induced with fear conditioning or estrogen replacement in rodent models.
Conclusions: These data suggest that perturbations in the PACAP-PAC1 pathway are involved in abnormal stress responses underlying PTSD. These sex-specific effects may occur via estrogen regulation of ADCYAP1R1. PACAP levels and ADCYAP1R1 SNPs may serve as useful biomarkers to further our mechanistic understanding of PTSD.
Disclosure: K. Ressler, Part 1: Extinction Pharmaceuticals: I am cofounder of this (virtual) company which exists to license d-cycloserine combined with psychotherapy for the treatment of anxiety disorders. I have received no financial support from Extinction Pharmaceuticals within the last 24 months. (note that nothing related to this relationship will be presented during this talk).

Cytosine Methylation and Expression of GR Related Genes in Association with PTSD Treatment Response

Rachel Yehuda*

MSSM/JJP VAMC, Bronx, USA

Background: This study sought to identify epigenetic and molecular mechanisms that may influence glucocorticoid alteration in PTSD, cytosine methylation of the human GR gene (NR3C1) and relevant transcription factors, and gene expression of the GR gene and FK506 binding protein 5 (FKBP506). These were measured in lymphocytes. All are influenced by environmental factors that can result in enduring differences in function, but we sought to determine if these molecular measures would change over time in association with symptom reduction following psychotherapy.
Methods: Cross-sectional observations of the above markers in association with PTSD and its psychological and biological risk factors in a sample of 90 combat veterans will be presented, followed by the results of repeated measures analyses before and after psychotherapy of PTSD in combat veterans.

Results: Though associated with enduring environmental risk factors such as childhood trauma, methylation of the GR gene in human lymphocytes can alter in response to therapy. Furthermore, there was a significant difference in the extent and pattern of methylation in psychotherapy responders when defined by stringent response criteria (i.e., no longer having PTSD). In responders, the degree of methylation per CpG methylated site decreased; whereas in non-responders methylation slightly increased ($F = 5.30$, $p = 0.034$). No changes in methylation could be detected using more liberal response criteria (e.g., a 12- or 25-point reduction in PTSD severity as measured by the Clinician Administered PTSD Scale).
Conclusions: This is the first study examining gene methylation and expression cross-sectionally in PTSD in association with treatment. It is also the first to provide an indication that molecular mechanisms associated with GR activity are involved in PTSD risk, pathophysiology, and resilience. Thus, the molecular biology of the GR should be considered a target for PTSD prevention and treatment.
Disclosure: R. Yehuda, Part 4: Eli Lilly Pharmaceutical Grant # F1D-US-X312 2008–2011 Glucococrticoid Dysregulation and Metabolic Syndrome in Patients with Schizophrenia Medicated with Second Generation Antipsychotics Role: PI.

Prospective Research in Military Cohorts: The Course of Stress-Related Biological Parameters in Response to Exposure to a War Zone

Eric Vermetten*

Military Mental Health/University Medical Center, Utrecht, Netherlands

Background: Development of PTSD symptoms is influenced by preexisting vulnerability factors. We prospectively determined trajectories of development of stress-related parameters and symptoms in response to deployment to a war zone. We focused on central, as well as peripheral, regulatory systems with a focus on amygdala function and activity of HPA-axis related parameters, in particular GR.
Methods: 1. Large, epidemiological study in which functional biological parameters were used in association with predicting PTSD prior to and 1 and 6 months after military deployment. 2. fMRI was used to assess long-term divergent influences on amygdala regulation in a cross-sectional study prior to and 3 and 12 months after deployment.
Results: 1. Before military deployment, GR number in PBMCs was significantly higher in the PTSD symptom group after deployment, relative to matched comparison subjects, and was maintained at 1 and 6 months after deployment. No group differences were observed in mRNA expression of GR subtypes and FKBP5. 2. Perceived threat leads to long-term divergent influences on amygdala regulation that may promote stress resilience in some and stress vulnerability in others. Amygdala reactivity appears to normalize after a period of severe stress, yet persistent changes in amygdala-dACC may continue to influence the response to subsequent stressors.
Conclusions: These studies have enhanced our ability to understand biological concomitants of risk, and demonstrate which risk factors might also be state-related. Preexisting high GR number in PBMCs was a vulnerability factor for subsequent development of PTSD symptoms. Combat stress also had sustained consequences on neural responsivity, in which appraisal of threat has a key role on amygdala-centered neural network.
Disclosure: E. Vermetten, None.

High Dose Hydrocortisone immediately After Trauma may alter the Trajectory of Posttraumatic Stress Disorder: Translational Interplay between Clinical and Animal Studies

Joseph Zohar*

Chaim Sheba Medical Center, Tel Hashomer, Israel

Background: The presentation focuses on translational research examining the clinical and morphological sequelae of administration of hydrocortisone immediately after trauma. A secondary prevention of PTSD, prospectively measured by CAPS via a single bolus of hydrocortisone given up to 6 hours after the exposure in patients with acute stress symptoms will be described, along with exploring the molecular trajectory of this technology in an animal model of stress-exposed animals.
Methods: The human study was a prospective double-blind placebo-controlled pilot study ($n = 24$). Follow-up included visits at 2 weeks, 1 month and 3 months, in which CAPS, VAS-Anxiety and VAS-Depression scores were recorded. The animal model examined the morphological arborization in dendrite gyrus in stress-exposed animals treated with hydrocortisone.
Results: Early single high-dose hydrocortisone intervention attenuated the core symptoms of both the acute stress and of subsequent PTSD in patients. Steroid-treated stressed animals displayed significantly increased dendritic growth and spine density, with increased levels of BDNF and obtunded PSD-95 levels.
Conclusions: These clinical and animal results suggest a potential value and potential mechanism of an early, single high-dose steroid as a treatment aimed at secondary prevention of stress-induced psychopathology.
Disclosure: J. Zohar, Part 1: Lundbeck - Grant support, Speaker honoraria Servier - Grant support, Consultancy fees Solvay/Abbot - Speaker honoraria Sanofi-Aventis - Consultancy fees Bristol-Myer-Squibb - Consultancy fees, Part 2: None, Part 3: None, Part 4: Lundbeck and Servier, Part 5: No.

Panel Session
Will We have New Drugs or Not? Addressing the Crisis in Neuropsychiatric Drug Discovery

Better Novel CNS Target Validation for Drug Development is Feasible

William Potter*

Philadelphia, USA

Background: The current state discourages investment in novel CNS drug development. Given costs and low success rates of recent agents alternative approaches to target selection and validation are needed to attract investment. More stringent criteria for selecting targets hold promise. These criteria rest on tools from basic and clinical neuroscience application of which will decrease clinical failure rates and focus efforts on higher probability target.
Methods: Methods: Genetic characterization, expression profiling, proteomics, metabolomics and receptor occupancy in CNS tissues can be coupled with assays of brain function (2DG, fMRI, EEG) and behavior. Degree of preclinical validation equals understanding of how these variables relate to one another. Target selection priority depends on extent of link between preclinical model/measures with those in human.
Results: Results: Target homology, receptor occupancy of an agent and its biochemical or functional effects from animals to

humans are only known for "me too" compounds. No novel agents (e.g. 5HT1A agonists, CRF1 antagonists,multiple drugs for Alzheimer's) that have failed in the clinic have met these or similar criteria.
Conclusions: Conclusions: Target selection based on a fuller understanding of biology coupled with requirements to show relevant drug effects in humans would have prevented most past failures. More rapid progress on target biology at preclinical and clinical levels requires more data sharing. To this end, standardization of methods across academia and industry with something approaching "open sourcing" of preclinical and clinical data can engage the field in a collaborative vs competitive effort to validate targets. Translational methods (PET, fMRI, EEG/MEG and MRS) provide a much firmer link between target specific drug action in animals and humans. Drug development should focus on finding the best molecule in terms of traditional pharmacologic attributes with target validation a shared risk across the field.
Disclosure: W. Potter, Part 1: Amgen Bristol Myers Squibb Elan En Vivo J & J Medavante Orasi Pfizer Roche Theravance, Part 2: Merck Stock, Part 3: N/A, Part 4: N/A, Part 5: Retired from Merck.

Who will Develop the Next Generation of Medications for Mental Illness? The NIMH Perspective
Thomas Insel*

NIMH, Bethesda, USA

Background: Current medications are necessary but not sufficient to reduce the burden of mental illnesses in this country. The time is right for a new generation of treatments based on (a) new molecular entities developed from an understanding of pathophysiology or (b) repurposing medications from careful clinical insights. As industry retreats from drug development for psychiatry, how can NIMH address the public health need for new and better medications?
Methods: NIH has created a new institute, the National Center to Advance Translational Science (NCATS), to study the discipline of translation, including new approaches to bridging the valley of death in drug development. NCATS will provide academic scientists with many of the tools, such as high throughput sequencing and medicinal chemistry, required for moving from target to treatment. Long before NCATS, NIMH was investing in drug development and repurposing of compounds. With industry in retreat, we believe that the best way to "turn the herd" is to demonstrate compelling opportunities for psychiatric medications.
Results: These opportunities can be found in several areas including (but not limited to) rapidly acting antidepressants, prosocial compounds for autism, combined therapies for psychotic illnesses, and early interventions for schizophrenia. Biomarkers will be an important guide to the next generation of treatments as we identify subgroups more responsive to specific interventions. But success will depend not only on what we do but how we do it. Standardization, integration, and sharing will help us to leverage investments for new discoveries of biomarkers and personalized treatments. Creating pre-competitive partnerships where data, methods, and compounds are shared will ensure the most efficient use of scarce resources. And including public participation in this process will improve public trust.
Conclusions: Scientific opportunity has never been better. As we focus on the most rigorous science, discoveries will lead to new treatments. But progress will require not only a change in our focus but a change in our culture.
Disclosure: T. Insel, None.

Taking Science Personally: A Non-Profit Research Foundation's Approach to Accelerating Therapeutic Development
Sohini Chowdhury*

The Michael J. Fox Foundation for Parkinson's Research, New York, USA

Background: The Michael J. Fox Foundation (MJFF) stands outside the traditional biomedical research system, yet sits at the hub of global Parkinson's research. Since its founding in 2000, MJFF has funded over $230 million in research. Acknowledging that the funds at its disposal are dwarfed by the resources available to government and industry, MJFF utilizes a de-risking strategy – invest to lower research risk and encourage ongoing investment in Parkinson's disease (PD) – to get the "biggest bang for its buck". In this session, MJFF will highlight several examples of innovative partnerships aimed at de-risking Parkinson's disease.
Methods: MJFF reviews more PD-specific grant proposals (~800 per year) than any other funder and has extensive outreach with hundreds of the world's top PD experts. Leveraging information we compile, we are able to assess the state of PD science and identify critical research roadblocks that can hinder on-going and/or new investment in PD. In recent years, MJFF has prioritized addressing the lack of critical research tools, such as biomarkers and animal models, and has built novel partnerships around tool development.
Results: Launched in 2010, the Parkinson's Progression Markers Initiative (PPMI) is a $40M five-year clinical study designed to identify progression markers for PD that can be used in clinical testing. PPMI is sponsored by MJFF and is jointly funded by MJFF and a consortium of industry partners. PPMI represents the first large-scale public-private-partnership in PD research. Also launched in 2010, MJFF has built an extensive Animal Models program with several partners aimed at addressing the issue of access to models. Working with partners, MJFF is generating and characterizing open source models and housing and distributing these models at-cost to academic and industry researchers. In this session, further detail will be provided on these and other novel collaborative research programs.
Conclusions: Unlike other stakeholders in the medical enterprise, MJFF has only one goal: find improved therapies or a cure for Parkinson's disease. Our mission and business model allows us to assume greater risk than other actors. As will be discussed, by working closely industry, academia and government and patients, MJFF can leverage its neutral platform to support, catalyze and fund research that is synergistic and complementary to drug development efforts undertaken by other groups.
Disclosure: S. Chowdhury, None.

Of Lazarus and Zombies: Looking for Life After Death in Discontinued Compounds
David Michelson*

Merck Research Laboratories, North Wales, USA

Background: One hypothesis about how to make more new drugs available for neuropsychiatric disorders holds that drug candidates aimed at novel targets are often insufficiently explored in the clinic by pharma, and that making such compounds more available to the academic and biotech communities would improve success rates, either through the use of improved methodologies to demonstrate effects in disorders where the drug has putatively failed, or by repurposing compounds to develop proof of concept in disorders other than those tested. A counter-position cites the law of diminishing returns to argue that expending scarce resources on compounds that have already failed at least once is most likely only to generate further failures. Unfortunately, to date, this discussion has been mostly conducted anecdotally, and absent

a systematic review of data that could support or refute either hypothesis.
Methods: Neuroscience is a highly prioritized therapeutic area for new drug discovery within Merck, and has brought forward many novel compounds into clinical development over the past 10 years. There is thus a sizeable database available to examine how compounds later discontinued were explored in the clinic, how adequately they tested their pharmacologic hypotheses, as well as how many were studied beyond their originally proposed indication with what success rate, either internally or by academic and/or biotech investigators working independently.
Results: In this presentation, we will review the experience with novel neuroscience compounds, with an emphasis on understanding the clinical testing strategy and methodology, the range of indications explored, the fate of compound, and the implications for the potential opportunity and probability of success related to further exploration.
Conclusions: A strategy aimed at extending clinical investigation of novel drugs beyond initial disease targets could yield important new therapies, but the probability of success for any individual molecule/mechanism is limited.
Disclosure: D. Michelson, Part 1: I am an empoloyee of Merck and Co., Part 2: Merck and Co., Part 3: Merck and Co., Part 4: N/A, Part 5: Merck and Co.

Panel Session
Epigenetic Modifications in Development, Aging and Mental Illness

Epigenetics and Gene Expression in the Human Brain
Mark Cookson*

Laboratory of Neurogenetics, Bethesda, USA

Background: Gene expression in the human brain is likely to be highly regulated due to the extreme biological complexity of the organ. To begin to address this, we have generated large scale datasets in a number of brain regions that include genotype, epigenetic marks (principally CpG methylation) and gene expression data with array based platforms.
Methods: We have analyzed these data in a series of studies, with progressively increasing numbers of samples, from $n = 150$ per brain region in our initial studies to $n \sim 400$ in the most recent versions. These data allow us to map gene expression as a quantitative trait relative to numbers of single nucleotide polymorphism variants within specific genomic regions.
Results: These expression quantitative trait loci (eQTLs) show that gene expression in the human brain can be genetically determined, with some relatively strong correlations ($R2 \sim 0.6$, $p < 10E\text{-}23$) for eQTLs that are shared across regions. We have also found significant mehtylation QTLs, again that are often shared across brain regions. Oneapplication of this data is in understanding how risk factor genes for age-related neurological diseases have proximal biological effects. For example, we have shown that variants at the MAPT locus on chromosome 17 increase lifetime risk of Parkinson's disease and are associated with increased expression of the gene encoding the neuronal microtubule binding protein tau. For other risk loci for Parkinson's, such as the HLA locus on Chr6, we can detect a significant signal for CpG methylation suggesting that genotype may modify the capacity of a gene to be expressed. We also have noted strong age-related effects in a subset of brain methylation markers. Both the expression and methylation datasets can be mined for associations with any disease or phenotype related genetic variant.
Conclusions: Ongoing studies include expansion of these dataset to more truly genomewide approaches, applying RNA sequencing (RNAseq) in the human brain to examine more complex events such as changes in RNA editing or splicing.
Disclosure: M. Cookson, None.

DNA Methylation Changes in Development and Schizophrenia
Barbara Lipska*

NIMH, Bethesda, USA

Background: DNA methylation is a critical process affecting gene expression during neurodevelopment. It has also been suggested to play a role in brain diseases. To elucidate the epigenetic signatures of the human dorsolateral prefrontal cortex (DLPFC), a brain region implicated in cognition and neuropsychiatric disorders, we conducted a genome-wide DNA methylation study across human brain development and aging and in patients with schizophrenia.
Methods: A genome-wide DNA methylation profiling using Illumina Infinium arrays (27,578 CpG dinucleotides spanning 14,495 genes) of the human DLPFC was conducted in a large cohort ($n = 269$) of well characterized specimens, including 30 fetal samples, combined with an analysis of genetic variance at $\sim$650,000 SNPs. Surrogate variable analysis was used to account for known and unknown factors, including batch effects. A general linear model was used to examine the effects of the primary variables: age, sex, race, diagnosis, diagnosis x sex, and smoking. The residuals were used to analyze associations with SNPs by PLINK.
Results: We found widespread epigenetic deregulation with aging. We observed high rates of methylation changes during the prenatal period (mostly decreases), whereas later in life changes were much slower and predominantly involved increases in methylation. Aberrant DNA methylation in schizophrenia was identified at 651 CpG sites (608 genes) and found to be more likely to occur at CpG sites outside CpG islands than in islands. A large number of cis- and trans- methylation quantitative trait loci (mQTL) (38,092 and 578 SNP-CpG pairs, respectively, at FDR < 0.05) were identified.
Conclusions: These results suggest that remarkable changes in DNA methylation occur during the 2nd trimester of gestation in the fetal prefrontal cortex and continue into old age. Altered DNA methylation may be involved in the pathophysiology of schizophrenia. A combination of genetic and epigenetic approaches will be useful to understand the molecular mechanism of this complex disorder.
Disclosure: B. Lipska, None.

Identifying Differentially Methylated Regions in Suicide Completers through Sequence Enrichment using MBD Protein and Next Generation Sequencing
Gustavo Turecki*

McGill University, Montreal, Canada

Background: Suicide is a complex and heterogeneous phenomenon. We have recently described a molecular subphenotype characterized by extreme low expression of astrocytic genes. In this study, we investigated the potential role of genomic DNA methylation in this subphenotype.
Methods: Prefrontal brain samples were screened using a combination of techniques to identify extreme low expressors (ELE) from 184 subjects. Of these, 21 (11.4%) suicides were ELE and were matched to 21 psychiatrically-unaffected controls according to gender, age, PMI and pH. Genomic DNA was sheared and methylated regions were isolated using methylated binding domain-2 (MBD) protein. Libraries were made using the Illumina ChIP-Seq library preparation protocol and each library used one

lane of Illumina GAIIx, 36 base pair single read sequencing. Using the DESeq software, a negative binomial test was implemented to obtain DMRs between suicides and controls. The most significant results were validated by EpiTyper.
Results: A total of ~250 million reads were produced per group and were used to assess differentially methylated regions (DMRs). Reads were trimmed, duplicates removed and the resulting data was mapped to the human genome (hg19) using BWA. The genome was tiled using overlapping 500bp windows at every 250bp and reads were counted for each 500bp interval. 989 DMRs passed multiple corrections using FDR. Within the top 20 most significant DMRs, sequences within or close to TXNRD3 ($P = 2.82E-07$), EFNB1($P = 9.80E-11$) and SLC25A18 ($P = 1.11E-06$) were among the most significant. The astrocytic associated solute carrier SLC25A18, a gene of particular interest given our approach, showed hypermethylation in suicide completers within 2 kb of the potential alternative promoter.
Conclusions: These results point to a large number of genomic DMRs that may play a role in the neurobiology of suicides. Several of these DMRs may help explain the ELE phenotype. Studies using functional models will be useful to characterize these findings.
Disclosure: G. Turecki, None.

Maturation of Prefrontal Cortex in Health and Disease- A Tale of Epigenomes in Transition

Schahram Akbarian*

University of Massachusetts Medical School, Worcester, USA

Background: Protracted development of human PFC, extending into or beyond the second decade, plays key role for normal human development and the neurobiology of schizophrenia and related disease. Little is known about the molecular mechanisms that fine-tune and regulate these developmentally regulated changes in gene expression and neuronal function. Here, we open up new perspectives to these long-standing questions and use novel approaches, including separate genome-wide profiling of neuronal and non-neuronal chromatin from postmortem PFC across the lifespan. Focusing on trimethyl-histone H3-lysine 4, a nucleosomal histone mark sharply enriched at transcription start sites and other regulatory sequences, we provide evidence for a highly regulated, "pre-programmed" remodeling of histone methylation landscapes in immature PFC neurons.
Methods: Neuronal nuclei from postmortem prefrontal cortex were immunotagged with NeuN antibody, and NeuN + and NeuN- nuclei sorted separately via fluorescence-activated "cell" (nuclei) sorting (FACS), and purified mononuclesomes enriched for H3K4me3 analyzed by massively parallel sequencing using an Illumina Solexa platform. In total more, neuronal H3K4me3 epigenomes were obtained from the PFC of 17 subjects from late prenatal to old (70yrs) of age, and compared to epigenomes of 16 subjects on the autism spectrum and 14 subjects with schizophrenia. In total, > 20 billion bp of H3K4me3-enriched sequence was analyzed.
Results: In comparison to input, promoter-associated sequences showed a 15–20 fold enrichment in the chromatin immunoprecipitates. Comparative analyses of various disease cohorts, including subjects diagnosed with schizophrenia or an autism spectrum disorder, reveals epigenetic dysregulation at hundreds of loci that are enriched for neurodevelopmental risk genes. These alterations, while highly variable between individuals, were particularly prominent in subjects on the autism spectrum. However, the large majority of diseased subjects did not show evidence for a generalized disruption of the developmentally regulated large scale chromatin remodeling that defines the normal PFC in early infancy.
Conclusions: Prefrontal neurons of subjects diagnosed with autism and related disease show epigenetic changes at hundreds of loci genome-wide, with considerable variability between subjects. There appears to be considerable overlap between genetic and epigenetic risk architectures.
Disclosure: S. Akbarian, None.

Panel Session
New Directions in Understanding the Neurocircuitry of Choice, Value, and Decision-Making

Contrasting Reward Signals in Orbitofrontal Cortex and Anterior Cingulate Cortex

Jon Wallis*

UC Berkeley, Berkeley, USA

Background: Over the last decade, converging evidence has shown the critical importance of orbitofrontal cortex (OFC) for valuation and decision-making. At the same time, neurons in other brain areas, notably anterior cingulate cortex (ACC), have been found to encode much of the same information as OFC neurons. A current challenge is to determine how these two populations are functionally distinct. I will discuss two lines of research that aimed to meet this challenge.
Methods: To address this challenge, we simultaneously recorded from OFC and ACC in awake, behaving monkeys while they performed simple decision-making tasks.
Results: In our first experiment, we examined whether the OFC and ACC differed with respect to the type of information that they were evaluating. Based on differences in the anatomical connections of the two areas, we hypothesized that ACC encoded value information as it related to internally-driven actions, while OFC encoded value information as it related to external stimuli in our environment. We found no evidence that this was the case: both areas were equally capable of encoding value information for actions or stimuli. Our second experiment examined whether the value signal itself differed between the areas. Two related value signals are the prediction (the value of what one expects to receive) and the prediction error (the difference between what one expects and what one actually receives). These two signals are typically highly correlated. However, we were able to show that OFC encoded predictions not prediction errors, while the reverse was true for ACC.
Conclusions: These results mark an important step in distinguishing different value signals in the frontal cortex.
Disclosure: J. Wallis, None.

The Neural Computation and Comparison of Values in Simple Choice

Antonio Rangel*

Caltech, Pasadena, USA

Background: A growing consensus in neuroscience suggests that the brain makes simple choices by assigning values to the options under considerations that are then compared to make a decision. This talk will describe recent human fMRI experiments showing how values are computed and compared in the orbitofrontal cortex at the time of choice, as well as the role that attention plays in such value signals.
Methods: The talk will describe the results of various studies using computational human fMRI, eye-tracking, and TMS.
Results: Recent human fMRI studies have shown that the medial orbitofrontal cortex (mOFC) encodes value signals at the time of decision making that seem to guide decision-making. These signals

are modulated by attention so that, at any given time, the mOFC encodes the relative value of attended minus unattended actions. Further work suggests that these relative value signals serve as the input to a value comparison process, implemented in a dynamic network that includes inferior parietal lobule and the dorsomedial prefrontal cortex, through which choices are made.
Conclusions: These results suggest that the human mOFC compute relative value signals that guide choices.
Disclosure: A. Rangel, None.

Human Ventral Striatal Neurons during a Gambling Task

Emad Eskandar*

Massachusetts General Hospital, Boston, USA

Background: The primary purpose is to present new data from human subjects illuminating the complex role of the ventral striatum during a gambling task with rewarding and aversive stimuli.
Methods: Single neurons were isolated from human subjects intra-operatively. Patients were undergoing deep brain stimulation surgery for the treatment of Major Depression or Obsessive Compulsive Disorder. As part of the surgery, we routinely obtain micro-electrode recordings. In these cases the subjects played a simple gambling task while were obtaining the recordings. The spikes were sorted and then analyzed after the surgery.
Results: Human ventral striatal neurons encode a number of variables. First, they convey information about the subjects' expectation of whether they will win or lose. Second they convey information about whether the subject actually won or lost. Third and most importantly, using receive operating curve analysis (ROC) we found that the neuronal responses actually predict whether the subject would make a conservative or a high risk choice well before the action is actually made. All the results were significant at ($P < 0.05$).
Conclusions: The ventral striatum encodes a complex array of information, regarding the subject's expection, rewarding and aversive stimuli, and predict their ultimate choice. Information at this level of detail would be impossible to obtain in any other way. Moroever, it give a more nuanced view of the ventral striatum and can illuminate its role in disorders such as depression, obsessive-compulsive disorder, and pathological gambling.
Disclosure: E. Eskandar, None.

Money, Value and Motivation in Cocaine Addiction: Unique Roles for the vmPFC, ACC, Striatum and Midbrain

Rita Goldstein*

Brookhaven National Lab, Upton, USA

Background: Adaptations of the reward circuit to intermittent and chronic supraphysiological stimulation by drugs increase reward thresholds. As a consequence, response to non-drug reinforcers in individuals with chronic drug use or addiction may be compromised. Clinical symptoms include anhedonia and compulsive drug use at the expense of the attainment of other rewarding experiences and despite detrimental consequences to the individual's functioning. While most addiction studies focus on this increased valuation of drug reward and drug-related cues, in this presentation we instead present studies in cocaine addicted individuals that target the mesolimbic and mesocortical dopaminergic circuit response to money, a secondarily generalizable reinforcer. Relevance to endogenous motivation (when behavior is not directly driven by monetary reward) is also explored.
Methods: A multimodal approach was used to study processing of monetary reward in individuals with cocaine use disorders (CUD) and matched healthy controls (HC). Imaging modalities included structural and functional MRI and recordings of event-related potentials (ERP, where we measured the P300, a component responsive to motivated attention/salience). Tasks targeted executive functions (e.g., inhibitory control) with or without monetary remuneration contingent on task performance. Self-reported ratings of monetary value and drug craving were also collected. Analyses included voxel based morphometry (VBM to assess neuronal structural integrity), whole brain functional activations, targeted region of interest analyses and cross-modality correlations.
Results: Our first fMRI study showed that sustained monetary reward was associated with a robust and complex neuronal activation pattern in the HC group (that encompassed the OFC, ACC, DLPFC but also midbrain). The CUD group did not display this complex pattern of activation to monetary reward, demonstrating either reduced regional fMRI signal in the between group analyses or less sensitivity to differences between monetary conditions in the within group analyses. These results of reduced cortical sensitivity to monetary reward were validated using ERPs where only HC showed a P300 response to reward vs. no reward as associated with respective behavioral changes (increased accuracy or faster speed of response to money). Importantly, a follow up study showed this cortical monetary sensitivity in HC (measured by the P300) was associated with structural integrity of the prefrontal cortical regions known to respond to reward (OFC, ACC, DLPFC, as measured with VBM). Again, CUD did not demonstrate such structure-function interdependence. Instead, hyperactivations to money in CUD were noted during a drug-cue task in the dorsal striatum as associated with deactivations in the vmPFC (with parallel structural results) and with enhanced cocaine over monetary wanting. Monetary gain on this task was predictive of midbrain recruitment during cognitive control breaking points, attesting to the importance of these results in understanding core deficits in sustained motivation in drug addiction.
Conclusions: Taken together, results suggest cortical hyposensitivity to monetary reward in drug addicted individuals as measured with both fMRI and ERP and associated with structural integrity of these regions. The opposite results for the dorsal striatum (putamen) suggest subcortical mechanisms that may be related to compulsive behaviors that override cortically-driven advantageous value assignment and decision making especially in a drug-related context. Combined, cortical hyposensitivity and subcortical hypersensitivity to a non-drug reward may underlie core deficits in drug addiction, encompassing the ability to sustain endogenous motivation, especially during cognitive control breaking points (e.g., during effort, stress or a salient drug-cue context). Future research should incorporate monetary loss to explore generalizability of results to negative reinforcement.
Disclosure: R. Goldstein, None.

Panel Session
A Convergence in Autism and Schizophrenia Genetics: The Conundrum of Shared Risks and Divergent Outcomes

Rare CNVs Reveal Genetic Overlap between Autism, Shizophrenia and Bipolar Disorder

Jonathan Sebat*

UCSD, La Jolla, USA

Background: I will present a series of new CNV findings in psychiatric disease, including unpublished family-based studies of bipolar disorder, schizophrenia and autism. This lecture will focus on the emerging genes and pathways implicated in mental illness with particular emphasis on the surprising number of genetic risk factors that shared between different psychiatric disorders.

Methods: Genome-wide scans of copy number variation were performed using a new high-resolution microarray platform consisting of 2.1 million probes. Data analysis was performed using custom software that we developed. CNVs that were associated with disease in case-control cohorts or in familes were validated by tiling resolution array CGH or FISH.
Results: Using a combination of genetic study designs, we have identified copy number variants (CNVs) that are associated with schizophrenia (SCZ). Using a family-based approach, we and others have observed a high rate of de novo copy number mutation in patients as compared with healthy controls. Using a case-control approach, we have identifiied multiple CNVs that confer high risk for schizophrenia, including large microduplications of 16p11.2 and microduplications of the Vasoactive intestinal Peptide Receptor-2 (VIPR2) on 7q36.3. We have applied similar approaches to related psychiatric disorders, including bipolar disorder (BD) and autism spectrum disorders (ASD).
Conclusions: Our findings suggest that virtually all CNVs that confer high risk of SCZ also confer risk for other psychiatric disorders. CNV Loci that overlap between ASD and SCZ include 1q21, 15q13, 16p11.2, NRXN1 and VIPR2. CNV Loci that overlap between SCZ and BD include 3q29 and 16p11.2. These results suggest that the phenotypic expressioin of CNVs is variable. Different clinical features can be observed in different individuals that carry that same mutation. Likewise, different clinical features can be observed in the same indivdual as a child and as an adult.
Disclosure: J. Sebat, None.

Large-Scale Follow up of Candidate Variants from Sequencing Schizophrenia, Epilepsy, and Autism Genomes

David Goldstein*

Duke University, Durham, USA

Background: One primary challenge in the interpretation of large-scale sequencing studies is the huge number of candidate variants that emerge. This occurs both because there are many functional variants in every sequenced genome and also because sequencing is associated with considerable artifact. One solution to this problem is large-scale follow up genotyping of candidate variants.
Methods: Here I describe an experiment in which 10,000 candidate variants are identified in over 200 patient genomes and genotyped in 7000 samples that are mixed between schizophrenia and epilepsy. The variants were selected on the basis of statistical enrichment in cases versus control and annotated function of the variants. In addition, any rare variants observed in regions of interested such as those corresponding to CNVs definitively associatd with disease were selected.
Results: Analyses are underway, but results will shed light on the advantages of performing large scale follow up genotyping of candidate variants.
Conclusions: Large scale follow up genotyping of candidate variants identified from sequencing studies is a effective approach to narrowing the number of variants with real association with the phenotype of interest.
Disclosure: D. Goldstein, None.

Findings from Number Variation and Whole Exome Sequencing in Autism Spectrum Disorders and the Overlap with Loci Implicated in Schizophrenia

Matthew State*

Yale University School of Medicine, New Haven, USA

Background: The presentation will provide new data on studies of copy number variation and whole exome sequencing in simplex autism spectrum disorders (ASD). The presentation will highlight the overlap of recent findings in ASD and schizophrenia.
Methods: Copy number variation studies were carried out on quartets from the Simons Simplex Collection (SSC), in which pedigrees consist of a single affected proband with ASD, an unaffected sibling snd two unaffected parents. The initial phase of the experiment involved 872 probands and 872 siblings evaluated with Illumina IM arrays. Phase 2 of the analysis consists of another 800 quartets hybridized on Illumina 2.5M probe arrays. In addition, whole exome sequencing was performed on 300 SSC quartets.
Results: Phase 1 analysis identified recurrent copy number variations at 16p11.2 and 7q11.23 associated with ASD at genome wide significance. 4 additional regions were found with both recurrent de novo CNVs and overlapping rare transmitted CNVs, a pattern not seen among controls. These regions implicate several previously identified in schizophrenia including 22q11, 1q21, and 15q13.2–13.3. Phase 2 genotyping involving another 1500 families is complete, analysis of structural variation is underway and CNV results will be presented. In addition whole exome sequencing has identified de novo sequence mutations in genes mapping within the 22q11 region and other loci previously implicated in schizophrenia.
Conclusions: Large scale studies of CNVs in ASD have led to highly reproducible findings, consistently implicating regions identified in studies of other neuropsychiatric and neurodevelopmental disorders, including schizophrenia. New data regarding this convergence of genetic risks will be presented along with a consideration of multiple alternative interpretations of these observations.
Disclosure: M. State, Part 1: I hold a patent with Yale regarding the contribution of CNTNAP2 to autism spectrum disorders licensed to Athena Diagnostics.

Connecting Genotype-Phenotype in Neurodevelopmental Disorders

Pat Levitt*

Keck School of Medicine of USC, Los Angeles, USA

Background: Genetic variants, both common and rare, are relevant to understanding pathogenesis of disorders by elucidating a biological understanding of their contribution to phenotypic disruptions. We study the function of the Met receptor tyrosine kinase, a gene carrying a 5′ promoter single nucleotide polymorphism (SNP) that increases risk for autism, autism-associated copy number variants, and SNPs reported for schizophrenia risk. Translational studies bring together a role for the Met receptor tyrosine kinase in developing synaptic architecture, a common theme in both autism and schizophrenia.
Methods: The signal-dead Met mutant mouse was analyzed for morphological disruption of dye-filled forebrain and for changes in local neocortical circuit connectivity using high resolution laser scanning photostimulation (LSPS) to uncage glutamate, generating a local input map to corticostriatal and corticopontine neurons. Statistical methods in neuroimaging genetics were applied to determine the relationship between the MET risk allele and brain activation patterns while viewing emotional faces.
Results: Elimining Met signaling results in reduced distal dendritic branching, spine volume increases, and a 2-fold increase in local connectivity between layer 2–3 and specific cortico-striatal output neurons of layer 5, but not cortico-pontine neurons. The neuroimaging studies show that the risk C allele has a dominant effect, correlating with atypical activation of non-relevant cortical regions, and altered activation of the amygdala.

Conclusions: Met is a new player at the developing synapse, regulating select aspects of neuronal morphology and developing local connectivity in the neocortex, which may be a potential shared etiology for autism and schizophrenia. The MET C allele correlates with abnormal circuit functioning in humans. The data indicate a new nerobiological foundation for the involvement of the MET receptor in neurodevelopmental risk through its role in synaptogenesis in specific forebrain circuits.
Disclosure: P. Levitt, Part 1: Pediatric Biosciences Puretech Bioventures.

Panel Session
Toward A Neuroimmune-Mediated Subtype of Autism Spectrum Disorders

Clinical Evidence for an Immune-Mediated Form of Autism
Christopher McDougle*

Indiana University School of Medicine, Indianapolis, USA

Background: The primary purpose of the presentation is to briefly review the clinical literature regarding immune involvement in autism spectrum disorders (ASDs). Results from a case-control study comparing the prevalence of autoimmune disorders in first- and second-degree relatives of probands with ASDs or an autoimmune disorder, as well as healthy controls will also be presented. Videotapes of two mothers of a child with autism that also have a number of relatives with autoimmune disorders will be shown. In addition, a videotape of a child with autism that received treatment with an immune modulating drug will be shown.
Methods: A literature review was conducted to identify articles published since 1943 that provide evidence for immune involvement in ASDs. For the case-control study, parents of child probands with ASDs or an autoimmune disorder or a healthy child ($N = 101$ probands per group) were asked to complete a 45-item questionnaire regarding which first- and second-degree relatives had received a diagnosis of specified autoimmune disorders. After obtaining voluntary written informed consent, two mothers of children with autism were videotaped to record their thoughts regarding familial autoimmunity as a contributing factor to their child's autism. After obtaining assent from a child with autism and voluntary written informed consent from her mother, a videotape of the patient, before and after treatment with an immune modulating drug, was obtained. Consent from all parties allows for showing the videotapes for educational purposes.
Results: There is evidence in the literature for immune involvement in ASDs dating back to Kanner's original description of the syndrome in 1943. The results of the case-control study show that the mean number of family members with an autoimmune disorder is significantly higher in those of parents of a child with an ASD ($1.87 +/- 1.6$) compared to those of parents of a child with an autoimmune disorder ($1.44 +/- 1.5$; $P = .03$) or a healthy child ($0.93 +/- 1.1$; $P = .000003$). Parents of children with ASDs were significantly more likely than parents of healthy children to have an autoimmune disorder ($\chi^2 = 10.97$; $P = .0009$). Among the various autoimmune disorders, hypothyroidism/Hashimoto's thyroiditis and rheumatic fever were more common in the families with children with ASDs than in the healthy control families. The videotapes illustrate that two mothers of children with autism that have a number of family members with autoimmune disorders attribute immune dysregulation to the etiology of their child's disorder. The videotape of the child with autism demonstrates that an immune modulating drug may result in improvement in symptoms of the disorder.
Conclusions: The results of the literature review, the case-control study and the videotapes of mothers of children with autism and a child treated with an immune modulating drug indicate that an immune-mediated form of autism may exist and that additional research in this area is necessary and warranted.
Disclosure: C. McDougle, Part 1: Bristol-Myers Squibb Co.: Consultant, Speakers Bureau, Research Grant Support; Forest Research Institute: Consultant; F. Hoffman LaRoche: Consultant, Part 2: Bristol-Myers Squibb Co., Part 3: Bristol-Myers Squibb Co., Part 4: Bristol-Myers Squibb Co., Part 5: N/A.

Modeling an Autism Risk Factor in Mice leads to Permanent Changes in the Immune System
Elaine Hsiao*

Caltech, Pasadena, USA

Background: While autism spectrum disorders (ASDs) are characterized by stereotyped behaviors and language and social deficits, recent studies highlight striking immune dysregulation in ASD individuals. Moreover, maternal infection is associated with increased incidence of ASD in the offspring. In a model of this risk factor, we inject pregnant mice with the double-stranded RNA, poly(I:C), to elicit maternal immune activation (MIA). This yields offspring with the mouse version of the cardinal behavioral abnormalities and neuropathology found in human ASD. We aim to elucidate the mechanism by which MIA leads to the development of ASD endophenotypes.
Methods: Pregnant C57BL/6J wildtype or IL-6 KO mice are injected with poly(I:C) or saline. Fetuses and placentas are examined by ELISA, Luminex, qPCR and immunohistochemistry. Adult offspring are tested in a series of behavioral paradigms, and evaluated for altered immunity by Luminex, in vitro stimulation assays and flow cytometry.
Results: By blocking its expression or function, our group determined that the cytokine IL-6 is an essential mediator of the effects of MIA on the fetus. Shortly after MIA, IL-6 is upregulated in the placenta and in subpopulations of neurons in the fetal brain. Maternally-derived IL-6 activates fetal placental cells and leads to altered IGFI, IGFBP3 and PLP levels. Adult MIA offspring display decreased T regulatory cells, hyperresponsive CD4+ T cells, altered immunophenotypes in the spleen and mesenteric lymph node, and altered cytokine profiles in the brain.
Conclusions: MIA induces endocrine changes in the placenta via IL-6. MIA offspring display permanent neural, peripheral and enteric immune dysregulation that is established in early postnatal life. Given the neuropathological, behavioral and immunological similarities of MIA offspring to human ASD, the MIA model is proving useful for exploring the mechanism of how maternal infection mediates the development of ASD-related endophenotypes.
Disclosure: E. Hsiao, None.

Gene Expression Signatures in Autism Spectrum Disorders
Louis Kunkel*

Children's Hospital Boston, Boston, USA

Background: Autism Spectrum Disorders (ASD) is a clinically and genetically heterogeneous group of disorders that are quite common within the human population. Large copy number variants and chromosomal rearrangements can be found in nearly 15% of ASD cases and another 5% of cases have inactivation mutations in a handful of genes. The remaining 80% of cases have genetic lesions in yet to be identified genes. These genetic lesions likely impact on key pathways in neuronal development and

should show marked changes in gene expression in the brains of children with ASD, as well as other tissues. The purpose is to test if gene expression changes occur in whole blood and can be used as a diagnostic test for ASD.
Methods: Peripheral blood RNA was prepared from 283 patients with ASD and 183 controls. The mRNA was converted to cDNA and labeled for hybridization to Affymetrix HG-U133 Plus 2.0 and Gene ST 1.0 arrays. A 245-gene prediction model with a sample cohort of 90 male patients with ASD and 55 age-matched controls was developed using a repeated cross-validation strategy. This prediction model was then used in the first and second validation sets of ASD patients and controls.
Results: The prediction model achieved 74% accuracy with the first validation set (Area Under the receiver operating characteristic Curve (AUC) 0.78, 95% confidence interval [CI], 0.73–0.84). The prediction model was further tested with the second validation dataset (AUC 0.80, 95% CI, 0.68–0.93). Within the predictor gene set, immune and neuronal signaling pathways were enriched. Neurotrophin signaling pathway was the most significant (hypergeometric test p-value 0.000018) among 16 significant pathways (p-value <0.05). There was clear perturbed immune signaling including chemokine signaling, notch signaling, and Fc epsilon RI-mediated signaling pathways.
Conclusions: Gene expression signatures may serve as integrated signals of genetic change, which can be used to develop a robust classifier across perturbed pathways in most patients. These results suggest that use of blood expression profiling for ASD might lead to a diagnostic test for these disorders.
Disclosure: L. Kunkel, Part 1: SynapDx has licensed the technology described from Childrens Hospital, Part 2: Consulting with SynapDx $30,000/yr, Part 3: Does not apply, Part 4: No grants from companies, Part 5: Not employed by pharmaceutical company.

Evidence for an Autoimmune Form of Autism

David Amaral*

UC Davis, Sacramento, USA

Background: There is now convincing evidence that a subset of children with autism have immune abnormalities. We have examined the blood of children with autism for the presence of brain-directed autoantibodies. Dr. Judy Van De Water has determined that 12% of women who have children with autism demonstrate autoantibodies to fetal brain proteins. To determine whether these may be pathogenic, we purified autoantibodies from the blood of women who have had children with autism and injected them into a nonhuman primate.
Methods: Immunohistochemistry was employed using sections from the brains of adult rhesus monkeys. We purified autoantibodies from the blood of women who have had children with autism or mothers of children who were typically developing. The IgG were injected into pregnant rhesus monkeys.
Results: We initially found that 22% of children with autism have autoantibodies that identify GABAergic neurons in tissue sections from macaque monkeys. These were not observed in typically developing children. In a replication study in younger children, similar patterns of immunoreactivity were observed. However, we found that an equivalent number of typically developing control children also demonstrated these antibodies. Interestingly, children with brain-directed autoantibodies, regardless of whether they had autism or not, scored significantly more poorly on a number of factors in the child behavior checklist. For the mother's autoantibody studies, we found that offspring from the monkeys treated with IgG from mothers of autistic children, but not mothers of typically developing children, developed profound stereotypies across several behavioral testing paradigms. We are now attempting to replicate this finding as well as extending our analysis using structural MRI and other behavioral testing paradigms.
Conclusions: These data add to the growing literature that a sizable subset of individuals with autism may have alterations of immune function.
Disclosure: D. Amaral, None.

Panel Session
Neurodevelopmental Pathology of Cortical Interneurons in Schizophrenia: Is it the Journey or the Destination that Matters?

Factors Determining Migratory Dynamics and Homing of Interneurons

Seong-Seng Tan*

The University of Melbourne, Parkville, Australia

Background: Interneurons undertake arduous journeys from their subcortical origins into the dorsal cortical wall. Apart from the long distances they have to travel, they encounter significant obstacles along the way including emerging axonal tracts and radially-migrating projection neurons. Above all, interneurons need to calibrate their speeds and directions in order to integrate synchronously with the waves of radially-migrating projecting neurons to produce properly-connected cortical circuits. Any abnormalities in their migratory capacity, or direction, speed, or final integration will lead to developmental neuropathology. This study will look at the factors that determine the migration and final destinations of interneurons in mouse models.
Methods: Live tracking of interneuron migration was performed using confocal video-imaging of cortical slices. Migrating neurons were imaged using fluorescent markers expressed by genetic reporter genes or following introduction of fluorescent markers by electroporation or retroviral infection. Birthdating of neurons was performed using BrdU, and interneuron subtypes identified using specific antibodies. To study the factors determining layer identity, interneurons were transplanted in utero into recipient cortices, and their fates followed using lineage markers.
Results: Interneurons have a number of distinguishing characteristics in their mode of migration. In addition, they have unique layering schedules although their integration into specific cortical layers appear to be cell-autonomous. While they appear to mimic aspects of projection neuron migration, there are certain unique features of interneuron migration that sets them apart, despite the sharing of a common migratory terrain.
Conclusions: This study demonstrates that interneuron migration is fraught with multiple opportunities for error and therefore vulnerable to neuropathology. Identifying and understanding the underlying forces that drive interneuron migration are important steps for preventing developmental abnormalities with psychiatric undertones.
Disclosure: S. Tan, None.

Excitatory Projection Neuron Subtypes Control the Distribution of Local Inhibitory Interneurons in the Cerebral Cortex

Paola Arlotta*

Harvard University, Boston, USA

Background: High-level cortical function including cognition, sensory perception and motor function relies on the coordinated assembly of local microcircuitry between glutamatergic projection neurons and GABAergic interneurons. The developmental events governing the building of the cortical microcircuitry are poorly

understood. We report that different subtypes of projection neurons differentially determine the laminar distribution of cortical interneurons, fundamentally affecting cortical activity.
Methods: We have examined the lamination of cortical interneurons in *Fezf2*$^{-/-}$ mice, where subcerebral projection neurons are replaced by another class of excitatory neurons, callosal projection neurons, within an otherwise normal cortex. In complementary gain-of-function experiments, we have used in vivo electroporation to experimentally generate ectopic populations of specific subtypes of excitatory neurons and have examined (i) whether projection neurons are sufficient to recruit cortical interneurons and (ii) whether the identity of the excitatory neurons generated determines the types of interneurons recruited.
Results: We find that in the *Fezf2*$^{-/-}$cortex, the exclusive absence of subcerebral projection neurons and their replacement by callosal projection neurons cause distinctly abnormal lamination of interneurons and altered GABAergic inhibition. We demonstrate that the experimental generation of either corticofugal neurons or callosal neurons below the cortex is sufficient to recruit cortical interneurons to these ectopic locations. Strikingly, the identity of the projection neurons generated determines the specific types of interneurons recruited.
Conclusions: These data demonstrate that in the neocortex individual populations of projection neurons cell-extrinsically control the laminar fate of interneurons and the assembly of local inhibitory circuitry.
Disclosure: P. Arlotta, None.

Postnatal Interneuron Development: Setting the Cellular Stage for Schizophrenia

Samantha Fung*

NeuRA & SRI, Sydney, Australia

Background: Primary Purpose: The onset of schizophrenia symptoms in late adolescence implies a neurodevelopmental trajectory for the disease and GABAergic deficits are widely replicated in post-mortem schizophrenia studies. However, the link between when the GABAergic inhibitory neurons mature in humans and when schizophrenia typically first strikes has not been made. Further, the developmental process involved in the GABAergic deficit has not been identified.
Methods: Using quantitative PCR, we examined mRNA expression of several interneuron markers across postnatal human prefrontal cortex development and in schizophrenia. We used immunohistochemistry for neuronal markers (NeuN, GAD-65, Dlx-1) and migration markers (PSA-NCAM and DCX) to study the white matter neurons in development and schizophrenia.
Results: Early in postnatal life, the human prefrontal cortex demonstrates dynamic expression of interneuron markers with genes following one of three general expression profiles: increasing (parvalbumin, cholecystokinin) or decreasing (somatostatin, calretinin, neuropeptide Y) in expression with most dramatic changes seen in the first few years of life before reaching a plateau; or increasing to peak expression in toddlers and then decreasing, following an inverted U-shaped pattern (calbindin, vasoactive intestinal peptide). The majority of these interneuron marker mRNAs were decreased in the dorsolateral prefrontal cortex of patients with schizophrenia, with the exception of calbindin, which was increased. In addition to the expected deficit in parvalbumin mRNA, we note that the largest reduction was for somatostatin mRNA (31%), expressed by dendrite-targeting subtype of interneurons. Interestingly, in our cohort of people with schizophrenia, we found that an increase in the density of interstitial white matter neurons was correlated with a decrease in somatostatin mRNA, suggesting that a deficit in neuron markers in grey matter may be related to the increased density of neurons in the white matter (including somatostatin + cells). Furthermore, we found neurons in the white matter to be positive for a variety of makers that implicates them as being immature GABAergic inhibitory interneurons (i.e. Dlx1 + , GAD-65 + , PSA-NCAM + and DCX +).
Conclusions: It appears that a heterogeneous population of interneurons is potentially dysfunctional in schizophrenia. The parvalbumin-containing interneuron subtype showed the most delayed developmental maturation reaching adult levels of expression just prior to adolescence, but this occurs on the background of other changes in interneuron markers throughout life. Our data suggest that altered neuronal migration of cortical inhibitory interneurons might contribute to the interneuron deficit in the grey matter found in people with schizophrenia.
Disclosure: S. Fung, None.

The Maturation of Neural Synchrony during Human Brain Development

Peter Uhlhaas*

MPI for Brain Research, Frankfurt, Germany

Background: Evidence has accumulated that points to the crucial role of synchronous oscillatory activity for coordinated brain functioning. Among the mechanisms underlying the generation of neural synchrony, precise inhbition mediated through GABAergic interneurons is of particular relevance, especially for the generation of oscillations in the beta/gamma-band range.
Despite the importance of neural synchrony for brain functions, little is known, however, about the course of synchronous oscillatory activity during the maturation of the cerebral cortex. The presentation summarizes the development of both task-related and resting-state EEG/MEG data during normal development from childhood to adolescence and relates changes in neural synchrony to maturation of GABAergic neurotransmission.
Methods: The development of synchronized, neural oscillations was investigated during perceptual integration in participants (N = 68) between 6–21 years with EEG. EEG-recordings were analysed for spectral power as well as for phase-synchronisation of induced oscillations. In a second study, visuo-spatial working memory (WM) was tested with MEG in a sample of n = 100 adolescent and adult participants (age range: 12–24 years). Estimates of sensor spectral power as well as a beamforming-technique was employed to locate the sources of oscillatory activity.
Results: The results showed profound, late-occurring modifcations in the amplitude and synchrony of neural oscillations during development. Specifically, the behavioural improvements in perceptual integration as well as visuo-spatial working memory during adolescence correlated with a strong increase in the amplitude of oscillations in the theta-, alpha and gamma-band range over frontal and parietal areas as well as with an increase in the precision of phase-synchrony in the theta- and beta-bands. Changes in task-related neural oscillations were accompanied by the maturation of resting-state activity. During adolescence, there was a reduction in amplitude of delta/theta-band oscillations while the amplitude of alpha- and gamma-band oscillations increased.
Conclusions: These data suggest close relations between the increase in neural synchrony and the maturation of functional networks during the transition from adolescence to adulthood. Specifically, full expression of precise temporal coding through neural synchrony is a late developmental phenomenon that only occurs during late brain maturation. The changes observed in neural synchrony parameters during adolescence are compatible with the contintued maturation of GABAergic neurotransmission as reflected in the changes of GABA A alpha 1/2-subunits and changes in dopamin D2-interneuron interactions.
Disclosure: P. Uhlhaas, None.

Wednesday, December 7, 2011.

Mini-Panel Session
Downstream Effects of Visual and Auditory Perceptual Impairment in Schizophrenia

To Find the Stream Follow the Waves: Neurophysiological Mechanisms of Downstream Dysfunction

Daniel Javitt*

Nathan Kline Institute, Orangeburg, USA

Background: Impairments in early auditory perception contribute to difficulties in prosodic processing, while deficits in early visual processing contribute to impairments in performance on tests of attention, working memory and object recognition. Mechanisms underlying early sensory deficits remain an area of active investigation, as do mechanisms by which early impairments lead to downstream effects.
Methods: Data will be presented from 2 studies investigating auditory and 1 study investigating visual processing dysfunction. In both sets of studies, behavioral, ERP and fMRI methods were used to investigate underlying impairments.
Results: Auditory studies investigated early sensory contributions to two aspects of social cognition: emotion perception and theory of mind. In behavioral experiments, patients showed reduced differentiation of responses to frequency modulated tones designed to elicit happy vs. sad vs. angry percepts. Deficits in FM tone identification correlated with deficits in both basic tone matching and higher-level emotion perception. In addition, patients showed deficits in mismatch negativity (MMN) generation at the level of both auditory cortex and amygdala to FM contour deviation. Parallel studies related these to deficits to impaired resting state connectivity. In the visual study, fMRI deficits were observed to magnocellularly bias visual stimuli, leading to pathway-specific deficits in feature attention. Deficits were associated with impaired reading ability, as well as impaired emotion processing, and with impaired modulation of ongoing rhythmic brain activity.
Conclusions: Early sensory deficits have become increasingly well documented over recent years. In addition to confirming prior reports of early sensory dysfunction, the present study demonstrates both neurophysiological and behavioral methods by which such deficits interfere with higher order brain functions, including effects at both the information transfer and oscillatory entrainment level.
Disclosure: D. Javitt, Part 1: Schering-Plough, Takeda, NPS pharma, Solvay, Sepracor, AstraZeneca, Pfizer, Cypress, Merck, Sunovion, Eli Lilly, BMS,Pfizer, Roche, Jazz, Promentis, Glytech, Part 2: Pfizer, Glytech, Part 3: Pfizer, Glytech, Part 4: Pfizer, Roche, Jazz, Part 5: No.

Effects of Visual Perceptual Organization Impairment on Later Cognitive Processing in Schizophrenia

Steven Silverstein*

UMDNJ-Robert Wood Johnson Medical School, Piscataway, USA

Background: This presentation will review the nature of impairments in visual perceptual organization in schizophrenia, and then discuss data and theory on its effects on later cognitive processing. The consequences that will be reviewed include problems with visual memory, face processing, and social cognition. Hypothesized links between reduced perceptual organization and delusion formation will also be noted.
Methods: A pattern recognition test, in which subjects had to determine, on each trial, whether the visual pattern they observed was novel or repeating, was used to explore the effects of stimulus grouping strength on the development of visual memory representations. A gender-discrimination task in which facial images were normal, or without low- or high-spatial frequency information, was used to explore the impact of global form information on face perception. Functional MRI data were collected during completion of this task, to identify regions of underactivity and compensatory overactivity during face processing in the different conditions. Potential links between impaired face processing and poor theory of mind ability were explored in 3 studies in which data from perceptual organization tasks were correlated with scores on face emotion identification and theory of mind measures.
Results: Perceptual organization impairments were related to impairments in the development of visual memory representations, face processing, facial affect recognition, and theory of mind. Functional MRI data suggest that underactivation in visual cortex regions subserving perceptual organization is associated with excess, perhaps compensatory, activity in higher regions dedicated to form processing.
Conclusions: These data suggest that visual perceptual organization impairments in schizophrenia are significant contributors to other impairments that are typically reported. They highlight the role of problems in intermediate-level visual functioning for attention, memory, face processing and social cognition. Perceptual organization may also be associated with delusion formation, as patients try to make sense of the change in their visual experiences. This possibility, and future directions for exploring the downstream effects of perceptual organization impairments in schizophrenia, will be discussed.
Disclosure: S. Silverstein, Part 1: I received grant funds from AstraZeneca from 2007–2011., Part 2: None, Part 3: None, Part 4: I received grant funds from AstraZeneca from 2007–2011., Part 5: N/A.

Downstream Ripples of Impaired Perceptual Processing in Schizophrenia

Michael Green*

UCLA, Los Angeles, USA

Background: There has been considerable progress in understanding the nature of perceptual abnormalities in schizophrenia, but few efforts to elucidate the downstream effects of these abnormalities on social cognition, negative symptoms and measures of functioning.
Methods: Data will be presented from three studies of visual processing and one study of auditory processing in schizophrenia. Methods include EEG and performance measures.
Results: Study 1 included a large sample of schizophrenia patients who received early stage (object formation), and later stage (4-dot) masking tasks. Structural equation modeling analyses showed good model fit when the visual masking tasks were connected to social cognition, and connected to negative symptoms indirectly through defeatist beliefs. Study 2 includes preliminary data from an EEG measure of steady-state adaptation of objects, which examines the degree to which a neural response increases from seeing the same object repeatedly to seeing two different objects alternated. Patients showed less differentiation between repeated and alternated objects and the degree of adaptation correlated with negative symptoms. For study 3, the Bubbles Task was used to isolate visual information needed to recognize emotion in faces. Patients used high spatial frequency information from the eyes significantly less often

than controls when identifying fear. Among the patients, those who utilized the eye region more (i.e. more like controls) performed better on measures of social perception and emotion perception. Study 4 examined the effects of mismatch negativity (MMN) on social cognition and functioning. We replicated findings of MMN abnormalities in schizophrenia and found that patients with more normal MMN showed better social perception and community functioning.
Conclusions: The results support the importance of downstream effects of visual and auditory processing in schizophrenia for social cognition, negative symptoms, and functional outcome.
Disclosure: M. Green, Part 1: Consultant to: Abbott Laboratories, Cypress, Dainippon Sumitomo Pharma, Lundbeck, Sanofi-Aventis, Takeda, and Teva Speaker for: Janssen Cilag, Otsuka, and Sunovion.

Mini-Panel Session
GABA, Glutamate and Neural Synchrony in Schizophrenia

Increasing Signal to Noise Ratio through Modulation of GABAB Receptors
Steven Siegel*

University of Pennsylvania, Philadelphia, USA

Background: To evaluate GABAB and GABAAα2/3/5 agonists as potential therapeutic agents for treatment-resistant negative (social deficits) and cognitive (working memory) impairments in mouse models of NMDA receptor hypofunction.
Methods: Our animal model consisted of mice with constitutive reductions in NMDAR1 ($NR1^{-/-}$) that have previously been demonstrated to display reductions in GABA-mediated inhibition (Halene *et al.*, 2009). Mice were tested for electrophysiological and behavioral abnormalities relevant to schizophrenia. Electrophysiological measures focused on background and auditory evoked gamma oscillations. Behavioral measures include social interactions, working memory (T-maze), prepulse inhibition of startle and locomotor activity. The effects of the GABAB agonist baclofen, and the GABAAα2/3/5 agonist L-838,417 were then tested for all measures. Additionally, the effects of risperidone were evaluated to assess negative predictive validity for treatment resistant working memory and social domains.
Results: $NR1^{-/-}$ mice demonstrate increased background and reduced evoked gamma activity as well as social, working memory and PPI deficits with hyperactivity. Baclofen, but not risperidone or L-838,417, reversed elevated baseline gamma-band activity and improved gamma signal-to-noise. Additionally, baclofen, but not risperidone or L-838,417 reversed social and working memory deficits. Baseline gamma-band activity was highly predictive of social and cognitive outcomes across $NR1^{-/-}$ and WT mice. Baclofen reversed PPI deficits and locomotor hyperactivity, although some these effects were seen with the other drugs as well.
Conclusions: The GABAB agonist baclofen was the only agent that was effective in reversing social and cognitive deficits following constitutive NMDA hypofunction. The GABAAα2/3/5 agonist L-838,417 was not effective in reversing such alterations, consistent with recent negative results from a large clinical trial using a similar compound in schizophrenia. Similarly, risperidone fulfilled negative predictive validity for lack of clinical efficacy on social and cognitive domains. These data suggest that GABAB agonists may have significant benefit for negative symptoms and cognitive deficits in schizophrenia.
Disclosure: S. Siegel, Part 1: NuPathe, Merck, Part 2: NuPathe, Merck, Part 3: Nupathe.

Glutamatergic Dysfunction in Schizophrenia: A Chemical Shift Imaging and Single Voxel H-MRS Study
Juan Bustillo*

University of New Mexico, Albuquerque, USA

Background: Glutamate-related excitotoxicity in schizophrenia (Sz) can be assessed in-vivo with proton magnetic resonance spectroscopy (H-MRS). H-MRS studies report reduced NAAc (a marker of neuronal viability) in mainly white or mixed gray/white matter; however, abnormalities in glutamate (Glu), glutamine (Gln; the metabolite of synaptic Glu) have not been consistently documented. Limitations in this H-MRS literature include poor spectral resolution (especially for Glu and Gln), reduced spatial coverage (for all metabolites) and small samples. We used chemical shift imaging (CSI; to improve coverage) and single-voxel H-MRS (to improve Glu and Gln resolution).
Methods: Data was acquired with a 3T Siemens Trio scanner. For CSI an axial tissue slab parallel to AC-PC and above the lateral ventricles was acquired with PRESS (TE = 40ms, TR = 1500 ms, slice thickness = 15 mm, FOV = 220 x 220 mm). For SV H-MRS a 12 cc voxel was placed superior to the anterior corpus callosum and acquired with PRESS TR/TE = 1500/40 msec. All voxels were partial volume/relaxation corrected for gray (GM), white matter (WM)and CSF composition.
Results: 54 Sz and 50 HCs were studied. For the CSI data, the Sz group had increased Glu + Gln (Glx) in the anterior medial frontal GM ($F(5, 438) = 2.6$, $p = 0.02$). For NAAc, there was a Group-xAgexTissue interaction ($F(1, 4679) = 5.9$, $p = 0.01$) consistent with WM NAAc reductions with age in the Sz group. These were mainly found in bilateral frontal WM. For the SV data, there were no group differences in Glu or Gln.
Conclusions: Elevations of medial frontal GM Glx are consistent with the NMDA hypo function model of schizophrenia which postulates a paradoxical increase in glutamate with resultant excitotoxicity (Olney *et al.*, 1999). Reduced frontal WM NAAc in older Sz subjects is consisted with an active process of axonal deterioration, perhaps a downstream effect of the glutamate-driven cortical process. Early use of agents that modulate a hyperglutamatergic state may prevent clinical deterioration in Sz.
Disclosure: J. Bustillo, Part 1: Member of a Data Safety Monitoring Board for a Novartis drug trial. Paid hourly for participation, Part 2: none, Part 3: none, Part 4: None, Part 5: N/A.

GABA and Glutamate-Glutamine in Schizophrenia Measured with Proton Magnetic Resonance Spectroscopy
Lawrence Kegeles*

Columbia University and the New York State Psychiatric Institute, New York, USA

Background: Postmortem studies have indicated abnormalities in GABA in prefrontal cortex in schizophrenia. The glutamate (Glu) N-methyl-d-aspartate receptor hypofunction hypothesis suggests abnormal brain Glu levels. These transmitters are thought to play a role in cognitive impairments in the illness. Our purpose was to measure GABA and glutamate-glutamine (Glx) levels in vivo in two prefrontal brain regions in medicated and unmedicated patients with schizophrenia and healthy control subjects.
Methods: Thirty-two patients (16 unmedicated and 16 treated with second-generation antipsychotic medications) and 22 matched healthy control subjects participated in MRS scans using a 3T GE Signa system and the J-edited spin-echo difference method with an 8-channel phased-array head coil. GABA and Glx were measured in the dorsolateral prefrontal cortex (DLPFC) and medial prefrontal cortex (MPFC) in each subject and quantified as the

ratio to the simultaneously acquired water signal within each voxel. Working memory was assessed with the n-back test.
Results: Regionally selective alterations were found in both GABA and Glx in the unmedicated but not the medicated patients. In the MPFC region, greater than 30% elevations were found in GABA ($p = 0.01$) and Glx ($p = 0.02$) levels in unmedicated patients compared to controls. There were no differences in the DLPFC, and medication-naïve patients did not differ from previously treated, unmedicated patients. GABA and Glx did not correlate with n-back performance.
Conclusions: These findings indicate a regionally selective baseline elevation in GABA and Glx in unmedicated patients. The Glx findings are in line with prior MRS studies in unmedicated and minimally medicated patients. The GABA findings are in contrast with expectations from postmortem data. MRS may detect GABA elevations rather than the deficits suggested by postmortem studies because the more global MRS measurement includes contributions from all interneuron subtypes. In summary, these findings suggest baseline GABA and Glx elevations in schizophrenia that may normalize with medication treatment.
Disclosure: L. Kegeles, Part 1: Amgen, research contract Pfizer, research contract, Part 2: Columbia University The New York State Psychiatric Institute, Part 3: None, Part 4: Amgen, research contract Pfizer, research contract.

Panel Session
Novel Functions of Prefrontal Cortex Regions in Motivated Behavior: Implication for Psychiatric Disorders

Effects of Cocaine Use on The Role of Orbitofrontal Cortex in Learning in Response to Violations In Reward Expectation

Geoffrey Schoenbaum*

University of Maryland School of Medicine, Baltimore, USA

Background: The orbitofrontal cortex has long been implicated in flexible behavior. Historically this has been viewed as reflecting a role in rapid, flexible recoding of new information *within* orbitofrontal cortex. However we have recently suggested that the involvement of orbitofrontal cortex in supporting flexible behavior may instead be due to this areas importance for facilitating rapid recoding of associative information elsewhere, through its contribution to error signalling mechanisms in the brain[1]. Here I will briefly review this evidence and also outline new work showing that orbitofrontal input regarding expected outcomes is necessary for error signalling by midbrain dopamine neurons.
Methods: Specifically we will use a Pavlovian over-expectation task to show that the orbitofrontal cortex is necessary for learning rather than using information[2]. This role will be linked to single unit recording data in the critical compound training phase of the task, showing that orbitofrontal neurons sum expectations for reward[3]. Further data will be presented indicating that this summed expectation is required for appropriate negative error signalling in midbrain dopamine neuron[4,5], providing a potential mechanism whereby estimates of expected reward signed by orbitofrontal cortex might facilitate extinction of previously acquired associative representations in other areas. Finally we will demonstrate that this function of orbitofrontal cortex is disrupted in rats that have a history of cocaine use[6]. Rats trained to self-administer cocaine over a month previously learned normally but failed to extinguish this learning as a result of over-expectation. These same rats extinguished normally in response to reward omission, suggesting a specific deficit in extinction when that learning relies on violations of estimates of expected rewards. Currently ongoing recording work will seek to confirm this deficit.
Results: See above.
Conclusions: These data indicate that orbitofrontal output is necessary for defining the expectancies used by dopamine neurons to generate reward prediction errors for instantiating learning. Further they suggest that this specific function is disrupted by experience using cocaine.
Disclosure: G. Schoenbaum, None.

When The Prefrontal Off Switch Is Broken: How Does Extinction Occur?

Jamie Peters*

VU University Medical Center, Amsterdam, Netherlands

Background: My presentation will focus on the neuropharmacological basis of extinction memory in both aversive (conditioned fear) and appetitive (self-administration/addiction) models. Extinction of conditioned fear requires NMDA receptor-mediated plasticity within the infralimbic medial prefrontal cortex (mPFC), as well as a BDNF input from the hippocampus. The extent to which these neural mechanisms of extinction overlap with those for drugs of abuse will be discussed with particular emphasis on the role of mPFC as a putative OFF switch for behavior.
Methods: Pharmacological agents were microinjected into discrete brain regions at various critical time-points in the models to dissect the neuropharmacology of the extinction memory circuits for fear and addiction. Endogenous BDNF protein levels were determined by ELISA.
Results: Extinction memory in both fear and addiction models requires NMDA receptor-mediated memory formation. The infralimbic mPFC is a critical locus in the extinction circuit for fear, and similarly controls expression of an extinction memory for cocaine. Heroin extinction appears to be unique in that it does not involve the infralimbic cortex, suggesting a broken OFF switch for behavior in mPFC. Yet preliminary data suggest a common role for hippocampal BDNF in turning OFF both fear and drug-seeking behaviors.
Conclusions: Extinction is a classic example of a prefrontal-dependent memory process that results in the turning OFF of a conditioned behavior, be it fear or drug seeking. Heroin self-administration appears to "break" this prefrontal OFF switch, and yet extinction can still occur. The hippocampus provides a critical BDNF input to mPFC for turning OFF behavior. Identifying where BDNF is working in the circuit when mPFC is broken will provide critical insight into alternative mechanisms of extinction and broaden the therapeutic spectrum for the application of pharmaceuticals that can enhance extinction memory in the clinic.
Disclosure: J. Peters, None.

Context-Induced Relapse To Heroin Seeking Is Controlled By Selectively Activated Neurons In Ventral But Not Dorsal Medial Prefrontal Cortex

Yavin Shaham*

IRP-NIDA, Baltimore, USA

Background: Peters, Kalivas, and Quirk (2009) proposed a dichotomy in medial prefrontal cortex (mPFC) function: dorsal (prelimbic, cingulate sub-regions) mPFC promotes fear expression and relapse to drug seeking, while ventral (infralimbic region) mPFC inhibits fear expression and drug seeking. This hypothesis was primarily based on fear extinction studies and extinction-reinstatement studies in cocaine-experienced rats. Our recent studies indicate that this dichotomy does not generalize to context-induced relapse to heroin seeking, which is controlled

by a minority of selectively activated neurons in ventral but not dorsal mPFC.
Methods: We used an established procedure (developed in our laboratory) of context-induced reinstatement of heroin seeking in combination with Fos immunohistochemistry, intracranial injections of muscimol and baclofen (to inhibit the majority of mPFC neurons), and a novel Dauno2 inactivation method (to selectively inhibit a minority of mPFC neurons activated by the heroin-associated context).
Results: We first found that while context-induced reinstatement of heroin seeking was associated with increased Fos (a neuronal activity marker) expression in both ventral and dorsal mPFC, reversible inactivation of ventral but not dorsal mPFC with muscimol + baclofen decreased context-induced reinstatement. We then used a novel pharmacogenetic inactivation method (cfos-lacZ transgenic rats injected with the pro-drug Dauno2 to selectively inactivate Fos-activated neurons in a behavioral task) to demonstrate that context-induced reinstatement of heroin seeking is controlled by a small subset of sparsely distributed selectively activated ventral mPFC neurons (Bossert *et al*. Nat Neurosci, 2011). I will present this study and data from an ongoing study in which we have been using an anatomical asymmetrical reversible inactivation procedure in combination with a FluroGold retrograde tracing + Fos activation procedure to study the role of ventral mPFC to nucleus accumbens shell projection in context-induced relapse to heroin seeking.
Conclusions: I will conclude the lecture by speculating on potential reasons for anatomical dissociations between circuits controlling cocaine seeking (and fear) versus heroin seeking.
Disclosure: Y. Shaham, None.

Cross-Cortical Phase Synchrony Between the Medial Prefrontal Cortex and Anterior Cingulate Cortex during Stimulus Expectancy

Bita Moghaddam*

University of Pittsburgh, Pittsburgh, USA

Background: Dynamic interaction between prefrontal cortex (PFC) sub-regions may be critical for executing goal-directed behavior and generating supporting cognitive processes, such as top-down attention. A potential physiological mechanism that enhances this interaction is cross-cortical spike and field potential phase synchrony during behaviorally relevant events. Based on the theory that PFC sub-regions communicate, rather than operate as distinct modules, we hypothesized that during a reward driven attention task, spike-field phase synchrony between the ACC and mPFC would be observed, and that this synchrony would be reduced on trials with a behavioral error.
Methods: During a reward-driven attention task, we recorded neuron spiking and local field potentials (LFP) from two rodent PFC sub-regions that are critical for decision-making and top-down attention, the anterior cingulate cortex and medial PFC. We focused our analysis on the pre-stimulus period of an attention task when a preparatory top-down bias is generated.
Results: During the pre-stimulus period of an attention task, we observed cross-cortical phase synchrony between spikes recorded in one PFC sub-region and LFP recorded in the other sub-region. Pre-stimulus synchrony was reduced on trials with a subsequent incorrect choice.
Conclusions: These data demonstrate that dynamic interactions across the PFC are necessary for top-down attention and goal-directed behavior. The findings suggest a mechanism for dysfunctional cortical connectivity observed in cognitive disorders such as schizophrenia and ADHD.
Disclosure: B. Moghaddam, None.

Panel Session
The Development of Novel Pain Therapeutics: New Strategies to Overcome Drug Discovery Barriers

Identifying Mechanisms Underlying Affective Components of Pain and Pain Relief in Rodents to Promote Discovery of New Therapies

Frank Porreca*

University of Arizona, Tucson, USA

Background: Preclinical evaluation of "pain" has relied on assessments of reflex behavioral responses to evoked stimuli. Pain differs from other sensations in that it is unpleasant at threshold. This dimension of pain has not been effectively captured preclinically and has been a major impediment to discovery of new therapies. These studies have explored mechanisms mediating and maintaining unpleasantness of pain and pain relief (PR).
Methods: Pain produces an aversive state that elicits motivation to seek relief. Relief of pain in humans is rewarding. We paired a context with PR in rats to elicit negative reinforcement and conditioned place preference (CPP). Aversiveness of pain is integrated partially in the rostral anterior cingulate cortex (rACC) and requires PKMz, a kinase that remains autonomously active once translated. PKMz is sufficient to establish late long-term potentiation (LTP) and long term memory.
Results: PR produced CPP and activated TH-positive cells in the ventral tegmental area (VTA) only in injured rats. Inactivation of the VTA blocked PR-induced CPP. Lesion of the rACC prevented PR-induced CPP without altering evoked thresholds. Increased inhibition (morphine, GABA-A agonist) elicited CPP selectively in injured rats but did not affect evoked responses. rACC D1 antagonist blocked PR-induced CPP. Inhibition of PKMz in the rACC did not affect sensory thresholds but produced a time-related reversal of PR-induced CPP in rats with chronic pain.
Conclusions: Clinically important affective dimensions of pain can now be measured and separated from sensory thresholds in rodents. Drugs that increase inhibition within the rACC (e.g. morphine) may produce PR in man primarily by modulating aversiveness. PR activates the reward circuit and likely promotes inhibition within the rACC via D1 receptors. Pain engages PKMz-dependent LTP within the rACC. While inhibition of PKMz and LTP can erase established memory, PKMz-dependent LTP may be reset by sustained afferent drive in chronic pain.
Disclosure: F. Porreca, Part 1: NeurAxon, Grunenthal, Part 4: NeurAxon, Grunenthal.

Imaging Opioid Effects on the Brain - from Preclinical to Postclinical

David Borsook*

Harvard University, Boston, USA

Background: CNS analgesic development is costly and inefficient in producing useful analgesics for chronic pain, and most CNS drugs fail in clinical development. Recent advances in functional imaging have allowed for more objective methods for evaluating or predicting drug efficacy.
Methods: To demonstrate the potential utility of imaging in drug development we used MRI to: (1) show the ability of imaging to define differences in opioids (on direct drug actions on the brain (pharmacological MRI); (2) demonstrate that such actions involve brain regions predicting analgesic as well as side effects; (3) demonstrate that small numbers of subjects can be used to evaluate drugs; (4) demonstrate that similar patterns of brain

activation can be observed across species, offering a unique "language of translation"; and (5) demonstrate that chronic use of prescription opioids may have deleterious effects on brain systems.
Results: For the direct effects of drugs, healthy male volunteers we administered 4–5 mg/70 kg of various opioids in a cross-over placebo controlled design. We compared differences in brain activations not only to the specific drug but also to manipulation of drugs that have multiple receptor targets (e.g., nalbuphine) where a small dose of naloxone can help dissect apart the drugs action. In rats, similar brain activations are observed for buprenorphine. For chronic opioids, significant changes in functional connectivity and brain structure (gray and white matter) that may provide insights into how opioids produce potential resistance to other analgesics.
Conclusions: Functional imaging provides insights into CNS processes on drug effects on CNS function. The potential for this emerging technology to be used as a method for defining biomarkers for pain and analgesia. The hope is that the successful identification of functional brain "signatures" for both drug action (analgesia) and disease state (chronic pain), when qualified, could provide objective biomarkers as a guide for drug development and clinical practice.
Disclosure: F. Porreca, None.

Neuroimaging as a Tool to Predict Analgesic Efficacy in Chronic Pain Patients and Determine the Significance of Expectation in Clinical Trial Design

Irene Tracey*

University of Oxford, Oxford, United Kingdom

Background: We desperately need novel approaches in patients that can better determine the potential for a new drug to succeed in the clinic. Neuroimaging is one such approach; it is sensitive to changes in neural processing relevant to a person's pain and relief, and as such can aid "go-no go" decisions at the transition from preclinical to clinical.
Methods: A small cohort of neuropathic pain patients were recruited (N = 16) into a double-blind, randomised study where they were given placebo, pregabalin or tramadol. Clinical measures and pain diaries were kept. All patients came for three neuroimaging sessions, where we evoked painful dynamic mechanical allodynia and measured the neuronal response. In a second study, healthy controls were given intravenous remifentanil and painful thermal stimuli during three manipulations of expection: none (i.e. hidden infusion), positive (i.e. open infusion), and negative (i.e. told infusion was stopped when it had not), while simultaneously undergoing neuroimaging and pain ratings.
Results: In the first study and prior to unblinding, we were not able to predict from the behavioural nor clinical measures whether patients were on placebo or drug, but from the neuroimaging data we successfully predicted when patients were on pregabalin, tramadol or placebo.
In the second study, the analgesia induced by intravenous infusion of remifentanil on thermal heat pain doubled in the "open infusion" compared to "hidden infusion", but during negative expectation pain ratings returned to baseline pre-drug levels. Neuroimaging data confirmed it wasn't report bias and allowed us to determine the neural mechanisms underpinning these powerful modulations.
Conclusions: By using neuroimaging data, we were able to distinguish drugs likely to work in the clinic from placebos, supporting the notion that neuroimaging should be incorporated as part of the analgesic drug discovery process. Further, our study examining the role of expectation highlights its significance for clinical trial designs.
Disclosure: I. Tracey, Part 1: Honorarium for educational talks and advisory board work from: Pfizer, Lilly, Grunenthal, Part 2: N/A, Part 3: N/A, Part 4: Non-restricted research grants from Pfizer and IMI consortium, Part 5: N/A.

Overcoming Scientific and Structural Barriers to Discovery of Therapies for Pain

Chas Bountra*

University of Oxford, SGC, Oxford, United Kingdom

Background: Discovery of pain therapies has been impeded for both scientific and structural reasons. Scientifically, we have limited understanding of mechanisms driving pain in heterogenous populations of patients or the influence of co-morbidities that negatively affect clinical trials. Early life experience elicits dramatic and persistent changes in expression of cassettes of genes influencing pain. Pharmacological modulation of epigenetic mediators is more likely to yield novel therapeutics than targeting a single gene product. However, exploration of this biology is limited by a lack of reagents, i.e., antibodies that recognise chromatin marks and selective inhibitors that prevent or reverse them. Structurally, more than 90% of targets fail in phase II proof of concept trials. We have created a public private partnership (PPP) to allow precompetitive generation of inhibitors and antibodies and are planning to take optimised assets into Phase II for clinical validation of novel targets.
Methods: Potent and selective inhibitors of epigenetic proteins have been made through 3D structure revealed by X-ray crystallography, structure-based drug design, and high-throughput and fragment-based screening.
Results: We have solved the structures of nearly 100 proteins (i.e., about a quarter of all known epigenetic proteins) and have produced potent and selective inhibitors of a bromodomain protein (BET4) and a methyl-transferase (SET7). These inhibitors are freely available without intellectual property (IP) barriers; all data are published. The BET inhibitor is effective in patient derived tumour cell lines and is being evaluated in neuronal hypersensitivity assays. Governments, charities and multiple pharma companies have contributed more than £30M to to develop probes to dissect disease pathways, including assessing their role in pain and inflammation precompetitively.
Conclusions: The environment, stress, inflammation, and injury elicit chromatin changes that influence chronic pain. Selective pharmacological tools have been developed that will catalyse understanding of how injury can lead to chronic pain. Probes are shared without competitive barriers to enable validation of more targets in phase II.
Disclosure: C. Bountra, Part 1: Grunenthal and Spinifex, Part 2: None, Part 3: None, Part 4: Grants from GSK, Novartis, Merck, Pfizer, Lilly, Part 5: No.

Panel Session
Neuroactive Cytokines: Critical Therapeutic Targets for Depression and Treatment Resistant Depression?

The Role of TNFalpha in Synaptic Scaling

Robert Malenka*

Stanford University, Stanford, USA

Background: Synaptic scaling is a homeostatic modification of synaptic efficacy used by neurons to adaptively moderate their excitability. I will review evidence that the increases in synaptic

strength at excitatory synapses during synaptic scaling are due to TNFalpha released from glia.
Methods: Cultured hippocampal neurons were pharmacologically silenced for two days and assays of excitatory synaptic assays were performed. To test whether synaptic scaling plays a role in vivo, in collaboration with the lab of Dr. Michael Stryker (UCSF), we examined ocular dominance plasticity in TNFalpha knockout mice.
Results: Treatment of cultures for 2 days with tetrodotoxin (TTX) increased surface expression of AMPA receptors and the amplitude of miniature excitatory postsynaptic currents (mEPSCs). Conditioned media from these cultures induced similar changes, an effect lost by addition of a soluble form of the TNFR1 receptor to the media. Chronic blockade of TNFalpha signaling also prevented the increase in mEPSC amplitude resulting from TTX treatment. Furthermore, cultures from knockout mice lacking TNFalpha did not exhibit synaptic scaling. To determine the source of TNFalpha that is required for synaptic scaling, mixed cultures from TNFalpha(-/-) and wildtype mice were prepared. TNFalpha(-/-) neurons grown on wildtype glia expressed normal synaptic scaling. In contrast, wildtype neurons grown on TNFalpha(-/-) glia did not exhibit synaptic scaling following activity blockade. In addition, although the initial loss of deprived-eye responses was normal in TNF alpha(-/-) mice, the subsequent increase in response to the open eye was absent. This increase was also prevented by pharmacological inhibition of endogenous TNFalpha.
Conclusions: Together these results suggest that TNFalpha is important for one prominent form of synaptic scaling, that contributes to experience-dependent plasticity in the developing visual cortex.
Disclosure: R. Malenka, Part 1: Pfizer, Inc (scientific advisory board), Part 2: Pfizer, Inc.

Cytokines-Neurochemiccals Interaction in Depression: Biochemical, Genetic and Structural Aspects
Aye Mu Myint*

Ludwig-Maximilians University, Munich, Germany

Background: Cytokines are the mediators of immune system which have pro- and anti-inflammatory effect. The pro-inflammatory type of cytokines at certain concentrations has negative impact on glial-neuron interaction. One of the mechanisms is through tryptophan degradation pathway, the kynurenine (KYN) pathway, which is composed of several metabolites. These metabolites have different actions on different receptors and neurotransmissions. The negative impact can finally result in depression and neurotoxic/degenerative changes if the elevation of pro-inflammatory cytokines and disturbances in KYN pathway continue chronically. This presentation will explain the mechanism by summarising the results of the studies carried out in human in terms of biochemical, genetic and morphological changes related to pro-inflammatory cytokines and major depressive disorder.
Methods: The above mentioned mechanism was studied in patients with major depressive disorder, interferon-a (IFNa) treated treated hepatitis-C patients, and post-mortem brain tissues of well characterized depressed patients. From studies on medication-naive and medication-free depressed patients, biochemical changes before and after antidepressant medication will be presented. From the genetic study on KYN related genes, the association with genetic aspect will be presented. From IFNa treated human study, gene-biochemical interaction related to KYN pathway will be presented. From post-mortem study, the immunoreactivity of neurotoxic quinolinic acid in anterior cingulate cortex area and hippocampus area will be presented.
Results: In medication naive depressed patients, the serum kynurenic acid (KYNA) level and the ratio between KYNA and KYN (KYNA/KYN) were significantly lower compared to healthy controls. The 6-week antidepressant medication could not efficiently improve the biochemical disturbance. In the genetic study, the kynurenine aminotransferase 3 (KAT III) gene polymorphism was shown to be associated with depression and anxiety symptoms. In patients with IFNa treatment, the kynureninase gene and KAT III gene polymorphisms are directly associated with the serum levels of KYN, 3-hydroxykynurenine (OHK) and KYNA. In the study of post-mortem brain tissues, the quinolinic acid immunoreactivity was shown to be increased in part of anterior cingulate cortex and hippocampus areas of suicidal depressed patients compared to those of healthy controls and suicidal patients with bipolar disorder.
Conclusions: The KYN metabolites play a role in the mechanism of depression associated with pro-inflammatory state. The disturbances in KYN pathway is partially related to polymorphism of some genes involved in KYN pathway. Those changes observed in the periphery might be associated with neurotoxic changes in the brain. Currently available antidepressants did not show satisfactory effect in terms of correction of KYN metabolites disturbances. Manipulation of KYN pathway could be a future therapeutic approach in treatment of major depressive disorder.
Disclosure: A. Myint, Part 1: -, Part 2: (1) European Large collaborative project MOODINFLAME: Early Diagnosis, Prevention and Treatment of Mood Disorders related to activated Inflammatory Response System. (2) Advanced Practical Diagnostics n.v., Turnhout, 2300 Belgium, Part 3: -, Part 4: No, Part 5: Advanced Practical Diagnostics n.v., Turnhout, 2300 Belgium.

Novel IL-1b Targets for Blockade of the Anti-Neurogenic and Behavioral Actions of Stress
Ronald Duman*

Yale University, New Haven, USA

Background: Atrophy and loss of neurons caused by repeated or traumatic stress exposure contributes to decreased hippocampal volume in depressed patients and certain mood disorder symptoms. Studies in rodent models demonstrate that blockade of interleukin-1b (IL-1b) blocks the inhibition of hippocampal neurogenesis and the anhedonic effects caused by chronic stress. The current study presents new data on novel targets and approaches for blocking IL-1b release and signaling.
Methods: The influence of an antagonist of the purinergic receptor P2X7, which controls the processing and release of IL-1b, on hippocampal neurogenesis was examined. Neurogenesis was determined by incorporation of BrdU, and basal and the response to acute immobilization stress was tested. The influence of the P2X7 receptor antagonist and peripheral administration of an IL-1b neutralizing antibody, on anxiety (open field) and anhedonia (sucrose consumption) was also determined.
Results: Pretreatment with a single dose of a P2X7 receptor antagonist resulted in a dose-dependent blockade of the anti-neurogenic effects of stress. P2X7 antagonist administration alone, in the absence of stress, increased levels of BrdU incorporation and produced an anxiolytic effect in the open field test. Preliminary studies demonstrate that IL-1b antibody neutralization blocks the anhedonic response caused by chronic unpredictable stress exposure.
Conclusions: The results provide further evidence that inhibition of IL-1b blocks the anti-neurogenic and behavioral actions of stress, and identify the P2X7 receptor as a novel target. The induction of neurogenesis and the anxiolytic response to the P2X7 receptor antagonist in the absence of stress indicates that basal levels of IL-1b release and signaling produce cellular and behavioral actions. Comparison with other approaches for

blockade of IL-1b, including IL-1R antagonists and null mice will be discussed.
Disclosure: R. Duman, Part 1: Lilly, Lundbeck, Wyeth, Johnson and Johnson, Taisho, Psychogenics, Pfizer, Bristol Myers Squibb,, Part 2: Taisho, Part 3: none, Part 4: Lilly, Repligen, Lundbeck, Johnson & Johnson.

Inflammation and Treatment Resistance in Major Depression: A Perfect Storm

Andrew Miller*

Emory University School of Medicine, Atlanta, USA

Background: Major depression is a common and devastating disorder associated with significant disability, morbidity and mortality. While conventional antidepressant medications are effective for many depressed patients, approximately one third are treatment resistant. Although the neurobiology of treatment resistant depression (TRD) is poorly understood, a number of factors have been associated with antidepressant non-response including obesity, early life stress, personality disorders, bipolar depression and medical co-morbidity. One pathophysiologic mechanism that may link these factors with TRD is inflammation.
Methods: The literature on markers of inflammation and factors associated with TRD was reviewed in conjunction with the impact of inflammatory cytokines on neurobiological pathways relevant to response to conventional antidepressants. The use of anti-inflammatory strategies to treat TRD was also examined.
Results: In addition to a direct relationship between inflammatory markers and TRD, increased inflammation was associated with depression in obesity, early life stress, and a number of medical illnesses as well as bipolar disorder. Activation of inflammatory cytokines was also found to interact with neurocircuits related to neuroticism, a common component of personality disorders. Studies in laboratory animals reveal that inflammatory cytokines inhibit neurogenesis, which is required in part for antidepressant efficacy, alter glutamate metabolism, which is not a target of conventional antidepressant medications, and increase monoamine transporter activity while decreasing monoamine synthesis, thus counteracting conventional antidepressant action.
Conclusions: Data indicate a unique relationship between inflammation and TRD borne out of the impact of inflammatory cytokines on neurobiological pathways that sabotage or circumvent the ability of conventional antidepressants to act effectively. Treatment strategies targeting inflammation may be especially relevant for patients with TRD.
Disclosure: A. Miller, Part 1: Abbott Laboratories, AstraZeneca, Centocor Inc., GlaxoSmithKline, Lundbeck Research USA, F. Hoffmann-La Roche Ltd., Schering-Plough Research Institute (now Merck)and Wyeth/Pfizer Inc., Part 2: None, Part 3: None, Part 4: Centocor Inc., GlaxoSmithKline, and Schering-Plough Research Institute (now Merck), Part 5: No.

Panel Session
Role of Phagocytes in Synaptic Plasticity and Remodeling of Tissues in the Nervous System

Pruning CNS Synapses: Role of Microglia and the Complement Cascade

Beth Stevens*

Children's Hospital Boston, Boston, USA

Background: During development, neural ciruitry undergoes a remodeling process in which excess synapses are eliminated or pruned and the remaining synapses are strengthened. We recently discovered that components of the classical complement cascade (C1q and C3), traditionally associated with innate immune system function, are necessary for synapse elimination in the normal, developing CNS; however, the mechanisms by which complement mediates synaptic pruning are completely unknown. The primary role of the complement cascade in the innate immune system is to opsonize or tag unwanted cells or debris for removal by *phagocytic macrophages*. We hypothesize that inappropriate synapses in the developing brain are similarly tagged by complement and then eliminated by microglia, the primary phagocytic cells in CNS.
Methods: To test the hypothesis that microglia prune inappropriate CNS synapses, we investigated the interactions between microglia and synapses in the mouse retinogeniculate system, a classic model for studying developmental synaptic remodeling. We used established anterograde tracing techniques to determine whether a specific disruption in the phagocytic capacity of microglia resulted in synaptic pruning and eye-segregation deficits. In addition, we developed an *in vivo* phagocytosis assay using anterograde tracers to visualize RGC inputs in the dLGN of transgenic CX3CR1::EGFP mice, in which all microglia express EGFP. We then used a combination of ImmunoEM, array tomography (AT) to determine whether microglia actively remove intact synapses in the healthy developing brain.
Results: We demonstrate that microglia phagocytose inappropriate synaptic inputs and are required for the formation of precise eye-specific maps in the brain. Furthermore, we identify a molecular mechanism underlying microglia-mediated pruning of CNS synapses which involves signaling between complement receptor 3 (CR3/CD11b/Mac1) expressed on the surface of microglia and its ligand C3, a complement cascade component developmentally localized to retinogeniculate synapses.
Conclusions: Our data provide important mechanistic insight into how CNS synapses are eliminated during normal brain wiring, and possibly in diseases thought to involve aberrant synapse loss and connectivity, such as epilepsy, schizophrenia, and autism.
Disclosure: B. Stevens, Part 4: Ellison Medical Foundation Dana Foundation Smith Family Foundation, Part 5: N/A.

Engulfment and Elimination of Synpases by Astrocytes

Ben Barres*

Stanford University School of Medicine, Stanford, USA

Background: Astrocytes are a major cell type in the mammalian brain that ensheath synapses. To understand the role of astrocytes at synapses, we have previously determined their transcriptome and found that they express several phagocytic pathways. Similarly previous studies have shown that Drosophila glia express one of these pathways and that this pathway is required to prune synapses during development. Thus in this project, we have investigated whether mammalian astrocytes are also able to phagocytose synapses.
Methods: To address this question, we used a combination of in vitro and in vivo methods to visualize astrocytes and synaptic engulfment. We developed a new method to prospectively purify mature brain astrocytes by immunopanning as well as serum-free culture conditions to maintain these cells at high viability in vitro where they maintain their in vivo gene profiles. We used these culture conditions to establish a quantitative assay of phagocytosis of synaptosomes by astrocytes. To investigate whether astrocytes engulf synapses in vivo, we injected retinal ganglion cells with a fluorescent dye that is anterogradely transported and examined the developing lateral geniculate nucleus during the period of synapse pruning to determine if astrocytes engulf synaptic terminals in vivo.

Results: Our findings demonstrate that mammalian brain astrocytes actively engulf synapses in vitro and in vivo.
Conclusions: These findings raise many questions. Do astrocytes control synapse turnover in the adult brain? Why do astrocytes express several different phagocytic pathways? Do different pathways engulf different types of synapses? What is the role of astrocyte engulfment of synapses in disease processes?
Disclosure: B. Barres, None.

In Vivo Studies of Microglial Function in Synaptic Plasticity

Wenbiao Gan*

New York University School of Medicine, New York, USA

Background: The focus of this presentation is to discuss the potential roles of microglia in regulating synaptic development and plasticity in the brain. Microglia are the resident immune cells of the central nervous system and display highly motile processes occupying a non-overlapping territory. Under physiological conditions, microglia may monitor the brain's microenvironment for damage signals and participate in the development and plasticity of neural circuits. Under pathological conditions, microglia undergo a series of morphological and functional changes, and may engage in containing tissue damage, phagocytosis and clearance of cellular debris, and/or the secretion of proinflammatory factors. Although microglia have been implicated in a multitude of physiological and pathological processes in the central nervous system, direct evidence of their roles in synaptic structure and functions remains elusive.
Methods: Hampering efforts to delineate the role of microglia is the lack of tools to specifically perturb microglial function in vivo. To overcome this difficulty, we have recently generated mice with a targeted gene insertion allowing for the expression of tamoxifen-inducible Cre recombinase in CX3CR1 expressing microglial cells. By crossing CX3CR1-CreER mice with mice harboring floxed alleles of the diphtheria toxin receptor (iDTR) under the control of the ubiquitous Rosa26 promoter, we have been able to specifically and efficiently ablate microglia in an inducible fashion. By ablating microglial cells and perturbing their functions in the living mice, we hope to elucidate the role of microglia in synapse development and learning-dependent synaptic plasticity.
Results: Our preliminary results suggest that deletion of CX3CR1 expressing microglial cells may cause a decrease in the turnover of postsynaptic dendritic spines in the living mouse cortex.
Conclusions: Our findings indicate that the CX3CR1-CreER mouse line provide a molecular handle for the *in vivo* manipulation of microglia including deletion and support an important role of microglia in synapse development and plasticity.
Disclosure: W. Gan, None.

Gene Targeting into the 21st Century: Mouse Models of Human Disease from Cancer to Neuropsychiatric Disorders

Mario Capecchi*

University of Utah/Howard Hughes Medical Institute, Salt Lake City, USA

Background: Gene targeting allows the designed modification of any gene in the mouse genome. Since genes impact all biological phenomena this methodology can be used to study any biological phenomena common to mammals in the mouse. We are using it to model human disease in the mouse. The models can be used to analyze the pathology of the disease at a level not feasible in humans and as a platform for the development of new therapeutic protocols. I will discuss modeling of a neuropsychiatric disorder, obsessive compulsive (OCD) spectrum disorder in the mouse. These studies provide the unexpected conclusion that microglia, the immune cells of the brain derived from bone marrow, are controlling behavior in the mouse.
Methods: The experimental approaches combine Cre/loxP conditional mutagenesis with bone marrow transplanation and automated behavior analyses.
Results: We demonstrate that microglia, the immune cells of the brain control a very specific behavioral output whose failure result in a disorder very similar to the human OCD spectrum disorder, trichotillomania.
Conclusions: Many neuropsychistric disorders, monopolar depression, bipolar depression, autism, obsessive compulsive disorder, OCD-spectrum disorder, schizophrenia and Alzheimer Disease have been associated with immune dysfunction but the relationship between the two is unclear. Is a patient depressed and therefore the immune system not optimal or is the immune system not functional and therefore the risk of depression is increased. Which is cause and which is effect. With respect to trichotillomania, like behavior in the mouse, we provide strong support that defective microglia are causational for the behavioral disorder.
Disclosure: M. Capecchi, None.

Panel Session
Translational Approaches to Understanding Negative Symptoms

Facilitating Novel Treatment Development and Neurobiological Research for Negative Symptoms: Findings from the Collaboration to Advance Negative Symptom Assessment in Schizophrenia (CANSAS)

William Horan*

University of California, Los Angeles, Los Angeles, USA

Background: Negative symptoms are key determinants of poor functional outcome and are only minimally responsive to available treatments. It is widely agreed that progress in treatment development is impeded by limitations in: the conceptualization and psychometric properties of existing assessment tools, our understanding of the underlying structure of negative symptoms, and accepted brain-based biomarkers and animal models for negative symptom sub-domains. The CANSAS project is addressing these issues through the development of a new instrument, the Clinical Assessment Interview for Negative Symptoms (CAINS), and complementary translational research approaches.
Methods: The CANSAS, a four-site NIMH-funded collaborative project, is following consensus-based, iterative, and data-driven scale development process in a combined sample of over 450 schizophrenia and schizoaffective disorder outpatients. Phase 2 focused on establishing the latent structure, inter-rater reliability, and initial validity of the CAINS in a diverse sample of 281 patients.
Results: Converging analyses indicated that the CAINS is comprised of two moderately correlated ($r = .39$) factors: experience-related and expression-related impairments. Within- and between-site rater agreement was generally high (ICC's $= .75$ to .94). The scale also demonstrated good convergent/discriminant validity with respect to other measures of clinical symptoms and neurocognition.
Conclusions: The CAINS provides a promising new approach to assess the two major domains of negative symptoms in multi-site studies. A revised, shorter version of the CAINS is now being tested in 160 patients to confirm its structure, psychometric properties, validity, and test-retest stability for use as an endpoint in clinical trials. We are also collaborating with preclinical researchers to investigate neurobehavioral processes that underlie

negative symptom sub-domains based on animal models of reward processing and motivation.
Disclosure: W. Horan, None.

Neural Substrates of Emotion Processing and Expressivity Deficits in Schizophrenia
Raquel Gur*

University of Pennsylvania, Philadelphia, USA

Background: Emotion expressivity deficits such as blunted affect are key features of negative symptoms in schizophrenia. The neurobiology of these impairments can be investigated by multimodal neuroimaging combined with clinical and neurobehavioral characterization. We present a series of studies showing abnormalities in amygdala activation and connectivity and establishing their relation to symptoms.
Methods: MRI (3T) was applied in patients with schizophrenia, first-degree relatives. sMRI used T1-weighted 3D MPRAGE and DTI. fMRI used BOLD contrast while performing emotion identification tasks. Participants were clinically characterized, including CAINS, and a computerized neurocognitive battery was administered. Initial studies used a standard set of facial expressions of actors and the last experiment incorporated individualized presentation where familiarity was manipulated: pictures of familiar staff, family members and self expressing neutral, angry and fearful emotions.
Results: Prior fMRI data indicated that abnormal amygdala activation to threat related facial expressions correlated with severity of blunted affect. There was an association of enhanced limbic response to threat with decreased cortical facial recognition memory response. Further evaluation of amygdala connectivity indicated abnormalities in reciprocal relations among limbic, frontal and ventrostriatal circuitry. Activation patterns were differentially modulated by familiarity in patients compared to controls. Impairment was evident in at-risk individuals.
Conclusions: The findings indicate that a systematic evaluation of parameters affecting limbic abnormalities can help delineate pathways through which negative symptoms related to expressivity are modulated. Such an understanding requires examination of both activation patterns and connectivity across tasks that vary in their demands and stimuli that vary in familiarity. Examination of patients, psychosis prone youths and family members can help identify vulnerability indices.
Disclosure: R. Gur, Part 4: Pfizer investigator initiated grant AstraZeneca investigator initiated grant.

Emotion Experience in Schizophrenia: Timing Matters
Ann Kring*

University of California, Berkeley, Berkeley, USA

Background: My colleagues and I have argued that the time course of emotional responding is important for understanding more precisely the nature of emotion deficits in schizophrenia. One of the important findings that has emerged in the last two decades is that people with schizophrenia can and do experience emotion in the presence of evocative stimuli. However, deficits in emotion experience, particularly those linked to anhedonia can be identified when considering the time course of emotional response. People with schizophrenia have difficulty in anticipating emotional experiences as well as maintaining emotional experiences. Drawing from psychophysiological and fMRI studies, I will illustrate how the timing of emotion matters in schizophrenia.
Methods: In Study 1, schizophrenia patients ($n = 23$) and controls ($n = 24$) participated in a slow event related fMRI study where BOLD activation was measured and reports of experience were collected during the presentation of evocative pictures and during a delay following picture presentation. In Study 2, schizophrenia patients ($n = 31$) and controls ($n = 28$) viewed evocative pictures and reported on their experience. Startle probes were presented during and after picture presentation, and blink magnitude was assessed.
Results: In Study 1, patients and controls exhibited comparable activation in emotion-related brain regions (e.g., PFC, amygdala) during picture presentation. However, only controls continue to exhibit activation during the delay Moreover, diminished activation during the delay among patients was significantly correlated with anhedonia. In Study 2, patients and controls exhibited comparable startle responses during picture presentation, but only controls continued to exhibit these responses during the delay. In both studies, patients reported experiencing comparable emotion as controls. However, patients reported experiencing more unexpected emotion than controls (more negative emotion to positive pictures and more positive emotion to negative pictures), perhaps reflecting the limited support of the PFC in making these difficult judgments.
Conclusions: Schizophrenia patients exhibit comparable experience and brain activation in the presence of evocative stimuli. Yet, they fail to maintain this experience, and this maintenance failure is linked to anhedonia.
Disclosure: A. Kring, None.

Preclinical Studies Investigating the Neurobiological and Genetic Underpinnings of Motivated Behavior
Jared Young*

University of California, San Diego, La Jolla, USA

Background: Negative symptoms remain a major contribution to dysfunctional behavior in patients with schizophrenia yet no efficacious treatments exist. One reason for the lack of treatments is a limited understanding of the neurobiological underpinnings of these symptoms. One negative symptom, avolition, reflects reduced motivation to act to obtain rewards. To begin to understand the genetic and neurobiological underpinnings of reward motivation, we have used the progressive ratio breakpoint paradigm (PRBP) in preclinical studies. The PRBP assesses the level of effort a subject is willing to make to obtain a reward and preliminary studies indicate patients with schizophrenia exert less effort in this paradigm than healthy volunteers. We assessed dopamine D1 receptor (drd1) and $\alpha 7$ nicotinic acetylcholine receptor (nAChR) mutant mice in the PRBP.
Methods: Drd1 & $\alpha 7$ nAChR mutant mice (knockout, KO; heterozygous, HT, and wildtype littermates, WT) were trained to nosepoke into a single aperture to gain a single food reward in a fixed ratio 1 (FR1) rate. Once nosepoking reliably, the mice were challenged in the PRBS by increasing the number of holepokes required to gain the same reward (1, 2, 4, 7, 11, 16…). The point where mice stop nosepoking for a reward is referred to as their breakpoint.
Results: Drd1 KO mice failed to acquire the FR1 rule. Drd1 HT mice exhibited comparable acquisition and breakpoint to WT mice. Modafinil- and GBR12909-induced increases in breakpoint were blunted in HT compared to WT mice however. $\alpha 7$ nAChR KO mice took longer to acquire the FR1 rule but exhibited a comparable breakpoint to WT and HT mice once acquired.
Conclusions: Complete Drd1 loss resulted in a failure of mice to acquire a rule associated with reward. Partial drd1 loss did not affect rule acquisition or motivation in a normal state, but blunted the increased motivation induced by the dopamine transporter inhibitors. Drd1 agonists may improve avolition in patients with schizophrenia.
Disclosure: J. Young, None.

Panel Session
Progress in Understanding the Role of GABA and GABAA Receptor Biology in Psychiatric Disease

GABA Signaling, Genetic Variation, Neurodevelopment, and the Molecular Pathology of Schizophrenia

Thomas Hyde*

Lieber Institute for Brain Development, Baltimore, USA

Background: GABA signaling molecules are critical elements for brain development and the pathophysiology of schizophrenia.
Methods: We studied three genes related to GABA signaling [*GAD1* (GAD67 and GAD25), *SLC12A2* (NKCC1), and *SLC12A5* (KCC2)] in the prefrontal cortex (PFC) and hippocampal formation of human non-psychiatric control brains across the lifespan (from 14–20 weeks gestational age in the fetus and birth through 80 years of age). We also studied schizophrenic transcript expression. Additionly, we examined if a schizophrenia risk-associated promoter SNP in *GAD1* (rs3749034) was related to transcript expression. The development of the PFC and hippocampal formation are characterized by progressive switches in expression from GAD25 to GAD67, and NKCC1 to KCC2. The former leads to GABA synthesis; the latter to switching GABA itself from excitatory to inhibitory neurotransmission.
Results: In hippocampus, the GAD25/GAD67 and NKCC1/KCC2 ratios are increased in schizophrenics, reflecting an immature GABA physiology. Remarkably, GAD25/GAD67 and NKCC1/KCC2 expression ratios are associated with rs3749034 genotype, with risk alleles predicting a less mature pattern. We next defined the alternative transcripts derived from KCC2 (*SLC12A5*. Besides the previously identified full length human (NM_020708.3) and truncated KCC2 transcripts (AK098371), we discovered previously unrecognized alternative transcripts. We selected four abundant truncated splice variants (EXON6, EXON2B, EXON6B, AK098371) for study in DLPFC. In SH-Sy5Y cells, these transcripts were translated into proteins at their predicted sizes. The transcript with a novel exon (6B) was increased in DLPFC of schizophrenics ($p = 0.032$) but was decreased in bipolar disorder ($p = 0.006$) and major depression ($p = 0.008$). Once again, rs3749034 genotype is significantly associated with transcript AK098371 expression.
Conclusions: These findings suggest that abnormalities in GABA signaling critical to brain development contribute to genetic risk for schizophrenia.
Disclosure: T. Hyde, None.

Circuit-Specific Alterations in Mediators of Cortical GABA Neurotransmission in Schizophrenia

David Lewis*

University of Pittsburgh, Pittsburgh, USA

Background: Impairments in cortical gamma oscillations might underlie the cognitive deficits in schizophrenia. Gamma oscillations depend on 3 physiological properties at the inputs from parvalbumin (PV)-containing GABA neurons to pyramidal cells: 1) the strength (IPSC amplitude) of GABA neurotransmission as determined by pre-synaptic GABA levels; 2) the kinetics (IPSC duration) of GABA neurotransmission as determined by the subunit composition of post-synaptic GABA-A receptors; and 3) the nature of inhibition (i.e., shunting or hyperpolarizing) as determined by Cl^- ion flow when GABA-A receptors are activated. Each of these physiological features is dependent upon the expression of particular sets of gene products.
Methods: Multiple methods were used to assess mRNA and protein levels of these gene products in the prefrontal cortex (PFC) of subjects with schizophrenia.
Results: In schizophrenia, 1) lower mRNA for GAD67, the enzyme responsible for most GABA synthesis in the cortex, is accompanied by less GAD67 protein; 2) the GAD67 protein deficit is ~10x greater in PV neuron axon terminals than in PFC tissue, suggesting that GABA is preferentially reduced in PV terminals; 3) opposed changes in GABA-A receptor $\alpha 1$ and $\alpha 2$ subunit levels, which differ in IPSC decay kinetics, are likely to alter the speed of GABA neurotransmission at inputs from PV-containing neurons; 4) $\alpha 1$ subunit mRNA deficits are especially pronounced in pyramidal neurons, suggesting that PV-containing basket cell inputs to pyramidal cells, which are mediated by $\alpha 1$-containing GABA-A receptors, are altered; and 5) disease-related differences in the levels of kinases that regulate Cl^- transporters may render GABA-A receptor activation of post-synaptic pyramidal cells less hyperpolarizing.
Conclusions: Alterations in the strength, kinetics and nature of GABA neurotransmission in the PFC of subjects with schizophrenia may provide a molecular basis for disturbances in cortical gamma oscillations.
Disclosure: D. Lewis, Part 1: AstraZeneca, BioLine RX, Bristol-Myers Squibb, Merck, Neurogen, SK Life Science, Part 4: BMS Foundation, Bristol-Myers Squibb, Curridium LTD, Pfizer.

Modifying $GABA_AR$ Clustering in the Prefrontal Cortex of Mice Induces Behavioral Deficits Reminiscent of Schizophrenia

Stephen Moss*

Tufts University School of Medicine, Boston, USA

Background: A common postmortem finding in the cortex of schizophrenic patients is that there are alterations in the size of GABAergic inhibitory synapses on the axon initial segments (AIS). These synapses are enriched in ã-aminobutyric acid type A receptor ($GABA_AR$) subtypes containing $\alpha 2$ subunits. Whether these modifications directly contribute to the pathology of schizophrenia is unknown.
Methods: We have demonstrated that the accumulation of $\alpha 2$ subunit-containing receptors on the AIS is dependent on a direct interaction with the inhibitory scaffold protein gephyrin. To directly address if alterations in $GABA_AR$ clustering contribute to the pathology of schizophrenia we have created adeno-associated viruses that express dominant negative reagents coupled to green fluorescent protein (DNα2GFP).
Results: When injected into the prefrontal cortex (PFC) of mice DNα2GFP but not viruses expressing GFP alone decrease the size of α2subunit gephyrin positive inhibitory synapses on the cell bodies/AIS of principle neurons. Reductions in the size of GAT-1 positive presynaptic terminals that innervate inhibitory synapses on the AIS are also evident. These morphological changes correlated with deficits in the efficacy of synaptic inhibition in neurons expressing DNα2GFP. This loss of GABAergic signaling in the PFC results in a profound deficit in pre-pulse inhibition. This deficit can be reversed by haloperidol an accepted antipsychotic. We are currently evaluating if modifying $GABA_AR$ clustering in the PFC induces other schizophrenic-like behaviors.
Conclusions: In summary our results suggest that modified GABAergic neurotransmission in the PFC contributes directly to the pathology of schizophrenia.
Disclosure: S. Moss, None.

GABAergic Regulation of the HPA Axis in Depression

Jamie Maguire*

Tufts University School of Medicine, Boston, USA

Background: A hallmark characteristic and diagnostic criterion of major depression is hyperexcitability of the hypothalamic-

pituitary-adrenal (HPA) axis. However, it has remained controversial whether HPA axis hyperexcitability is a cause or a consequence of this disorder. It is known that the output of the HPA axis is governed by corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus (PVN), the activity of which is regulated by GABAergic inhibition. Here we test the novel hypothesis that the pathophysiology depression involves alterations in the GABAergic control of CRH neurons and that GABAergic dysregulation of the HPA axis is sufficient to induce depression-like behavior in mice.
Methods: Using a model of acquired depression-like behavior, the social defeat model, we are able to measure changes in GABAergic inhibition in CRH neurons associated with the development of depression in mice, using biochemical and electrophysiological approaches. Further, we utilize a newly generated, novel floxed *Gabrd*$^{-/-}$ mouse model to eliminate $GABA_AR$ δ subunit expression specifically in CRH neurons and assess the effect on depression-like behaviors.
Results: Our data demonstrate that GABAergic deficits in neurons controlling the HPA axis is sufficient to induce depression-like behavior in mice. Following social defeat, submissive animals exhibit elevated levels of the stress hormone corticosterone and depression-like behavior in the tail suspension and forced swim tests compared to dominant animals and control animals which did not fight in the social defeat paradigm. Submissive animals demonstrating depression-like behaviors exhibited a decrease in $GABA_AR$ δ subunit expression in the PVN which is associated with deficits in the tonic GABAergic control of CRH neurons in the PVN.
Conclusions: These data suggest that pathological alterations in the GABAergic control of CRH neurons plays a role in depression-like behavior induced by social defeat. Further, these data suggest that GABAergic dysregulation of the HPA axis is sufficient to induce depression-like behaviors in mice. This study supports the GABAergic deficit hypothesis of major depression.
Disclosure: J. Maguire, None.

Panel Session
Novel Approaches to Therapeutic Development in Alzheimer's Disease

Apolipoprotein-E: An Obvious Target for Alzheimers Disease
Michael Vitek*

Duke University Medical Center, Durham, USA

Background: Beyond age, presence of the APOE4 gene (apoE4 protein) is the largest risk factor for susceptibility to Alzheimer's Disease (AD) where at least half of all AD patients carry this epsilon 4 allele. The mechanism by which APOE4 exerts this susceptibility to AD is a prime target for therapeutic intervention in AD. In researching how apoE inhibits inflammation, we found that apoE3 was a significantly better anti-inflammatory than apoE4, where apoE4 was about equivalent to controls lacking apoE. To duplicate the anti-inflammatory activity of apoE3, we created small peptides from residues 133–149 of apoE's receptor binding region that mimic apoE-holoprotein by showing robust anti-inflammatory activity.
Methods: We used apoE-mimetic peptide as an affinity probe to define the binding partners and mechanism of action of apoE. We also used this apoE peptide as a candidate drug to treat CV and CVND mice and measured their behavior, neuronal loss, amyloid and tangle-like pathologies.
Results: ApoE and mimetic peptides bind to SET/I2PP2A resulting in antagonism of SET, which then leads to an increase of Protein Phosphatase 2A activity levels. Subcutaneous administration of apoE-mimetic peptides increased PP2A activities resulting in improved behavioral performance, reduced neuronal loss, reduced phospho-tau, and reduced amyloid deposition.
Conclusions: Numerous reports confirm APOE4's association with at least half of all Alzheimer's patients. Our discovery connecting the mechanism of apoE action to PP2A activity creates a novel and druggable target for creation of Alzheimer's therapeutics. Using published transgenic mouse models, we show that peripheral administration of apoE-mimetic peptides in animals with active disease resulted in a robust decrease of disease pathology and improvement of behavioral performance. We now need to evaluate the safety profile of these compounds as a prelude to proof-of-concept experiments in human clinical trials of apoE-mimetic peptides and Alzheimer's patients.
Disclosure: M. Vitek, Part 1: I am a part-time professor at Duke and a part-time CEO of Cognosci and recieve salary from both. All of my salary at Duke and at Cognosci is from NIH grants to the respective institutions., Part 2: All of my income derives from NIH and IRS grants, some of which were to Duke and others to Cognosci, Inc. I have no other sources of income., Part 3: As stated above, I am a part time professor at Duke and a part time CEO of Cognosci, Inc. Both provide more than 5% of my personal income as compensation for employment services at those institutions., Part 4: Matters of conflict of interest for Michael P. Vitek are managed by the Duke University Conflict of Interest Committee. As part of this management plan, I do not receive subcontracts at Duke that are from NIH grants awarded to Cognosci. I do not receive subcontracts at Cognosci that are from NIH grants awarded to Duke., Part 5: Cognosci, Inc. which is totally supported by NIH SBIR/STTR and IRS QTDP grants.

Proinflammatory Cytokine Overproduction: A Contributor to CNS Pathophysiology that is a Viable Target for Disease Progression Modification
Linda Van Eldik*

University of Kentucky, Sanders-Brown Center on Aging, Lexington, USA

Background: Glia proinflammatory cytokine up-regulation contributes to synaptic dysfunction and neuronal death in neurodegenerative disease progression. Targeting relevant glia signaling pathways that are up-regulated is a viable approach to new therapies. The goal is selective restoration of excessive production back towards basal through appropriate dosing with novel drugs designed for oral bioavailability, CNS penetrance and low adverse pharmacology potential.
Methods: We used a validated discovery engine for the design, synthesis and development of novel drug candidates of high quality with appropriate in vivo properties.
Results: Two classes of orally bioavailable, CNS-penetrant, selective, novel small molecule drugs that attenuate excessive proinflammatory cytokine production and yield improved neurologic outcomes were generated. Preclinical efficacy testing was done in animal models of AD, TBI, MS, neurologic sequelae of seizures, and "two-hit" susceptibility models that test how intervention in response to a CNS injury affects increased susceptibility to late-in-life events. Attenuation of proinflammatory cytokine overproduction improved neurologic outcomes; one drug provided a pharmacological "priming" effect in which susceptibility to later in life injury was attenuated. The preclinical data demonstrate that targeting distinct signaling pathways converging on the same cellular endpoint can in some cases provide similar neurologic outcomes.
Conclusions: Disease-modifying treatments for complex neurodegenerative disorders will require a combination of compatible

drugs as part of a multi-target approach. Pathology progression involves a changing, aberrant, glia-neuronal cycle. Targeting complementary glial and neuronal pathways in this cycle at discrete pathology progression stages is a viable approach in future therapeutic development.
Disclosure: L. Van Eldik, None.

Histone Acetyltransferase (HAT) Activators as Chromatin Remodelers in the Treatment of Alzheimer's Disease

Ottavio Arancio*

Columbia University Medical Center, New York, USA

Background: The normal physiological roles of tau, Aβ precursor protein (APP), its fragments and processing enzymes might present a problem in providing effective and safe approaches to Alzheimer's disease (AD) therapy. Epigenetic mechanisms such as histone acetylation play a key role in memory formation. We have therefore investigated whether agents enhancing histone acetylation represent an alternative approach downstream of Aâ to counteract the disease progression.
Methods: The main strategy currently used to up-regulate histone acetylation involves histone deacetylase (HDAC) inhibitors. However, the pleiotropic effect of nonspecific HDAC inhibition may hamper their therapeutic potential. Use of activators of histone acetyltransferases (HATs) might constitute an alternative avenue to enhance histone acetylation. To this end, we have designed and tested novel HAT activators by combining biochemical, electrophysiological and behavioral techniques.
Results: We have discovered that Ab reduces acetylation of specific histone lysines that are important for memory formation. We have also found that Ab reduces endogenous expression of CREB binding protein (CBP) and p300/CBP associated factor (PCAF), two HATs that are relevant for memory formation. Most importantly, AD patients were found to have a decrease in acetylation of histone residues important for memory. Using a SAR approach, we designed and synthesized a series of compounds that lead to YF2, a novel potent activator of memory related HATs, CBP, p300 and PCAF. YF2 was found to be soluble, membrane permeable and blood-brain barrier permeable. YF2 was also found to rescue the reduction in histone acetylation following Ab elevation, and rescue synaptic and memory deficits induced by Ab exposure.
Conclusions: Current AD therapies have limited efficacy. Use of HAT activators up-regulating histone acetylation downstream of Aâ elevation might effectively counteract memory loss in AD.
Disclosure: O. Arancio, None.

Targeting Neuronal Protein Indigestion as a Therapeutic approach for Alzheimer's Disease

Ralph Nixon*

Nathan Kline Institute/New York University Langone Medical Center, Orangeburg, USA

Background: Signature pathogenic proteins in adult-onset neurodegenerative diseases only accumulate late in life implying a failure of protein quality control mechanisms as the brain ages. In Alzheimer's disease (AD), abeta and tau accumulation occurs in the context of a more generalized profound buildup of waste proteins in dystrophic neurites throughout affected brain regions, rivaling the degree of neuronal "storage" in some congenital lysosomal storage disorders. Recent genetic and biochemical studies of AD identify a failure of neuronal autophagy, the principal pathway by which cells eliminates organelles and long-lived proteins including aggregates. Modulation of autophagy and lysosomal proteolysis is now being actively investigated as a possible therapy for AD and other proteinopathies.
Methods: We and others are investigating sites within the autophagy pathway and lysosome that can be safely modulated using pharmacologic, dietary, and genetic approaches to achieve disease modification in mouse models of AD and other neurodegenerative diseases.
Results: As proof of principle for therapeutic effects of remediating marked lysosomal proteolytic dysfunction in an FAD-APP model of AD, we rescued lysosomal proteolytic activity by deleting cystatin B, an endogenous inhibitor of cysteine proteases in lysosomes, This specific intervention prevented cognitive decline, while decreasing intracellular and extracellular amyloid and other AD-related pathologies. Promising effects of small molecule modulators of autophagy and lysosomal function in several models of amyloidosis and tauopathy further validate the importance of autophagy failure in AD pathogenesis and as a therapeutic target. Targeting different specific points along the autophagy pathway have distinct implications for the therapeutic success of autophagy/lysosomal modulators.
Conclusions: Autophagy/lysosomal failure has been implicated in the development of all of the major features of AD pathology and is consistent with newly appreciated cellular roles of FAD-related genes and AD risk factors. Studies to date consistently validate the therapeutic potential of remediating deficits in lysosomal efficiency and autophagy.
Disclosure: R. Nixon, None.

Panel Session
Novel Synaptic Targets in Depression Emerging from Clinical, Biochemical, and Circuit Based Approaches

Is Synaptic Plasticity Involved in the Mechanism Underlying the Rapid Antidepressant Effects of N-Methyl-D-Aspartate Receptor Antagonists?

Carlos Zarate*

National Institute of Mental Health, Bethesda, USA

Background: Recent clinical studies have demonstrated the rapid antidepressant effect of ketamine, an NMDA receptor antagonist in treatment-resistant major depressive disorder (MDD) and bipolar depression. A study indicates that ketamine injections in rat prefrontal cortex produces an increase in synaptic strength, as reflected by enhanced postsynaptic protein signaling, increased synaptogenesis and enhanced excitatory postsynaptic currents. Other investigations in rats showed that injections of ketamine induced increases in slow wave activity (SWA, EEG activity between 1 and 4 Hz) during non-rapid eye movement (NREM) sleep. A relationship between SWA and cortical function using high-density EEG in humans demonstrated how SWA and individual slow wave parameters are sensitive markers of cortical synaptic strength and network synchronization. This finding suggests a link between SWA, a sensitive marker of sleep pressure, and NMDA channel blockade, possibly mediated by increased synaptic strength.
Methods: We tested the effects of a single infusion of ketamine followed by double-blind administration of either placebo or riluzole on sleep EEG and mood in 30 treatment-resistant MDD patients. We specifically examined the acute effects of ketamine and riluzole on SWA and individual slow wave parameters during the first and second nights after treatment.

Results: Ketamine's mood effect at 230 min was correlated with the effect on high amplitude slow waves, consistent with an association between these parameters and synaptic change ($p < 0.05$). Additional analysis on individual slow wave parameters showed a significant increase in the occurrence of high amplitude waves during the first NREM sleep episode, consistent with a net increase in synaptic strength.
Conclusions: These results suggest that the strengthening of cortico-cortical connections, reflected by an increase in SWA and slow wave amplitude, may be the physiological mechanism underlying the rapid antidepressant effects of NMDA antagonists.
Disclosure: C. Zarate, Part 1: Dr. Zarate is listed as a co-inventor on a patent for the use of ketamine in major depression. Dr. Zarate has assigned his patent rights on ketamine to the U.S. government., Part 2: None, Part 3: None, Part 4: All research funding is from the National Institute of Mental Health, Part 5: National Institute of Mental Health.

NMDA Receptor Blockade at Rest Triggers Rapid Behavioural Antidepressant Responses

Lisa Monteggia*

UT Southwestern Medical Center, Dallas, USA

Background: Clinical studies consistently demonstrate that a single sub-psychomimetic dose of ketamine, an ionotropic glutamatergic N-methyl-D-aspartate receptor (NMDAR) antagonist, produces fast-acting antidepressant responses in patients suffering from major depressive disorder (MDD), although the underlying mechanism is unclear. Depressed patients report alleviation of MDD symptoms within two hours of a single low-dose intravenous infusion of ketamine with effects lasting up to two weeks, unlike traditional antidepressants (i.e. serotonin reuptake inhibitors), which take weeks to reach efficacy. This delay is a major drawback to current MDD therapies, leaving a need for faster acting antidepressants particularly for suicide-risk patients. Ketamine's ability to produce rapidly acting, long-lasting antidepressant responses in depressed patients provides a unique opportunity to investigate underlying cellular mechanisms.
Methods: Using a variety of behavioral techniques we examined the ability of ketamine, and other NMDAR antagonists, to trigger a rapid antidepressant response. We also used genetically modified mice to examine the requirement of brain-derived neurotrophic factor (BDNF), as well as TrkB, for fast acting antidepressant responses. We utilized biochemistry and pharmacology to identify a specific intracellular signaling cascade that is important for rapidly acting antidepressant action. We also examined the impact of ketamine on electrophysiological responses to further validate the specific signaling pathway that is important for the antidepressant response.
Results: We show that ketamine, as well as other NMDA receptor antagonists, produce fast-acting behavioural antidepressant-like effects in mouse models that are dependent on rapid synthesis of BDNF. We find that ketamine mediated NMDA receptor blockade at rest deactivates eukaryotic elongation factor 2 (eEF2) kinase (also called CaMKIII) resulting in a reduction of eEF2 phosphorylation and de-suppression of BDNF translation. Furthermore, we find inhibitors of eEF2 kinase trigger fast-acting behavioural antidepressant-like effects.
Conclusions: Our findings suggest a behavioural and clinically relevant correlate of protein translational regulation that may serve as a viable therapeutic target for the development of fast-acting antidepressants.
Disclosure: L. Monteggia, None.

Fast Optical Probing of Mechanisms underlying Depression-Related Behaviors

Melissa Warden*

UT Southwestern Medical Center, Dallas, USA

Background: We will present both published and unpublished data probing the high-speed circuit manifestations of depression-related behaviors. First we describe the use of both fast circuit imaging in vitro and multi-unit recording in vivo to identify neurons and circuits that may be causally involved in depression related behaviors. Then we describe the application of optogenetic tools to causally control candidate circuit elements, and we report on changes in depression-related behaviors and circuit properties.
Methods: We describe both high-speed readouts and high-speed optical control to probe circuit properties of the depressed brain. The high-speed imaging is carried out with voltage-sensitive or calcium dyes, and with in vivo tetrode recordings. The high-speed control is implemented by optogenetics, with single-component microbial opsins delivered by viral vectors to targeted circuit elements.
Results: We find altered circuit properties in depression-related states, including altered activity of neurons in prefrontal cortex and hippocampus, and present evidence suggesting a causal significance of these cells (and projections to and from these cells) in depression-related behaviors.
Conclusions: Fast optical methods are used to illuminate aspects of circuit function and dysfunction in depression-related states, with optogenetics employed for causal information.
Disclosure: Melissa Warden, None.

Synaptic Mechanisms in Models of Depression

Roberto Malinow*

UCSD, La Jolla, USA

Background: To discuss recent findings relating synaptic function in the lateral habenula to models of depression.
Methods: Electrophyiological analysis in brain slices from the lateral habenula and related structures are examined; in normal animals and those demonstrating learned helplessness, a model of depression.
Results: We have found increased excitatory synaptic input to lateral habenula neurons in brain slices obtained from animal models of depression (learned helplessness).
Conclusions: Enhanced excitatory transmission onto lateral habenula neurons may contribute to aspects of depression.
Disclosure: R. Malinow, None.

Panel Session
The Autism Sequencing Consortium (ASC): Unraveling the Genetic and Functional Architecture of Autism Spectrum Disorders

Applying Biological Pathways to Next-Generation Sequence Data in Autism Spectrum Disorders

Joseph Buxbaum*

Mount Sinai School of Medicine, New York, USA

Background: We will present an update on novel approaches to gene discovery in autism spectrum disorders, using pathway and network analyses applied to whole-exome data from over 2000 samples.

Methods: The ARRA-funded next-generation sequencing grant [PIs: M Daly (communicating PI), J Buxbaum, B Devlin, R Gibbs, G Schellenberg, J Sutcliffe] has completed whole exome sequencing in 1000 individuals with autism spectrum disorders (ASDs) and 1000 controls, matched on gender and ancestry. Ancestry matching was carried out using existing genome-wide SNP data. In addition, the ARRA group is in the process of sequencing exomes in 300 trios. The individual groups are using methods to assess gene associations, using multiple methods including burden tests, C-alpha etc. At Sinai, in addition to these approaches, we are also using an additional novel testing strategy (reported in *PLoS Genet* 2011 Feb 3;7(2):e1001289). We are also examining genes previously implicated in Mendelian and X-linked forms of autism (e.g., *Brain Res* 2011 Mar 22; 1380:42–77) or intellectual disability for rare variant that might be associated for high risk for ASD. In parallel, we are making use of these lists of genes previously implicated in Mendelian and X-linked forms of autism to identify gene lists that are enriched for ASD genes, focusing on synaptic gene lists, Gene Ontology (GO) categories for Biological Process (BP), and within the Mouse Phenome database, and we are using the enriched categories to determine whether there are enrichments of rare variants in the in the whole-exome date for these pathways.
Results: We have observed that expanding our lists of genes previously implicated in Mendelian and X-linked forms of autism using protein-protein interaction information we can create lists of genes that are significantly enriched for intellectual disability genes ($P < 0.01$) supporting the use of the ASD gene list for novel gene discovery. We show that the ASD gene list is strongly enriched for subsets of synaptic genes, particularly genes in glutamate receptor complexes (P 1E-4–1E-6). In addition we asked where there might be enrichment within the Gene Ontology (GO) categories for Biological Process (BP) and within the Mouse Phenome and MP database. Multiple GO:BP and MP categories showed very significant enrichment (P 1E-2–1e-7). Some of the categories were specific enough (between 100 and 300 genes in the list) that they could reasonably be used to test for enrichment of rare variants in those pathways in ASD. These studies are underway, using both C-alpha (in collaboration with B Devlin and K Roeder) and using our novel testing strategy (in collaboration with I Ionita-Laza). All the pathway analyses will be presented as well as the ongoing application of the resultant gene lists to gene discovery in whole-exome data.
Conclusions: The use of pathway and network analysis can facilitate gene discovery in whole-exome data in autism spectrum disorders and additional neuropsychiatric disorders.
Disclosure: J. Buxbaum, None.

The Genetic Architecture of Autism Spectrum- and Related- Neurodevelopmental Disorders Revealed through High-Resolution Genome Analysis

Stephen Scherer*

Hospital for Sick Children, Toronto, Canada

Background: To provide an update of our Canadian efforts aimed at cataloguing all highly-penetrant copy number variation (CNV) and DNA sequence-level variants in autism spectrum and related neurodevelopmental disorders.
Methods: We are using the highest-resolution genomic technologies to (i) examine a new Ontario-wide ASD consecutive case cohort for the impact of CNVs in autism and related neurodevelopmental disorders for diagnostic validity assessment and discovery and (ii) through next generation sequencing (NGS) define the allelic "mutation" architecture in ASD creating a resource for translational research and validated diagnostics.
Results: We will present our latest data from the first few hundred sequenced exomes/genomes describing both characteristic and new findings. We will discuss our NGS sequencing on individuals carrying potentially pathogenic large CNVs under the hypothesis that there may be additional sequence changes or other contributing loci. Further characterization of the variants identified is achieved by assessment of segregation in families and by determining frequencies in independent case and control populations, as well as through functional studies.
Conclusions: We speculate that our approach will not only yield novel genes and variants influencing ASD susceptibility, but will also shed light on the broader issues of allelic variant architecture, variable penetrance and expressivity, and phenotypic heterogeneity in complex disorders.
Disclosure: S. Scherer, None.

Examples of Recessive and Oligogenic Disease

Richard Gibbs*

Baylor College of Medicine, Houston, USA

Background: This presentation will present examples of Mendelian disease analyses that illustrate the opportunities to identify the genetic underpinning of complex phenotypes, beginning with the study of single gene diseases. This will be contrasted with evolving models of the genetic basis of autism.
Methods: Families with genetic diseases with simple modes of inheritance are studied using state of the art targeted capture enrichment and next generation DNA sequencing methods. Large sample sets of patients with autism are also under analysis in consortia projects.
Results: Single family studies in neurological phenotypes clearly illustrate that the individual alleles that contribute to recessive disease can also be associated with phenotypes. The data for studies of autism are currently in analysis.
Conclusions: Previous and recent studies of Mendelian disease, with simple patterns of inheritance, show that we may yet validate models for complex disorders like autism by studying genetic variants that have high penetrance and produce clear phenotypes, in at least some genetic backgrounds.
Disclosure: R. Gibbs, Part 1: SeqWright, Inc; Life Technologies, Part 2: Baylor College of Medicine; SeqWright Inc, Part 3: SeqWright, Inc.

Transcriptome and Genome Analysis of ASD

Daniel Geschwind*

UCLA, Los Angeles, USA

Background: Autism Spectrum Disorder (ASD) is a genetically heterogeneous disorder in which several dozen disease causing mutations have been identified, none of which account for more than 1% of cases. Thus, a major question is whether these myriad pathways coalesce into one or more major pathways at a molecular level.
Methods: We performed gene expression profiling from 3 brain regions, the cerebellar vermis and frontal and temporal lobes in autism and controls. We also performed RNA seq and analysis of splicing to identify splicing dysregulation. These data were integrated with genetic polymorphism data to assess the genetic versus environmental contribution to dysregulation.
Results: We identified shared pathways in ASD post mortem brain and validated these patterns in independent cases. Using network analysis, we further identified 2 major co-expression modules associated with autism. One of these, a neuronal module had as its hub, the neuronal splicing factor A2BP1/Fox1. To test whether Fox1-dependent exons were altered we performed RNA

seq in 3 ASD cases with low Fox1 and 3 controls with normal Fox1 levels, identifying several hundred differential splicing events between autism and controls. Many of these reflect bioinformatically identified Fox1 targets. Lastly, we found that the neuronal module had an enrichment for genetic association signals, indicating that its dysregulation is likely causal.
Conclusions: These data show that despite heterogeneity at the genetic level, there is convergence of transcriptional and splicing abnormalities in autism.
Disclosure: D. Geschwind, Part 2: Biological Psychiatry (the journal as deputy editor), Part 4: Repligin 2009–2010.

Panel Session
Gimme Another Hit of Chocolate. Is Food Addictive?

Neurobiology of Compulsive Eating: Role for Striatal Dopamine D2 Receptors
Paul Kenny*

The Scripps Research Institute, Jupiter, USA

Background: Hedonic mechanisms contributing to obesity remain poorly understood. We investigated the effects of extended access to a palatable high-fat diet on the sensitivity of brain reward systems and on compulsive-like eating in rats. We also examined the role for striatal dopamine D2 receptors (D2Rs) in these behavioral deficits.
Methods: Rats were trained in the brain stimulation reward (BSR) procedure, and given restricted (1-h) or extended (18–23-h) daily access to a palatable high-fat diet. We also tested whether the restricted or extended access rats continued to eat palatable food when they were exposed to a light (conditioned stimulus) previously paired with delivery of foot shocks. Finally, we developed a lentivirus vector to deliver a short interfering RNA against D2Rs into the striatum of rats to knockdown their expression, and assessed BSR thresholds and compulsive eating in the restricted and extended access rats.
Results: Rats with extended but not restricted access to palatable food for 40 days gained significant amounts of weight ($\sim$150g), an effect closely associated with a worsening deficit in brain reward function, reflected in elevated BSR thresholds ($\sim$30% elevation). The obese extended access rats, but not restricted access rats, also demonstrated compulsive-like consumption of palatable food, reflected by the fact that their intake was resistant to disruption by an aversive conditioned stimulus. Finally, knockdown of striatal D2Rs accelerated the emergence of reward deficits and compulsive eating in extended access rats (detected after 14 versus 40 days).
Conclusions: Development of obesity is associated with reward dysfunction and compulsive-like eating in rats, regulated in part by striatal D2Rs. Similar behavioral deficits also occur in rats with extended access to heroin or cocaine. These findings suggest that obesity and drug addition may share common hedonic mechanisms.
Disclosure: P. Kenny, None.

Overeating of Sugars and Fats: Links to Addiction and Obesity
Nicole Avena*

University of Florida, Gainesville, USA

Background: Overeating of palatable food (PF) can, in some cases, result in addiction-like behaviors and changes in the reward-related brain areas. However, less is known about the distinct roles that specific nutrients play in the manifestation on addiction-like responses to PF.
Methods: Sprague-Dawley rats were given limited (binge) or *ad libitum* access to (1) a 10% sucrose solution and standard chow, (2) a fat-rich food, or (3) standard chow alone. Signs of anxiety and opiate-like withdrawal were assessed, and pharmacological probes, *in vivo* microdialysis, and immunohistochemistry were used to investigate the contributory role of specific neurotransmitter systems and genes.
Results: Sugar-overeating rats were normal weight and signs of opiate-like withdrawal were noted (somatic signs; elevated-plus maze anxiety = 52 vs 75 s, $p<0.05$). Sugar-overeating rats showed alterations in dopaminergic, cholinergic and opioid systems, as well as increased DeltaFosB immunoreactivity in the nucleus accumbens (835 vs 408 cells, $p<0.05$); these findings are similar to the effects of some drugs of abuse. However, when rats overate the fat-rich diet they gained excess body weight, but opiate-like withdrawal signs were not seen. Systemic injection of the opioid antagonist naloxone had a suppressive effect on PF intake for both sugar- and fat-bingeing rats. However, AM 251 (CB1 inverse agonist) suppressed overeating of a fat-rich food (.85 mg/kg, -30 kcal vs saline) compared with *ad libitum* access to the fat-rich food (-15 kcal) or standard chow (-10 kcal), and the GABA-B agonist baclofen selectively reduced intake of a fat-rich, but not a sugar-rich, binge food (1.8 mg/kg, 50 vs 25 kcal, $p<0.05$).
Conclusions: The findings suggest aberrant behaviors and brain changes can ensue when rats overeat PF, and highlight the differences that emerge when body weight and the type of PF are considered. These findings may be of use in understanding the concept of "food addiction" and extending it to the study of overeating in humans.
Disclosure: N. Avena, None.

Excessive Over- and Under-Eating Differentially Determine Brain Reward Learning in Humans
Guido Frank*

University of Colorado Anschutz Medical Campus, Aurora, USA

Background: To test whether underweight (anorexia nervosa, AN), or overweight (obesity, OB) individuals would have functional brain response abnormalities in a dopamine (DA) anchored reward-learning paradigm, the temporal difference (TD) model. TD model tasks are based on differential brain response to unexpected receipt (US+) or omission (US−) of reward stimuli, creating a prediction error (PE), that is the difference between expected and received reward values. Brain dopamine (DA) response is directly related to the PE.
Methods: Twenty-one AN (age M 21.0, SD 5.1 years, body mass index [BMI, weight in kg/m^2] M 16.1 SD 0.9), nineteen OB (age M 30.0, SD 6.7 years, BMI M 34.8, SD 4.9) and 24 healthy control women (CW, age M 23.5, SD 4.3 years, BMI M 24.3 SD 0.9) underwent functional magnetic resonance brain imaging (fMRI) while performing the TD task. For each subject and trial the PE value was computed based on current and past task trial experience. PE values were regressed with brain activation in a parametric modeling approach on the single subject level, and regression strengths compared across OB, AN and CW groups to test sensitivity and reward learning of brain DA pathways.
Results: All brain maps were FDR corrected at $p<0.05$, with 50 voxel contiguity. AN individuals showed significantly greater brain response compared to CW for US+ and more negative response to US− conditions, as well as significantly stronger brain regression with PE data, in midbrain, ventral putamen, and insula. In contrast, OB individuals showed opposite results, with significantly reduced activation to US+ and less negative response to US−

conditions, as well as reduced regression strength with model derived PE values.
Conclusions: Consistent with limited data, these findings suggest that individuals with AN and OB have opposite function of neural food reward pathways. Whether cause or consequence, these processes are likely to be important for driving disordered eating behavior.
Disclosure: G. Frank, Part 4: 1. NIMH K23 award, Anorexia Nervosa and Computational Modeling 2. Klarman Foundation award, Brain Reward Pathway Function in Anorexia Nervosa, Bulimia Nervosa and Binge Eating Disorder, and its Relationship to Dopamine Genotype, Part 5: No.

Is Food Restriction in Anorexia Nervosa caused by Reduced Reward and/or Increased Inhibition?

Walter Kaye*

UCSD Department of Psychiatry, La Jolla, USA

Background: Individuals with anorexia nervosa (AN) restrict eating to such an extreme that they can die from emaciation. They tend to be anhedonic, anxious, inhibited, and over controlled. We present new data that supports the hypothesis that such symptoms are due to an imbalance within and/or between ventral limbic reward circuits and inhibitory dorsal cognitive circuits.
Methods: To avoid the confounding effects of malnutrition, 10 to 15 recovered AN (RAN) were compared to control women (CW) using 3 imaging studies that interrogate these pathways: 1) Amphetamine challenge and positron emission tomography (PET) [11C]raclopride paradigm to assess endogenous striatal DA transmission. 2) A delay discounting task, that chooses between monetary rewards that varied by temporal delivery, with functional magnetic resonance imaging (fMRI). 3) Response to tastes of sucrose using fMRI.
Results: 1) RAN had an increase of anxiety after amphetamine administration compared to CW ($p = 0.01$). Endogenous DA values in the ventral striatum was associated with euphoria ($p = 0.03$) in CW and anxiety in the dorsal caudate ($p = 0.05$) in the RAN. 2) As expected, RAN demonstrated a preference for the delayed option ($p < 0.001$). For trials in which the early temporal choice was immediate, CW showed task-related activation ($p < 0.01$) in the ventromedial prefrontal cortex and left parahippocampus but RAN failed to activate these regions. 3) RAN had diminished ($p = 0.01$) right anterior insula response to tastes of sucrose.
Conclusions: These data provide further evidence that a failure of life-sustaining drives in AN may be due to impaired ability to engage limbic circuitry that contributes to reward as well as exaggerated dorsal cognitive circuit function that correlates with an anxious response to stimuli that is normally rewarding. Understanding AN may provide new treatment targets for inhibitory and reward brain mechanisms that could improve the ability to restrain the eating of palatable foods.
Disclosure: W. Kaye, Part 1: Astra Zeneca small grant, Part 2: none, Part 3: none, Part 4: Astra Zeneca, Part 5: no.

Panel Session
Neural Mechanisms of Environmental Risk for Psychiatric Disorders

Risk, Resilience, and Gene-Environment Interplay in Primates

Stephen Suomi*

NICHD/NIH, Bethesda, USA

Background: This presentation will present data clearly demonstrating that risk for developing axniety-like, depressived-like, and impulsive-related behavioral and biological symptoms in rhesus monkeys is the product of gene-environment interplay.
Methods: Prospective longitudinal studies of rhesus monkeys reared from birth in randomly assigned different social environments are carried out, during which a variety of behavioral, neuroendrocrine, neurotramsmitter, neuroimaging, and genome-wide expression data are repeatedly collected from infancy to early adulthood.
Results: Differential early social experience (mother-rearing [MR] vs. peer-rearing [PR]) for the first 6–7 months of life profoundly influences development over a wide range of levels of analysis, including behavior expression and emotional regulation (PR more fearful and aggressive than MR), neuroendocrine functioning (greater HPA activation in PR), neurotransmitter metabolism (chronically lower CSF 5-HIAA concentrations in PR), brain structure and function (significant differences in structural MRI and PET images of PR and MT brains), and genome-wide methylation patterns (approximately 1/5 of rhe entire rhesus monkey genome is differentially methylated, both in pfc and lymphocytes, as a function of differential social experience duringnthe first 6–7 months of life. The magnitude of these early experience effects differ significantly as a function of allelic status of multiple "candidate" genes.
Conclusions: Individual differences among rhesus monkeys in their risk for and resilience to the effects of adverse early social experiences are a product of gene-environment interplay for a wide range of behavioral and biological functions expressed at multiple levels of analyses.
Disclosure: S. Suomi, None.

Neurobiology of Gene-Environment Interactions in Mediating Child Abuse Associated Risk for Mood and Anxiety Disorders

Charles Nemeroff*

University of Miami Miller School of Medicine, Miami, USA

Background: In the past several years there has been a plethora of data generated supporting the hypothesis that early life trauma, in the form of child abuse and neglect, is associated with an increased risk for major psychiatric disorders in adulthood including depression, PTSD, bipolar disorder and schizophrenia. In the past few years, a number of candidate gene polymorphisms have been convincingly demonstrated to mediate the vulnerability (and resilience) for the development of these psychiatric syndromes. The primary purpose of this presentation will be to summarize the current state of this field including the work from our group and others. This includes most specifically the genetic polymorphisms that have been shown to mediate child abuse and neglect associated vulnerability for major depression and PTSD. Functional and structural brain imaging studies in patients with a history of early life trauma will also be reviewed, as will our recent unpublished findings on cognitive function in a well characterized cohort of men and women evaluated in our NIMH funded Conte Center. In addition, a variety of measures have also demonstrated increases in inflammatory markers in patients with early life trauma.
Methods: Several patient populations will be included in this presentation. They include the large cohort of patients studied in the Grady Memorial Hospital PTSD study which now totals more than 1000 patients, as well as all of the patients studied in the 10 year Conte Center for the Psychobiology of Early Life Trauma. Methods employed ranged from standard measurement of genetic polymorphisms to standardized dimensional measures of psychopatholgy, eg. HAM-D, Beck Depression Inventory, Childhood Trauma Questionaire. Structural and Functional MRI was used to assess changes in the volume of the hippocampus and neural circuit responses to provocative stimuli. Inflammatory markers

including IL-6 were measured in patients with early life trauma and the results are consistent with persistent inflammation in such patients.
Results: In the face of certain genetic polymorphisms such as FKBP5, PAC1 and the CRF-1 receptor, patients exposed to early life trauma exhibit a marked increase in vulnerabiltity to certain mood and anxiety disorders. Patients with a history of child abuse and neglect and current major depression or PTSD exhibit a number of persistent neurobiological alterations including reduced hippocampal size, cognitive dysfunction, functional brain imaging alterations and persistent neuroendocrine and inflammation.
Conclusions: The neurobiological consequences of child abuse and neglect in increasing risk for adult psychopathology is a classic example of gene-environment interactions of complex medical disorders.
Disclosure: C. Nemeroff, Part 1: Board of Directors: Novadel Scientific Advisory Board: Cenerx, Pharmaneuroboost Consultant: Xhale, Takeda, SK Pharma Equity: Reevax, Part 2: American Psychiatric Publishing, Astra Zeneca, SK Pharma, Novadel Pharma, Cenerex, Xhale, Pharmaneroboost.

How does Cannabis increase Risk of Schizophrenia?

Robin Murray*

Institute of Psychiatry, London, United Kingdom

Background: To elucidate the mechanisms whereby cannabis increases risk of schizophrenia
Methods: 1) Case-control study of 499 first episode psychotics and 350 controls. 2) Experimental study administering intravenous tetrahydrocannabinol (THC) to normal subjects.
Results: 1) First episode psychotics had used cannabis for longer, more frequently, and preferentially used skunk cannabis compared with matched controls. Skunk is much more potent than traditional UK cannabis and contains about 16% THC and $<1\%$ canabidiol (CBD). Age of onset of psychosis was brought forward by frequency of cannabis use and by use of high potency cannabis. All subject were genotyped and the results of the GxE interactions between cannabis and a) COMT, b) AKT1 will be presented. 2) Effects of THC 2.5 mg v placebo intravenously showed an increase in PANSS positive and negative scores, and decrements in cognitive function such as memory. Pretreatment with CBD appeared to diminish the psychotogenic effects of THC. Effects on THC on MRI during various cognitive challenges will also be reviewed as will the effects of THC on striatal dopamine.
Conclusions: Cannabis has a risk increasing effect on schizophrenia depending on duration and amount of use and potency of type of cannabis used. Age of onset of psychosis is brought forward by the same factors. THC can induce an acute psychosis and preliminary evidence suggest that this may be partly ameliorated by pretreament with CBD. THC appears to have a modest effect on striatal dopamine but it is unclear whether this is the mechanism whereby it induces psychotic symptoms. We shall show data confirming one of the two previously suggested gene x cannabis interactions.
Disclosure: R. Murray, Part 1: Honoraria for lectures from AZ, BMS, Lilly, Janssen, Novartis, Part 2: NOne, Part 3: None, Part 4: None.

Neural Mechanisms for Environmental Risk related to Urbanicity and Migration

Andreas Meyer-Lindenberg*

CIMH, Mannheim, Germany

Background: Urban upbringing and migration are clearly related to risk for schizophrenia in epidemiological studies, but the unterlying mechanisms are unclear. Here, we pursue the hypothesis that these risk factors may relate to social evaluative stress processing and their neural mechanisms.
Methods: We use an innovative social stress paradigm together with functional magnetic resonance imaging: where participants solve arithmetic tasks under time pressure. Difficulty was varied adaptively to keep success rates, continuously visually presented on a "performance scale", between 25–40%. Study investigators provided further negative feedback after each test segment via headphones. Subjective stress levels were measured before and after the session via visual analogue scale, and effects of the MIST on salivary cortisol, heart rate, and blood pressure were recorded repeatedly. Urbanicity was quantified as follows[4]: 1-rural, 2-towns more than 10000 and 3-cities of more than 100000 inhabitants. For urban upbringing, these numbers were multiplied by years lived in the area up to age fifteen and added. We studied 32 participants of German origin and an additional sample of second-generation Turkish migrants. To demonstrate specificity to social stress, a sample of 80 subjects were examined using cognitive paradigms (an n-back working memory and face matching task).
Results: Urban upbringing and city living had dissociable impacts on social evaluative stress processing in humans. Current city living was associated with increased amygdala activity, while urban upbringing impacted the perigenual anterior cingulate cortex, a key region for regulation of amygdala activity, negative affect and stress. These findings were regionally and behaviourally specific, since no other brain structures were affected and no urbanicity effect was seen during control experiments invoking cognitive processing without stress. In Migrants, the same area of perigenual cingulate was specifically more active than in German inhabitants and showed an interaction with earl-life urbanicity.
Conclusions: Our results identify distinct neural mechanisms for an established environmental risk factor, link the urban environment for the first time to social stress processing, suggest that brain regions differ in vulnerability to this risk factor across the life span, and indicate that experimental interrogation of epidemiological associations is a promising strategy in social neuroscience.
Disclosure: A. Meyer-Lindenberg, Part 1: Roche Astra Zeneca J + J Pfizer Novartis.

Panel Session
Drug of Abuse during Adolescence: A Development Period of Vulnerability or Resilience?

The Relationship between Substance Use and Brain Development in Human Adolescents: Insights from Neuroimaging

Adriana Galvan*

UCLA, Los Angeles, USA

Background: The series of studies described in this presentation will illustrate how neurodevelopmental changes in human adolescents in regions critical for reward processing, risk-taking and behavioral regulation are related to risky behavior and substance use during adolescence.
Methods: Functional magnetic resonance imaging (fMRI), coupled with behavioral tasks, was used to probe brain function in healthy children, adolescents and adults. Self-report data and diagnostic interviews on risky behavior and substance use were also collected.
Results: The first set of studies revealed exaggerated neural activation in adolescents compared to children and adults in the nucleus accumbens during reward processing. This hyperactivation in adolescents was coupled with relatively immature

engagement of prefrontal cortex regulatory regions as compared to adults. More recent work in a sample of adolescent smokers examined neural correlates of risk-taking and response inhibition. These data revealed an association between nicotine dependence and neural activity during an impulse control task, with less engagement of the prefrontal cortex related to severity of cigarette use and dependence. Further, risk-taking behavior was also associated with nicotine dependence and atypical activation in the dorsolateral prefrontal as compared to nonsmoking counterparts.
Conclusions: Collectively, this body of work suggests that the adolescent brain undergoes significant, normative neural changes in frontostriatal circruity that are associated with a heightened propensity to engage in risk-taking behavior. Further, these results suggest that adolescents who engage in substance use show abnormal neurofunctioning as compared to adolescents who are not substance users. Given the important neurodevelopmental changes during adolescence, understanding the long-term effects of substance use on brain and behavioral development is of critical importance.
Disclosure: A. Galvan, Part 1: Philip Morris USA funded a part of the data presented.

Adolescence Is a Period of High Risk for Addiction: The Role of the Prefrontal Dopamine System and Cocaine Cues in Rats
Susan Andersen*

McLean Hospital/Harvard Medical School, Belmont, USA

Background: Adolescents are at a high risk to abuse substances, however little is known about mechanisms that contribute to this risk. This study investigated the role that the D1 dopamine receptor in the prefrontal cortex plays in how adolescent rats respond to cocaine-associated environments.
Methods: Male Sprague-Dawley rats were tested with non-biased place conditioning at postnatal day 23 (juvenile), 44 (mid-adolescence), and 100 (adult) days of age. Subjects were tested for sensitivity to cocaine-associated environments, extinction of these associations, and reinstatement of place preferences following a priming injection of cocaine. Age differences in the expression of D1 receptors in prelimbic cortex was determined receptors on glutamate neurons retroactively traced from the accumbens. To provide a causal link between behavior and D1 expression, we produced a lentiviral vector that expressed D1 specifically in glutamate neurons and tested a separate group of adult transduced subjects.
Results: Adolescent rats developed a significant preference for cocaine cues at 10, 20, and 40 mg/kg cocaine, whereas juveniles and adults consistently demonstrated significant preferences at 40 mg/kg only. When tested in environments where the absence of cues was not explicitly paired, adolescent rats took 75% longer to extinguish place preferences than adults and reinstated these preferences to a greater extent. In contrast, age differences were diminished when cues were explicitly paired with the absence of cocaine. Transient over-production of D1 receptors on glutamate outputs from the prelimbic prefrontal cortex paralleled these adolescent sensitivities, which were recapitulated in adult rats transduced with the D1 lentivirus. Moreover, these rats demonstrated high impulsive choice, without a change in locomotor activity.
Conclusions: Taken together, high D1 enhances motivational salience that increases a constellation of behaviors that may render adolescents more vulnerable to addiction.
Disclosure: S. Andersen, None.

Mechanisms of Adolescent (in)Vulnerability
Kyle Frantz*

Georgia State University, Atlanta, USA

Background: Drug abuse is prevalent among teenagers, and early-onset drug use might predispose individuals to drug addiction. Prior work from our laboratory suggests, however, that adolescent male rats are actually less sensitive than adults to some reinforcing effects of cocaine and the opiates, morphine and heroin. Our current work aims to explore potential behavioral or molecular mechanisms for age differences in long-term sensitivity to drugs or drug-paired cues.
Methods: Using the intravenous drug self-administration model, adolescent vs. adult rats are allowed to acquire lever-pressing maintained by infusions of cocaine, morphine, or heroin. After abstinence periods of various durations and environmental conditions, reinstatement of drug-seeking is tested, animals sacrificed, and gene expression analyzed. In adjunct studies, signs of drug withdrawal are quantified and motor sensitization tested after repeated drug injections.
Results: Regardless whether amounts or patterns of drug self-administration differs across age groups, we have observed attenuated reinstatement of drug-seeking after abstinence among the younger cohorts, as triggered by either discrete drug-paired cues or re-exposure to the drug itself. Some evidence rules out differential responsivity across age groups to the abstinence environment, but less robust drug withdrawal in younger subjects could contribute significantly to these surprising age differences in reinstatement. Ongoing studies probe expression of plasticity-related genes that might inherently protect younger subjects from some of the long-term effects of early drug exposure.
Conclusions: Ultimately, adolescent male rats may serve as a model for some elements of natural protection from at least some of the long-term effects of early drug exposure.
Disclosure: K. Frantz, None.

Unique Effects of Nicotine on Adolescent Limbic System Function
Frances Leslie*

UC Irvine, Irvine, USA

Background: Epidemiological studies have shown that adolescent smoking is associated with health risk behaviors, including high-risk sexual activity and illicit drug use. Using rat as an animal model, we have determined whether nicotine, the psychoactive component of tobacco, induces alterations in limbic function which may contribute to these changes in adolescent behavior.
Methods: Adolescents, aged postnatal day (P) 28 and P38, and adult male Sprague Dawley rats were treated for 4 days with saline or nicotine (60 μg/kg; i.v.). Separate groups of animals were pretreated with the 5-HT1A receptor (5-HT1AR) antagonist, WAY 100635, alone or in combination with nicotine, or with the 5-HT1AR agonist, 8-OH-DPAT. Animals were then tested for self-administration of cocaine, or for locomotor and penile erectile effects of quinpirole in the absence and presence of the D2 receptor (D2R) antagonist, L-741,626.
Results: Nicotine pretreatment significantly increased acquisition of cocaine self-administration, quinpirole-induced locomotor activity, and penile erection in adolescent rats, aged P32. These effects were long-lasting, remaining evident 10 days after the last nicotine treatment, and were seen when nicotine pretreatment was administered during early adolescence (P28–31), but not late adolescence (P38–41) or adulthood (P86–89). Nicotine-enhancement of cocaine self-administration and quinpirole-induced locomotor activity, but not penile erection, was mediated by

5-HT1AR activation. Pharmacological analysis further indicated an increased functional sensitivity of D2Rs.
Conclusions: These findings indicate that early adolescent nicotine exposure uniquely alters limbic function, in part by modulation of 5-HT neuronal activity and activation of 5-HT1ARs. The resulting enhancement of quinpirole activity is mediated by increased sensitivity of D2Rs. Our data suggest that nicotine-induced alterations in monoamine function uniquely alters adolescent limbic function, increasing sensitivity to dopaminergic drug action.
Disclosure: F. Leslie, None.

Panel Session
From Genome to Macro-Connectome: Integrating High-Dimensional Genetic, Imaging and Behavioral Data, with Application to Large-Scale Studies of Alzheimer's Disease, Schizophrenia, and Substance Abuse

The Statistical Challenges of High Dimensional Neuroimaging and Genetic Data Analyses
Jean-Baptiste Poline*

Neurospin, I2BM, Gif-sur-Yvette cedex, France

Background: In this talk I will review the challenges of neuroimaging genetics statistical analysis. I will take as an example the Imagen study in which 2000 adolescents were fully characterized with behavioural tests, neuroimaging (fMRI, DTI, T1) and genotyping. A specific emphasis will be given to the data analysis using sparse multivariate techniques such as sparse Partial Least Square techniques.
Methods: We investigated the use of genome wide analysis for several tens of region of interest in which BOLD data was measured. Sparse Partial Least Square (sPLS) was combined with univariate feature selection and cross validation to measure the appropriate degree of sparsity, and permutations were used to evaluate the significance of the association. Alternative techniques that construct regions clustered both in the brain and on the genome were also tested.
Results: Multivariate techniques such as sPLS were able to find associations between BOLD signals and SNPs that could not be found using simpler genome wide associations with each brain region, hence demonstrating a greater sensitivity. Clustering techniques also showed promising results.
Conclusions: The neuroimaging genetics data analyses are still in their infancy. More integrated techniques able to account for behavioural as well as neuroimaging and genetic data, such as three-way multivariate analyses, are still to be developed. Sparse regression or sparse Partial Least Square techniques show very promising results for neuroimaging genetic data analyses.
Disclosure: J. Poline, Part 4: IPSEN Laboratory.

New Findings in Schizophrenia via Robust Identification of Linked Genetics Factors and Functional Brain Regions within a Multivariate Framework
Vince Calhoun*

The Mind Research Network/UNM, Albuquerque, USA

Background: To highlight the advantages of multivariate approaches to identify relationships between functional magnetic resonance imaging (fMRI) phenotypes and polygenic components of single nucleotide polymorphisms (SNPs) using parallel independent component analysis (ICA). Due to the gene–gene interaction effect, a multivariate approach is very useful for analyzing genomic properties of complex diseases.
Methods: In study one, fMRI data from a sensorimotor task and genome-wide SNPs from an Illumina Human1M-Duo assay were collected for 75 schizophrenia (SZ) patients and 96 healthy controls (HC). We first selected 5291 SNPs due showing a weak group difference. An additional 1542 SNPs were also included due to their previously reported relationship to SZ. Parallel-ICA was applied to the fMRI-SNP dataset to extract maximally independent functional brain networks and polygenic SNP components simultaneously, while emphasizing the correlation between the two modalities. In study two, we present a guided ICA method which is of particular use for studies with smaller sample sizes and when a priori information is available. We used auditory oddball fMRI data from the fBIRN study and 308,330 SNP loci in autosomes using the Illumina Infinium HumanHap300 from 43 SZ patients and 45 HC. We first select SNPs reflecting a prominent feature of interest and use the location of these SNPs as a guide to an ICA with reference approach which is then applied to the full array. After extracting the genetic component, we test its association with brain networks from fMRI data extracted using a regular ICA approach. We evaluate our method on both real and simulated datasets.
Results: Study one: Parallel-ICA detected one linked and robust fMRI-SNP pair. The fMRI-SNP correlation was -0.33 (P-value = 1.41E-05) and showed significant group differences between SZ patients and healthy controls. The fMRI component mainly included regions of pre/post central gyri and superior temporal gyrus. Results further indicated that most consistently detected coding-region SNPs were related to SZ risk genes, including: rs17526697 (HTR7), rs660464 (NRG3), rs1669191 (MYH14) and rs17038674 (CX3CR1). Study two: Simulation results demonstrate that ICA with reference can extract the disease-related genetic loci, even when the variance accounted for by such loci is so small that a regular ICA fails. Using the initial genetic reference, we extracted a maximally independent genetic component with a significant group difference ($p < 4E-17$) and focused our attention on the maximally contributing SNP loci. These SNPs fall into two clusters centered at chromosome 7q21–22, gene CDK14 and chromosome 5q35, gene GRM6. The regions in our functional network mainly locate in the thalamus, anterior cingulate and posterior cingulate gyri.
Conclusions: We demonstrate two examples showing unique findings in two SZ data sets. Results demonstrate the advantages of multivariate approaches. Overall, these preliminary results provide us with a point from which we can further explore the underlying mechanisms leading to the linkage between the identified SNPs and regional brain function and its underlying relationship to SZ. Results from both studies support the notion that a breadth of brain functional abnormalities and genetic variations are linked with SZ, but more importantly, they indicate that the approaches used can be particularly useful to explore the whole genome to find factors that contribute to illness.
Disclosure: V. Calhoun, None.

A Large Scale Multivariate Parallel ICA Method reveals Novel Imaging Genetic Relationships for Alzheimer's Disease in the ADNI Cohort
Godfrey Pearlson*

Yale University/IOL, Hartford, USA

Background: The underlying genetic etiology of late onset Alzheimer's disease (LOAD) remains largely unknown, likely due to 1) its polygenic architecture and 2) lack of sophisticated analytic methods to evaluate complex models. We aimed to overcome these limitations in a bi-multivariate fashion by linking endophenotypic

quantitative trait loci (sub-cortical/cortical volume/thickness measures) with a genome-wide sample of common single nucleotide polymorphism (SNP) variants, both derived from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort.
Methods: We studied 209 healthy controls, 367 subjects with amnestic mild cognitive impairment (MCI) and 181 with mild, early-stage LOAD, all European-American. Imaging was performed on comparable 1.5T scanners and protocols at over 50 sites in the continental USA and Canada. We evaluated associations between 94 FreeSurfer-derived different brain regions of interest and 533,872 genome wide SNPs using parallel-independent component analysis in the above cohort.
Results: Results revealed four primary "genetic components" associated significantly with a single structural network that included all regions involved neuropathologically in LOAD. Pathway analysis suggested that all four components included several genes already known to contribute to LOAD risk and additional previously unidentified risk genes, involved in multiple processes contributing to the disorder, including inflammation, diabetes, obesity and cardiovascular disease. In addition to APOE4, some of the most significant novel genes identified included ZNF673, VPS13, SLC9A7, ATP5G2 and SHROOM2.
Conclusions: Unlike conventional analyses, this multivariate approach identified distinct groups of LOAD risk genes that would not survive conventional GWAS, and are plausibly linked in physiologic pathways, perhaps epistatically. Further, the study exemplifies the value of this novel approach to explore large-scale data sets involving high-dimensional gene and endophenotype data.
Disclosure: G. Pearlson, None.

Substance Use Disorders: Linking Genes, BOLD Response, and Clinical Phenotypes
Kent Hutchison*

University of Colorado, Boulder, USA

Background: Given that the etiology of alcohol dependence is related to changes in the neuronal systems involved in the anticipation of reward and executive control, genetic markers that are associated with individual differences in these mechanisms may be important in terms of predicting the effects of treatments that also target these mechanisms. We recently developed an approach that emphasizes brain-based phenotypes that can be used to link changes at the molecular level (e.g., genetic variation), to changes in neuronal function, and ultimately to changes in clinical outcomes.
Methods: Extensive neuroimaging and clinical data as well as genome wide data using the Illumina 1M Duo were collected on a sample of 326 individuals with alcohol use disorders. Because replication is critical, the sample was split in half to demonstrate replication. SNPs identified in the first half of the sample were used to form an aggregate genetic risk (AGR) score that was tested in the second half of the sample. In addition, the AGR score was tested in separate large population based sample of 6327 individuals who had both genome wide data and a quantitative alcohol use phenotype.
Results: In the first analysis, SNPs were tested for an association with BOLD response to alcohol cues and loss of control over consumption. Results suggested that 302 SNPs were associated with whole brain cluster size at a genome wide significance level, with significant activation in a critical region of interest (dorsal striatum), and with a clinical measure of loss of control over drinking. The second analysis in an independent sample indicated that the AGR score based on these SNPs was significantly associated with BOLD response and loss of control over consumption. Finally, analyses suggested that the AGR score was significantly associated ($r = .19$, $p < 0.0001$) with the quantitative measure of alcohol abuse in the large community sample.
Conclusions: In summary, this research has uncovered a number of novel genetic variations that are related to functional brain changes and suggest that these genetic variations are associated with alcohol use phenotypes in large clinical samples. Perhaps most importantly, the results suggest that the findings replicate in small datasets with precise neuroimaging phenotypes as well as large population based datasets with quantitative measures of alcohol abuse.
Disclosure: K. Hutchison, None.

Special Session

An Oral History of Neuropsychopharmacology
Samuel Gershon*, Martin Katz, Edward Shorter, Fridolin Sulser, Barry Blackwell, Donald Klein, David Janowsky, Herbert Kleber, Carl Salzman, John Davis, Thomas Ban

Emeritus Professor, Aventura, USA

Reflective epilogues on the 10 volume ACNP series "An Oral History of Neuropsychopharmacology" highlight the past and guide us into the future. This multidisciplinary set of 213 interviews presents the history of the new science, tracing developments in each principal branch during the past 50 years. Our members contribute a great stream of research that led to the foundation of present-day neuropsychopharmacology. The final volume speaks directly to the history of the ACNP during this period. Each volume focuses on a specific science and is comprised of member interviews associated with that field. The interviews cover scientific advances, the member's career, and views on developments in the science during the past 50 years. The sum of these 200 interviews – including remarkable detail from senior and young investigators and four Nobel Laureates – offers an extraordinary account of the evolution of our discipline from its early days to the present. Major events in each science, the controversies across clinical and basic research, and progress during 50 years are summarized by the Editor and a representative interviewee. Success in identifying drugs that revolutionized treatment of mental disorder, and triumphs in uncovering mechanisms of selective drug actions, are contrasted with failures to solve other critical mechanism issues, to significantly advance understanding of mental disorder, and to produce new, more effective drugs. From the 213 interviews, Editors draw conclusions about where our science has come over the past 50 years and where it is headed. It is clear that major controversies remain, and the wisdom of interviewees will help resolve these. The mission of our College in this new age requires reflection, and the lessons of the past drawn from these interviews may light our way into the future. There is a concerted effort to resolve current problems between clinical and basic scientists, and to move to a more productive translational state. Unique Data Description Information: This experiment in "oral history" is unique. Nowhere previously has such a critical mass of clinicians and neuroscientists reflected about the evolution of the field and their contributions as leaders to it. The interviews reach across all fields to detail the foundation and growth of the new science of neuropsychopharmacology. These interviews help tell us whether the mission of the College defined by its Founders has kept pace with progress in the basic and clinical sciences. No other intellectual venture has ever brought together so many participants and vantage points from which to assess the past growth of neuropsychopharmacology and its future prospects. This is the raw material of history, and it gives perspective for the College in planning an even more productive future.
Disclosure: S. Gershon, None.

Thursday, December 8, 2011

Mini-Panel Session
The Use of Intraoperative Techniques to Assess the Physiology of the Anterior Cingulate Cortex

Boundaries of Anterior Cingulat Cortex and the Midcingulate Concept

Brent Vogt*

SUNY Upstate Medical University, Syracuse, USA

Background: Brodmann proposed the anterior/posterior dichotomy for cingulate cortex based on cyto- and myeloarchitecture and he stated that his map was preliminary. Human imaging has failed to identify any unifying function for his anterior cingulate cortex (ACC). The difficulty with concepts like rostral, caudal and dorsal ACC is they are not based on any structural principles and the boundaries implied are unknown. It has been apparent that ACC is not uniform and a regionalized system is needed that submits to the detailed analyses from modern neuroscience. The system that evolved from our work is the four-region neurobiological model and the key innovation is the midcingulate concept. Midcingulate cortex (MCC) is qualitatively unique from ACC and the so called caudal or dorsal ACC are not ACC in this model. This model can be applied to other mammals including rodents for direct comparative studies with human brain.
Methods: The ligand binding study is based on postmortem autoradiography of ACC/MCC for 15 neurotransmitter receptors. The results are shown in coronal sections and polar plots to compare statistical differences in ACC and MCC. For the probability map, 10 postmortem brains were magnetic resonance imaged before histological processing, prepared with silver stains and each area in ACC plotted. A coregistration of all brains was then performed and a probability map generated for the position of each area with pseudo-color coding.
Results: The MCC is differentiated from ACC. 1) Every layer of ACC shows differences from MCC in neuron sizes/densities including layer Vb of area p24c' which has large, corticospinal projection neurons. 2) Afferents from the amygdala and inferior parietal cortex in monkey show variations within MCC. 3) Resting glucose metabolism correlated with seeds in different parts of ACC show correlated metabolism throughout ACC which has a resting metabolism distinct from MCC. 4) Multireceptor binding of 15 neurotransmitter receptors show that ACC does not have a uniform pattern of binding; $GABA_A$ and AMPA binding show low levels of the former rostrally and very high levels caudally, while AMPA receptors had the reverse pattern. Eight classes of receptors differed significantly between these cortices and GABAergic organization plays a more prominent role in MCC than in ACC. 5) The distribution of emotion activity generated with pictures/scripts shows different parts of ACC are highly active during sadness and happiness, while MCC is almost devoid of such activity. Fear activates the anterior MCC and there is a part of pregenual ACC activated by emotional awareness independent of specific emotions. 6) The rat has an ACC and MCC, although each contains fewer areas than primates, and it will be compared to the human and monkey.
Conclusions: ACC is comprised of areas 25, 24 and 32 based on cytoarchitecture and a systematic study of postmortem brains provides a probability map for locaizing each area. Functional maps including receptor binding whether ex vivo or in vivo need to be couched in terms of cortical structure because this is the essential substrate employed by cingulate cortex to implement specific functions. As we become more precise in determining the neural bases of psychiatric diseases and drug therapeutics, these probability maps will play are larger role in localization problems including in vivo receptor binding and postmortem neuropathology. The validity and bases for the four-region neurobiological model have been reviewed including its many psychiatric impairments (Cing Neurobiol Dis, 2009; Oxford Univ. Press). The challenge for future imaging of cingulate functions and psychiatric diseases is to achieve higher resolution to identify each area. Thus, this model does not simply refer to places in the cingulate cortex but provides structure/function substrates for making predictions about the organization and functions of parts of cingulate cortex and for evaluating neuropathological alterations evoked by psychiatric diseases.
Disclosure: B. Vogt, None.

Intraoperative Physiologic Evidence of Anterior Cingulate Cortex Modulation of Autonomic Arousal and Neuroimaging Correlations

Andre Gentil*

Department of Psychiatry, University of Sao Paulo Medical School, Sao Paulo, Brazil

Background: Neuroimaging studies attempting to map autonomic-specific activity within the anterior cingulate cortex (ACC) have produced conflicting results: while most reported increased rostral ACC (rACC, or "emotional" subdivision) activity correlating with autonomic measures, others have implicated the dorsal ACC (dACC, or "cognitive" subdivision) in autonomic function. To inform this debate the present study investigated autonomic responses to direct brain stimulation during stereotactic limbic surgery in humans.
Methods: Skin conductance activity (SCA) and accelerative heart rate responses (HRR) to multi-voltage stimulation at the transition of rACC/dACC ($N = 7$) and paralimbic subcaudate ($N = 5$) regions were continuously recorded during bilateral anterior cingulotomy (BAC) and bilateral subcaudate tractotomy (BST, in patients that had previously received a lesion in the ACC), respectively.
Results: Stimulations in both groups were accompanied by increased autonomic arousal. SCA was significantly increased during r/dACC stimulations compared with paralimbic targets at 2 volts (2.34 0.68 [score in microSiemens SE] vs. 0.34 0.09, $p = 0.013$) and 3 volts (3.52 0.86 vs. 1.12 0.37, $p = 0.036$), exhibiting a strong "voltage-response" relationship between stimulus magnitude and response amplitude (difference from 1 to 3 volts = 1.15 0.90 vs. 3.52 0.86, $p = 0.041$). HRR was less indicative of between-group differences.
Conclusions: This study provides direct support for the rACC/dACC region as a crucial node within the neuroanatomical circuitry responsible for central autonomic modulation. The blunted autonomic responses during electrical stimulation of BST targets in patients previously submitted to BAC suggest that the areas connected (i.e. ventral prefrontal cortex) or in close proximity (i.e. subgenual ACC and surrounding rACC) are not independently capable of producing autonomic output, but subserve a larger neuroanatomical network that necessarily includes dACC engagement.
Disclosure: A. Gentil, None.

Human Anterior Dorsal Cingulate Neuronal Activity During a Stropp Interference Task

Emad Eskandar*

Massachusetts General Hospital, Boston, USA

Background: The human anterior dorsal cingluate cortex (dACC) has been implicated in Stroop interference tasks using functional imaging techniques such as functional MRI. The purpose of this

presentation is demonstrate the role of individual human neurons during a Stroop interference task.
Methods: Single neuronal data was obtained form subjects undergoing cingulotomy to treat severe Major Depression or Obsessive Compulsive Disorder. We routinely obtain micro-electrode recordings during these operations. During these surgeries, the subjects play a Stroop interference task, while the electrodes were recording single neuron data from the dACC. Spike sorting and analysis were completed after the procedure.
Results: Neurons in the dACC exhibit several interesting properties. They signal degree of interference in their firing rates. Moreover, the neurons exhibit priming effects in that harder trials (more interference) tended to speed-up responses on subsequent hard trials. Conversely, hard trials tended to slow-down responses on subsequent easy trials (less interference). Finally, these priming effects were behaviorally abolished after ablation of the dACC suggesting a causative role in this process.
Conclusions: These data suggest that the cingulate gyrus detects conflicts and recruits other areas to resolve the conflict. This process maybe appropriate in the case of hard trials followed by other harder trials, or inappropriate in the case of hard trials followed by easier trials.
Disclosure: E. Eskandar, None.

Mini-Panel Session
Genes, Fear and Anxiety: From Mice to Humans

Genetic Factors Driving Corticolimbic Mediation of Fear in Mouse Models

Andrew Holmes*

NIAAA, Rockville, USA

Background: Mood and anxiety disorders develop in some but not all individuals following exposure to stress and psychological trauma. However, the factors underlying individual differences in risk and resilience for these disorders, including genetic variation, remain to be determined. Isogenic inbred mouse strains provide a valuable approach to elucidating these factors. As an example of this approach, we have examined the extinction-impaired 129/SvImJ (S1) inbred strain for behavioral, autonomic, neuroendocrine, corticolimbic neuronal morphology, and prefrontal single-unit activity phenotypes.
Methods: This presentation will discuss data showing that S1 exhibited fear overgeneralization to ambiguous context and cues, impaired context extinction and impaired safety learning, relative to the reference C57BL/6J (B6) strain.
Results: These behavioral phenotypes are associated with autonomic disturbances in S1 including poor recovery of fear-induced suppression of heart-rate variability, and exaggerated corticosterone responses to stress, insensitivity to dexamethasone challenge and reduced hippocampal CA3 glucocorticoid receptor mRNA. The latter suggesting downregulation of negative feedback control of the hypothalamic-pituitary-adrenal axis. Analysis of neuronal morphology in key regional nodes within the fear and extinction neural circuit revealed enlarged dendritic arbors in basolateral amygdala neurons in S1, but normal infralimbic and prelimbic dendritic arborization. In addition, single-unit recordings showed that ventromedial prefrontal cortical neuronal firing and bursting during extinction was poorly coupled to fear in S1, as compared to B6.
Conclusions: These data provide support for the utility of the S1 strain as a tractable model of excessive fear. More generally, this provides an illustration of how inbred mouse models can be effectively leveraged to perform multi-level analyses to provide convergent evidence of neural, molecular and genetic factors driving individual differences in fear. Ideally, these models should inform, and be informed by, other strategies in other animal models and human subjects to facilitate cross-fertilization across fear research.
Disclosure: A. Holmes, None.

Genetic Dissection of the Role of Phasic Dopamine in Fear Processing and Generalized Anxiety

Larry Zweifel*

University of Washington, Seattle, USA

Background: To discuss the role of dopamine in contingency awareness processing during aversive conditioning and illuminate a connection between phasic dopamine neurons activation, fear conditioning, and generalized anxiety.
Methods: This presentation will discuss genetic inactivation of phasic dopamine neuron activity and single unit recordings from dopamine neurons in freely moving mice during aversive conditioning. Two combinatorial viral-based approaches for functional mapping of projection targets of dopamine neurons in mice will be discussed. This first involves the conditional expression of the genetically encoded calcium indicator, GCaMP3 in select dopamine projections that allows for activity-dependent mapping. The second approach is a combinatorial DNA recombinase strategy for selective gene inactivation in specific dopamine projection neurons that allows for functional genetic dissection of neural circuits.
Results: Inactivation of functional NMDAR receptor signaling in dopamine neurons (DA-NR1 KO mice) impairs phasic activation during aversive conditioning, impairs acquisition in a fear potentiated startle paradigm, results in sensitization of the acoustic startle reflex, and leads to the development of generalized anxiety-like behavior. Deep-brain calcium imaging has revealed that aversive stimuli elicit activity-dependent calcium transients in dopamine neurons that can be used for functional mapping. Preliminary results indicate that conditional gene manipulation can be achieved in select dopamine projection neurons using a combinatorial recombinase approach.
Conclusions: These data provide evidence in support of a phasic dopamine signal important for contingency processing during an aversive experience. Disruption of this phasic dopamine-dependent process can be an underlying cause generalized anxiety. Development of new strategies for selectively mapping dopamine neurons in a target-dependent manner allows us to better understand the basic neural mechanisms of generalized fear and anxiety disorders.
Disclosure: L. Zweifel, None.

Neural Correlates of Impaired Fear Inhibition and Extinction in PTSD

Tanja Jovanovic*

Emory University School of Medicine, Atlanta, USA

Background: This presentation will examine physiological and fMRI correlates of PTSD in a highly traumatized population based on work from Dr. Tanja Jovanovic, Bekh Bradley, Seth Norrholm, and colleagues at the Grady Trauma Project. Additionally we will examine the extinction of conditioned fear, and its augmentation with a BDNF agonist, 7,8-dihydroxyflavone, in mouse models based on work from Dr. Raul Andero.
Methods: Human studies examine fear conditioning and response inhibition in highly traumatized human subjects. Acoustic startle reflex is measured during extinction of fear and fear inhibition.

fMRI approaches are used to examine cortical BOLD signal during a response inhibition task. Mouse models of fear conditioning and extinction are used with freezing behavior as the primary output.
Results: We find that subjects with PTSD have impairments in their ability to inhibit fear to a non-reinforced conditioned stimulus and that they have deficits in extinction to conditioned fear stimuli. Additionally, we find in a mouse model of PTSD, that mice with a history of immobilization stress show deficits in extinction of fear that can be rescued with a BDNF agonist.
Conclusions: This work in PTSD patients implicates diminished prefrontal inhibition in the resistance of fear extinction. Additionally, we find a role for activation of BDNF-dependent plasticity in facilitating the rescue of mouse models with enhanced fear/diminished extinction. These findings suggest novel approaches to enhancing treatment for subjects with PTSD.
Disclosure: T. Jovanonic, None.

Panel Session
Sex Differences in Brain and Behavior: Emerging Genetic and Cellular Mechanisms

Parent-of-Origin Effects in the Male and Female Mouse Brain
Christopher Gregg*

University of Utah, School of Medicine, Salt Lake City, USA

Background: A wide variety of psychiatric and neurological disorders exhibit sex differences in prevalence and/or symptoms, such as eating disorders, mood disorders, and autism spectrum disorders. Recently, novel genetic and epigenetic mechanisms have been suggested to contribute to differences in the underlying biology of males and females, including genomic imprinting. Genomic imprinting is a heritable epigenetic mode of gene regulation that results in preferential expression of the paternally or maternally inherited allele for a subset of genes in the genome. A role for imprinted genes in neuropsychiatric diseases has been proposed. My talk will discuss recent studies in mice that have uncovered evidence for complex parent-of-origin effects that influence gene expression, physiology and behavior in one sex, but not the other.
Methods: My work, with colleagues at Harvard, has developed a genome-wide approach to uncover parent-of-origin allelic effects in the adult mouse brain. Our approach uses RNA-Seq to profile gene expression in specific brain regions of male and female F_1 hybrid mice generated by reciprocal crosses of the distantly related mouse strains, CASTEiJ and C57BL6J. Base calls at single nucleotide polymorphisms are used to distinguish expression from maternally versus paternally inherited alleles across the transcriptome. The approach yields a high-resolution analysis of parental effects influencing gene expression according to sex and brain region.
Results: Our study identified a paternal gene expression bias in the adult brain and maternal bias in the developing brain. Preferential selection of the maternally inherited X chromosome was found in glutamatergic neurons of the female cortex. Moreover, analysis of the cortex and hypothalamus identified 347 candidate autosomal genes with sex-specific imprinted features. In the hypothalamus, sex-specific imprinted genes were mostly found in females, suggesting parental influence over the hypothalamic function of daughters. We show that Interleukin 18, a gene linked to diseases with sex-specific prevalence, is subject to complex, regional, and sex-specific parental effects in the brain.
Conclusions: These studies indicate novel sexually dimorphic pathways and mechanisms capable of influencing brain function and disease.
Disclosure: C. Gregg, None.

Sex Chromosome Genes and Hormones Interact to Mediate Behavior
Emilie Rissman*

University of Virginia, Charlottesville, USA

Background: To determine whether differences in sex chromosome complement organize sexual dimorphisms in brain and behavior.
Methods: Mice with sex chromosome rearrangements and other knockouts are used for this work. A combination of data was collected using methods including behavioral tests, gene expression arrays, and validations with qPCR and western protein blots.
Results: When adult gonadectomized mice are treated with testosterone and then tested with receptive females both males and females mount and thrust. The timing and frequencies of these motoric behaviors are sexually dimorphic. Using two sex chromosome mouse models we have found that genes on the X-chromosome cause the sex difference in this dimorphic behavior. Another reflexive motor behavior that is essential for mating is the lordosis response shown by hormone-primed females. This behavior is only noted at very low levels in hormone primed adult males. We have examined the interactions between estradiol and sex chromosome complement using a sex chromosome model mouse crossed with an aromatase knockout mouse. We will present data showing that sex chromosome complement influences expression of lordosis in females. Finally both behaviors have a large motor component. We asked if the motor coordination center of the brain, the cerebellum, is sexually dimorphic. Calbindin is a calcium binding protein and in the cerebellum is expressed only in Purkinje cells, the main motor output neurons. We have found that XX animals have more calbindin mRNA and protein than XY animals in the cerebellum using several mouse models. Regulation of this protein by X-chromosome genes is currently underway.
Conclusions: More than simple differences in hormones between males and females mediate sexually dimorphic motor behaviors. In addition X-chromosome genes are involved. These data may have important implications for other motor behaviors as well as cognitive behaviors that require the cerebellum such as ADHD and dyslexia.
Disclosure: E. Rissman, None.

Sex Differences in Stress Responses: From Molecules to Mood
Debra Bangasser*

The Children's Hospital of Philadelphia, Philadelphia, USA

Background: Stress-related psychiatric disorders are more prevalent in women than in men. As dysfunction of the stress neuromediator, corticotropin-releasing factor (CRF) has been implicated in these disorders, sex differences CRF sensitivity could underlie this disparity. This presentation will highlight recently discovered sex differences in CRF receptor (CRFr) signaling and trafficking that render neurons of females more sensitive to low levels of CRF and less adaptable to high levels of CRF.
Methods: In rats, immunoprecipitation was used to evaluate sex differences in CRFr coupling to G_s, a signaling molecule, and βarrestin, a protein required for CRFr internalization. Electron

microscopy visualized CRFr localization in locus coeruleus (LC)-norepinephrine cells following acute stress in rats and in CRF overexpressing (CRF-OE) mice. Electrophysiology tied these cellular changes to functional sex differences.
Results: CRFr-G_s coupling was greater in female rats and this translated to enhanced LC sensitivity to CRF. Also, stress-induced CRFr-βarrestin binding was decreased in females and this translated to a failure to internalize CRFr. Likewise, in CRF-OE mice, CRFr was internalized in males, but not females. This effect was associated with a higher tonic LC neuronal firing rate in female CRF-OE mice. These sex differences were unrelated to circulating gonadal hormones.
Conclusions: Sex differences in CRFr signaling and trafficking result in increased neuronal sensitivity to CRF and inability to adapt to excess CRF in females. At the level of the LC, which mediates the arousal expression of the stress response, this would translate to hyperarousal and inability to focus attention. Excessive CRF and increased activity of the LC-norepinephrine arousal system are implicated in many stress-related psychiatric disorders, including affective disorders and PTSD. Thus, these results provide potential molecular and cellular mechanisms underlying the higher incidence of these disorders in females.
Disclosure: D. Bangasser, None.

Sex-Specific Signaling Mechanisms in Schizophrenia
Eugenia Gurevich*

Vanderbilt University, Nashville, USA

Background: Documented sex differences in the prevalence and course of schizophrenia are likely based on the sex-specific activity of the brain signaling pathways. Animals with the neonatal ventral hippocampal lesion (NVHL) demonstrate altered responsiveness to stress and various drugs reminiscent of that in schizophrenia. Post-pubertal onset of abnormalities suggests a role for sexual maturation in the development of the NVHL psychopathology and the possibility that sex differences in the NVHL effects model sex differences in schizophrenia.
Methods: The behavioral responsiveness to novelty and drugs in male and female rats with NVHL was compared to sham-operated rats. Alterations in the ERK and Akt signaling and the expression of GRKs and arrestins were examined in male and female rats following NVHL using quantitative Western blotting.
Results: The novelty- and MK-801-induced hyperactivity was evident in both male and female NVHL rats, whereas only NVHL males were hyperactive in response to apomorphine. The basal activity of the ERK and Akt was higher in females than in males. NVHL reduced the level of phosphorylation of ERK1/2, Akt, and GSK-3 in both sexes. Females had higher levels of G-protein-coupled kinases (GRK) 3 and 5, whereas the concentrations of other GRKs and arrestins were the same. In the nucleus accumbens, the concentration of GRK5 in females was elevated by NVHL to the male level. Although wild type arrestin expression was similar, male and female mice lacking arrestins displayed differential sensitivity to dopaminergic drugs, which suggests a role for arrestins in sex-specific signaling.
Conclusions: The data demonstrate profound sex differences in the expression and activity of signaling proteins that might modulate susceptibility to schizophrenia. A comprehensive comparative assessment of signaling modifications induced by neonatal stress in males and females will improve our understanding of the molecular mechanisms involved in schizophrenia.
Disclosure: E. Gurevich, None.

Panel Session
The Putative Role of ER Stress in Neuropsychiatric Illnesses

Impact of Endoplasmic Reticulum (ER) Signaling on Neuroplasticity and Neuronal Survival
John Reed*

Sanford-Burnham Medical Research Institute, La Jolla, USA

Background: ER-initiated signal transduction pathways impact neuronal survival and neuroplasticity (Kim I *et al.*, *Nature Drug Disc Rev* 2008). Bcl-2-family proteins regulate cell survival, as well as modulating neuroplasticity through affects on synaptic events. Polymorphisms in BCL-2 are associated with Bipolar Disorder, correlating with reduced Bcl-2 protein expression.
Methods: Various experimental approaches were employed to interrogate the role of ER signaling in neurological and neuropsychiatric disorders, including genetic engineering of mice and screening for chemical modulators of ER signaling.
Results: Bcl-2 and Bcl-XL associate in ER membranes with Bax Inhibitor-1 (BI-1), a cytoprotective protein that enhances cellular resilience (Xu & Reed *Molecular Cell* 1998; Chae HJ *et al Mol Cell* 2004). BI-1 regulates ER Ca2+ in a manner that phenocopies Bcl-2 and Bcl-XL (Xu C *et al.*, *J Biol Chem* 2008). Transgenic mice over-expressing BI-1 in neurons show resistance to acute brain injury (stroke; seizure; traumatic brain injury) (Krajewska M *et al., Brain Res* 2011), and also display increased resilience in behavioral models of affective disorders, including depression resulting from learned helpless (LH) conditioning and from serotonin or catecholamine depletion (Hunsberger JG *et al.*, submitted). ER-initiated signaling events occur when unfolded proteins accumulate in this organelle (Unfolded Protein Response [UPR]). Pro-apoptotic proteins Bax and Bak directly bind the UPR protein IRE1 and stimulate it, while BI-1 binds IRE1 and inhibits it (Lisbona, F *et al.*, *Mol Cell* 2009; Bailly-Maitre B *et al.*, *J Biol Chem* 2010). In mouse models of acute brain injury, BI-1 regulates UPR signaling events within the IRE1 pathway (Krajewska M *et al.*, *Brain Res* 2011). Polymorphisms in the IRE1 target *XBP1* are correlated with Bipolar Disorder, suggesting that altered signaling in the IRE1/XBP1 pathway impacts neuropsychiatric disorders. Chemicals modulating ER signaling were identified that show neuroprotective activity, rescuing cultured neurons from ER stress-induced apoptosis (Kim I *et al.*, *J Biol Chem* 2009).
Conclusions: The role of ER signaling in neurological diseases merits further investigation, including explorations of the role of ER stress-modifying compounds in models of brain disorders.
Disclosure: J. Reed, None.

A Possible Role of XBP1 in Neural Plasticity
Tadafumi Kato*

RIKEN Brain Science Institute, Wako, Japan

Background: XBP1 is known to play a pivotal role in the unfolded protein response (UPR) that is important to protect the cells from the endoplasmic reticulum (ER) stress. Whereas most studies of ER stress have focused on the cellular homeostasis against the cellular stress, we have focused on the physiological role of UPR in neural development and plasticity.
Methods: Role of XBP1 in neurons were examined using primary cultured neurons obtained from wild type or XBP1 knockout mice.

Results: In situ hybridization and immunocytochemistry revealed that XBP1 mRNAs and proteins are distributed throughout the neuronal process. Application of BDNF (brain derived neurotrophic factor) induced splicing of XBP1. This was partly inhibited by rapamycin. This was verified in isolated neurites. Locally translated XBP1 proteins translocate to the nucleus. In XBP1 knock out neurons, neurite extension in response to BDNF was compromised. Transcriptome analysis using DNA microarrays showed that BDNF induced upregulation of GABergic marker genes such as Neuropeptide Y, Somatostatin, and Calbindin, were attenuated in neurons lacking XBP1.
Conclusions: These findings altogether suggest that BDNF stimulation causes ER stress-like conditions in neurons, and activates XBP1. Activation of XBP1 plays a role in neurite extension, and possibly in differentiation into GABergic neurons.
Disclosure: T. Kato, Part 1: Consultant: Taisho Toyama Pharmaceutical Co., Ltd. GlaxoSmithKline Otsuka Pharmaceutical Co. Ltd. Honorarium for educational lecture: Asahi Kasei Corporation Astellas Pharma Inc. Dainippon Sumitomo Pharma Co., Ltd Eli Lilly Japan K.K. GlaxoSmithKline Janssen Pharmaceutical K.K. Kyowa Hakko Kirin, Co., Ltd. Meiji Seika Kaisha, Ltd. Otsuka Pharmaceutical Co. Ltd. Pfizer Inc. Taisho Toyama Pharmaceutical Co., Ltd. Yoshitomiyakuhin Corporation. Manuscript fee: Eli Lilly Japan K.K., Part 2: None, Part 3: None, Part 4: Grant support: NARSAD (National Alliance for Research on Schizophrenia and Depression) Takeda Science Foundation Mitsubishi Pharma Research Foundation, Part 5: No.

Oxidative Damage to Biomolecules as a Potential Therapeutic Target for Bipolar Disorder

L. Trevor Young*

University of Toronto, Toronto, Canada

Background: A growing body of evidence suggests that oxidative damage occurs in patients with bipolar disorder (BD). A recent meta-analysis of studies of oxidative markers in blood from BD patients indicated increased levels of a free radical nitric oxide as well as increased lipid peroxodation as demonstrated by elevated levels of thiobarbituric acid reactive substances. Furthermore, postmortem studies have identified protein oxidative damage in prefrontal cortex, lipid peroxidation in cingulate cortex and oxidative DNA/RNA damage in hippocampus from patients with BD. Additionally, decreased levels of reduced glutathione have been reported in post-mortem pre-frontal cortex from patients with bipolar disorder. Taken together these findings support the involvement of oxidative damage to biomolecules, in brain and periphery in BD. Although the precise mechanisms remain unclear, one of the most accepted hypotheses is that mitochondrial complex I impairment resulting in ROS production causes oxidative stress in this patient group. Recently, We have demonstrated that decreased activity of complex I correlates negatively with the increase of protein oxidation and tyrosine nitration-induced damage in patients with BD.
Methods: Post mortem prefrontal cortex from subjects with BD or SCZ, and from non-psychiatric comparison controls was generously provided by the Harvard Brain Tissue Resource Center. Mitochondrial and synaptosomal fractions were isolated using the percoll gradient method. The quality of extraction was verified by electron microscopy followed by western blotting analysis. The oxidative damage to protein was assessed by measuring carbonyl levels and nitration-induced damage to tyrosine residues was assessed by measuring 3-nitrotyrosine levels using immunoblotting analysis.
Results: We continue to investigate the oxidative stress alteration in post mortem prefrontal cortex from subjects with BD or SCZ, and from non-psychiatric by isolating mitochondrial and synaptosomal fractions; our results showed increased levels oxidative damage to synaptosomal proteins in BD, but not in SCZ. Whereas, 3-nitrotyrosine levels was increased in mitochondrial protein in patients with BD or SCZ. Interestingly, we did not find alterations for carbonyl levels in mitochondrial proteins as well as 3-nitrotyrosine levels was equal for all groups in synaptosomal proteins.
Conclusions: Together these data might provide new targets for development of neuroprotective strategies and may help elucidate a better understanding of the pathophysiology of BD.
Disclosure: L. Young, Part 3: occasional speaker for Eli Lilly and Astra-Zeneca.

Roles of ER Stress Modulators, Bcl-2 and BI-1, in Stress Coping and Action of Antidepressant

Guang Chen*

J & J, San Diego, USA

Background: Recent studies revealed modulation of ER stress pathways by mood stabilizers and antidepressants and ER stress pathway dysfunction in mood disorders. For instance, mood stabilizers, antidepressants, and ECT up-regulate Bcl-2 (B cell lymphoma protein 2) levels in the brain. Human postmortem brain study found downregulation of Bcl-2 and up-regulations of Bax and Bad in cerebral cortical tissues of mood disorder patients. Bcl-2 is known to regulate ER calcium release and ER stress signaling. BI-1 (Bax inhibitor-1) inhibits a key ER stress signaling molecule, inositol-requiring protein 1, a protein with intrinsic protein kinase/endoribonuclease activity. These data suggest ER stress pathways as one of contributors of mood regulatory system. However, the behavioral role of ER stress pathways in mood regulation is still largely unknown.
Methods: Both chemical and genetic methods are used to manipulate Bcl-2 and BI-1 levels in the brain. The behavioral consequences of Bcl-2 and BI-1 manipulations are studied using a battery of tests for alterations related to mood disorders and behavioral actions of mood stabilizers and antidepressions.
Results: Bcl-2 heterozygous knockout mice and mice with ICV infusion of Bcl-2 inhibitor displayed increased response to helplessness induction by uncontrollable and unavoidable foot shocks. The mice also showed decreased spontaneous recovery from the helplessness. As well, the mice showed reduced respond to citalopram. Monoamine depletions are known to cause depression relapses in human. BI-1 over-expressing mice displayed reduced responses to the induction of anhedonia-like deficits by monoamine depletions.
Conclusions: The components of ER stress pathways, Bcl-2 and BI-1, sufficiently alter the behavioral displays related to depression and actions of antidepressants and mood stabilizers. Whether the effects of Bcl-2 and BI-1 on the behavior displays are exclusively through ER stress pathways remain to be further elucidated. Current behavioral data are coherent with the treatment data and clinical data and the data together support dysfunction of ER stress is one of contributing factors of mood disorders.
Disclosure: G. Chen, Part 1: Full time employee of Johnson & Johnson, Part 2: Full time employee of Johnson & Johnson, Part 3: Full time employee of Johnson & Johnson, Part 4: Full time employee of Johnson & Johnson, Part 5: Full time employee of Johnson & Johnson.

Panel Session
Glutamate Targets for CNS Therapy: Insights Obtained from a Potential Dynamic Duo

Glycine Transporter-1 and Metabotropic Glutamate Receptor mGluR5 as Potential Therapeutic Targets for Schizophrenia

Kenji Hashimoto*

Chiba University Center for Forensic Mental Health, Chiba, Japan

Background: Glycine transporter-1 (GlyT-1) on glial cells plays a role in the regulation of glycine levels at NMDA receptors. Furthermore, the metabotropic glutamate receptor subtype 5 (mGluR5) is a closely associated signaling partner of the NMDA receptor, and may play a significant role in setting the tone of NMDA receptor function in the forebrain. Therefore, GlyT-1 and mGluR5 are potential therapeutic targets for schizophrenia.
Methods: The effects of GlyT-1 inhibitor NFPS and positive allosteric modulator of mGluR5 CDPPB on cognitive deficits in mice after repeated administration of NMDA receptor antagonist phencyclidine (PCP) were examined. Furthermore, we measured the levels of GlyT-1 protein and amino acids (glycine, D-serine) in the frontal cortex and hippocampus of mice. In addition, we developed a novel radioliagnd [^{3}H]CHIBA-3007 for studying GlyT-1 in the brain.
Results: Cognitive deficits in mice after the repeated administration of PCP were significantly improved by subsequent subchronic administration of NFPS. The effects of CDPPB on PCP-induced cognitive deficits will be presented. Repeated administration of PCP significantly increased the levels of GlyT-1 protein in the hippocampus, but not frontal cortex. *In vivo* microdialysis showed that repeated administration of PCP significantly decreased the extracellular levels of glycine, but not D-serine, in the hippocampus, but not frontal cortex. Scatchard analysis of [^{3}H]CHIBA-3007 binding to rat brain membranes revealed a $K_d = 1.61$ nM and $B_{max} = 692.8$ fmol/mg protein. The potency of GlyT-1 inhibitors for blocking specific binding of [^{3}H]CHIBA-3007 correlated significantly with the potency of these inhibitors for inhibiting [^{14}C]glycine uptake.
Conclusions: These findings suggest that GlyT-1 inhibitors are potential therapeutic drugs for schizophrenia. Furthermore, [^{3}H]CHIBA-3007 should prove useful as a radioligand for studying GlyT-1 in the brain.
Disclosure: K. Hashimoto, None.

Modulation of Glutamatergic and Dopaminergic Transmission by RG1678, a Novel and Potent Glycine Reuptake Inhibitor

Daniela Alberati*

F. Hoffmann-La Roche, Basel, Switzerland

Background: Deficient signaling through the N-methyl-D-aspartate (NMDA) receptor has been hypothesized to be a key factor underlying symptoms of schizophrenia. Targeting the glycine site of the NMDA receptor has been proposed as an approach to compensate for the reduced receptor signaling. RG1678 is a novel glycine reuptake inhibitor (GRI) presently in clinical phase 3 for the treatment of negative and sub-optimally controlled positive symptoms in schizophrenia. We report here the in vivo efficacy of RG1678 in rodents, its ability to modulate the efficacy of antipsychotics in animal models of schizophrenia and the relationship between target receptor occupancy and plasma exposure.
Methods: The main behavioral outcome measured was locomotor activity in both mice and rats. For combination experiments a low dose of RG1678 was added to graded doses of antipsychotics. For *in vivo* binding, Wistar rats pretreated with vehicle or RG1678 received [^{3}H]RO5013853 i.v. and were sacrificed 30 min later. Brain sections were exposed to tritium-sensitive imaging plates in a phosphor imager and [^{3}H]RO5013853 bound in regions of interest quantified.
Results: RG1678 attenuated the hyperlocomotion induced by d-amphetamine or L-687,414 and the hyper-response to d-amphetamine in animals chronically-treated with PCP. When low doses of RG1678 were added to risperidone and olanzapine a more than additive effect was observed. Plasma concentrations of RG1678 measured at behaviorally-active doses produced a maximal GlyT1 occupancy of about 54%.
Conclusions: The potent GRI RG1678 modulates both glutamatergic and dopaminergic tone. *In vivo* binding studies in the rat indicates that brain GlyT1 occupancy of less than 60% is needed to attain efficacy in models of schizophrenia. The finding that RG1678 enhanced the effect of risperidone and olanzapine suggests that it may be beneficial in ameliorating sub-optimally controlled positive symptoms in schizophrenia.
Disclosure: D. Alberati, Part 5: F. Hoffmann-La Roche.

Imaging Biomarkers for Glutamate: Focus on mGluR5/GlyT1

Dean Wong*

Johns Hopkins Medical Institution, Baltimore, USA

Background: There is a dire need for imaging biomarkers for the glutamate (Glu) as an increasing number of novel glutamatergic agents have entered clinical trials, targeting mGlu(2,3,5) and modulating the function of NMDA receptors. Recently Roche's glycine reuptake inhibitor (GRI) enhancing NMDA neurotransmission has been studied in early trials in schizophrenia (SCZ) with imaging biomarkers playing an important role in monitoring drug action and in dose selection. We will review PET and related imaging methods for high profile Glu targets.
Methods: Three novel radiotracers with the potential to contribute uniquely to translational studies of novel Glu drugs are highlighted. For $mGluR_5$ PET: Healthy volunteers (HV) were each studied twice with either [^{11}C]ABP688 ($n = 8$; 6 male (M); age: 44 ± 9 years; 19 ± 1.3mCi; mass: 0.5 ± 0.3 μg) or [^{18}F]FPEB ($n = 8$; M; age: 41 ± 8 years; 4.8 ± 0.4mCi; mass: 0.2 ± 0.1 μg). Distribution volume (V_T) and binding potential (BP_{ND}) in MRI-defined volumes of interest (VOIs) were obtained by 2-tissue compartment model (TTCM, K1/k2 ratio set to cerebellum), plasma reference (Ref) graphical analysis (PRGA), Ref tissue graphical analysis (RTGA) and multi-linear Ref tissue method (MRTM2, CB as reference). Regional test-retest variability (TRV) was determined. GlyT1 PET: Baboon and human PET included IV [^{11}C]RO5013853 (90 min scan), with metabolite HPLC correction of input function, and occupancy (OCC) studies in HVs were also performed. PET data were analyzed and the outcome variables transport (K_1) and (V_T) calculated. For OCC, HV received up to 175 mg of the test drug, RG1678, a potent GRI, qd for 10–12 days, 3 PET scans ($\sim$30 mCi [^{11}C]RO5013853) were done; at baseline, on the last day of RG1678, and 2 days after RG1678 discontinuation.
Results: mGluR5: Ref methods yielded more robust V_T and BP_{ND} for both tracers and showed TRV was lower than with plasma input: MRTM2. Although the regional BP_{ND} of the 2 tracers correlated ($ABP = 0.38$ FPEB + 0.182; $R^2 = 0.897$), [^{11}C]ABP688 had lower BP_{ND} (< 0.8 in highest BP_{ND} regions; insula, cingulate, and frontal cx) and larger TRV (10–20%) compared to [^{18}F]-FPEB (BP_{ND}, 1.5–1.6, TRV < 10).
GlyT1: Kinetic analysis confirmed the reversible binding of [^{11}C]RO5013853 to human and baboon GlyT1. Highest baboon uptake was in brain stem, pons, thalamus, and CB, consistent with rodent autoradiography. In HV ($n = 7$; aged 23–56 years) confirmed the ability of [^{11}C]RO5013853 to image regions expressing GlyT1. V_T was highest in the pons (2.59 ± 0.22) and lowest in the temporal cx (0.85 ± 0.04). For TRV, HVs [$n = 5$] received 2 PETs; V_T had a TVR of 3–6%. The RG1678 plasma to GlyT1 OCC relationship exhibited an E_{max} of $\sim$90%, an EC_{50} of $\sim$190 ng/mL,

and an ED_{50} of ~15 mg. OCC values derived by 2T5P and SRTM were comparable.
Conclusions: [^{18}F]FPEB demonstrated lower TRV and higher regional BP_{ND} OCC values than [^{11}C]ABP688. For human $mGluR_5$ [^{11}C]RO5013853 is a novel, effective PET human ligand for imaging of GlyT1 occupancy which may be used to support clinical development of a GRI.
Disclosure: D. Wong, None.

Pharmacological Strategies For Nmdar Enhancement
Daniel Javitt*

Nathan Kline Institute, Orangeburg, USA

Background: Over recent years, both glycine transport inhibitors (GTIs) and mGluR5 positive allosteric modulators have been proposed as potential treatments for schizophrenia. GlyT1 inhibitors were initially developed based upon rodent models, and have subsequently entered clinical phase II testing. Glycine-based approaches, while promising, are limited by potential saturation of the glycine binding site and receptor downregulation. mGluR5 agonism provides an alternative mechanism for enhancement of NMDAR through receptor-level interaction.
Methods: This presentation will review relative basis for targeting of the glycine site of the NMDAR vs. the mGluR5 receptor, and will review both preclinical and clinical data for relative effectiveness of the two approaches.
Results: Glycine-site based approaches are based upon preclinical effects of glycine, D-serine and prototypic GTIs. These compounds reverse PCP-induced impairments in animal models related to schizophrenia pathology, including amphetamine-induced DA release and rodent auditory ERP generation. In clinical studies, improvements are seen in negative and total symptoms, while studies of neurophysiological effects remain ongoing. mGluR5 are co-localized with NMDAR in many brain regions such that negative allosteric modulators (NAMs) reduce and positive allosteric modulators (PAMs) enhance NMDAR function in processes such as long-term potentiation and receptor plasticity. Although clinical data are not yet available with mGluR5, NAMs show activity in animal models of fragile-X, which PAMs show effectiveness in a range of preclinical schizophrenia models.
Conclusions: Both glycine-site- and mGluR5-based approaches are exciting and viable potential approaches for reversal of glutamatergic dysfunction in schizophrenia. Effects of combination treatments should be evaluated early in the drug development pathway to evaluate potential synergies across therapeutic approaches.
Disclosure: D. Javitt, Part 1: Schering-Plough; Takeda; NPS; Solvay; Sepracor; AstraZeneca; Pfizer; Cypress; Merck; Sunovion; Eli Lilly; BMS; Pfizer; Roche; Jazz; Promentis; Glytech, Part 2: Pfizer; Glytech, Part 3: Pfizer; Glytech, Part 4: Pfizer; Roche; Jazz, Part 5: No.

Panel Session
Serotonin Signaling during Development: Unexpected Sources, Large Neuron Heterogeneity, Limited System Plasticity and Big Impact on Physiology and Behavior

Developmental and Physiological Properties of Raphe Neuron Subpopulations
Sheryl Beck*

Children's Hospital of Philadelphia, Philadelphia, USA

Background: Anxiety is a normal response to stress, but when it becomes excessive it is pathological and considered a psychiatric disorder. A critical period for anxiety behavior development is between postnatal days 12–21 and involves the the $5\text{-}HT_{1A}$ autoreceptor of the raphe. The dorsal raphe is the principal site where serotonin cell bodies are located and they provide the majority of the 5-HT innervation of the forebrain. Traumatic events that occur early in life during critical periods of brain development are important factors in the etiology and development of mood disorders that can occur immediately or manifest later in life in adulthood. The normal physiological development of the raphe is unknown during this critical time period.
Methods: Whole cell recording techniques were used to record from serotonin neurons that were YFP tagged to the Pet-1 transcription factor. Active and passive cell characteristics, receptor mediated responses, and GABAergic synaptic activity were recorded from the serotonin raphe subfields at postnatel days 4, 12 and 21. In addition recordings were obtained from serotonin neurons in $5\text{-}HT_{1A}$ receptor knockout mice at postnatal days 12 and 21. Immunohistochemical techniques were used to confirm the serotoninergic identify of the neurons and to map the development of serotonin, glutamate and GABAergic neurons.
Results: In the YFP tagged serotonin neurons, the physiological properties and $5\text{-}HT_{1A}$ autoreceptor mediated response matured towards adult values between postnatal days 4 and 21 and the time course of development was not the same across subfields. Surprisingly GABAergic synaptic activity developed slowly and only became apparent between postnatal days 12 and 21, in concert with the slow appearance of GABA neurons as demonstrated immunohistochemically. In contrast, during development the serotonin neurons of the $5\text{-}HT_{1A}$ knockout pups had some characteristics that resembled adult values, but others that developed properties that resembled those of the immature YFP P4 characterisitcs, i.e., depolarized resting membrane potential, increased membrane resistance and increased number of action potentials elicited by depolarizing current injection. The neurons were very excitable. An additional major difference was enhanced GABAergic activity present at both postnatal days 12 and 21.
Conclusions: During postnatal days 4 to 21, serotonin neurons develop mature active and passive membrane properties as well as $5\text{-}HT_{1A}$ autoreceptor mediated responses. This development time course provides opportunity for disruption by traumatic events, leading to altered behavior as adults. In contrast the serotonin neurons from the $5\text{-}HT_{1A}$ receptor knockout mice exhibited some adult like properties, and others that developed properties of immature serotonin neurons. A major difference was the lack of GABAergic activity in the YFP tagged serotonin neurons until postnatal days 12–21, whereas the neurons from the $5\text{-}HT_{1A}$ knockout mice had greatly enhanced GABAergic activity. These data lead to the hypothesis that the immature physiological properties and enhanced GABAergic activity of the serotonin neurons from the knockout mice may be responsible for alterations in forebrain regions and underlie their anxiety phenotype.
Disclosure: S. Beck, None.

The Placenta, Serotonin and Developmental Programming
Pat Levitt*

Keck School of Medicine of USC, Los Angeles, USA

Background: Serotonin (5-hydroxytryptamine; 5-HT) has been implicated in the regulation of neurodevelopmental processes through maternal-fetal interactions. Dogma states that beyond fetal 5-HT neurons, there are significant maternal contributions to fetal 5-HT during pregnancy, but this has not been tested empirically. Studies with mutant mice and a new ex vivo method were undertaken to examine extra-embryonic sources of 5-HT and brain regions that are most sensitive to these sources.

Methods: To examine putative central and peripheral sources of embryonic brain 5-HT, HPLC measures of 5-HT and 5-HIAA were obtained in Pet-1$^{-/-}$mice, lacking most dorsal (DR) raphe neurons, Sert$^{-/-}$mice, lacking maternal blood 5-HT, and Mao-A$^{-/-}$ mice, lacking normal metabolic capacity for 5-HT. The placentometer, a new technology, ex vivo perfusion of mouse placenta, was developed to assess the de novo synthetic capacity of 5-HT and direct transfer of 5-HT from the maternal to fetal sides.
Results: Measures of 5-HT revealed previously unknown differences in accumulation between the fore- and hindbrain during early and late fetal stages in the mouse, through an exogenous source of 5-HT. Genetic model analyses show that this source is not of maternal origin. Trypophan hydroxylase assays were performed to show the synthetic capacity of 5-HT from tryptophan in the human and mouse placenta. The placentometer studies show rapid synthesis of 5-HT from maternal tryptophan, which is then transported to fetal circulation within minutes. 5-HT itself is not transferred from the maternal to fetal side. Direct inhibition of placental neosynthesis of 5-HT showed that fetal forebrain, but not hindbrain, levels of 5-HT are altered.
Conclusions: The study implicates a new, direct role for placental metabolic pathways in modulating fetal brain development, which may be involved in the variety developmental factors that increase risk for mental illnesses.
Disclosure: P. Levitt, Part 1: Pediatric Biosciences Puretech Bioventures.

Redefining Brain Serotonergic Neurons by Genetic Lineage and Selective In Vivo Silencing
Russell Ray*

Harvard Medical School, Boston, USA

Background: Central serotonin-producing neurons are heterogeneous and differ in embryonic origin, final location, morphology, gene expression profiles, firing properties, and associated clinical disorders. However, the underpinnings of this heterogeneity are largely unknown, as are the molecular markers capable of distinguishing among functional subtypes.
Methods: To further understand the role of heterogeneous gene expression in serotonergic system development, fate determination and impact on behavior, we have developed a set of mouse genetic tools that allow multiple features of a neuron type to be delineated and linked in vivo, from its embryonic and molecular origins to its fate in the adult and ultimately function in particular circuits as related to behavior and physiology. Our starting point has been development of a dual recombinase-based molecule delivery system that allows most any genetically-encoded lineage tracer or effecter molecule to be incorporated and delivered in vivo to most any neuron type. Neuron types are defined by combinatorial gene expression, making cell-type specificity high.
Results: Using these tools, we have generated a new classification scheme for serotonin neurons that is based on genetic programs differentially enacted throughout development. This new classification scheme, based on molecular expression, represents a more mechanistic view of serotonergic neuron heterogeneity than offered by anatomical segregation. In addition to this approach and resultant scheme, we will present new neuronal silencing tools used to assay freely moving adult animals that are now being used to layer functional identities onto our newly defined serotonergic genetic lineages.
Conclusions: Through intersectional genetics, we are able to redefine serotonergic neuron subtypes, linking molecularly defined 5-HT neuron subtypes to specific behavioral and physiological functions in the adult mouse, thus furthering the understanding of serotonergic neuron dysfunction and specific consequences on behavior.
Disclosure: R. Ray, None.

Serotonin Signaling during Development - Impact on Raphe Function, Limbic Circuitry and Behavior
Mark Ansorge*

Columbia University, New York, USA

Background: Developmental serotonin transporter (5-HTT) blockade increases anxiety/depression-like behavior in rodents, but mechanistic insight remains scarce and the impact on other behaviors relevant to neuropsychiatric disorders is largely unknown. Our presentation aims at filling these gaps of knowledge by providing data on the consequences of increased 5-HT signaling on raphe function, limbic circuitry, cognitive behavior and aggression.
Methods: Genetic and pharmacologic manipulations were deployed to alter monoamine signaling during specific developmental periods in mice. Consequences of altered developmental monoamine signaling were assessed at the level of behavior (emotional, cognitive and aggressive behavior), neurochemistry (monoamine and -metabolite levels, HPLC), cell morphology (Golgi and GFP-based tracing), and electrophysiology (in vivo extracellular and whole cell recordings from acute slices).
Results: Increased 5-HT signaling during early postnatal development leads to increased neophobia and conflict anxiety, altered stress reactivity and impaired spatial and fear conditioned learning. Altered behaviors are associated with molecular, morphological and electrophysiological changes in the raphe, mPFC and hippocampus. MAOA blockade but not 5-HTT blockade during adolescence increases adult aggressive behavior. Data on the effects of norepinephrine and dopamine transporter blockade on aggressive behavior and correlations of altered behaviors to altered monoamine levels will be presented.
Conclusions: The 5-HT system is developing from the early fetal stages all the way to adolescence and factors altering 5-HT signaling during critical periods of development can impact brain maturation and alter behavior in mice. In humans, developmental 5-HT signaling can be affected by genetic factors, drug treatment (e.g. SSRIs during pregnancy) and more natural environmental conditions. The relevance of our findings to the human situation will be discussed.
Disclosure: M. Ansorge, None.

Panel Session
Beyond Genome-Wide Association Studies: New Approaches to Risk of Psychiatric Illness

De Novo Copy Number Variants Confer Risk for Bipolar Disorder, Schizophrenia and Autism
Jonathan Sebat*

UCSD, La Jolla, USA

Background: We tested the hypothesis that de novo CNVs are enriched in bipolar disorder (BD) and in BD subjects with early age-at-onset (AAO18), and we sought to confirm the strong association of de novo CNVs in schizophrenia (SZ) and autism spectrum disorders (ASD).
Methods: Genome wide scans for copy number variation were performed using a high-resolution microarray platfom consisting of 2.1 million probes. Data analysis was performed using software that we developed. CNVs that were associated with disease in families were validated by tiling resolution microarray CGH.
Results: High-resolution microarray CGH was performed in blood derived DNAs from 833 subject-mother-father trios from multiple disease cohorts. De novo mutations were detected in 0.9% of

healthy controls and at significantly increased rates in BD (4.3%, P = 0.009), SZ (4.5%, P = 0.007) and ASD (8.9%, P = 0.003). In BD, the observed effect was greatest in subjects with AAO18 (5.6%, P = 0.006), though the same affect was not observed in SCZ.
Conclusions: Our findings provide evidence that de novo structural mutations are associated with BD, particularly in cases with an early disease onset. Genes identified in this study may help to elucidate the neurobiological basis of mood disorders and other psychiatric disorders.
Disclosure: J. Sebat, None.

Genomic Studies of Rare and Common Variation in Schizophrenia and Bipolar Disorder

Shaun Purcell*

Massachusetts General Hospital, Boston, USA

Background: Genome-wide association studies (GWAS) of common single-nucleotide polymorphisms (SNPs) and rare copy number variants (CNVs) have pointed to a complex genetic basis for schizophrenia and bipolar disorder. A large number of loci determine disease risk and no individual variant, common or rare, explains even a moderate fraction of heritability. We build on these findings with new data and analyses, driven by next-generation sequencing technology.
Methods: We describe results from several studies: a Bulgarian family-based GWAS (collaboration with Cardiff University, UK) for both polygenic SNP and de novo CNV analysis, pooled and individual targeted sequencing using next-generation sequencing in hundreds of samples (collaboration with Karolinska Institutet, Sweden). Analytic and computer simulations inform genetic architecture.
Results: We have proposed a highly polygenic component of common variation. Here we show, a) robustness to population-stratification in a family-based study, explaining a similar proportion of risk, ~5%, b) intersecting post-mortem expression data with schizophrenia GWAS, that brain-expressed eQTLs are enriched and c) via coalescent simulation, incompatibility with models of ubiquitous low frequency, "synthetic" association. Building on earlier work on rare CNVs, we report a) a family-based study of de novo CNVs, finding 34 in 662 patients, that are also enriched in independent cases, b) a pooled sequencing experiment of ~1,700 individuals and 503 genes, to follow-up deletion regions and other candidates, which implicates calcium channel genes and rare variants at a GWAS hit at 3p21 and c) an ongoing whole-exome sequencing study of over 500 individuals.
Conclusions: We can now begin to assess more comprehensively different forms of rare variation previously not assayed by earlier technologies. Taken together, our data are consistent with a highly multifactorial genetic basis for schizophrenia and bipolar disorder, in which different approaches are pointing to genes and pathways.
Disclosure: S. Purcell, None.

Brain eQTLs and Function-based GWAS of Bipolar Disorder Identified Novel Disease Risk Genes

Chunyu Liu*

University of Chicago, Chicago, USA

Background: Genome-wide association studies (GWAS) with psychiatric diseases have yielded limited findings, including a few genome-wide significant associated genes, one polygenic model, and a few rare copy number variations. Functional annotation of SNPs may be able to assist the re-evaluation of the genome-wide association results to identify novel disease risk genes, to recover some lost heritability. One major function of SNPs is to affect gene expression.
Methods: We used the genome-wide association study to map quantitative trait loci of gene expression in two human brain regions. Extensive statistical analyses take into account covariates (such as brain pH) and batch effects in the microarray data. Functional SNPs are then used to perform function-based GWAS analysis to identify novel disease susceptibility genes of bipolar disorder.
Results: We have identified thousands of SNPs that are associated with gene expression levels, including different splicing isoforms in two brain regions. We are applying these data into the re-evaluation of existing genome-wide association studies of bipolar disorder and schizophrenia, and have identified novel genes that are associated with disease but have been missed in previous gene hunting. Three genes showed genome-wide significant associations ($p < 1E\text{-}6$) in one sample set and were replicated at nominal significance level in a second dataset.
Conclusions: Biological understanding of genetic variants could lead to new understanding of GWAS data, and to the discovery of novel disease risk genes.
Disclosure: C. Liu, None.

Biologic and Epidemiologic Approaches to Risk of Psychiatric Illness

Elliot Gershon*

University of Chicago, Chicago, USA

Background: Epidemiologic data supports contributions to genetic risk of Bipolar Disorder and Schizophrenia by de novo CNVs and polygenic inheritence, which are not detected by genome-wide association with individual common SNPs. We will present data that can serve to analyze these findings to identify specific genes associated with disease.
Methods: Population Attributable Risks (PARs), as derived from case-control studies, are an estimate of effect of an exposure on overall risk of illness in a population. Some significant PARs are not directly interpretable biologically, such as polygenic determinants of risk and increased risk from de novo CNVs. Genes involved in de novo CNVs and in polygenic inheritance may potentially also be parsed into expression modules to guide biologically-based association studies. Aggregation of genotypes has been developed to test association of a disease with multiple rare polymorphisms of the same gene, and this approach can be applied to association analysis of genotypes of genes in a pathway. To date, pathways have been largely based on curated associations of genes in the scientific literature. Based on the advent of whole-genome expression methods, we present here new network analyses based on correlational analysis of expression in the entire genome in a specific tissue, through weighted co-expression network analysis (WGCNA). This analysis generates transcriptional modules that can be used as condensed molecular phenotypes in association analyses.
Results: We estimate the joint PAR of significant associations from GWAS (based on largest published meta- or mega-analyses) as: Schizophrenia (SZ) 25%, Bipolar (BD) 8%. PAR of de novo CNVs (calculated on findings presented by Sebat): SZ = 3.6%, BD = 3.4%. Attributable variance (R^2) of polygenic factors (presented by Purcell) depends on model, 3% to 34%. Analysis of transcriptional modules in human brain may serve as a tool to identify gene networks involved in de novo CNVs and polygenic inheritance. We hypothesized that coordinated gene expression networks identified through WGCNA may have stronger and more robust changes in patient brains than individual gene expression. We studied gene expression microarray data from Cerebellum (CB), parietal cortex (PC) and prefrontal cortex (PFC) in several data sets of Schizophrenic and control postmortem brains. An adjacency matrix was derived from the correlations of gene expression with

each other, and modules of expression were then developed. After adjusting for covariates including brain pH, we found one module, consisting largely of members of the metallothioneins (MT) gene family, was differentially expressed in Schizophrenia and control brains (FDR $q < 0.05$, by both Wilcoxon rank-sum test and module eigengene-based correlation test).
Conclusions: Transcriptional modules are dimensionally condensed expression phenotypes, which can be tested for differential expression in brain tissue of patients vs. controls, and for preferential inclusion in de novo CNVs of patients vs. controls. These modules might also be used to parse large sets of SNPs with modest association with illness, such as sets of SNPs that show polygenic association with illness.
Disclosure: E. Gershon, None.

Panel Session
Rapid Acting Antidepressants Increase Synaptogenesis

Scopolamine Produces A Rapid Antidepressant Response: Comparison with Ketamine

Wayne Drevets*

Laureate Institute for Brain Research, Tulsa, USA

Background: The cholinergic muscarinic receptor (MR) system is implicated in the pathophysiology of depression, with physiological and clinical evidence indicating this system appears overactive or hyper-responsive in depression, and with genetic evidence associating variation in the CHRM2 gene with higher risk for depression (particularly in females). We assessed the antidepressant efficacy of the MR antagonist, scopolamine (SCOP).
Methods: In a series of randomized, double-blind, placebo-controlled studies, 54 subjects with unipolar or bipolar depression underwent blocks of three infusions of SCOP (4.0 ug/kg IV) or placebo administered at 3 to 5 day intervals in a cross-over design.
Results: SCOP induced robust antidepressant effects versus placebo, which manifested 12 to 24 hours after the initial infusion, several hours after side effects subsided. Placebo-adjusted remission rates were 56% and 45% for the initial and subsequent replication studies, respectively. While effective in males and females, the change in depression ratings was greater in females. Clinical improvement persisted $\geqslant$two weeks, and in some cases lasted several months. Subjects who previously had failed to respond to SSRI agents showed the same response rate ($\sim$70%) as subjects who were SSRI-naive.
Conclusions: The timing and persistence of the antidepressant response to SCOP suggests a mechanism beyond that of direct MR antagonism. These temporal relationships instead suggest MR antagonist-induced changes in gene transcription or synaptic plasticity may confer the therapeutic mechanism.
Disclosure: W. Drevets, Part 1: Served as consultant for Pfizer, Eisai, Rules Based Medicine, Johnson & Johnson, Part 2: Oklahoma University Health Sciences Center (Primary employer), Part 3: N/A, Part 4: N/A, Part 5: N/A.

Rapid-Acting Antidepressants Require mTOR Signaling and Synaptic Protein Synthesis

Ronald Duman*

Yale University, New Haven, USA

Background: The rapid antidepressant actions of NMDA receptor antagonists (i.e., ketamine) require stimulation of the mammalian target of rapamycin (mTOR) pathway, synaptic protein synthesis, and increased synaptogenesis. The aim of the current study was to determine if another rapid acting antidepressant, scopolamine (see Drevets) a muscarinic receptor antagonist, acts via the same downstream signaling pathway.
Methods: The influence of scopolamine administration on the phosphorylated and activated components of mTOR signaling and synaptic proteins (i.e., GluR1, PSD95, synapsin I) in the rat prefrontal cortex (PFC) was determined. The requirements for glutamate/AMPA receptor and mTOR in the behavioral actions of scopolamine (forced swim and novelty suppressed feedings) were also tested.
Results: Scopolamine administration resulted in a dose- and time-dependent stimulation of mTOR signaling and synaptic proteins in the PFC. Activation of mTOR signaling was observed at 1 hr, followed by induction of synaptic proteins at 6 hr; the increase in synaptic proteins was sustained for 3, but not 7 d. Pretreatment with inhibitors of the AMPA receptor or mTOR blocked the induction of synaptic proteins and the behavioral actions of scopolamine.
Conclusions: The results demonstrate that scopolamine, like ketamine, rapidly stimulates mTOR signaling and synaptic protein synthesis, and that the behavioral actions of this agent are dependent on AMPA and mTOR signaling. Ketamine-elevation of synaptic proteins was longer compared to scopolamine (7 vs 3 d), consistent with the longer therapeutic actions of ketamine and the induction of mature and stable mushroom spines (see Aghajanian). These findings provide further evidence that rapid-acting antidepressants increase the number and function of synaptic connections and reverse the atrophy caused by of chronic stress exposure (see Morrison).
Disclosure: R. Duman, Part 1: Lilly, Lundbeck, Wyeth, Johnson and Johnson, Taisho, Psychogenics, Pfizer, Bristol Myers Squibb, Part 2: Taisho, Part 3: None, Part 4: None, Part 5: No.

Synaptogenesis and Rapidly Acting Antidepressants: Comparison between Ketamine and Scopolomine

George Aghajanian*

Yale School of Medicine, New Haven, USA

Background: Previous studies indicate that the rapid antidepressant action of a single subanesthetic dose of the NMDA antagonist ketamine is associated with an increase in synapse formation/maturation (synaptogenesis) in medial prefrontal cortex (mPFC). The purpose of the present study was to determine if a single dose of the muscarinic antagonist scopolamine, which also has a rapid antidepressant effect (Drevets, this panel), has similar synaptogenic effects.
Methods: Adult rats were given a single injection of scopolamine at 25 mg/i.p., a dose which has a rapid antidepressant effect in animal models. Twenty-four hours later whole-cell patch clamp recordings were made from layer V pyramidal cells in medial prefrontal brain slices. Serotonin (5-HT)- and hypocretin (Hcrt)/orexin-induced EPSCs were recorded and later imaged by 2-photon a laser scanner.
Results: Similar to ketamine, 24-hours after a single dose of scopolamine there was a marked increase in the frequency of 5-HT and Hcrt-induced EPSCs. However, in contrast to ketamine, there was no increase in mean EPSC amplitude. In the same cells there was a small increase in dendritic spine density but not spine diameter; in contrast to ketamine, the increase was confined to the distal portion of the dendritic tuft.
Conclusions: We conclude that the scopolamine-induced increase in EPSC frequency occurs primarily through a conversion of silent synapses to functional synapses in association with an increase in synaptic proteins (see Duman, this panel). The relatively small increase in spine density induced by scopolamine suggests it does not promote the formation of new synapses as robustly as ketamine. The two drugs also differ with respect to a second stage

of synaptogenesis, maturation into stable mushroom spines that are dramatically increased by ketamine. These functional and morphological differences may account for the relatively greater persistence of ketamine's action to increase synaptic protein levels (see Duman, this panel).
Disclosure: G. Aghajanian, None.

Aging alters Stress-Induced Structural Plasticity and Recovery in Medial Prefrontal Cortex

John Morrison*

Mount Sinai School of Medicine, New York, USA

Background: The prefrontal cortex has been implicated in the effects of stress on future behavior. We have demonstrated that stress induces morphological plasticity reflected by dendritic retraction and spine loss in medial prefrontal cortex (mPFC) of young animals, and this behavior-induced plasticity is reversible with a rest period. PFC is also vulnerable to aging. The present study was designed to investigate whether or not age affects the nature of stress-induced plasticity in mPFC, as well as the capacity to recover from stress-induced alterations.
Methods: Young, middle-aged, and aged male rats were exposed to 3 weeks of chronic stress, with additional groups for each age given an opportunity to recover from stress. Pyramidal neurons in mPFC from all six groups were loaded with Lucifer Yellow, followed by detailed morphometric analyses of both dendritic arbor and spine density and morphology.
Results: With respect to dendritic arbor, young animals showed stress-induced dendritic retraction and recovery. However, while both middle-aged and aged animals displayed stress-induced dendritic retraction, they lacked the capacity for recovery. With respect to spines, stress resulted in dendritic spine loss and altered patterns of spine morphology in young rats. In contrast, spines from middle-aged and aged animals did not display any behavior-induced alterations, though there were robust age-related reductions in spine density.
Conclusions: Aging alters the neuronal response to stress in mPFC, both with respect to stress-induced plasticity and capacity for recovery. With respect to spines, the age-induced spine loss is accompanied by diminished capacity for spine plasticity in response to stress. With respect to dendritic arbor, while the stress-induced retraction is unaffected by age, the capacity for recovery is lost. Both observations reflect a profound age-related loss in the capacity for experience-dependent structural plasticity.
Disclosure: J. Morrison, None.

Panel Session
APOE and Alzheimer's Disease: Neurosusceptibility, Neuroprotection and New Treatments

Brain Imaging, Genomics, and the Prevention of Alzheimer's Disease

Eric Reiman*

Banner Alzheimer's Institute, Phoenix, USA

Background: To provide a foundation for the presymptomatic detection, tracking and scientific understanding of Alzheimer's disease (AD) and the accelerated evaluation of presymptomatic AD treatments in cognitively normal people at differential genetic risk for AD.
Methods: We have been using fluorodeoxyglucose positron emission tomography (FDG PET), fibrillar amyloid-β (Aβ) PET, volumetric magnetic resonance imaging (MRI), and cognitive measurements to detect and track brain changes associated with the predisposition to AD in cognitively normal people with two copies, one copy and no copies of the *apolipoprotein E (APOE) ε4* allele, the major late-onset AD susceptibility gene. We have recently extended our efforts to the study of cognitively normal presenilin 1 E280A mutation carriers carriers and noncarriers from the world's largest early-onset AD kindred in Antioquia, Colombia. We have estimated the sample sizes needed to rapidly evaluate presymptomatic treatments in *apolipoprotein E (APOE) ε4* carriers using different brain imaging and cognitive endpoints; used our brain imaging measurements as presymptomatic endophenotypes to investigate suggested genetic and non-genetic risk factors for AD; and provided a springboard for other studies. We have proposed an Alzheimer's Prevention Initiative (API) to evaluate presymptomatic amyloid-modifying treatments in people who, based on their age and genetic background, are at the highest imminent risk of symptomatic AD.
Results: Our findings have helped characterize some of the earliest brain changes associated with the predisposition to AD, provided new information about genetic and non-genetic risk factors, and provided a foundation for the use of biomarker endpoints in rapid evaluation of presymptomatic AD treatments.
Conclusions: Now is the time to initiate presymptomatic treatment/surrogate marker development trials in people at the highest imminent risk of symptomatic AD.
Disclosure: E. Reiman, Part 1: Amnestix/Sygnis, AstraZeneca, Bayer, Eisai, Elan, Eli Lilly, GlaxoSmithKline, Intellect, Link Medicine, Novartis, Siemens, Takeda, Part 4: AstraZeneca, Avid, Kronos Life Sciences National Institute on Aging, Anonymous Foundation, Nomis Foundation, Banner Alzheimer's Foundation, state of Arizona.

APOE2 and Neuroprotective Responses: Molecular, Biomarker, and Cognitive Findings

Terry Goldberg*

Feinstein Institute/AECOM, Manhasset, USA

Background: APOE is triallelic at two loci in exon 4. It is well known that the E4 variant is the major risk factor for late onset AD (OR = 3.8). It is less well known but perhaps equally important that the E2 variant is neuroprotective (OR = .54). However, the E2 variant has received little attention in the literature. In this presentation we will present new data on E2 modulatory effects in molecular, biomarker, and cognitive experiments.
Methods: In studies of post mortem human cortical tissue we used RT-qPCR to measure expression and Western blots using polyclonal and monoclonal antibodies to measure protein level in cases with known E2/E3 and E3/E3 genotypes. In a large ADNI cohort we examined the impact of E2 in healthy controls (HCs) and Mild Cognitive Imairment (MCI) on biomarkers, including regional brain volumes, CSF measures of $A\beta_{42}$ and total tau, and cognition. Critically, these analyses were stringent, as they compared E2/E3 carriers to E3 homozygotes (the neutral variant).
Results: We found that expression levels did not differ between the two genotypes. However, protein levels were higher for E2 cases. In the ADNI sample, Compared to E3/E3 healthy subjects, E2 healthy subjects had higher levels of cognition, most notably in episodic memory after delays, greater entorhinal and lateral temporal lobe cortical thicknesses, and higher (i.e., healthier) CSF $A\beta_{42}$ ($ps < 0.01$, effect sizes .40 to .82). In the MCI sample E2 was protective against MCI subjects converting to AD ($p = .003$ by X^2). The profile was characterized by larger differences in favor of E2 subjects in cognition, and smaller differences in biomarkers.
Conclusions: Differences in protein level between isoforms in the context of equivalent expression implicate post translational

mechanism and replicate transgenic mouse work. Results are in keeping with that higher APOE levels promote lipid transport and/or membrane repair and modification and so may increase neuronal functional integrity. Thus, APOE2 was a key neuroprotective factor in preventing conversion from MCI to AD. It was also robustly associated with more advantageous biomarker and cognitive markers.
Disclosure: T. Goldberg, Part 1: GSK (consultant) Merck (consultant), Part 2: None, Part 3: Neurocog Trials, Part 4: Pfizer Eisai (investigator initiated), Part 5: No.

Apolipoprotein E4: A Causative Factor and Therapeutic Target in Neuropathology, including Alzheimer's Disease

Robert Mahley*

The J. David Gladstone Institutes, San Francisco, USA

Background: Apolipoprotein (apo)E4 is the major genetic risk factor for Alzheimer's disease and other neuropathological disorders. It causes detrimental effects and neuropathology directly, so "second hits" can later precipitate disease. Unique structural features are responsible for apoE4-associated neuropathology. Specifically, intramolecular domain interaction between the amino- and carboxyl-terminal domains causes the protein to assume a neuropathological conformation. In response to CNS injury, neurons synthesize apoE. ApoE4 undergoes neuron-specific proteolysis to yield neurotoxin fragments that enter the cytosol, alter the cytoskeleton, disrupt mitochondrial energy metabolism and cause cell death.
Methods: Small-molecule "libraries" were screened with a GFP/FRET assay to identify structure correctors, compounds that block apoE4 domain interaction. Positive compounds were assessed by measuring mitochondrial respiratory complex expression and activity levels, mitochondrial motility, neurite outgrowth, and neurotoxic fragment generation in culture neurons.
Results: ApoE4, but not apoE3, results in a decrease in the levels of several mitochondrial respiratory enzymes, impairs mitochondrial motility, retards neurite outgrowth, and results in an increase in toxic apoE4 fragments. Mutant apoE4(R61T) lacking domain interaction behaves like apoE3 in neurons, further indicating that the detrimental effects of apoE4 depend on domain interaction. Likewise, treatment of apoE4 with structure-correcting small molecules that block domain interaction prevents apoE4's detrimental effects.
Conclusions: Our findings suggest potential therapeutic strategies, including the use of structure correctors to convert apoE4 into an apoE3-like form, protease inhibitors to prevent generation of toxic apoE4 fragments, and "mitochondrial protectors" to prevent cellular energy disruption.
Disclosure: R. Mahley, Part 1: Consultant to MERCK on CV drug, Part 2: The J. David Gladstone Institutes, Part 3: None, Part 4: Research collaboration between MERCK and The J. David Gladstone Institutes involving the Mahley laboratory, Part 5: N/A.

ApoE and the Molecular Pathogenesis of Alzheimer's Disease: Therapeutic Implications

Steven Paul*

Weill Cornell Medical College, New York, USA

Background: The apoε4 allele is a very well-established genetic risk factor for late-onset AD, where homozygotes have been shown to have a >15-fold risk of developing AD and also develop the disease approximately a decade earlier than apoε3 homozygotes. It has been estimated that inheritance of the apoε4 allele accounts for approximately 30–50% of AD in the general population. By contrast, the apoε2 allele is protective, reducing the risk of developing AD by approximately 50%. The robust impact of apoE genotype on the risk of developing AD strongly suggest that apoE plays a pivotal role in the etiology and pathogenesis of AD. By inference, understanding how these two apoE alleles so dramatically (and bidirectionally) alter the risk of developing AD could lead to an effective therapeutic intervention for either slowing disease progression and (or) even preventing its onset entirely.
Methods: Recent work in my laboratory employing several transgenic mouse models of AD have demonstrated a profound impact of the three apoE isoforms on brain Aβ accrual and amyloid burden (E4 > E3 > E2). Our data on the cellular and molecular mechanisms underlying apoE's effect on Aβ clearance support a pivotal role for astrocytes and microglia in the apoE-dependent catabolism and clearance of Aβ, which likely explains how apoE isoforms so profoundly influence amyloid plaque burden in brain. The role of other cholesterol-regulating proteins (e.g. ABCA1 and clusterin) on AD neuropathology will also be presented. Collectively, these data have prompted several novel therapeutic strategies including the discovery of small molecule drugs that stimulate LXR/RXR receptors to increase apoE expression/secretion from astrocytes as well as ABCA1 expression in brain. Finally, the use of gene delivery strategies to facilitate apoE2 expression in brain will be discussed. The latter dramatically reduces AD neuropathology in APP transgenic mouse models and may offer another therapeutic approach to preventing or treating AD.
Results: Our results demonstrate that apoE alters the clearance of Aβ in a isoform-dependent manner (E2 > E3 > E4). The apoE isoform-dependent clearance of Aβ most likely accounts for the increase in brain amyloid burden in apoE4 carriers. Small molecule drugs which increase apoE expression in brain may prove useful in the treatment and prevention of AD.
Conclusions: ApoE remains an important therapeutic target for treating and potentially preventing AD.
Disclosure: S. Paul, Part 1: Sigma Aldrich-Board of Directors Alnylam Pharmaceuticals-Board of Directors Constellation Pharmaceuticals-Board of Directors Seaside Therapeutics-Board of Directors Third Rock Ventures-Venture Partner Karuna-Board of Directors, Part 2: All of the above including Weill Cornell Medical College.

Panel Session
Translating Pharmacogenetics into Clinical Utility: Optimizing the Phenotype

Common and Rare Variation in the POMC Pathway contributes to Antipsychotic Drug-Induced Weight Gain

Anil Malhotra*

The Zucker Hillside Hospital, Glen Oaks, USA

Background: Neuropsychiatric pharmacogenetic studies have traditionally focused on the identification of variants that predict drug efficacy. Challenges with this approach include the reliance on patient report to infer biological response, difficulties in standardizing ratings across groups large enough for genetic analysis, and the modest effects of single variants on this complex phenotype. Assessment of adverse events in pharmacogenetics represents a complementary approach. Side effects may be defined based upon established and quantifiable criteria, measurement error is minimal, and biological hypotheses can be informed by studies from other branches of medicine.

Methods: We have comprehensively characterized a discovery cohort of antipsychotic-naïve pediatric patients undergoing initial clinical treatment with the second generation antipsychotic drugs. Subjects were confirmed to be receiving antipsychotic drug by plasma blood levels, and were weighed at baseline, 4, 8 and 12 weeks of treatment. DNA was collected via blood sample and genotyping conducted with the Illumina 1M OmniQuad platform. Regression analysis of weight at 12 weeks of treatment versus baseline was conducted using both additive and recessive models. Subsequently, we assessed two additional cohorts of subjects treated with second generation agents and genotyped SNPs implicated in the discovery cohort, as well as conducted a rare variant analysis of our discovery cohort focused on missense SNPs.
Results: QTL analysis revealed a region of the genome near the melanocortin 4 receptor gene (MC4R) that significantly associated with antipsychotic induced weight gain. SNPs in this region were also associated with weight gain in the two additional cohorts, with the same allele relationships and effect sizes observed across all three cohorts. Rare variant analysis also revealed a relationship between an amino acid substitution in the PCSK1 gene, also located in the proopiomelanocortin (POMC) pathway, and significant weight gain.
Conclusions: Pharmacogenetic studies of antipsychotic-induced weight gain may be most powerful if comprised of previously untreated patient populations with documented adherence to treatment. These data suggest that specific genes in the POMC pathway may be associated with antipsychotic induced weight gain in these populations.
Disclosure: A. Malhotra, None.

Genetic Variation in CYP450s Impacts Clearance of Antipsychotics

Kristin Bigos*

National Institute of Mental Health, Bethesda, USA

Background: Antipsychotics have a high rate of discontinuation due to inefficacy and/or adverse effects. This study aimed to identify genetic variants in CYP450 drug metabolizing enzymes that impact antipsychotic clearance using pharmacokinetic and genetic data from the CATIE trials.
Methods: Clearance of atypical antipsychotics (olanzapine, risperidone, quetiapine, and ziprasidone) was estimated using nonlinear mixed-effects modeling. Single nucleotide polymorphisms (230 SNPs) in CYP450 metabolizing enzymes (CYP1A, CYP2A, CYP2B, CYP2C, CYP2E, and CYP3A families) were tested as possible predictors of antipsychotic clearance.
Results: CYP3A43 genotype (rs472660), which previously predicted olanzapine clearance, also significantly predicted 30% of risperidone clearance ($p = 4e\text{-}18$). Of the 230 SNPs in CYP450s, the CYP3A43 SNP (rs472660) was the most significantly associated with both risperidone and olanzapine clearance, and predicted most and the entire previous race effects in drug clearance, respectively. This CYP3A43 SNP did not predict either quetiapine or ziprasidone, which was predicted based on the lack of racial effects on their clearance. The most significant predictors of ziprasidone and quetiapine clearance were SNPs located in the CYP2A/2B families of genes on chromosome 12. SNPs in other CYP gene families were also associated with clearance of one or more of the antipsychotics.
Conclusions: Genetic variation in CYP450 drug metabolizing enzymes are significantly associated with clearance of antipsychotics, which likely contributes to the wide variability in response and side effects to these medications.
Disclosure: K. Bigos, None.

Rare Outcomes and Rare Alleles in Treatment-Resistant Depression

Gonzalo Laje*

NIMH, Bethesda, USA

Background: Candidate gene, genome-wide and meta-analyses have identified multiple markers associated with antidepressant response. However, these common genetic variants have small effect and no current clinical utility. It is possible that rare variants with large effect sizes may offer additional insight into the pathophysiology of this complex phenotype. For this purpose we undertook a pilot whole exome sequencing experiment in individuals selected from the Sequenced Treatment Alternatives to Relieve Depression study (STAR*D).
Methods: We selected a limited number of Caucasian European-ancestry individuals from the STAR*D sample ($n = 8$) who represented treatment-resistant cases and sustained treatment remitters. Extreme non-responders (NR), derived from Level 4, had failed 4 treatment strategies. The control group, sustained remitters (SR), were remitters at Level 1 endpoint and did not relapse in the 6 months post Level 1 completion (QIDS-SR < 11). Additionally, groups were matched on gender, anxious depression status, treatment adherence, drug and alcohol abuse, and ancestry. All samples were sequenced at EdgeBio using a SOLiD platform with $\geqslant 10$X coverage.
Results: Results pertaining to single nucleotide variants (SNVs) revealed that each participant had about 90,000 SNVs on initial annotation. We used SIFT (Sorting Intolerant From Tolerant), an algorithm that predicts whether an amino acid substitution affects protein function, to analyze both novel and known variants. There were no differences in the mean number of known variants between groups. However, the mean number of novel variants, predicted as damaging, had a small but significant difference in non-responders (89.8 + /-7.2) and sustained remitters (100.8 + /-3.8) ($p < 0.05$).
Conclusions: The detection of rare variants through high throughput sequencing may shed new insight into the pathophysiology of treatment-resistant depression.
Disclosure: G. Laje, None.

The Consortium on Lithium Genetics (ConLiGen): Phenotypic Characterization and Genome-Wide Association Study of Lithium Response

Thomas Schulze*

Depts. of Psychiatry, Johns Hopkins University & Georg-August-University Göttingen (Germany), Baltimore, USA

Background: Co-authored by F.J. McMahon (Bethesda, MD) for the ConLiGen consortium Lithium remains a mainstay in the long-term treatment of bipolar diorder (BD). Response to lithium is variable. About 30% of patients treated with lithium have fewer illness episodes over time, while about 20% have no response. Data from pharmacogenetic studies of lithium are comparatively sparse, and these studies have generally employed small sample sizes and varying definitions of response. Genetic markers of lithium response would be valuable for treatment planning and could provide insights into the biological mechanism of lithium action. To put that idea into practice, the international Consortium on Lithium Genetics (www.ConLiGen.org) was established.
Methods: *ConLiGen* has now collected over 1400 lithium-treated BD patients. All patients have been characterized for lithium response with an 11-point treatment response scale ("Alda Scale", Grof *et al.*, 2002). The Alda Scale assesses clinical improvement attributable to lithium, taking into account the history and frequency of episodes, duration of treatment, medication

adherence, and concurrent treatment. Phenotype definitions were developed by consensus within *ConLiGen*. The whole sample has been genotyped using Illumina arrays to perform a genome-wide association study (GWAS) of lithium response.
Results: Inter-rater reliability of lithium response assessment was good, with kappa values >0.7. Given a responder rate of 35%, the ConLiGen sample has $>80\%$ power to detect a common allele that confers a genotype relative risk of response of 1.5 at genome-wide significance. At the time of abstract submission (4/2011) genotyping is largely complete, and preliminary quality control data indicates excellent call rates. ($>99\%$ of samples have a call rate $>98\%$), GWAS completion is expected for 05/2011.
Conclusions: Genetic findings from *ConLiGen* could have important implications for treatment planning and for developing new drugs that mimic the action of lithium but are better tolerated and more effective.
Disclosure: T. Schulze, None.

Panel Session
Enhancing Cognititve Performance: Molecular, Pharmacological, and Experiential Strategies

Cognitive Enhancement in Animal Models of Neuropsychiatric Disorder
Trevor Robbins*

University of Cambridge, Cambridge, United Kingdom

Background: The search for novel cognitive enhancing drugs has both preclinical and clinical aspects. We report new data on cognitive enhancing effects in rodents, with particular reference to fronto-executive functions relevant to schizophrenia and ADHD. These functions include response inhibition and cognitive flexibility, measured in tests having parallels in human cognition.
Methods: We measured cognition in rats with a test battery including reversal learning and intra- and extra-dimensional shift learning (digging paradigm) and 5-choice reaction time performance. In some experiments we used sub-chronic PCP and E17 treatment with MAM, the developmental neurotoxin, as perturbations producing some elements of schizophrenia pathology. The main test drug was a novel mGLU-R5 potentiator, LSN2463359, and some informative comparisons were also made with the reference "cognitive enhancer" modafinil.
Results: Activation of mGLU5 receptors reversed MAM but not PCP-induced deficits in cognitive flexibility as measured by reversal learning in the texture/olfactory digging task. The drug specifically reversed perseverative behaviour produced by MAM, but had no effect on the form of reversal learning deficit induced by PCP which was characterised by interference rather than perseveration. By contrast, modafinil is known to reverse many of the deficits in this task produced by chronic PCP. We also found that modafinil improved the accuracy of performance under certain conditions at low doses (10mgkg) in the 5-choice serial reaction time task.
Conclusions: Effects of cognitive enhancing drugs can readily be measured in experimental animals in tests of executive function having some affinity to humans. However, the effects of such drugs may differ according to the precise profile of functions studied and to the type of model employed of the psychiatric impairment under study. There are strong potential implications for the predictive validation of such paradigms for cognitive enhancers in schizophrenia.
Disclosure: T. Robbins, Part 1: Consulted for Cambridge Cognition, Pfizer, Lilly, Lundbeck, GlaxoSmithKline, Part 2: Pfizer, Cambridge Cognition, Part 3: Pfizer, Cambridge Cognition, Part 4: Research Grants from Lundbeck, GlaxoSmithKline, Lilly.

The Role of Insulin-Like Growth Factors and Insulin in Memory Enhancement
Cristina Alberini*

Mount Sinai School of Medicine, New York, USA

Background: We recently found that the insulin-like growth factor II (IGF-II) enhances inhibitory avoidance (IA) memory when injected into the rat hippocampus immediately after training or retrieval (Chen *et al.*, *Nature* 2011). IGF-II is part of the IGF superfamily, which includes the structurally similar IGF-I. These proteins have high sequence similarity to insulin. We investigated the role of IGFs, insulin and relative receptors on memory retention.
Methods: We used fear conditioning as well as non-aversive tasks in rats to investigated whether intracerebral or, in specific cases, systemic administrations of IGFs or insulin affect memory retention.
Results: We found that IGF-II significantly and persistently enhances hippocampal-dependent memories, but has no effect on amygdala-dependent memory. Insulin enhances hippocampal-dependent memories when injected into the hippocampus and result in a trend toward memory enhancement when injected into the amygdala. In contrast, IGF-I showed no effects in any conditions tested.
Conclusions: Taken together, these results suggest that IGF-II is a potent memory enhancer that also produces a persistent effect on memory retention in hippocampal-dependant memories, whereas insulin has a milder and transient effect, suggesting that the two factors act on different mechanisms.
Disclosure: C. Alberini, None.

Brain Plasticity Perspective about the Origin & Treatment of Psychiatric Illness
Michael Merzenich*

UCSF, San Francisco, USA

Background: Studies demonstrating the fundamentally reversible nature of brain plasticity processes, and the implications that this bears for understanding the origins of, and possible treatments of schizophrenia shall be briefly reviewed.
Methods: Reversible-plasticity studies conducted in animal models have been extended to studies of patients with chronic schizophrenia.
Results: A broad series of neurologically- and behaviorally-expressed "corrections" have been recorded, as predicted, in chronic patients.
Conclusions: This non-drug treatment strategy shall have an important future role in the treatment of this (and other psychiatric) patient population(s).
Disclosure: M. Merzenich, Part 1: DNA, Part 2: Posit Science Corporation, San Francisco, CA, USA, Brain Plasticity Institute, San Francisco, CA, USA, Scientific Learning Corporation, Oakland, CA, USA, Part 3: DNA, Part 4: DNA, Part 5: Posit Science Corporation, San Francisco, CA & SA.

Cognitive Enhancing Drugs and Society
Barbara Sahakian*

University of Cambridge, Cambridge, United Kingdom

Background: Cognitive enhancing drugs are needed to treat the cognitive impairments associated with debilitating neuropsychiatric disorders, such as Alzheimer's disease, schizophrenia and Attention Deficit Hyperactivity Disorder (Sahakian and Morein-Zamir, 2007). Such treatments will improve the quality of life and

wellbeing for patients and their families and reduce the financial burden on society (Beddington *et al.*, 2008).
Methods: Double-blind acute studies using cognitive enhancing drugs such as methylphenidate, atomoxetine and modafinil were conducted.
Results: These studies have demonstrated enhancement in forms of cognition in both neuropsychiatric patients and healthy volunteers.
Conclusions: Cognitive enhancement is of great interest to the general public and has implications for society, particularly in regard to the increasing use of cognitive enhancing drugs in school age children, and in young adults and academic staff at University (Greely *et al.*, 2008; Maher, 2008; Sahakian & Mohamed, 2010; Sahakian and Morein-Zamir, 2007). Therefore, it is important to consider the potential harms of lifestyle use of these drugs, for example substance abuse, the unknown effects on the developing brain or coercion at school or work. Nevertheless, with the rapidly developing field of pharmacogenomics we may be able to gain maximum benefits with minimum harm to the individual and society as a whole. Certainly, the benefits of safe and effective cognitive enhancing drugs to society, including the ageing population and people with neuropsychiatric disorders and brain injury, are great (Beddington *et al.*, 2008; The Academy of Medical Sciences Report, 2008). It is therefore important to engage the public in discussions of these neuroethical issues (Sahakian and Morein-Zamir, 2009; Morein-Zamir and Sahakian, 2010).
Disclosure: B. Sahakian, Part 1: Professor Barbara Sahakian consults for Cambridge Cognition. She has consulted for Novartis, Shire, GlaxoSmithKline, Lilly, Boehringer-Ingelheim and Hoffmann-La Roche. She has also received honoraria for Grand Rounds in Psychiatry at Massachusetts General Hospital (CME credits) (Boston, 27 April 2007) and for speaking at the International Conference on Cognitive Dysfunction in Schizophrenia and Mood Disorders: clinical aspects, mechanisms and therapy (Brescia, 17–19 January 2007). She was on the Medical Research Council Neurosciences and Mental Health Board (2010) and on the Science Co-ordination Team for the Foresight Project on Mental Capital and Wellbeing, 2008 (Office of Science, The Department of Innovation, Universities and Skills). She is currently on Panel LS5 for the European Research Council. As an Associate Editor, she also receives an honorarium from the Journal of Psychological Medicine.

Panel Session
Optogenetic Dissection of Cortico-Limbic Circuit Function and Dysfunction

Optogenetic Probe of the Role of Cholinergic Neurons in Cocaine Conditioning
Karl Deisseroth*

Stanford, Stanford, USA

Background: To describe an optogenetic probe of the causal role of cholinergic neurons in cocaine.
Methods: The first behavioral loss of function optogenetic experiment in mammals is described here, in which a halorhodopsin was multiply engineered for safe high-level expression and targeting in freely moving mice. This allowed selective inhibition of the nucleus accumbens cholinergic neurons both during behavior and during slice physiology experiments to probe circuit mechanisms of cocaine conditioning.
Results: The NAcc cholinergic neurons were found to be specifically involved in responding to cocaine, inhibiting NAcc medium spiny neurons, and allowing the implementation of cocaine conditioning in mice, without affecting other aspects of locomotion, place aversion, contextual memory, or other behaviors.
Conclusions: This talk will conclude with a review of optogenetic history, with behavioral gain of function experiments now matched with loss of function experiments, opening the door to versatile and generalizeable studies of neural circuit function.
Disclosure: K. Deisseroth, None.

Optogenetic Tuning of Cholinergic Inputs to Basolateral Amygdala
Lorna Role*

Stony Brook Univeristy, Stony Brook, USA

Background: Cholinoceptive circuits of the amygdala play a critical role in normal and pathological mechanisms of the formation and extinction of emotional memories. Our ongoing studies examine the role of *endogenous* cholinergic inputs from the Nucleus Basalis of Meynert (NBM) and other basal forebrain areas (BF), with and without exogenous nicotine exposure, in the modulation of cortical and hippocampal inputs to basolateral amygdala (BLA).
Methods: Cortico-amygdala circuit activity is assayed by patch clamp recording from target BLA pyramidal neurons in acute slice preparations. Optogenetic stimulation of cholinergic inputs and electrical stimulation of cortical /hippocampal projections to the BLA is used to assess the effects of endogenous ACh +/- nicotine on cortico-amygdala synaptic plasticity.
Results: With as little as 10 Hz light-activated stimulation of NBM inputs to BLA, pyramidal neurons reliably increase their bursting activity. The NBM-elicited bursting activity requires the activation of nicotinic, but not muscarinic, AChRs**.** Preliminary studies combining optogenetic stimulation of cholinergic inputs with voltage clamp of BLA neurons reveal underlying changes in both excitatory and inhibitory inputs as well as occasional direct depolarization of the BLA neurons. The latter effects of endogenous ACh appear to be mediated by *both* nicotinic and muscarinic receptor activation. Pharmacological isolation of glutamatergic inputs in conjunction with optogenetic stimulation of NBM fibers ± stimulation of cortical – BLA afferents reveals increased mini frequency and increased amplitude of paired pulse responses consistent with nAChR mediated enhancement of synaptic transmission.
Conclusions: These studies support an important role of presynaptic nAChRs in the modulation of cortico-amygdala circuitry. Ongoing studies of the fine tuned activation and inhibition of cholinergic inputs from NBM to BLA using optogenetic probes will reveal how nAChR activation via endogenous and exogenous ACh regulates cortico-amygdala transmission *in vivo*.
Disclosure: L. Role, None.

Optogenetic Dissection of Development and Function of Newborn Neurons in the Adult Brain
Shaoyu Ge*

SUNY Stony Brook, Stony Brook, USA

Background: Neurogenesis has obtained more and more attentions for its potential role in therapeutic applications. My laboratory is interested in examining the function and mechanisms regulating the sequential integration of newborn neurons in the adult brain.
Methods: Using retroviral, imaging, physiological, optogenetic and behavioral approaches, we are studying different aspects on how newborn neurons successfully integrate into pre-existing neural circuits and determined the behavioral roles of newborn neurons in the adult brain.

Results: In this panel, I will present our recent studies on functional synapse formation of newborn hippocampal neurons with their targets using optogenetic approaches.
Conclusions: I will also present our work demonstrating the behavioral role of newborn neurons in the adult animals using optogenetic activation and silencing of the newborn neurons to alter performance in specific learning tasks.
Disclosure: S. Ge, None.

Optogenetic Manipulation of Cortical Activity alters Behavioral Flexibility in Making and Breaking Habits

Ann Graybiel*

Massachusetts Institute of Technology, Cambridge, USA

Background: Goal-directed and habitual behaviors represent two poles of behavioral control and are thought to depend on different neural circuits. For habits to form, goal-directed exploratory behavior gradually declines until repetitive sequences of behavior emerge as habits that become notoriously difficult to break. These behavioral changes are thought to require a transition in predominant behavioral control from prefrontal associative-limbic circuitry to sensorimotor cortico-basal ganglia circuitry. Yet it is still unclear what activity patterns in these circuits underlie their effects on behavior, nor mechanistically how transitions between behavioral strategies actually occur.
Methods: We approached these issues by capitalizing on a reward devaluation strategy that allows testing for the presence of habitual behavior. We trained and then overtrained rats on a conditional T-maze task, and then devalued one of the task rewards. We recorded simultaneously from cortical (infralimbic cortex) and striatal (sensorimotor striatum) parts of the brain's habit circuitry as the rats learned the task and then encountered devaluation.
Results: We found that the striatal habit region developed a habit-related neural activity pattern and maintained it after devaluation, but that the cortical component flexibly shifted its habit-related pattern after devaluation. These findings suggested that within the habit system itself, the striatal component inflexibly maintains its habit-related activity pattern despite a change in reward value, whereas the cortical component's activity can flexibly shift. We then tested the hypothesis that this cortical flexibility is necessary for behavioral flexibility in making and breaking habits. We optogenetically inactivated the cortical component during performance by using light-driven halorhodopsin to silence neurons there. We were able to block habitual performance reversibly in sequential light-on/light-off experiments.
Conclusions: We conclude that the cortical habit system is poised to override the striatal habit system and to control the expression of habits flexibly on-line.
Disclosure: A. Graybiel, None.

Panel Session
Functional Connectivity in Neural Systems as a Developmental Abnormality in Creating Risk for Bipolar Disorder

Anterior Limbic Abnormalities in Youth with a Bipolar Parent

Melissa DelBello*

University of Cincinnati, Cincinnati, USA

Background: Recent findings suggest that adolescents with bipolar disorder exhibit structural and functional abnormalities in prefrontal and amygdala brain regions. We investigated whether these abnormalities are also present prior to the onset of bipolar disorder in order to determine whether these alterations may be useful predictors of illness.
Methods: Seventy-seven youth (ages 10–20 years) with a bipolar parent ("at-risk") and 42 demographically-matched healthy comparison subjects (HC) without any first- or second-degree relatives with a mood disorder underwent functional MRI while performing a Continuous Performance Test with Emotional and Neutral Distracters (CPT-END). Structured and semi-structured interviews were conducted, by interviewers blind to subject group, to confirm DSM-IV diagnoses (or absence), in parents and study participants, respectively. Only youth with at least one biological parent with bipolar I disorder and with no history themselves of a major depressive disorder, bipolar disorder or a psychotic disorder were included in the bipolar offspring group. Functional images, analyzed using Analysis of Functional Images (AFNI), included motion correction, spatial smoothing, normalization and random effects analysis of activation data.
Results: Compared with HC subjects (mean age = 14.0 years, 52% boys), at-risk (mean age = 13.3 years, 54% boys) subjects exhibited decreased activation in left amygdala and bilateral prefrontal regions (Brodmann areas (BA), 24, 10 and 47) as well as increased activation in right middle temporal and fusiform gyri (BA 37) during emotional stimuli. Moreover, at-risk youth exhibited increase amygdala-prefrontal (BA 10, 11, and 47) connectivity compared with HC.
Conclusions: Our preliminary findings indicate that adolescents at risk for developing bipolar disorder exhibit alteration in ventral lateral prefrontal-amygdala coupling prior to illness onset, suggesting that these abnormalities may serve as predictors of incipient mood episode.
Disclosure: M. DelBello, Part 1: BMS, Eli Lilly, Pfizer, GSK, Amylin, Johnson and Johnson, Somerset, Merck, Schering Plough, Astra-Zeneca, Pfizer, Part 2: BMS, Merck, Part 3: BMS, Merch, Part 4: Eli Lilly, Astra Zeneca, Amylin.

Abnormal Structural and Functional Integrity of Emotion Regulation Neural Circuitry differentiate Healthy Adolescents at High Risk for Mood Disorders from Healthy Low-Risk Adolescents

Mary Phillips*

University of Pittsburgh, Pittsburgh, USA

Background: Offspring of parents with bipolar disorder (BD) are at increased risk for BD and other psychiatric disorders. Abnormalities in the structure and function of neural circuitry supporting mood regulation most likely mediate familial risk for these disorders, given the onset of many psychiatric disorders in adolescence, and the important maturational changes in brain structure and function that occur during this developmental period.
Methods: We examined white matter (WM) connectivity, with tract-based spatial statistics, and activity in emotion processing and emotion regulation circuitry using fMRI in 20 healthy youth offspring of bipolar parents (HBO) and 25 healthy youth without family history of bipolar disorder (HC) (9–17 years). We also used pattern recognition and Gaussian Process Classifier to determine whether HBO could be distinguished case by case from HC based on wholebrain activity during face emotion processing.
Results: Relative to HC, HBO showed: abnormal developmental trajectories of WM in fronto-temporal tracts relative to HC, that parallel abnormalities in WM in youth with BD; and abnormally reduced activity in lateral prefrontal cortex during an emotion regulation task. Pattern recognition analyses using wholebrain fMRI data during face emotion processing accurately differentiated HBO from HC on a case by case basis, and predictive probabilities were significantly higher for HBO who subsequently developed an Axis I disorder than for HBO remaining healthy at follow-up.

Conclusions: Altered development of WM in fronto-temporal tracts, together with abnormal functional integrity in prefrontal cortical regions supporting mood regulation, in HBO relative to HC may represent vulnerability markers for future BD and other psychiatric disorders in HBO. A combination of machine learning and neuroimaging not only discriminates HBO from HC, but also accurately predicts which HBO subsequently develop Axis I disorders.
Disclosure: M. Phillips, Part 2: I receive an annual consultant stipend from Cardiff University, UK.

Brain Activation Predicts Clinical Change Following Family Focused Therapy in Youth at High-Risk for Bipolar Disorder

Amy Garrett*

Stanford University, Palo Alto, USA

Background: Previous studies have found that FFT is effective in stabilizing symptoms of mania and depression in adolescent patients. Better understanding of the mechanisms of positive therapeutic change in adolescents at risk for bipolar disorder may lead to improved treatments and prevention.
Methods: A subset of youth participating in a clinical trial assessing the efficacy of FFT versus Enhanced Care (EC) were scanned before and after 4 months of treatment. Twelve subjects with BD-NOS or MDD were included: 6 received FFT and 6 received EC. Functional magnetic resonance imaging scans were acquired using a spiral pulse sequnce on a 3T GE scanner. Subjects viewed fearful, neutral, and calm faces and indicated the gender of each face. Whole brain data were processed in SPM5 using a repeated measures ANOVA. Data from the contrast of fearful-scrambled faces was examined. Clusters of activation that changed significantly from baseline to follow-up were identified. and activation in clusters that fell in a priori hypothesized regions was extracted to SPSS.
Results: First, activation in the right amygdala significantly decreased from baseline to 4-mo. post-treatment follow-up. Correlations with clinical scores showed that higher activation in the right amygdala at baseline was found in youth who showed the greatest improvement in depressive symptoms at follow-up, but only in the FFT and not the EC group. Second, the right DLPFC increased activation from baseline to follow-up. For all subjects, greater increases in right DLPFC activation from baseline to follow-up were associated with greater improvement in manic symptoms following treatment.
Conclusions: Clinical improvement is associated with changes in the function of specific brain regions that have been implicated in emotion perception and regulation. These data also suggest that fMRI may help us to predict who will respond most significantly to FFT rather than EC treatments.
Disclosure: A. Garrett, None.

Functional Connectivity Abnormalities in Youth at High-Risk for Bipolar Disorder

Kiki Chang*

Stanford University, Stanford, USA

Background: It may be that abnormal development in neural *networks*, and thus functional connectivity (FC), create risk for maladaptive patterns of mood regulation, which then lead to fully developed bipolar disorder (BD). We performed fMRI on symptomatic offspring of parents with BD and compared results with healthy controls (HC). We hypothesized that subjects at high-risk for BD would have abnormalities in FC between prefrontal cortical structures and amygdala, and that subjects with the 5-HTTLPR *s*-allele would have increased amygdalar activation than those without.
Methods: 52 youth 9–17 years old, who had at least one parent with BD I or II, were included, along with 30 age, gender, and IQ-matched HC. Bipolar offspring were diagnosed using the WASH-U-KSADS as having either major depressive disorder (MDD), or ADHD, with at least moderate affective symptoms. Subjects were scanned on a 3T GE MRI scanner after completing mock scanning. We used an fMRI task of implicit emotional face recognition, using NimStim faces (Calm, Fearful, Neutral, Scrambled), with subject pressing buttons corresponding to gender of person shown. 4 offspring and 1 HC were not used due to excessive movement. SPM8 and PPI were used to analyze data, using ROIs from the AAL for VMPFC, VLPFC, amydgdala, and DLPFC. Data are shown for the Fearful – Neutral contrast.
Results: Offspring had increased amygdala and VMPFC activation compared to HC. Multiple regression showed levels of left amygdala and left sgACC (BA25) activation were moderated by 5-HTT genotype. Offspring with an *s*-allele ($n = 33$) had higher left amygdalar ($p < 0.05$) and sgACC activation ($p = 0.018$) than those without ($n = 11$). When seeding the right amygdala, offspring had higher FC with the right VLPFC compared to HC (Figure, left). Furthermore, the HC group had an inverse FC between right amygdala and right DLPFC, which was not found in the Offspring group.
Conclusions: Youth at high-risk for BD may have genetic predispositions that lead to overactivation of amygdala in response to affective probes. Furthermore, the Bipolar Offspring group appeared to activate VLPFC in higher positive synchrony with amygdala, a finding echoed in adults with BD (Chepenik *et al.*, 2010). PO subjects did not have an inverse FC between right amygdala and DLPFC, which could represent impaired inhibition of amygdalar activation from DLPFC in youth at high-risk for BD, consistent with adults data in BD probands (Yurgelen-Todd, 2001). Thus, genetic influences may lead to aberrant neural connectivity in prefrontal-subcortical circuits which then lead to BD development. Longitudinal assessment of these subjects is necessary to clarify if these are state or trait findings.
Disclosure: K. Chang, Part 1: GlaxoSmithKline, Lilly, Bristol-MyersSquibb, Merck, Part 4: GlaxoSmithKline.

Panel Session
Contribution of Genetic Epidemiology to Identifying Genetic and Environmental Risk Factors for Neurologic and Psychiatric Disorders

Sources of Heterogeneity of Migraine: Longitudinal Stability and Comorbidity with Mood Disorders

Kathleen Merikangas*

National Institute of Mental Health, Bethesda, USA

Background: The major goals of this presentation are: (1) To present data on the longitudinal stability of migraine and patterns of comorbidity across the life span; and (2) to investigate familial patterns of manifestation and co-aggregation of mood and anxiety disorders with migraine in the NIMH-Lausanne collaborative family study of comorbidity of affective spectrum disorders and migraine.
Methods: Data on the stability of common headache subtypes will be presented from the Zurich Cohort Study of young adults that includes 591 young adults enrolled at ages 18–19 followed for 30 years with comprehensive clinical evaluation of mental disorders and migraine at 7 interviews over time. Familial aggregation and co-aggregation of migraine and mood disorders will be examined in data from a collaborative family study of affective spectrum disorders that includes 1500 probands with the full range of mood disorders (bipolar I and II, major depression, anxiety disorders),

migraine and controls, and their first degree relatives including children ages 8–18. Comparable semi-structured diagnostic interviews for mood and anxiety spectrum disorders were conducted by clinically experienced interviewers and diagnoses were derived from a best estimate procedure for diagnostic ratings based on interviews, medical records and family informants.
Results: The results of the Zurich Cohort Study reveal a lack of stability of longitudinal manifestations of the current subtypes of migraine in the diagnostic nomenclature, with 40% cross-over across the distinct headache subtypes over time. There was a gradient of severity across discrete headache subtypes based on clinical indicators of severity including age at onset, impairment, symptom severity and recurrence. The aggregate family study analyses revealed: (1) a three-fold increase in migraine in relatives of those with migraine, net of comorbid disorders (odds ratio = 3.4; 1.9–6.3); (2) independence of familial co-aggregation of anxiety disorders and migraine (odds ratio = 1.0; 0.6–1.7); and (3) evidence for common familial factors underlying migraine and bipolar disorder (odds ratio = 2.3;1.1–5.1), particularly the atypical subtype of depression (odds ratio = 1.4 ; 1.1–1.9).
Conclusions: These findings suggest that the lack of successful identification of genes underlying migraine may be in part attributable to heterogeneity attributable to phenotypic definitions, sex differences in expression, and heterogeneity associated with comorbid mood disorders. Future studies of broader phenotypes stratified by comorbid disorders may enhance our power to identify genetic susceptibility factors underlying migraine and associated mood disorders.
Disclosure: K. Merikangas, None.

Genetic Studies of Schizophrenia in Sweden: Population-Based Samples, Discordant Mono-Zygotic Twins and Co-Morbidity with Bipolar Disorder and Infantile Autism
Christina Hultman*

Department of Medical Epidemiology and Biostatistics, Stockholm, Sweden

Background: To investigate the co-aggregation of schizophrenia and bipolar disorder and to identify common and unique neurodevelopmental factors associated with these disorders.
Methods: Data derived from linking national registry data from (1) The Swedish Patient Registry (psychiatric treatment) (2) the Medical Birth Registry (obstetric/delivery/birth complications) (3) the School and Education Registry (cognitive function) and (4) the Twin and the Multi-generation Registry (family history, fertility, paternal age). n molecular epidemiological studies, we have combined register-based design and clinical recruitment of patients with DNA-sampling of large nation-wide cohorts to incorporate the inevitable combination of molecular genetics and psychiatric epidemiology.
Results: We have shown shared genetic etiology in schizophrenia and bipolar disorder in family studies of over 2 million nuclear Swedish families. All classes of biological relatives of probands with bipolar disorder had increased risk for schizophrenia, and the genetic correlation (the correlation between the genetic effects that determine the liabilities for the two disorders) was 0.60. Adopted children, whose biological parents had one of these disorders, had a significant increase in risks for the other disorder. Paternal age and other birth difficulties were associated with both schizophrenia and bipolar disorder, whereas low birth weight was uniquely associated with schizophrenia.
Conclusions: We have found compelling evidence from our extensive family register data, from our molecular genetic data, and from neurodevelopmental data that schizophrenia and bipolar disorder partially share a common genetic etiology.
Disclosure: C. Hultman, None.

Genetic Heritability, Shared Environmental Factors, and Sex Differences Among Twin Pairs With Autism
Neil Risch*

University of California at San Francisco, San Francisco, USA

Background: To provide estimates of the genetic heritability and the effects of common and unique environmental factors in the etiology of autism, and to examine concordance and cross-concordance and sex differences in broad and narrow autism phenotypes and associated features in a population based sample of autism.
Methods: A large twin study from a population-based sample was conducted using twin pairs born between 1987 and 2004 identified through the California Department of Developmental Services, with at least one twin diagnosed with an autism spectrum disorder (ASD). Structured diagnostic assessments (ADI-R and ADOS) were completed on 192 twin pairs including 54 monozygotic and 138 dizygotic twin pairs. Concordance rates were calculated and parametric models fitted for two definitions of autism, one narrow (strict autism) and one broad (ASD). Differences in concordance were also calculated after stratification on common and unique exposures and risk factors, as well as potential endophenotypes of autism.
Results: For strict autism, probandwise concordance for male twins was 0.58 for 40 monozygotic pairs (95% Confidence Interval [CI] .44 to .74) and 0.21 for dizygotic pairs (95% CI .09 to .43); for female twins the concordance was 0.60 for 7 monozygotic pairs (95% CI .28 to .89) and 0.27 for 10 dizygotic pairs (95% CI .09 to .69). For ASD, the probandwise concordance for male twins was 0.77 54 for 45 monozygotic pairs (95% CI .65 to .86) and 0.31 for 45 dizygotic pairs (95% CI .16 to .46); for female twins the concordance was 0.50 for 9 monozygotic pairs (95% CI .16 to .84) and 0.36 for 13 dizygotic pairs (95% CI .11 to .60). The best fitting models for both autism and ASD suggest that a large proportion of the variance in liability can be explained by shared environmental factors – 55% (95% CI 9% to 81%) for autism and 58% (95% CI 30% to 80%) for ASD, in addition to moderate genetic heritability – 37% (95% CI 8% to 84%) for autism and 38% (95% CI 14% to 67%) for ASD. Cluster analysis of quantitative variables (IQ, VABS composite score, PPVT composite score, SRS T-score, and ADOS severity score) suggest that there is a single continuous severity gradient affecting all aspects of behavior characteristic of ASD among affected individuals. Correlations are relatively equal in MZ and DZ twins indicating that severity itself is not inherited but likely due to environmental factors shared by twins.
Conclusions: These results suggest that future studies should investigate common environmental factors, particularly prenatal and early developmental environmental exposures, that may interact with susceptibility genes to enhance the risk for development of autism.
Disclosure: N. Risch, None.

Interaction between Genetic Susceptibility and Infections in the Etiology of Narcolepsy
Emmanuel Mignot*

Stanford University, Palo Alto, USA

Background: This presentation will describe recent developments in our understanding of the etiology of narcolepsy-cataplexy, a disease caused by the loss of hypocretin neurons, as a unique model to address neuronal autoimmunity in other neuropsychiatric disorders with a recently reported HLA association such as Parkinson's disease and schizophrenia. In addition to the established role of HLA DQB1*06:02 in narcolepsy and identification of an association between narcolepsy and a T Cell Receptor

(TCR) Alpha J segment polymorphism as another susceptibility factor, there has been indirect evidence for infectious causes/triggers of narcolepsy. This presentation will report new evidence on the role of winter infections in the trigger of narcolepsy.
Methods: Compilation of data from several sleep centers in the U.S., France, Canada and China will be presented to evaluate potential links with H1N1 vaccinations and infections, the presence of antistreptolysin-O (ASO) antibody (a marker of streptococcal infection), seasonality of onset, and HLA DQB1*0602. Further evaluation of Incident cases of narcolepsy over the past 9 years from China will be presented to assess the strength of these associations.
Results: In 2 independent and successive samples from the United States and Europe, we found that 63% (of 23) and 67% (of 16) of recent onset narcolepsy (<1 year) had increased antistreptolysin-O (ASO) antibody titers (>200) respectively (versus 15–20% in matched controls) ($p<0.01$). This together with an epidemiological study from Seattle indicating the condition was 5.4-fold more common, among people reporting a physician-diagnosed strep throat before the age of 21 years ($p<0.01$) suggests a role for this pathogen. Following on a report suggesting that narcolepsy may be triggered by H1N1 vaccination (a 9X fold increased risk is suggested with Pandremix by the WHO) in Scandinavia, we collected cases in France, Canada and the US following the 2009–2010 season. We identified 16 cases and found that vaccination with the Pandremix vaccine was more likely to increase narcolepsy risk than US vaccinations with non-adjuvanted H1N1 US vaccines. Most cases developed narcolepsy abruptly 2–8 weeks following vaccination and were atypical, displaying a sudden and explosive onset and an unusual age range (older or younger than usual). We also noted a large increase in recent onset case referrals to Stanford following the 2009–2010 season (most not reporting any infections, 14 cases versus 0–3 the years prior), suggesting that H1N1 infections and not only Pandremix vaccination could be involved in triggering narcolepsy. These findings were next used to analyze data on 626 patients with narcolepsy diagnosed in China in the last 13 years. This unique set of data is remarkable as most Chinese cases are diagnosed very close to onset, allowing documentation of the precise month of onset in a majority ($n=623$) of cases. Analyzing the month of onset, we found a 5 fold increase in sudden onset around spring-early summer (March-July) following the winter season) when compared to early winter (lowest point November-February). Further, a 3 fold increased in incident cases was found following the H1N1 2009–2010 pandemic season versus prior years ($p<0.001$), suggesting a link with the H1N1 pandemic.
Conclusions: Our results indicate that winter infections trigger narcolepsy on a specific HLA genetic background. We are now testing the hypothesis that a specific H1N1 epitope presented by DQB1*06:02 is involved and recognized by a specific TCR, with additional non specific factors activating the immune system or facilitating blood brain barrier penetration of T-cells (such as other infections, streptococcal superantigens, fever, or adjuvants). As very few autoimmune disorders are known to directly target neurons, and immune-brain interactions are only starting to be studied, a better understanding of the pathophysiology of narcolepsy is likely to illuminate the cause of other neuropsychiatric disorders with a neuroimmune component as well. These results also suggest the importance of the use of genetic information in risk estimation regarding susceptibility to infectious causes of human neuropsychiatric diseases.
Disclosure: E. Mignot, Part 1: Jazz Pharmaceutical Merck GSK.

Panel Session
From Transcription to Oscillations: How Sick Interneurons create a Schizophrenia-Like Phenotype

A Critical Role for PGC-1α in the Transcriptional Control of Parvalbumin-Positive Interneuron Function
Rita Cowell*

University of Alabama at Birmingham, Birmingham, USA

Background: Recent findings suggest that abnormalities in parvalbumin-positive (PV+) interneurons give rise to working memory deficits in schizophrenia. Our lab previously found that the expression of PV in cortical interneurons depends on the presence of the transcriptional coactivator PGC-1α (peroxisome proliferator activated receptor gamma coactivator 1 alpha), a gene within a region of chromosome 4 associated with schizophrenia. We sought to investigate the influence of PGC-1α ablation on interneuron transcription, function, and behavior.
Methods: To identify novel putative targets for PGC-1α, we performed an oligonucleotide array on neuroblastoma cells transfected with PGC-1α and tested a number of upregulated genes for changes in PGC-1α knockout mice. We then evaluated the electrophysiological characteristics of interneurons and pyramidal neurons in PGC-1α knockout mice and deleted PGC-1α specifically from interneurons to determine the involvement of interneuron-specific PGC-1α in performance on tasks of learning and memory.
Results: Novel PGC-1α putative targets included the developmentally-regulated genes synaptotagmin II, complexin I (synchronous neurotransmitter release), and neurofilament heavy chain (structural protein). Whole body and PV+ neuron-specific deletion of PGC-1α resulted in interneuron-specific reductions in the expression of PV and these targets, alterations in interneuron firing rate and post-synaptic responses to tetanic stimulation, and impairments in learning and memory.
Conclusions: PGC-1α is required for normal PV+ neuron function and memory processing in mice. Disruption in PGC-1α signaling in disorders such as schizophrenia could contribute to alterations in interneuron-specific gene expression and function. A further clarification of the mechanisms by which PGC-1α regulates interneuron gene expression could reveal novel targets for therapeutic intervention aimed at counteracting deficiencies in interneuron gene expression.
Disclosure: R. Cowell, None.

What's Wrong with Cortical Disinhibition? Exploring the Role of GABAergic Interneuron Dysfunction in Distinct Neuropsychiatric Disorder-Like Phenotypes
Kazu Nakazawa*

NIMH, Bethesda, USA

Background: Accumulating evidence links the pathophysiology of neuropsychiatric disorders to a cortical excitation/inhibition imbalance resulting from a dysfunction in proper GABAergic inhibition. However, the attribution of specific diseases to the abnormal function of cortical GABAergic neurons is far from established. Here we compared the behavioral and physiology phenotypes of three genetically-engineered mouse models, all of which exhibits impaired cortical inhibition.
Methods: We performed slice and in vivo unit recordings from mPFC and somatosensory cortex, respectively, in three conditional knockout lines (NMDA NR1 KO, Gad67 KO and ErbB4 KO). These lines were generated using Ppp1r2-Cre line in which cre

recombination was confined to a subset (~50%) of cortical and hippocampal, largely parvalbumin-positive, interneurons. (Belforte *et al*., 2010).
Results: Reduced GABA release and/or impairments in principal neuron rebound excitation were found in all three mutant lines. Despite these commonalities, however, they displayed distinct phenotypes. NR1 mutants were deficient in spatial working memory and prepulse inhibition (PPI), showed enhanced amphetamine-induced accumbens dopamine release, and had impaired tone-evoked gamma oscillations in the auditory cortex. Gad67 mutants showed reduced home-cage running wheel activity, attenuated hedonic behavior (in female urine sniff test and in saccharine preference), impaired social behavior, and marked weight gain. In contrast to the NR1 mutants, Gad67 mutants showed reduced amphetamine-induced dopamine release and no impairments in PPI or spatial working memory. ErbB4 mutants displayed no obvious behavioral phenotypes.
Conclusions: Cortical disinhibiton was common to all three mutants yet they each displayed unique phenotypes, therefore an imbalance of cortical excitation/inhibition alone is insufficient to explain the abnormal behavior. It is important to explore other, more subtle, gene-specific phenotypes, such as impaired synchronized firing.
Disclosure: K. Nakazawa, None.

Recurrent Excitation-Inhibition in Local Cortical Circuits: Synaptic Properties relevant for Gamma Oscillations
Guillermo Gonzalez-Burgos*

University of Pittsburgh, Pittsburgh, USA

Background: Alterations of perisomatic inhibition from parvalbumin-positive basket cells (PVBCs) may contribute to gamma oscillation deficits in schizophrenia. Whereas the normal mechanisms recruiting PVBCs during gamma rhythms are poorly understood, recent studies favor the Pyramidal Interneuron Network Gamma (PING) model, which relies on recurrent excitation-inhibition between pyramidal neurons (PNs) and PVBCs. We explored the role of excitatory and inhibitory synaptic connections between PVBCs and PNs in the circuit mechanisms of gamma oscillations in prefrontal cortex (PFC).
Methods: Single-cell and paired whole-cell recordings from PNs and PVBCs visualized with infrared differential interference contrast and epifluorescence microscopy to detect GFP-labeled PVBCs in the adult mouse PFC. Inhibitory and excitatory postsynaptic currents (IPSCs and EPSCs) were recorded from PNs and FSBCs. Mathematical tools were used to simulate gamma oscillations in a model network.
Results: IPSCs at PVBC->PN connections had fast decay kinetics and were highly sensitive to zolpidem, a modulator of fast-decaying a1 subunit-containing $GABA_A$ receptors. The IPSCs had low variability in latency during action potential firing at gamma frequency. EPSCs at PN->PVBC connections had fast decay kinetics, low sensitivity to NMDA antagonists and high AMPA receptor contribution. EPSP-to-action potential coupling in PVBCs was fast and predominantly mediated by AMPA receptors.
Simulations in a computational model network of PVBCs and PNs showed that the production of gamma oscillations via PING mechanisms was critically dependent on the properties of the model synapses. Specifically, fast AMPA receptor-mediated EPSCs at PN->PVBC connections and fast $GABA_A$ receptor-mediated IPSCs at PVBC->PN connections were crucial for optimal gamma band synchronization.
Conclusions: Our study revealed properties of recurrent excitation-inhibition that seem crucial for the mechanisms of gamma synchrony in PFC circuits and its alterations in schizophrenia
Disclosure: G. Gonzalez-Burgos, None.

Cell Type Selective Reduction in NMDAR1 leads to Cognitive and Negative Symptom-like Deficits
Steven Siegel*

University of Pennsylvania, Philadelphia, USA

Background: To determine selective contributions of NMDA receptor dysfunction on either inhibitory or excitatory neurons to electrophysiological, cognitive and negative symptom-related behavioral phenotypes of schizophrenia.
Methods: NMDAR1 (NR1) was selectively reduced in either parvalbumin (PV)-containing interneurons or CamKIIa-containing forebrain pyramidal cells using cre-lox knockout mice. Mice were tested for electrophysiological and behavioral abnormalities relevant to schizophrenia. Electrophysiological measures focused on background and auditory evoked gamma oscillations. Behavioral measures include social interactions, novel object recognition, contextual fear conditioning and working memory (T-maze). The effects of the GABAB agonist baclofen, the GABAAá2/3/5 agonist L-838,417, and the mGluR5 antagonist MPEP were then tested for all measures. Additionally, the effects of risperidone were evaluated to assess negative predictive validity for treatment resistant domains.
Results: Data suggest that both PV- and CamKIIa-selective reduction in NR1 leads to EEG abnormalities in gamma inter-trial coherence similar to those in schizophrenia. However, pharmacological reversal of these deficits differed depending on the mechanism by which that abnormality was induced. Specifically, MPEP selectively reversed deficits in PV cre-lox mice, while baclofen reversed deficits in CamKIIa cre-lox mice. Neither the GABAAá2/3/5 agonist LY838417 nor risperidone were effective in either mouse line, consistent with the lack of effect for similar agents in schizophrenia patients. Importantly, all three cognitive domain measures (CFC, NOR and T-maze) were selectively disrupted in CamKIIa cre-lox mice, while the PV cre-lox mice showed greater impairments in negative symptom models including social interactions and nest building.
Conclusions: Data suggest that the predominant cellular localization of NMDA receptor dysfunction in patients may influence the relative expression of cognitive and negative symptoms. Furthermore, MPEP may be more effective in treating negative symptoms, consistent with current clinical trials testing its efficacy in Fragile X. Alternatively, GABAB agonists may be more appropriate for cognitive deficits.
Disclosure: S. Siegel, Part 1: Ortho McNeil Janssen Pharmaceuticals Part 2: Ortho McNeil Janssen Pharmaceuticals.

Panel Session
Is Love Epigenetic? Transformative Effects of Social Experiences and of Oxytocin

Social Monogamy as a Model for Love: Does Oxytocin explain the Protective Effects of Love?
Sue Carter*

University of Illinois at Chicago, Chicago, USA

Background: Oxytocin (OT) has been implicated in social bonding, a component of most definitions of "love." Yet the behavioral and neuroendocrine factors regulating the release and actions of endogenous OT remain poorly understood. Factors associated with both the protective effects of sociality and OT are examined to address these issues.
Methods: Consequences of differential early handling and exposure to OT or an OT antagonist are measured in prairie voles, including effects on pair bonding and alloparenting, and changes in neuropeptides (by EIA) and their receptors (by autoradiography). In adult voles, short-term effects of OT or

exposure to an infant are measured using heart rate variability and central and peripheral OT. EIA measurements of plasma levels of OT in typical humans and those diagnosed with schizophrenia and Williams Syndrome.
Results: In prairie voles handling once during the first days of life is associated with long-lasting increases in social behavior and OT and reductions in the expression of OT receptor (OTR). In male prairie voles exposure to an infant caused a transient release of OT, facilitates social bond formation and creates a physiological state defined by increases in sympathetic and parasympathetic activity. High blood levels of OT are associated with increased sociality and reductions in symptom presentation.
Conclusions: The study of OT is offering insights into what humans call "love." A model is described illustrating some of the novel properties of OT during development and in adults. These may be mediated by long-lasting changes in OT and the OTR. OT acts on brain regions that regulate social behavior, emotion and the autonomic nervous system. These findings may help to explain the health benefits of positive social interactions and love and also may suggest novel therapeutic approaches to the treatment of mental disorders.
Disclosure: S. Carter, None.

The Epigenetics of Social Behavior and the Oxytocin Receptor
Jessica Connelly*

University of Virginia, Charlottesville, USA

Background: Molecular evidence suggests that the oxytocin receptor (*OXTR*) is epigenetically regulated in the human temporal cortex (BA 41/42) at least in part by DNA methylation. Individuals diagnosed with autism spectrum disorder are more heavily methylated at the *OXTR* gene in both the brain and the blood when compared to controls suggesting an epigenetic link to social behavior. In order to better understand the impact of epigenetic regulation of the *OXTR* gene and its affect on social behavior we have characterized *OXTR* epigenetic response to early experience in a model system of social behavior. Additionally, to further elucidate a role for *OXTR* DNA methylation in the human brain phenotype we have examined the relationship b.
Methods: Pyrosequencing of bisulfite treated DNA was performed on *OXTR* in vole brain and human blood. Level of methylation in the brain was compared in a vole-handling paradigm that results in measurable social behavioral changes with handled voles being less anxious and more social. In addition, level of DNA methylation was correlated with activation of the superior temporal sulcus during presentation of social behavioral stimuli in human typicals. Oxytocin receptor methylation is modulated by early experience and methylation level correlates with response of the social brain. Significantly higher levels of DNA methylation were detected in the *OXTR* gene in the brain of handled voles compared to voles that were not handled. This epigenetic outcome is consistent with decreases in the oxytocin receptor levels in handled voles suggesting that the receptor level may decrease in response to moderate stress. In studies of human typicals, variation in brain response to social stimuli can be explained in part by DNA methylation of the oxytocin receptor.
Conclusions: Atypical social behavior and anxiety are features of many human disorders such as autism spectrum disorder, social anxiety disorder, and schizophrenia. The study of the epigenetic differences that accompany these behaviors may bring us closer to understanding the mechanisms involved in protecting against and treating mental dysfunction.
Disclosure: J. Connelly, None.

Epigenetic and Transgenerational Transmission of Individual Differences in Maternal Behaviour: Role of Estrogen Receptor Alpha, BDNF and Oxytocin - Dopamine Interactions in Regulation of Maternal Mood.
Michael Meaney*

McGill University, Montreal, Canada

Background: The extent of maternal care in humans is associated with positive enhancement of affective states but also with increased risk for affective illness. This paradox underscores the importance of understanding the biological basis for individual differences in maternal states.
Methods: We used ICC to label oxytocin (OT)-positive neurons and double-labeling to trace projections to the ventral tegmental area (VTA). Site-specific infusion was used to examine the effects of OT receptor blockade in the VTA on pup licking (LG) and dopamine release in the nucleus accumbens (nAcc) using in vivo voltammetry. We examined brain-derived neurotrophic factor (BDNF) expression using in situ hybridization and qRT-PR, and studied the effect of BDNF on pup LG using BDNF antibody infusion and shRNA knockdown.
Results: The transgenerational transmission of variations in pup LG are estrogen receptor alpha (ER-α) dependent. ER-α activation increases OT-positive cells in the MPOA and OT projections to the VTA. OT infusion into the VTA stimulates nAcc dopamine release. Differences in pup LG and dopamine release between High and Low LG mothers are eliminated by infusion of an OT receptor antagonist into the VTA. BDNF expression in the hippocampus and VTA is increased High in LG dams; differences in pup LG between High and Low LG mothers are eliminated by BDNF antibody infusion into the nAcc. OT increases the expression of BDNF.
Conclusions: Pup LG is associated with both ER-á and with OT – dopamine interactions (that appear to be regulated by BDNF). Increased dopamine signaling in the nAcc parallels human neuroimaging studies showing increased activation of VTA and nAcc in mothers in response to infant stimuli. In humans, chronic stress attenuates this activation signal and chronic stress decreases the frequency of pup LG in the rat. These studies provide a potential intergenerational model for the study of the dynamic regulation of human affective states.
Disclosure: M. Meaney, None.

The Cross Generation Transmission of Social Affiliation in Humans: Oxytocin, Brain, and Interactive Synchrony
Ruth Feldman*

Bar-Ilan University, Ramat-Gan, Israel

Background: Oxytocin (OT) is one element of the core neural/somatic system that leads to the formation of pair bonds and parent-infant bonding. OT is synthesized in the brain and throughout the body including the heart, thymus, and GI tract. OT receptors are even more widely distributed. We investigated possible transgenerational effects of maternal care as well as the importance of peripheral measures of OT.
Methods: Six prospective longitudinal studies of new parents and new lovers were conducted involving plasma and salivary OT, intranasal administration of OT, genotyping of the OXTR gene, functional neuroimaging, and micro-coding of dyadic (mother-infant, father-infant, romantic partners) and triadic (mother-father-infant) interactions.
Results: IResults show that 1. OT increases during periods of bond formation including transition to parenthood and falling in love. 2. OT is individually stable across lengthy period and is inter-related among parents and romantic partners. 3. OT is related to

the maternal and paternal gender-specific repertoire and to interactive synchrony between parents and child, romantic partners, and in triadic interactions.
4. Evidence is found for cross-generation transmission between parent and child's OT that is moderated by the degree of interaction synchrony. 5. Peripheral OT is linked with variations on the OXTR and CD38 and with maternal and paternal gender-specific activations of brain areas related to parenting (NA and amygdale in mothers, mPFC in fathers). 6. Intranasal administration of OT to fathers improved fathers' and infants' vagal tone, durations of social engagement behavior, and markedly increased father and infant's salivary OT.
Conclusions: Evidence suggests that the OT system in humans is activated during periods of bond formation, its functioning is consistent with that of other mammas, the cross-generation transmission of OT is behavior-based, peripheral OT is linked with central markers, and that the human OT system supports the expression of unique bonding-related synchronous interactions between parents and infants and among lovers.
Disclosure: R. Feldman, None.