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Mini Panel
1. Renaissance in Opioid Biology: From Preclinical Concepts to Clinical Practice

1.1 Molecular Basis for Kappa Opioid Receptor Antagonism: Implications of Ligand-directed Signaling for the Development of Novel Antidepressants

Charles Chavkin*

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Background: Preclinical studies have demonstrated the role of stress-induced dynorphin release in mediating a component of the anxiogenic and aversive effects of stress exposure. These results suggest that kappa opioid receptor (KOR) antagonists or low efficacy partial agonists may promote stress-resilience in humans and may potentially be useful in treating certain forms of mood disorders exacerbated by stressful experience. In rodent models, stress-induced stimulation of dynorphin release produces aversion, reduces open arm time, potentiates cocaine and nicotine conditioned place preference, inhibits social interaction and reinstates extinguished drug seeking. Each of these responses have been shown to be blocked by prior treatment with KOR antagonists or gene deletion of either KOR or prodynorphin. Two classes of KOR antagonists have been developed and clinical trials of both types have begun, but important differences in the molecular mechanisms of antagonism have been revealed. In addition, partial agonists that activate KOR without stimulating p38 MAPK may also effectively promote analgesia and stress-resilience. Understanding these ligand-directed signaling differences has important implications for the optimization of future therapeutics that manipulate the functioning of the KOR system to affect mood.
Methods: Mouse genetic and behavioral data were generated using conditional gene deletion approaches and behavioral stress response assays.
Results: Long-acting KOR antagonists including norBNI, JDTic, and GNTI fail to evoke Gbg signaling typical of KOR agonists, but do initiate a Protein Kinase C (PKC) cascade resulting in C-Jun N-terminal Kinase (JNK) activation. Inhibition of PKC by G06976 blocked norBNI increase in phospho-JNK-ir, and inhibition of JNK by SP600125 or gene deletion of JNK1 isoform blocked the long duration of norBNI antagonism. The JNK phosphorylation site has been localized to the KOR signaling complex, and ongoing site-directed mutagenesis are being used to determine the regulatory site within KOR. Short acting antagonists (e.g. naloxone and buprenorphine) failed to activate PKC/JNK regulation of KOR. Interestingly, KOR agonists (U50,488 and dynorphin A(1-17)) activate phospho-JNK-ir following ligand binding, but do so through a G-protein kinase 3/beta arrestin dependent mechanism that does not result in long-lasting receptor inactivation. These strong agonists also activate p38a MAPK mechanisms resulting in aversion. KOR partial agonists have distinctly different signaling properties. For example, the mixed MOR/KOR partial agonist, pentazocine was significantly more potent in activating p38-MAPK in hKOR than rKOR in transfected HEK293 cells. In contrast, pentazocine was equally potent in arrestin-independent activation of ERK1/2 in hKOR and rKOR. We confirmed that pentazocine was a partial agonist at both receptors for both signaling pathways. Although pentazocine is reported to produce dysphoria in humans, its lower efficacy at p38 activation of rKOR suggests that it may be unlikely to produce aversion in rodents. Consistent with this prediction, pentazocine (10 mg/kg i.p.) produced analgesia and a MOR-dependent place preference but did not produce KOR-dependent aversion.
Conclusions: The signaling differences identified in this study have important implications in screening different KOR agonists and antagonists having distinctly different ligand directed signaling properties.
Disclosure: C. Chavkin, **Part 1:** Consulting for Trevena 2010-2011, **Part 2:** Contract with Trevena 2010-2011.

1.2 The Place of Opiates in the Cortico-basal Ganglia Reward Circuit

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Background: Reward is a central component for driving incentive-based learning, appropriate responses to stimuli, and habit formation. Pathology in this circuit is associated with several diseases including addition. The ventral medial prefrontal cortex (vmPFC), orbital prefrontal cortex (OFC), dorsal anterior cingulate cortex (dACC), striatum, ventral pallidum, and midbrain dopamine neurons are at the center of the circuit associated with reward in general and, in particular addiction. Connectivity between these areas forms a complex neural network that mediates different aspects of reward processing, that can lead to habit formation. The opiates are differentially distributed through the striatum and basal ganglia output structures. The goal of this study was to gain a better understanding of place of opiates within this network.
Methods: Tracing methods were used to define the projection pathways from the vmPFC and OFC through basal ganglia structures in non-human primates. Specific nodal points convergence of these connections and opiate distributions were identified.
Results: Previous results have shown a convergence between vmPFC, OFC, and dACC terminals in the striatum, suggesting functional integration. Moreover, inputs from the amygdala also converge with these inputs in the ventral striatum. These convergent regions appear to have high levels of enkephalin immunoreactivity (Enk ICC). Of particular note is that convergent inputs and high levels of opiates are also found in the rostral dorsal striatum, particularly along the medial wall of the caudate nucleus, an area not typically included in the ventral striatum. Interestingly, the dense patches of mu receptor ICC is somewhat more limited, and concentrated in the ventral part of the rostral caudate and putamen. Projections from these striatal areas were followed to the globus pallidus and substantia nigra and compared to the opiate distribution in those structures. There was a clear convergence between the limbic projections and high levels of Enk ICC, in both the external and a portion of the internal globus pallidus, in addition to the ventral pallidum.
Conclusions: The data illustrate the relationship between cortical networks and the opiates outside the conventional ventral striatal system. These results suggest that by focusing research and development on the conventional ventral striatum and ventral pallidum may too limiting and that opiates within the rostral dorsal striatum may play a key role in modulating behavior. The rostral striatum also receives a major input from the dorsal prefrontal cortex, including areas 9 and 46, suggesting a striatal region involved in not only limbic processing, but also top-down control.
Disclosure: S. Haber, **Part 1:** Pfizer.

1.3 New Clinical Research in Opioid Modulation Indicates Novel Utility in Treating Resistant Depression

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Background: The endogenous opioid system is thought to play a key role in the regulation of mood. Indeed, the "opium cure" was a pharmacologic mainstay of depression therapy prior to the advent

of tricyclic and monoamine oxidase inhibitor anti-depressants in the 1950's. The precise mechanism of endogenous opioids in mood regulation, however, is uncertain.

The contemporary use of opioids for depression is limited by abuse potential, presumably a result of mu opioid agonism. ALKS 5461 consists of buprenorphine (BUP), a partial mu agonist, combined with ALKS 33, a counter-acting mu antagonist, co-formulated for sublingual administration. The ALKS 33 component was designed to be highly potent and sublingually bioavailable with the latter two properties being essential for sublingual co-formulation.

Initial clinical evaluation included a remifentanyl challenge study to establish mu opioid blockade by ALKS 33, and a pharmacokinetic and pharmacodynamic interaction study of BUP and ALKS 33 to ascertain appropriate ratios of the two ALKS 5461 components. Subsequently a pilot assessment of safety and efficacy of ALKS 5461 in treatment resistant depression (TRD) was conducted.

Methods: 1. Remifentanyl challenge study in N = 20 non-addicted opioid-experienced volunteers. Serial remifentanyl or saline challenges were performed pre & up to 7 days post dose of 10 and 20 mg doses of ALKS 33. Mu agonist effects were assessed by physiologic and subjective VAS assessments.2. Double-blind two period randomized crossover interaction study assessing single doses of 0, 1, 4, 8 and16 mg ALKS 33 co-administered with 8 mg buprenorphine in N = 16 volunteers. PK, physiologic and subjective assessments were obtained. <3. Double-blind, placebo-controlled pilot study in N = 32 patients with TRD randomized to two cohorts. The ALKS 5461 8:1 (BUP: ALKS 33) ratio cohort was treated with escalating doses of 2:0.25 mg and 4:0.5 mg for 7 days. The 1:1 ratio cohort received escalating doses of 4:4 mg and 8:8 mg. Efficacy was measured using the 17-item Hamilton Rating Scale for Depression (HAM-D-17) and the Montgomery-Åsberg Depression Rating Scale (MADRS). Visual analog scales were used to assess drug liking, subjective drug effects and standard AE assessments.

Results: The remifentanyl challenge study demonstrated that ALKS 33 is a potent mu antagonist with complete blockade of mu agonist effects lasting >24 hours following a single dose. The BUP – ALKS 33 interaction study demonstrated that a 8:1 ratio was associated with partial blockade of subjective and physiologic mu agonist effects of BUP whereas complete blockade was observed with a 1:1 ratio of the two agents. In the pilot study in patients with TRD, changes from baseline to day 7 in HAM-D-17 scores were -1.0 (4.2), -5.0 (6.1), and -6.7 (3.4), [mean (SD), placebo, 8:1 ratio, and 1:1 ratio respectively; p = 0.032 for 1:1 ratio vs. placebo]; changes in MADRS scores were -3.5 (5.8), -8.5 (7.4), and -11.4 (6.6), respectively (p = 0.054 for 1:1 ratio vs. placebo). Patients receiving the 8:1 ratio experienced greater subjective scores of "Feeling High" and "Feeling Sedated" compared to the 1:1 ratio. The most common AEs were dizziness, nausea, vomiting, and sedation, which occurred most frequently in patients receiving the 8:1 ratio.

Conclusions: In patients with TRD, ALKS 5461 showed evidence of clinically important efficacy vs. placebo with rapid onset at both dose ratios. Greater efficacy was observed with the 1:1 ratio, i.e. with complete mu blockade. More favorable safety and subjective drug effect profiles were also observed for the 1:1 ratio as compared with the 8:1 partial mu blockade ratio in the pilot study. ALKS 5461 may represent a novel treatment of TRD with a rapid onset of effect.

Disclosure: E. Ehrich, **Part 1:** Alkermes plc, Full time employee, **Part 2:** Alkermes plc, Full time employee, **Part 3:** Alkermes plc, Full time employee.

Mini Panel

2. Interaction of Ontogeny and Environment in Adolescent Substance Abuse

2.1 Adolescent Response To Reward And Adversity

Cynthia Kuhn*

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Background: Early substance use in adolescence is a strong predictor of the development of substance abuse,although how the individual, brain development and drug action intersect to create this vulnerability is controversial. This risk may be especially great for individuals who manifest extremes of the adolescent behavioral phenotype of being sensitive to reward and/or less sensitive to the aversive experience. Adolescent rats will consume more ethanol, nicotine and cocaine than adults. In most individuals, this excess consumption declines as the animals mature. For example, we have shown that adolescent rats that are self-administering cocaine or drinking ethanol decrease consumption as they enter adulthood. However, specific individuals do not show this normal developmental decline: these may provide an animal analog of at-risk humans. We have been exploring the relative contribution of immature neural circuits and individual differences to the pattern of behaviors that confers addiction vulnerability to adolescents.

Methods: Our laboratory has used behavioral and neurochemical approaches to studying the responses to addictive drugs in adolescent (PN 28-45) and adult rats. To study reinforcement circuits, behavioral strategies including cocaine locomotion and self-administration and alcohol ingestion and fast-scan cyclic voltammetry (FSCV) to study dopamine release. Wehave used conditioned taste aversion (CTA) and c-fos labeling to study behavioral and neural response to aversive stimuli.

Results: Our behavioral studies of cocaine responsivity suggest that reported developmental differences in the reinforcing effects of drugs of abuse reflect in large part the high responsivity of dopamine (DA) systems to pharmacologic stimuli in adolescence. Using FSCV, we have found that after cocaine, both electrically-stimulated and spontaneous DA release exhibit much greater increases in adoleslcents than adults. Weaker autoreceptor regulation in adolescents permits these greater increases in DA relative to adults, which may support the greater reinforcing effects that are reported. Studies of DA function in highly responsive individuals are underway. CTA studies have shown that adolescents respond less to the aversive properties of every addictive drug that has been tested including alcohol, cocaine and THC. Again, extreme individuals (non-averse adolescents) skew the adolescent data higher. Preliminary findings indicate that c-fos activation in central amygdala in response to anxiogenic or aversive pharmacologic stimuli (8-OHDPAT, lithium) is significantly less than is observed in adults.

Conclusions: These findings suggest that neural circuits which mediate the reinforcing effects of drugs of abuse are more responsive in adolescents, while those circuits mediating behavior responses to aversive experiences are less responsive. Furthermore, individuals who exhibit extremes in either of these characteristics may be at higher risk of initiating drug use and developing substance abuse. Identifying developmental differences in neural circuits supporting enhanced drug reinforcement and/or less drug aversion should provide insight into the development of substance abuse in vulnerable adolescents.

Disclosure: C. Kuhn, Nothing to Disclose.

2.2 Intersection of Environment, Individual and Drug in Development of Substance Abuse in Adolescence

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Background: Adolescence is a vulnerable period associated with a high incidence of drug abuse initiation and an increased risk for developing dependence and addiction. Drug use during this critical developmental period has been associated with higher incidence of cocaine abuse later during adulthood. Factors such as age and sex, as well as a variety of environmental variables (e.g. social conditions, physical conditions, type of food available) all influence drug reward during adolescence and can persist into adulthood. Factors that modulate the effects of drugs of abuse during this critical period of development will be discussed, as will underlying neurochemical changes that mediate these effects. The purpose of these studies was to determine (1) whether there are differential effects of drugs during adolescence

depending upon sex, social and physical environment, and food and (2) whether these factors influence the effects of cocaine both soon after drug exposure and later during adulthood.
Methods: Rats were housed on postnatal day (PND) 23 in either an isolated impoverished (alone with no toys, II), isolated enriched (alone with toys, IE), social impoverished (3/cage with no toys, SI3), or social enriched (3/cage with toys, SE3) condition. In some studies, rats had a choice of a high fat diet or regular chow available either starting on PND 29 or starting later, during adulthood, while others had only regular chow available. Starting on PND 29, THC (delta-9-tetrahydrocannabinol), nicotine, or MDMA (Ecstasy) was injected daily for 5 days and locomotor activity was measured daily. The following week, or 30 days later in adulthood, cocaine conditioned place preference (CPP) began.
Results: The data show that social and environmental enrichment interact to differentially alter drug effects in male and female adolescent rats. Further, the effects of enrichment differ across drugs as a function of sex. The data also show that basal and cocaine-mediated levels of proteins involved in dopaminergic transmission, as well as some inflammatory factors are impacted by enrichment as a function of sex, age and diet.
Conclusions: Social and environmental enrichment differentially alter drug reward in males and females during adolescence and these changes are mediated by distinct neurochemical changes. These factors should be considered when developing or implementing preventions or treatments for substance abuse.
Disclosure: S. Izenwasser, Nothing to Disclose.

2.3 Environmental Stressors and Risk for Alcohol Problems: A Longitudinal GxE GWAS in Community Samples

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Background: Gene-environment interaction (GxE) may be understood as the genetic control of sensitivity to the environment. As part of the NIDA-funded Gene-Environment-Development Initiative, we conducted a GxE genomewide association study to identify which genes predict problematic substance use in context of recent stress exposure.
Methods: The data come from 4 community studies that have followed children prospectively into early adulthood, collecting repeated data on substance use, related problems, and stress exposure. The phenotype of latent alcohol involvement was harmonized across studies using indicators of recent alcohol-related symptoms, quantity of use and frequency of use. All studies also collected information on recent exposure to stressful life events. Blood samples were collected from subjects in each study and genotyped using the Illumina 660KW-Quad v1 DNA Analysis BeadChip. All genetic data was processed through the Psychiatric GWAS Consortium quality control. Principal components were derived from a limited SNP set to be used as covariates in all analyses adjusting for racial admixture.
Results: Successive models tested the effects of each SNP independently. All models predicted problematic alcohol involvement from each genetic marker, recent stressful life events, and an interaction term between the marker and life events. All models were run using PROC MIXED in SAS to account for multiple observations of individual subjects. Individual results from each study were combined using meta-analytic techniques. Analytic models were run for 866,099 autosomal SNPs in 2124 total subjects. The results focused on the main effect of the SNP or the interaction term between the SNP and stressful event exposure. The minimum p values were $9.2 \times 10\text{-}7$ and $2.3 \times 10\text{-}8$ for the main effect and interaction terms, respectively. Two markers met the criteria for significance and another sixteen interaction terms met the suggestive threshold with notable findings of markers in NTRK2, DNMT1, SEC23B, KIAA0564, and FOXN3 gene. No main effect findings met either the significant or suggestive threshold. Two marker sets were of particular interest for stress susceptibility to alcohol problems. The first involved intronic SNPs in the NTRK2 gene which codes for the TRkB recepter that binds neurotrophins including BDNF. The second involved intronic markers in the gene that codes for DNA methyltransferase 1. DNA methyltransferase 1 has a role in the establishment of tissue-specific patterns of methylated cytosine residues.
Conclusions: These results suggest limited involvement of large or intermediate genetic effects in the alcohol involvement phenotype, but some modest effects may affect risk through stress susceptibility, perhaps through mediating neurotropic binding or tissue-specific methylation. This study supports recent calls for prospective, longitudinal studies with careful assessment of both genetic and environmental risk.
Disclosure: W. Copeland, Nothing to Disclose.

Panel
3. Neuroplasticity Deficits in Neuropsychiatric Illness: New Targets for Cognitive Enhancement

3.1 Learning Mechanisms In Obsessive-Compulsive Disorder: Bias To Stimulus-Response Habit Learning

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Background: Despite considerable evidence for orbitofrontal-striatal impairment in OCD, how this leads to characteristic obsessive-compulsive symptoms remains unclear. We recently showed with a points reward-based set of conditional visual discrimination learning tasks that patients with OCD are biased towards the use of stimulus-response, habit-based learning mechanisms, being deficient in the flexible use of action-outcome (or response-reinforcer) associations, and impaired in remembering them subjectively (Gillan et al 2011 Amer. J Psychiat. 168, 718-726). These findings suggest an imbalance in the neural systems mediating these two forms of instrumental conditioning, favoring a dorsal striatal (putamen)-cortical circuitry in OCD. A new study has investigated habit-based active avoidance learning in patients with OCD.
Methods: 25 patients with DSM-IV diagnosed OCD were compared with age- and IQ-matched healthy volunteers on an active shock-avoidance learning task. Subjects received scheduled shocks through electrodes on fingers of each hand which they could avoid by pressing a compatible right or left foot pedal following a warning conditioned stimulus. After either brief or extended training, all subjects received an instructed shock devaluation procedure for shocks to one hand only, prior to a test of habit-based responding.
Results: Patients with OCD learned the avoidance task at the same rate as controls but selectively persisted in foot-pedal responding formerly appropriate to shock avoidance on the now-devalued contingency. This was accompanied by a greater tendency to report subjective 'urges' to respond in this way, but by normal GSR responses, suggesting no greater level of motivating fear or anxiety.
Conclusions: These findings suggest that OCD behavior has the inflexible qualities of habit learning and can be considered to exemplify deficits in behavioral plasticity. These findings are discussed in terms of a biased engagement of the neural system of habit learning in OCD and possible remediation strategies, including serotoninergic, dopaminergic and glutamatergic medications.
Disclosure: T. Robbins, **Part 1:** Consultancy: regular: Cambridge Cognition; E. Lilly Inc, Lundbeck, GlaxoSmithKline, Merck, Pfizer, ChemPartners Shanghai, Shire Pharmaceuticals. Royalties for CANTAB (Cambridge Cognition), Editing the journal 'Psychopharmacology' (Springer-Verlag), **Part 2:** Cambridge Cognition, Pfizer (2010 only), **Part 3:** Cambridge Cognition, **Part 4:** E. Lilly, Lundbeck, GlaxoSmithKline.

3.2 Experience Dependent Cortical Long-term Synaptic Potentiation (LTP) and Sequelae in the Intact Visual System

Mark Bear*

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Background: Impaired cognitive function is a core phenotype in many psychiatric diseases, and it may arise from disturbances in the elementary molecular mechanisms of experience-dependent cortical synaptic plasticity. It is therefore of interest to devise assays to assess these mechanisms in the intact organism, (1) for understanding the pathophysiology of the diseases, (2) for assessing the *in vivo* efficacy of potential treatments, and (3) to provide a bridge from animal to human studies. We discovered a form of synaptic plasticity in the mouse visual cortex, called stimulus-selective response potentiation (SRP), that has all the hallmarks of naturally occurring long-term potentiation (LTP). SRP is induced by brief daily exposure of the animal to visual grating stimuli, and manifests as a ~2x increase in the magnitude of the cortical visual evoked potential (VEP) to the familiar stimulus. It requires for induction cortical NMDA receptor activation and trafficking of GluR1-containing AMPA receptors. SRP occludes and is occluded by LTP induced with electrical stimulation of the lateral geniculate nucleus, and like LTP is reversed by local cortical injection of the ZIP peptide.
Methods: Our objectives in the current study were threefold: (1) determine the behavioral significance of SRP in mice, (2) investigate the influence of SRP on cellular responses in primary visual cortex, and (3) assess the contribution of plasticity of intracortical inhibition to the expression of SRP. We discovered that mice have a reflexive motor response to visual stimulation that can be measured with a piezoelectric device. This response, we call a "vidget", was monitored to novel and familiar stimuli after induction of SRP. Discharge properties of neurons in primary visual cortex before and after SRP were measured in awake animals using conventional extracellular recording techniques. A role for inhibitory plasticity was investigated using Pv-NR1 mutant mice in which NMDARs are genetically deleted in parvalbumin-containing interneurons.
Results: We found that the vidget accurately reports visual contrast and spatial frequency sensitivity. Of particular interest, the vidget amplitudes were decreased to the familiar stimulus following induction of SRP in visual cortex. This behavioral habituation was eliminated by local cortical infusion of the ZIP peptide. Thus, a functional consequence of SRP (cortical LTP) is the recognition of familiarity, an aspect of cognition that is impaired in several psychiatric diseases. Unit recordings suggest that the cellular correlate of SRP is a redistribution of spikes evoked by the familiar stimulus, rather than a simple increase in number. After SRP, spikes occur in a narrow temporal envelope that coincides with the maximal negativity of the VEP. This finding suggests the possibility that SRP reflects sculpting by inhibition, and indeed SRP is absent in the Pv-NR1 mutant mouse and it is temporarily erased by systemic ketamine.
Conclusions: Our experiments show that a consequence of SRP is the behavioral recognition of familiarity, and that this learning involves recruitment of NMDA receptor-dependent plasticity of inhibition, a process that may be selectively impaired in schizophrenia.
Disclosure: M. Bear, Nothing to Disclose.

3.3 Induction of Neuroplasticity in Humans by Transcranial Direct Current Stimulation: Clinical Applications and Methodological Advancements

Michael A. Nitsche*

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Background: Neuroplasticity is the physiological basis of cognitive processes in the human brain. Its Pathological alterations of plasticity is a contributing factor in neuropsychiatric diseases. Non-invasive brain stimulation allows the induction of plasticity in humans. Transcranial direct current stimulation (tDCS) induces neuroplastic alterations of cortical excitability, lasting for more than one hour. These excitability alterations depend on the glutamatergic system, are affected by neuromodulators, and thus share similarities with plasticity induced in animal models. tDCS is thus an attractive tool to explore pathological alterations of neuroplasticity in neuropsychiatric diseases, as well as to treat these alterations. However, since the time course of plasticity induced by tDCS is still relatively restricted, advanced stimulation protocols are needed.
Methods: For identifying pathological plasticity alterations in schizophrenia, tDCS was applied to the motor cortex of patients and healthy controls. tDCS-induced alterations of cortical excitability were monitored by transcranial magnetic stimulation (TMS)-induced motor evoked potentials (MEP). For the treatment of major depression, anodal or sham tDCS was applied to the dorsolateral prefrontal cortex (DLPFC) of patients suffering from major depression in double-blinded and open studies, since it is speculated that a pathological diminution of long term potentiation, and an underactivation of the DLPFC contributes to clinical symptoms. Improvement of clinical symptoms was monitored for up to 4 weeks after stimulation by HAMD, and BDI. For optimizing tDCS protocols, the effects of repeated and stronger tDCS, and protocols including neuromodulatory substances were compared to standard stimulation protocols in healthy humans. tDCS was performed over the primary motor cortex, and plasticity monitored by TMS-induced MEP.
Results: For schizophrenia it was shown that dependent on the duration of the course of the disease, long-term potentiation and depression-like plasticity are pathologically reduced. Specific genetic polymorphisms differed in the effects on plasticity. In depression, pilot studies have shown that excitability-enhancing anodal tDCS reduces clinical symptoms, and that the effects might be similar to those of pharmacological treatment, but evolve faster. For improving the efficacy of tDCS, it was shown that after-effects of stimulation are prolonged for over 24 h by repetitive tDCS tDCS within specific intervals, or by addition of neuromodulatory agents, whereas enhancing the strength of stimulation resulted in partially non-linear effects.
Conclusions: Neuroplasticity induction by tDCS is a promising approach to identify and treat disease-related pathological alterations of plasticity, and new stimulation protocols might be able to enhance the efficacy of stimulation.
Disclosure: M. Nitsche, **Part 1:** Advisory Boards: UCB, Eisai, GSK, Starstim.

3.4 Neurophysiological Basis of Auditory/Motor Plasticity Deficits and tDCS Effects in Schizophreina

Daniel Javitt*

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Background: Cognitive dysfunction represents a core feature of Sz and a major predictor of impaired outcome. Deficits localize to sensory and motor areas in addition to higher cortical regions. Across regions, patients show reduced cortical plasticity, limiting their ability to benefit from cognitive retraining. tDCS is a recently developed brain stimulation technique with potential for improving local brain plasticity. Present studies investigate effects of tDCS on brain plasticity in Sz.
Methods: Data are presented from controls and patients in two paradigms: 1) an auditory plasticity paradigm initially developed for assessment of developmental dyslexia; 2) a motor plasticity paradigm known to be sensitive to tDCS. In addition to behavior, ERP were obtained during tDCS to evaluate mechanism of effect. Parallel rodent studies investigated tDCS-associated intracortical activity.
Results: In the auditory task, patients showed reduced plasticity, which correlated with deficits in both phonological function and emotion recognition. As opposed to developmental dyslexia, patients showed deficits in both "roving" and "fixed" standard condition, reflecting both bottom-up and top-down components. Patients also showed reduced motor plasticity. In controls, tDCS enhanced motor learning in association with modulation of premotor potentials, reflecting greater recruitment of frontal brain

regions (pre-SMA). In patients, impaired plasticity was associated with reduced frontal recruitment. In rodents, tDCS modulated local theta/gamma rhythms, providing potential substrates for enhanced auditory plasticity.
Conclusions: Plasticity deficits are a core feature of Sz and a critical barrier to cognitive remediation. tDCS leads to objective changes in brain function and may provide a novel and practical method for enhancement of remediation response. Auditory and motor systems provide key brain systems in which to evaluate approaches for combined tDCS/cognitive remediation.
Disclosure: D. Javitt, **Part 1:** Solvay, Sepracor, AstraZeneca, Pfizer, Cypress, Merck, Sunovion, Lilly, BMS, Takeda, Glytech, AASI, Promentis, **Part 2:** Pfizer, Glytech, **Part 3:** Glytech, **Part 4:** Pfizer, Roche.

Panel
4. Common Neural Mechanisms across Dimensions of Pediatric Psychopathology

4.1 Human Amygdala Development Following Early-life Stress
Nim Tottenham*

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Background: Early caregiving adversity can have long-lasting effects on behavioral developmentthat are associated with alterations of amygdala development. Childrenwho experience caregiving deprivation (e.g., previously institutionalized (PI) children) are at heightened risk for emotional disturbances, with anxiety beingone of the most common. This talk will describe the anxiety-related behaviorsand associated amygdala function and connectivity in a population of PIchildren.
Methods: Data were obtained from children with and without a history of early institutional care. Functional magnetic resonance imaging (fMRI), salivary cortisol, computerized tasks, and parent reports were used to examine the anxiety-related phenotypes in PI children.
Results: PI children are at a significantly elevated risk for anxiety. At the grouplevel, PI children are more likely to show elevated amygdala activity and alteredbasal salivary cortisol levels. Mediation analyses show that amygdalaactivity explains a significant amount of the variance between early neglectand subsequent anxiety in childhood. Moreover, early neglect isassociated with developmentally atypical functional connectivity between theamygdala and ventromedial prefrontal cortex.
Conclusions: These findings are consistent with animal models that show heightened and possiblypremature development of the amygdala following early-life maternaldeprivation, which has significant consequences for subsequent anxiety. Ourfunctional connectivity analyses suggest that the effects of maternaldeprivation extend beyond the amygdala to include connectivity with corticalregions.
Disclosure: N. Tottenham, Nothing to Disclose.

4.2 Childhood Disruptive Behavior Disorders and Risk for Adolescent Substance Use
Iliyian Ivanov*

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Background: It is hypothesized that the development of substance abuse (SA) may be due to imbalance in functions of the motivation-reward and behavioral inhibition systems in the brain. This speaks to the search for biological risk factors for SA in drug-naïve children who also exhibit motivational and inhibitory control deficits, however, this type of research is currently lacking. The objective of this study was to establish a neurobiological basis for addiction vulnerability using functional magnetic resonance imaging (fMRI) in drug-naïve youth with attention deficit/hyperactivity disorder (ADHD). We hypothesized that children with ADHD alone would show higher activity in regions of the motivation-reward and behavioral inhibition systems than children with ADHD and a parental history of SA.
Methods: Towards this goal we designed a novel hybrid reward/conflict task that can index activation in the motivational and behavioral inhibition systems. Twenty drug-naïve children with ADHD ages 8-13 while performing this event-related reward task. High ($N = 10$) and low ($N = 10$) risk subjects were identified, based on parental history of SA. The effects of anticipation, conflict, and reward were assessed with appropriate linear contrasts and between group differences were assessed using statistical parametric mapping.
Results: The two groups did not differ on behavioral measures of the task. The fMRI results show heightened activation in the brain motivational-reward system, particularly in right insula and orbitofrontal cortex, in high-risk compared to low - risk yours. In contrast, reduced activation of the inhibitory control system including medial frontal gyrus was documented in high-risk compared to low-risk children.
Conclusions: These results suggest that that a functional mismatch between these two systems may represent one possible biological underpinning of SA risk, which is conferred by a parental history of addiction.
Disclosure: I. Ivanov, **Part 1:** Memeber of Data Safety Monitoring Board for Lundbeck, **Part 4:** Co-investigator on a neuroimaging grant from Shire awarded to Dr. Jeffrey Newcorn at Mont Sinai School of Medicine.

4.3 Functional and Structural MRI Studies of the Neural Circuits that Mediate Self-regulation over Development in Bulimia Nervosa
Rachel Marsh*

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Background: Bulimia Nervosa (BN) is characterized by recurrent episodes of binge-eating followed by self-induced vomiting or another compensatory behaviorto avoid weight gain. These episodes of binge-eating are associated with a severe sense of loss of control. Impulsive behaviors are also common inindividuals with BN, indicating more pervasive difficulties in behavioral self-regulation. Our fMRI findings from women (Marsh et al, 2009) and adolescents (Marsh et al, 2011) with BN suggest that self-regulatory processes are impaired due to their failure to engage fronto-striatal circuits appropriately. These data will be presented along with unpublished surface-based analyses of anatomical MRI data to explore the relationship of structure and function in the same patients and controls.
Methods: We compared fMRI BOLD response in 20 adults with BN and 20 age-matched controls during performance of the Simon task, and in 18 adolescents with BN and 18 controls during performance of a shorter task version, more suitable to younger individuals. Surface-based analyses werethen used to map features of the cortical surface in 34 of the BN patients and 34 controls (age range, 13 to 45 yrs).
Results: When responding correctly on conflict trials of the Simon task, both adults and adolescents with BN failed to activate fronto-striatal circuits to the same degree as controls. The adolescents instead deactivated left IFG, as well as a neural system encompassing the PCC and SFG. These group differences were driven by the abnormal processing of preceding conflict in the adolescents with BN. Our preliminary structural analyses revealedreductions in volumes of the frontal cortex localized to the left DLPFC and IFG in the patients compared to controls. Thenumber of objective binge episodes in the patients correlated inversely with volumes of bilateral IFG and ACC, suggesting the greatest reductions in those with the most severe symptoms. Finally, diagnosis-by-age interactions were detected in bilateral DLPFC and the left ACC. Scatterplots of these interactions indicated that local volumes of the PFC increased with increasing age in the controls but not in the BN patients.
Conclusions: When engaging the self-regulatory control processes necessary to resolve conflict, both adults and adolescents with BN displayed abnormal patterns of activation in fronto-striatal circuits. These functional disturbances may contribute to, and possibly perpetuate, the binge-eating behaviors and the conflict-

ridden binge-purge cycle in adolescents and adults with BN. Our structural findings suggest that individuals with BN do not show the normaldevelopmental increases in local prefrontal volumes and that this abnormal trajectory may underlie self-regulatory deficits that persist over development in BN. Together, these multimodal imaging findings enhance our understanding of BN pathogenesis by pointing to abnormalities within aspecific neural system that may contribute to the poor self-regulatory control over feeding behaviors across development in BN.
Disclosure: R. Marsh, Nothing to Disclose.

4.4 Neural Response to Social Threat: Disease Specificity in Adolescents with Generalized Anxiety Disorder, Social Phobia and at Risk Populations

Jarcho M. Johanna*

National Institutes of Health, Bethesda, Maryland

Background: Generalized anxiety disorder (GAD) and social phobia (SP) are prevalent, often comorbid, share similar risk factors, and typically arise in adolescence. Such commonalities may stem from the same altered brain mechanisms; however few imaging studies directly compare disorders. Two studies assessing neural correlates of SP and GAD using a "chatroom task" are reported here. Study 1 compared SP, GAD, and healthy controls, whereas Study 2 compared adolescents at high and low risk for developing SP.
Methods: Subjects believed they would chat online with peers after an fMRI scan. Prior to scanning they sorted photos of peers based on their desire to interact with them, and were told their peers would do the same. While scanning, subjects rated how much they thought peers wanted to chat with them, then received positive (acceptance) or negative (rejection) peer feedback, and rated how surprising they found the feedback. Study 1 included 37 adults and 36 adolescents, approximately half diagnosed with GAD or SP. Study 2 included 17 adolescents at high and 22 at low risk for developing SP based on childhood temperament.
Results: Study 1. Regardless of diagnosis, patients indicated their peers would be less interested in interacting with them than healthy subjects, and had greater amygdala activation when providing these ratings. Patients were also less surprised by rejection feedback, which evoked greater activity in ventrolateral prefrontal cortex (vlPFC) than healthy subjects. No effects of age or specific diagnosis were found. Study 2. Adolescents at high and low risk for anxiety did not differ in subjective ratings, or in brain activity while evaluating peer interest. However, in response to feedback, there was reduced activity in caudate among high vs. low risk adolescents.
Conclusions: While GAD and SP are distinct diagnoses, they share common dysregulation in brain mechanisms that process social threat. Vigilance to social threat may be linked with amygdala responding, while decreased efficacy of self-regulatory processes may be associated with vlPFC activity. Risk-related dysregulation may reflect diminished reward from positive social feedback, evidenced by less striatal activity. These findings have important implications for the definition of psychopathology and provide critical insight into the dimensionality of anxiety disorders.
Disclosure: J. Johanna, Nothing to Disclose.

Panel
5. Unraveling the Genetic Architecture of Mental Illness with Whole Genome Sequence Data

5.1 The Utility of Whole Genome Sequencing in Human Pedigrees for Identifying Genes Underlying Human Quantitative Trait Loci

John Blangero*

Texas Biomedical Research Institue, San Antonio, Texas

Background: The results across many different genome-wide association studies, with their focus on common genetic markers, generally have captured only a small component of the observed genetic variation segregating for human quantitative traits and brain-related diseases. However, data are rapidly accumulating that rare variants likely have a large cumulative effect on normal phenotypic variation and are important in mental illness. Whole genome sequencing (WGS) in large extended pedigrees represents the optimal design for identifying rare causal variants because Mendelian transmission from parents to offspring increases the chance that multiple copies of rare variants will be observed in a pedigree. A pedigree-specific rare functional variant with small relative effect size in relation to population attributable risk or locus-specific heritability, but with a larger absolute effect size, can be sufficient to verify that a given gene is causally involved in relevant phenotypic variation. Thus, the human genetics field is once again returning to its roots in the study of large families.
Methods: In this talk, I describe how WGS can be used in large pedigrees to rapidly identify genes causally involved in human disease-related variation. The analysis of such data in pedigrees requires special methods that can take into account the non-independence amongst family members due to shared genetic kinship. The use of pedigree-based variance component mixed models provides a general framework for the analysis of rare variants in extended pedigrees. I will summarize our general methods for such analysis and detail a new approach that dramatically speeds up the exact analysis of quantitative trait genetic association even in the largest most complex human pedigrees available. Using some of the world's very first WGS data from large Mexican American pedigrees, I'll provide examples of the utility of both the sampling design and analytical approaches for gene discovery.
Results: In the San Antonio Family Study, we now have WGS on approximately 1000 individuals who have been studied for a wide array of phenotypic dimensions including imaging-derived measures of brain structure and function. The amount of rare (and even private) genetic variation seen in these pedigrees is striking. We have currently observed approximately 25 M sequence variants. Because of the power-leaching effects of testing so many potential variants, I provide strategies for identifying high quality prior hypotheses for reducing the causal search space. For example, restricting our focus to protein-altering coding variation, we observed an average of $\sim$9600 non-synonymous variants per individuals of which 15% are predicted to be potentially damaging to the focal protein. These numbers suggest the substantial likelihood that rare coding variants play a significant role in the phenotypic variation observed in any given biological pathway. Similarly, likely rare regulatory variation identified by both bioinformatic and empirical transcriptional profile data are also found in substantial numbers and can serve as a useful hypothesis-driven filter for testing. Examples of human quantitative trait loci (QTLs) identified using this approach in the remarkable data set will be provided.
Conclusions: WGS studies of very large pedigrees are now economically feasible. Although it requires substantial analytical effort, this approach has the greatest potential of all genetic paradigms to rapidly capture rare functional variants and then identify causal players in human brain-related phenotypic variation.
Disclosure: J. Blangero, **Part 1:** Eli Lilly and Co.

5.2 Endophenotypes, Normal Variation and Whole Genome Sequence Data in Pedigrees: Insights into the Genetics of Psychotic Illnesses

David C. Glahn*

Yale University, Hartford, Connecticut

Background: Although several genome-wide significant loci have been localized for psychotic disorders, these findings explain a fraction of the genetic variance predisposing psychotic illnesses and have yet to result in gene identifications. Yet, progress in elucidating the pathophysiology of disorders like schizophrenia and bipolar disorder is predicated on causal gene identification. Focusing on quantitative endophenotypes, traits that index genetic liability for an illness, rather

than diagnoses alone, provides a complementary strategy for identifying risk genes. Quantitative endophenotypes established in family-based studies of clinical samples typically vary within the normal population, providing the opportunity to localize genes influencing these traits in unselected pedigrees. Such genes are then validated in case-control samples. This normal endophenotypic variation strategy has been successfully applied to identify disease-risk genes for heart disease, obesity, and diabetes. Here, we provide a successful example of this approach using a spatial working memory endophenotype.
Methods: The "Genetics of Brain Structure and Function" study involves acquisition of behavioral, neurocognitive and neuroimaging endophenotypes in 1500 Mexican Americans from randomly-selected extended pedigrees. All participants have high-density SNP arrays and 502 individuals have 60-fold genome-wide sequence data. Our approach involves localizing loci for an endophenotype via linkage, identifying the non-synonymous or regulatory variants diving that effect with sequence data, and then demonstrating pleiotropy with published association studies.
Results: We localize a linkage peak at 12q24 (LOD = 3.28) for a spatial delayed response task that is sensitive to liability for schizophrenia and psychotic bipolar disorder. Using sequence data, we found common and rare non-synonymous variants on the P2RX7 gene that influence task performance and cortical thickness in frontoparietal brain regions. Variants on the P2RX7 gene associated with schizophrenia or bipolar disorder ($p < 0.05$) are pleiotropic with task performance.
Conclusions: Our results suggest a role for purinoceptors in psychotic disorders and provide a clear example of how endophenotypes can provide novel genetic insights for mental illness.
Disclosure: D. Glahn, Nothing to Disclose.

5.3 Rare Variants in Genes Involved in Neurotrophin Signaling Identified by Genome Sequencing in Bipolar Disorder

John R. Kelsoe*

University of California, La Jolla, California

Background: Neurotrophin signaling has been implicated in bipolar disorder by previous studies that showed that lithium and SSRI administration turned on expression of BDNF. We have also previously reported evidence suggesting association of the BDNF receptor gene, NTRK2, with both lithium response and risk for bipolar disorder. Recently, we identified a family co-segregating for bipolar disorder and medullary cystic kidney disorder where linkage studies implicated the 1q region containing the gene for NTRK1, which codes for TrkA, the receptor for NGF.
Methods: We have explored the role of NTRK1 in bipolar disorder using a two phase strategy. Whole genome sequencing was conducted in the proband of the above described family to identify possibly putative variants in the linkage regions. Variants were then validated using Sanger sequencing in the entire family. Genes identified were then further validated by targeted sequencing in a sample of 1000 bipolar I subjects and 1000 controls.
Results: The whole genome sequence data was filtered for novel non-synonymous coding variants in the linkage region in the affected family. A coding sequence variant resulting in a glutamic acid to lysine substitution in the juxtamembrane region of the NTRK1 gene was identified. This variant results in a charge change and is predicted to be deleterious and may impact the nearby SHC binding domain. In the followup targeted deep sequencing study, 13 nonsynonymous SNPs were identified suggesting a greater mutational burden in cases.
Conclusions: These data implicate rare variants in the NTRK1 gene as conveying vulnerability to bipolar disorder. They demonstrate the value of whole genome sequencing and targeted deep sequencing, and the likely role of rare variants in bipolar disorder. They further suggest that multiple variants in multiple genes involved in neurotrophin signaling may convey risk to bipolar disorder and modulate response to lithium.
Disclosure: J. Kelsoe, Nothing to Disclose.

5.4 Transcriptional Profiling in ASD: A Systems Biology Approach

Daniel H. Geschwind*

University of California, Los Angeles, California

Background: Progress in autism genetics has revealed exceptional heterogeneity. The initial results of exome sequencing predict potentially over 1000 genes that may contibute to overall population risk for autism spectrum disorder (ASD). Genetic effect sizes range from very small (for common variants), to rare mutations that are essentially causal. A major question is whether there are common molecular mechanisms that link ASD cases with diverse etiologies.
Methods: We performed expression profiling in patients with idiopathic and major gene forms of ASD, using microarrays and RNA-seq in post-mortem brain tissue, in lymphoblasts from peripheral blood, and in induced pluripotent stem cell (iPSC)-derived neural progenitor cells and differentiated neurons. We used gene network analysis to identify core elements shared in cases and not found in unaffected subjects.
Results: We find striking evidence of simillar molecular phenotypes that appear to converge on common pathways in the ASD brain. The signal in blood is much weaker, providing a significant challenge to biomarker development. Not surprisingly, there is a very strong signal in iPSC-derived neural cells in patients with monogenic forms of ASD. In some cases, genes of interest were only expressed in neural progenitors and not in more differentiated neurons, indicating that studying different developmental stages may be powerful. Additionally, we find a significant overlap in transcriptional alterations between certain monogenic forms of ASD.
Conclusions: Our data support the existence of convergent molecular pathways in ASD, based on analysis of monogenic and more polygenic forms of ASD. Investigation of patient-derived cells provides greater power, but consideration of early developmental stages is important.
Disclosure: D. Geschwind, **Part 1:** Synapdx - scientific advisory board/consultant Roche - *ad hoc* advisory board, **Part 2:** Synapdx 2011-2012.

Panel
6. De-risking the Pathway of Treatment Development for Autism Spectrum Disorders

6.1 Measures of Clinical Meaningful Change, a Summary of the Recent Meeting on Outcome Measures Consensus Statements for Clinical Trials in ASD

Evdokia Anagnostou*

Holland Bloorview Kids Rehabilitation Hospital, Toronto, Ontario, Canada

Background: Despite the increased prevalence of autism spectrum disorders, and emerging data from genomics/neuropathology/ animal models about potential molecular targets, there have been no medications shown to be effective in the treatment of core symptomdomains. Challenges in this area are many, but a key one identified is the relative paucity of validated outcome measures targeting core domains. In the area of behavioral, psychosocial interventions, great gains have been made, but challenges still remain and include the lack of measures that capture change across age span and cognitive abilities. In this context, Autism Speaks convened a panel of experts in 2011 to evaluate availablemeasures that assess social communication and repetitive behaviors/anxiety, in an effort to identify established or promising measures, make recommendations to investigators and regulatory bodies, and indentify areas for future work.
Methods: Two groups were formed based on the core symptom domain of interest: social communication and restrictive repetitive behaviors (RRB)/anxiety, and met monthly over a year. More than 50 measures described in the literature were evaluated. The measures

were assessed and scored in 3 main categories: reliability, validity and clinical relevance. Other indices considered included demonstrated sensitivity to change, burden, and ability to use across IQ and age ranges. Ultimately measures were classified as 1) Appropriate/ Appropriate with conditions; 2) Potentially Appropriate; 3) Unproven; 4) Not Appropriate; 5) Not relevant. The data was presented to the group of experts, other collaborators, community stakeholders and FDA in March 2012.
Results: Six measures were identified to be appropriate/Appropriate with conditions for the measurement of the social communication domain (e.g. Aberrant Behavior Checklist – Social Withdrawal). The RRB/anxiety group, after extensive discussion of the construct of interest, decided to split the RRB discussionfrom the anxiety discussion and two separate lists were produced. Four RRB measures (e.g. CYBOCS-PDD) and 4 anxiety measures (e.g. CASI-anxiety) were identified as appropriate/ appropriate with conditions A series of gaps were identified and a plan to address them has been proposed.
Conclusions: We have identified a series of measures that are appropriate for use as outcome measures in clinical trials targeting social communication, repetitive behaviors and anxiety in ASD. Areas of further work have been delineated.
Disclosure: E. Anagnostou, **Part 1:** Consultation fee from Sea Side Therapeutics Consulted without fees to Novartis, **Part 2:** Forest has provided free drug and placebo for a clinical trial.

6.2 Quantifying Social Deficits in Autism via Eye-tracking Measures of Social Engagement

Warren Jones*

Holland Bloorview Kids Rehabilitation Hospital, Toronto, Ontario, Canada

Background: Autism is a neurodevelopmental disorder of genetic origins, although how genetic liabilities convert into symptoms is not known. In our studies, we hypothesize that symptomatology results from the disruption of normative, evolutionarily highly conserved and developmentally early emerging mechanisms of socialization. The focus of our methods is on quantification of social entrainment, here defined as the moment-by-moment visual engagement with social signals as they occur in naturalistic social scenes of mother-child and peer group interactions. This presentation will focus on 3 studies: 1. Eye-tracking measurements of social entrainment in toddlers with autism viewing peer interaction scenes; 2. Eye-tracking measurements of blink inhibition as autonomic indices of individual child's perception of scene salience in the same cohort; and 3. Prospective eye-tracking measurements of visual fixation relative to caregiver's approaches, obtained from 2 to 24 months in a densely sampled design involving babies at risk for autism.
Methods: In the 3 studies we used eye-tracking technology and lab-unique data analytic strategies to measure infants and toddlers visual responses during natural viewing of dynamic social scenes: peer interactions in studies 1 and 2, and caregiver's infant-directed speech in study 3. In study 1, we analyzed data for toddlers seen in Time 1 (24 months) and Time 2 (40 months). Using kernel density space-time distributions revealing statistically significant "funnels of attention, we were able to segregate the group with autism from controls, and demonstrate the clinical utility of these measures. In study 2, we demonstrate the clinical utility of blink inhibition patterns as autonomic indices of children's relative engagement with what they view. In study 3, we use visual fixation measures to areas of interest in the viewed scenes to trace "growth charts" of social visual engagement during the first 2 years of life.
Results: In study 1, we demonstrate the diagnostic and prescriptive value of our measurements of social entrainment as quantifiers of social disability. Further, we demonstrate that social entrainment serves as a measure of social learning given its strong predictive power relative to levels of social disability, cognitive and language function close to 1 $\frac{1}{2}$ years after the experimental measurement was taken, during a time of greatest variability in outcome trajectories of children with autism. In study 2, we demonstrate that blink inhibition patterns can be used as indices of engagement with what the child views, and highlight the potential utility of this autonomic measure, relative to other autonomic measures, given that it is intrinsic to the visual system. In study 3, we demonstrate that it is possible to detect risk for autism in infants as early as 6 months of age, raising the possibility of a cognitive science, performance-based method for early screening for autism in babies.
Conclusions: A new generation of cognitive science measures promises to provide the quantification necessary to serve as early biomarkers for the condition, as more helpful descriptors – both diagnostic and prescriptive, and as measures of change resulting from clinical trials.
Disclosure: W. Jones, Nothing to Disclose.

6.3 Electrophysiological Signatures of Language Impairment in Autism Spectrum Disorders - Biomarkers, Neurobiological Insight and Potential Early Signals of Efficacy: Magnetoencephalographic (MEG) Investigations

Timothy Roberts*

Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

Background: Language impairment is acore aspect of the autism phenotype. Abnormal auditory processing has beensuggested as a neural underpinning of such impairment. Novel "brainwave"scanning technology (MEG) can be used to probe brain functional activity, non-invasively and with sub-millisecond "real-time" resolution.
Methods: MEG determination of evokedresponse latencies to (i) isolated tones, and (ii) oddball paradigms ofdiffering sounds is used to index successive stages of auditory processing. Separately, diffusion tensor imaging (DTI) is used to assess integrity ofauditory pathway white matter tracts (acoustic radiations). 100 children (6-15 years; ~40 typically developing (TD); ~60 ASD) underwent MEG recording andDTI study using a 275-channel biomagnetometer and a 3T Siemens MRI and adiffusion weighted imaging sequence with 30 encoding directions and 2 mm isotropic spatial resolution. Clinical determination of LI was assessed using the CELF-4 test, using a standardized score of 85 to indicate impairment.
Results: Main findings are: (1)delayed M100 response latencies (~10-20 ms) in children with ASD vs. TD peers;this does not distinguish children with ASD with and without LI. (2) Delayed "normalization" of M100 latency with developmental age in children-with ASD vs. TD peers - M100 latency shortens with age in both groups, but at aslower rate in ASD. The age-modulated M100 latency in TD is associated withincreasing fractional anisotropy of the acoustic radiations (interpreted as anindex of white matter integrity/ maturation). (3) MMF latencies are prolonged inchildren with ASD, but this effect is especially pronounced in children withASD with LI (~50 ms). An age-covaried mixed-model found a main effect of group, with pair-wise comparisons all significant indicating resolution of ASD + LI fromASD-LI and from TD.
Conclusions: Electrophysiologicalmeasures of auditory processing at various stages may offer diagnostic insightwith early responses discriminating ASD *per se*, and later responses showingresolution of LI sub-groups.
Disclosure: T. Roberts, **Part 1:** Research Support received from Seaside Therapeutics Consulting, Prism Clinical Imaging, **Part 2:** Research Support received from Seaside Therapeutics.

6.4 Using DSM-5 Criteria to Assess Core Symptoms of Autism Spectrum Disorders for Diagnosis and Evaluation of Treatment Outcomes

Susan Swedo*

National Institute of Mental Health, Bethesda, Maryland

Background: Publication of DSM-5 will occur early in 2013 bringing significant changes in the diagnostic criteria for autism

and related disorders. As a start, the DSM-5 Neurodevelopmental Disorders (ND) Workgroup has recommended merging autism, PDD-NOS and Asperger disorder into a single disorder - autism spectrum disorder (ASD). The current criteria are also changed by the recommendation that three criteria [communication, social interactions and repetitive behaviors/restricted interests (RRB's)] should become two criteria (social communication and RRB's). In addition, the ND Workgroup is recommending improvements to the age at onset criterion and diagnosis across the lifespan, as the previous criteria focused only on grade-school aged children. Perhaps the most significant change is the inclusion of severity measures for both social communication and RRB's. It is hoped that these scales will render DSM-5 criteria useful for assessing core symptom severity, as well as establishing a clinical diagnosis of ASD.
Methods: The recommended DSM-5 criteria were subjected to field trials of inter-rater reliability at two sites. The results of those field trials will be presented and discussed. In addition, the ND Workgroup is planning to examine videotapes from the interviews to examine the validity of the proposed criteria, as well as to evaluate the utility of the severity rating scales. The results of these analyses should be available for discussion at the session.
Results: Proposed changes to the DSM diagnostic criteria for autism and related disorders are expected to have significant effects not only on the diagnosis of ASD, but also on the evaluation of severity of the core symptoms. The proposed criteria will provide a means of standardizing clinical assessments of core symptom severity and serve as a starting point for developing measures that can measure outcome effects with reliability and validity.
Conclusions: In combination with other develpments in the field, the DSM-5 diagnostic criteria promise not only to improve the clinical assessment and diagnosis of individual patients (of all ages and developmental stages) with ASD, but also to provide a platform for the development of research-reliable measures of treatment outcome.
Disclosure: S. Swedo, Nothing to Disclose.

Panel
7. Links between Activity, Sleep and Mental Function: Translational Models

7.1 Objective Assessment of Rhythms and Inter-relationships of Activity, Sleep and Mood in a Community Based Family Study of Affective Spectrum Disorders

Kathleen R. Merikangas*

National Institutes of Health, Bethesda, Maryland

Background: Although our population studies of both adults and adolescents reveal that increased activity is the most common manifestation of mania in both adults and adolescents, most evidence regarding activity, sleep, reactivity to stressful life events and mood fluctuation are based on self-reported retrospective assessment. The goal of this presentation is to examine objective assessment of patterns of activity and their inter-relationship with sleep, mood and anxiety as core features of mood disorder subtypes.
Methods: Mobile technologies including digital diaries administered 4 times per day and actigraphy were used for two weeks in 380 participants from a community based family study of affective spectrum disorders, with an age range from 10-80.
Results: Latent profile analysis of the aggregate actigraphy data yielded four broad groups of stable activity patterns including a highly active class; 2 classes of moderate activity; and a fourth and most prevalent class with low activity. Lagged analyses using functional linear modeling indicated that sleep efficiency is associated with large differences in afternoon and evening activity, sleep duration has a potent effect on both early morning and evening activity, whereas sleep onset latency is not related to circadian patterns of activity. People with bipolar disorder showed greater variability in activity and sleep parameters, as well as stronger relationships between activity with subsequent changes in energy and mood. Distinct patterns emerged among those with subtypes of bipolar disorder and major depression.
Conclusions: These findings highlight the importance of activity as a core component of affect regulation in both normative samples and bipolar disorder. The pronounced age effects on activity, sleep and mood also highlight the importance of investigation of neurodevelopmental correlates of the inter-relationships of regulatory patterns.
Disclosure: K. Merikangas, Nothing to Disclose.

7.2 Links between Anxiety and Activity in Nonhuman Primates

Judy Cameron*

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: Nonhuman primates (NHPs) serve as excellent models for human psychopathology as they share many similarities in behavior, neuroanatomy, and genetics. To determine whether infant monkeys reared in a naturalistic setting could serve as useful models for the development of normally-occurring childhood anxious behaviors, we adapted assessments designed to test anxiety in children. To begin to understand other physiological links to anxious behavior we measured level of physical activity in infancy through pubertal development.
Methods: We studied infant rhesus macaques ($n = 640$) raised in a naturalistic outdoor setting. Between 3 and 6 months of age, monkeys were given modified assessments that are routinely used to evaluate behavioral inhibition in children. When monkeys were 3 years of age (just post-pubertal), anxiety tests were again performed and activity was measured by accelerometer.
Results: Factor analysis revealed three factors related to anxiety (behavioral inhibition, reticence, impassivity) accounting for 52.8% of variance in observed behaviors. Behavioral inhibition was decreased exploration in the presence of the mother (as defined in children). Reticence was decreased exploration when alone and impassivity was decreased behavioral expression in the face of a threatening or frightening stimulus. Activity measured in infancy was correlated with reticence ($r = 0.685$, $p < 0.001$) and impassivity ($r = 0.728$, $p < 0.001$), and remained correlated with these anxious behaviors in late adolescence, such that monkeys that were more reticent to explore and showed greater impassivity when confronted with novel or frightening stimuli were less active. Both anxious behavior and activity measures were significantly heritable. Anxiety traits were strongly associated with physiological correlates of anxiety (i.e., responsiveness to alcohol, CSF monoamine levels, heart rate and sleep).
Conclusions: These findings show that monkeys reared in a naturalistic setting exhibit a spectrum of anxious behaviors that parallel what is observed in clinical settings. Level of physical activity is strongly correlated with the display of several anxious behaviors.
Disclosure: J. Cameron, Nothing to Disclose.

7.3 Sleep-wake Cycle, Patterns of Physical Activity and Circadian Rhythm Disruption in Young People with Emerging Mood Disorders

Ian Hickie*

Brain & Mind Research Institute, The University of Sydney, Sydney, Australia

Background: Episodic bipolar and unipolar mood disorders are characterized by disruptions in sleep-wake cycle, patterns of physical activity and circadian rhythms. These phenomena are most evident in those who experience recurrent mania or 'atypical' depressive episodes. Those with disrupted circadian function are also at high risk of obesity, metabolic syndrome and premature cardiovascular disease.
Methods: The sleep-wake cycle, physical activity and circadian features of young persons with emerging mood disorders were investigated in two large clinical samples of young persons with

emerging mood disorders (n = 307, 30% bipolar-type, mean age = 19 years; n = 1797, 16% bipolar-type, mean age = 18 years) as well as a longitudinal study of adolescent twins (n = 2459, mean age = 16 years). Measures included objective and prolonged actigraphic-derived assessments of 24-hour sleep-wake cycles and daytime physical activity, early evening melatonin secretion patterns and relevant metabolic function parameters in selected sub-groups.
Results: In clinical samples, there is evidence of delayed onset and offset of sleep-wake cycles, reduced day-time physical activity and disrupted onset of night-time melatonin release in up to half of young persons with emerging mood disorders. These features are more pronounced in those with more severe conditions and those with a history of mania or hypomania episodes. Relationships with disturbed metabolic function, independent of treatment parameters are being explored. In twins, sleep-wake cycle phenotypes are predicted by shared genetic characteristics and appear to have their own longitudinal associations with the 'atypical' as distinct from more classical 'anxious depression' illness-type.
Conclusions: Disrupted circadian function is a characteristic of a major subgroup of young people with emerging bipolar or unipolar mood disorders and is likely to be a key pathophysiological aspect of these conditions. While under strong genetic control, it is exacerbated by other environmental and illness-related factors. These studies provide unique data concerning its relationships with the onset and course of depression, and other comorbidities, in young persons during the early phases of illness. The data support an increased focus on objective measurement of changes in patterns of daytime physical activity and 24-hour sleep-wake cycles as key biomarkers of the onset, course and response to treatment of major mood disorders in humans. Circadian systems may represent a key target for behavioural or pharmacological treatments of depression independent of other illness characteristics.
Disclosure: I. Hickie, **Part 1:** Paid Educational Seminars/Resources: Servier, Astra Zeneca, Pfizer, Eli-Lilly; Travel Support from Pharmaceutical or Business Companies: Servier, Astra Zeneca, Price Waterhouse Cooper; Research Support from Pharmaceutical Companies: Servier, Pfizer; **Part 2:** Professor of Psychiatry, University of Sydney; Executive Director, Brain & Mind Research Institute, University of Sydney; Clinical Consultant in Psychiatry, Sydney Local Health District (NSW Government Services) Bupa Australia (Private Health Insurance) - Member of the Medical Advisory Panel; Headspace: the National Youth Mental Health Foundation- Director on behalf of the University of Sydney, which is a member of the Company. (ENDED JAN 2012), **Part 3:** Hickie I. (2011-2012) Project: Does circadian disturbance predict response to sleep-wake interventions in early-onset depression. Servier, $100,000.

7.4 Seasonal Effects on Sleep, Activity and Behavior in Migratory Birds

Ruth Benca*

University of Wisconsin-Madison, Madison, Wisconsin

Background: The goal of this presentation is to examine associations between seasonal changes in sleep, activity and behavior in migratory sparrows as a model for bipolar disorder. Bipolar disorder is characterized by dramatic reductions in sleep during episodes of mania and a tendency for increased sleep during periods of depression, as well as a seasonal pattern of mania and depression in some patients.
Methods: Migratory and non-migratory subspecies of white-crowned Sparrows (Zonotrichia leucophrys gambelii and Z. l. nutalli) were captured in California and brought to the University of Wisconsin where they were exposed to photoperiods that approximated seasonal changes in day length or were fixed day length for a 1-2 year period. Activity monitoring, operant testing and sleep deprivation were performed. EEG sleep recordings were obtained in migratory sparrows.
Results: Dramatic changes in sleep amount occurred in response to changes in photoperiod duration as well as during endogenously driven migratory periods in the spring and fall in migratory sparrows, with sleep in the summer and during migratory periods reduced to about half the amount in the winter. Furthermore, during migratory periods, sleep shifted from a diurnal temporal organization to an almost complete temporal fragmentation of sleep, suggesting potential alterations in circadian rhythms. Changes in sleep amount and organization across seasons were associated with behavioral differences; overall responding rates on operant tasks and impulsivity increased significantly during migratory periods in comparison to winter or summer. Seasonal effects on activity and operant responding were significantly lower in non-migratory sparrows. We also assessed the effects of enforced sleep deprivation on both subspecies. As in mammals, even short-term sleep deprivation results in significantly decreased responding rates in both subspecies of sparrows. The effects of migration-related sleep reduction and exogenous sleep deprivation were additive, such that when sparrows were sleep-deprived while in a migratory state, operant responding and impulsivity decreased to levels similar to those in non-migratory periods. These findings suggest that sleep loss due to migration is not equivalent to sleep deprivation. Furthermore, during migration gene expression patterns in the telencephalon showed increases in genes involved in coping with cellular stress and increased energy demands, and differed from patterns seen during both normal waking and enforced sleep deprivation.
Conclusions: Migratory behavior in birds shares a number of features with manic episodes in bipolar disorder, including a seasonal component; decreased sleep; increases in activity, goal-directed behaviors and impulsivity; and circadian dysregulation, suggesting possible shared mechanisms for seasonal changes in behavior in migration and bipolar disorder. Seasonal influences on sleep and behavior may be important factors in the development of mood disorders and provide opportunities for sleep- and circadian-based interventions in vulnerable populations.
Disclosure: R. Benca, **Part 1:** Consultant to Merck and Sanofi-Aventis.

Panel
8. Glial Regulation of Synaptic Pathology: Novel Mechanisms of Neuropsychiatric Disease and Avenues for Repair

8.1 Role of Astrocytes in Synaptic Development

Cagla Eroglu*

Duke University Medical Center, Durham, North Carolina

Background: Excitatory circuits in the rodent CNS develop primarily during the first three postnatal weeks. During the first week, dendrites produce actin-based dynamic cell protrusions called filopodia. Dendritic filopodia are thought to mediate early target recognition events at points of contact between glutamatergic axons and dendrites. It is also postulated that stabilized filopodial connections recruit pre- and postsynaptic specializations and eventually mature into dendritic spines. The majority of excitatory synapses in the CNS are compartmentalized into dendritic spines by the end of third week and this compartmentalization is crucial for the regulation of basic circuit physiology and plasticity. However, the cellular and molecular interactions that regulate spine development are largely unknown. In recent years, astrocytes emerged as regulators of synaptogenesis. Astrocytes secrete thrombospondin family proteins (TSPs) that strongly induce excitatory synapse formation. Recently, we identified another astrocyte-secreted synaptogenic protein, hevin. Hevin is a synaptic cleft protein that is localized to excitatory synapses in the CNS. We found that hevin robustly stimulates excitatory synaptogenesis between neurons in culture. Astrocytes also express a close homolog of hevin called SPARC. Intriguingly, SPARC is not synaptogenic but specifically inhibits hevin-induced synaptogenesis.
Methods: Using *in vitro* and *in vivo* approaches such as glia free retinal ganglion cell cultures or analysis of synaptic development in hevin and SPARC null mice we have unraveled a converging

mechanism through which TSPs, hevin and SPARC orchestrate the formation of dendritic spine synapses *in vivo*.
Results: We found that TSPs, which are produced during the first postnatal week by immature astrocytes, stimulate formation of dendritic filopodia through interactions with their neuronal receptor calcium channel subunit alpha2 delta-1. By the end of the second postnatal week maturing astrocytes start secreting hevin and SPARC. Using Golgi-cox staining and serial construction of electron microscopical images we found that hevin is required for stabilization of dendritic filopodial connections and their subsequent maturation into spines, whereas SPARC negates this function of hevin therefore controls the rate of spinogenesis.
Conclusions: In summary, our results show that astrocytes actively contribute to the formation of excitatory circuits by regulating spine formation and maturation.
Disclosure: C. Eroglu, Nothing to Disclose.

8.2 Lactate-mediated Coupling between Astrocytes and Neurons Controls Memory Consolidation

Cristina Alberini*

New York University, New York, New York

Background: Astrocytes have generally been believed to have mainly a supportive role for neurons in the central nervous system. However, in the last 2 decades, a growing body of evidence suggests that they play many more active roles, including information processing, signal transmission and regulation of neural and synaptic plasticity. Thus, brain functions, including perhaps cognitive ones, may result from the concerted action of neuron–glia networks.
Methods: We have found that inhibitory avoidance (IA) learning in rats leads to a significant increase in hippocampal extracellular lactate derived from astrocytic glycogenolysis. We also found that disrupting either glycogen metabolism or lactate transport from astrocytes into neurons impairs long-term, but not short-term memory and several related underlying molecular changes required for memory formation. Similarly, blocking glycogenolysis disrupts the maintenance but not induction of *in-vivo* hippocampal long-term potentiation (LTP). Furthermore, we have investigated the crosstalk between the lactate metabolic coupling and the stress pathway, and specifically noradrenaline, which is known to modulate memory consolidation. We find that training-related lactate release requires beta-adrenergic receptors and lactate rescues the memory impairment produced by the beta-adrenergic receptor antagonist propranolol.
Results: Our results suggest that the metabolic coupling between astrocytes and neurons is required for memory consolidation and its modulation.
Conclusions: Astrocytes and astrocytic-neuronal metabolic coupling mechanisms mediated by lactate play a critical role in memory consolidation.
Disclosure: C. Alberini, Nothing to Disclose.

8.3 Control of Drug Seeking Behavior by Modulation of Astroglial Glutamate Transport

Kathryn Reissner*

Medical University of South Carolina, Charleston, South Carolina

Background: A downregulation of astroglial high affinityglutamate transporter EAAT2/GLT-1 in the nucleus accumbens has been reportedfollowing self-administration of cocaine, heroin, nicotine, and alcohol. These findings indicate that impairedglutamate transport may be an important common mechanism of addiction, andfurther suggest that the effects of drugs of abuse on astrocytes may representan important cellular response. Studiespresented herein are designed to investigate the functional significance ofGLT-1 downregulation and astrocyte reactivity in the behavioral and neuralpathology of addiction.
Methods: Male Sprague-Dawley rats were trained in theself-administration of cocaine (or heroin), followed by extinction training inthe absence of drug or drug cues. Reinstatement of drug seeking was induced by the reintroduction of lightand tone drug-paired cues. All manipulationsdesigned to test xCT and/or GLT-1 manipulations on reinstatement were made bymicroinjection into the nucleus accumbens. Suppression of xCT and/or GLT-1 protein expression was performed usingantisense versus control sequence vivo-morpholinos. Overexpression of GLT-1 was performed usingan AAV viral vector expressing GLT-1 under the control of a GFAP promoter.
Results: The compound N-acetylcysteine (NAC) has beenthoroughly demonstrated to reduce reinstatement to cocaine, heroin, andnicotine in the rat reinstatement model. We find that while NAC restores cocaine-depleted expression of both xCTand GLT-1 in the nucleus accumbens, restoration of GLT-1 expression is ofparticular importance to the efficacy of NAC. Moreover, robust reinstatement observed in the presence of NAC whileGLT-1 is suppressed can be blocked by a sub-threshold dose of an mGluR5negative allosteric modulator, suggesting glutamatergic overflow as a mechanismof action. Similarly, antagonism ofextrasynaptic NR2B receptors impairs heroin reinstatement, suggesting thatglutamate overflow is a contributing mechanism to relapse-related behaviors forboth classes of drug. In addition to NAC, the glial modulatorpropentofylline (PPF) impairs cocaine reinstatement and restores expression ofGLT-1. Ongoing experiments are designedto investigate whether, like NAC, the restored expression of GLT-1 is required forthe behavioral effect of PPF on reinstatement.
Conclusions: Drug self-administration and extinction trainingresults in altered astroglial protein expression, particularly a decrease inGLT-1. Pharmacological targeting ofastrocytes (NAC, ceftriaxone, propentofylline) can ameliorate these astroglial-changes, as well as measures of drug seeking.
Disclosure: K. Reissner, Nothing to Disclose.

8.4 Schizophrenia and Astrocytes: The Importance of System xc- to Preclinical Models of PFC Dysfunction

David A. Baker*

Marquette University, Milwaukee, Wisconsin

Background: Altered network activity within subregions of the frontal cortex likely contributes to impaired cognition in schizophrenia. Astrocytes are well-positioned to regulate network-wide activity levels since these cells are capable of influencing synaptic activity through the release of glutamate and other molecules. These studies examined the role of system x_c-, which is a source of astrocytic glutamate, to cognitive deficits observed in the methylazoxymethanol (MAM) neurodevelopmental model of schizophrenia.
Methods: Pregnant Sprague Dawley rats received vehicle or MAM (22 mg/kg, IP) on gestational day 17. Some rats also received the cysteine prodrug N-acetylcysteine (0-60 mg/kg, IP) either in utero, acutely during adulthood or chronically during adulthood. Additional groups of rats received the system x_c- inhibitor sulfasalazine (0-24 mg/kg, IP) either *in utero* or acutely during adulthood. Juvenile or adult male offspring were examined for a) changes in cognitive performance using attentional set shifting or b) indicators of system x_c- activity including ^{14}C-cystine uptake in tissue punches, extracellular glutamate or cysteine levels, and tissue glutathione levels.
Results: Male adult offspring exhibited deficits in reversal learning and abnormal system x_c- activity in the medial prefrontal cortex evident as altered ^{14}C-cystine uptake in tissue punches reduced tissue glutathione levels. Interestingly, cognitive deficits were attenuated by acute or chronic administration of N-acetylcysteine during adulthood, and completely reversed by *in utero* N-acetylcysteine. Rats receiving sulfasalazine alone during adulthood exhibited deficits in reversal learning similar in nature to rats that had received MAM administration *in utero*.
Conclusions: These data link cognitive deficits produced by *in utero* administration of MAM to abnormal system x_c- activity. Interestingly,

MAM-induced changes in system x_c^- mirror what has been detected in schizophrenia, including a reduction in glutathione and a change in system x_c^- at the functional or protein level. *In utero* administration of N-acetylcysteine was the most effective regimen in normalizing cognition in MAM-treated rats, although acute or chronic administration in adults attenuated the effects of MAM. These data indicate that the regulation of glutamate release from astrocytes may contribute to impaired cognition observed in schizophrenia. Given that these cells regulate the activity of up to 2 million synapses in the human cortex, astrocytes may be important, unrecognized component of synchronized cortical network activity in the normal or disease state.
Disclosure: D. Baker, **Part 1:** Promentis Pharmaceuticals (2010-present), **Part 2:** Promentis Pharmaceuticals (2010-present), **Part 3:** Promentis Pharmaceuticals (2010-present), **Part 4:** Promentis Pharmaceuticals (2010-present).

Study Group
9. 'If We Thought our Field was in Trouble Before…' Is Ethical Mental Health Care

9.1 Possible in the Second Decade of the 21st Century?
Ellen Frank*, John G. Csernansky, Kenneth L. Davis, Howard H. Goldman, William Z. Potter, Alan F. Schatzberg

University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Increasingly marginalized from every direction, those engaged in treating the mentally ill and those attempting to discover new and better ways of doing so are finding that the challenges are becoming almost insurmountable. Medicaid and Medicare reimbursement so substantially underfunds mental health programs as to make them fiscally unsustainable with the result being the closure of programs. Where programs have not been closed, many institutions severely limit access so as to limit their losses. Private mental health care is not in an appreciably better state. Even in major cities, well-reputed clinicians and programs typically have waiting lists that are so long as to make access when it is needed nearly impossible. The pharmaceutical industry, for a variety of reasons, appears to have lost its incentive to develop new drugs for mental disorders. Finally, funding agencies have shifted their priorities away from the testing of new interventions, especially for disorders of mid-life. All of this means that as adults our children will likely receive the same only partially efficacious treatments their parents did if they are able to access treatment at all. At the time of this writing, the fate of the Affordable Health Care Act is unknown, but whatever the Supreme Court's decision, it will have profound effects on the accessibility of mental health care for almost all Americans. This panel will focus on this perfect storm of challenges to the provision of ethical treatment of mental disorders. Dr. Davis will address the public mental health care crisis, taking Study Group participants through an economic analysis based on real data and making the case for putting existing programs on a financially stable base so that services do not continue to disappear. Dr. Potter will address the incentives and disincentives for pharma to return to investment in psychiatric drug discovery, while Dr. Schatzberg will discuss the relative disappearance of funding for academic research and its impact on the field's ability to grow a cadre of new researchers in this area. Finally, Dr. Goldman will focus on the implications of whatever the fate of the Affordable Health Care Act proves to be for the provision of ethical mental care in the United States.
Disclosure: E. Frank, **Part 1:** Servier International (Consultant)-Vanda Pharmaceuticals (Consultant) Guilford Press and American Psychological Association Publishing (Royalties), **Part 2:** Servier International (Consultant) Spouse: Consultant to the American Psychiatric Assocation; J. Csernansky, **Part 1:** Membership on a Data Monitoring Committee for Eli Lilly and Co.; K. Davis, **Part 1:** Wife, Bonnie Morrison Davis, MD, is a patent holder on the use patent for galantamine for Alzheimer's disease and dementias that has been licensed to Janssen-Pharma, a subsidiary of Johnson & Johnson. She receives royalty income from this license, **Part 2:** Wife, Bonnie Morrison Davis, MD, is a patent holder on the use patent for galantamine for Alzheimer's disease and dementias that has been licensed to Janssen-Pharma, a subsidiary of Johnson & Johnson. She receives royalty income from this license, **Part 3:** Wife, Bonnie Morrison Davis, MD, is a patent holder on the use patent for galantamine for Alzheimer's disease and dementias that has been licensed to Janssen-Pharma, a subsidiary of Johnson & Johnson. She receives royalty income from this license, **Part 4:** None; H. Goldman, Nothing to Disclose; W. Potter, **Part 1:** AgeneBio, Amgen, Astellas, AstraZeneca, BMS, EnVivo, Envoy, J&J, Medavante, Orasi, Otsuka, Pfizer, Sonkei, Takeda, Theravance, **Part 2:** Merck Stock; A. Schatzberg, Nothing to Disclose.

Study Group
10. Practical, Societal, Ethical and Legal Challenges for Modern Brain and Biobanking: Experiences from America and Europe
Thomas Schulze* Francine M. Benes, Thomas Insel, Joel E. Kleinman, Camilla Stoltenberg, Peter G. Falkai, Shawn HE. Harmon, Robert H. Ring

University of Göttingen, Göttingen, Germany

Future success in biological psychiatric research will critically hinge on the availability of large and adequately characterized samples of patients and control individuals. Also, epidemiological cohorts are gaining more and more popularity for biological studies. Not only will ample phenotype data be needed but various types of biomaterial collected from study participants will be crucial for 21st century research. Biomaterial to be collected may include any type of tissue, in particular brain tissue or fibroblasts, whole blood, plasma, serum etc. For brain research, collection of whole brains has been and will always be key to successful research in neuroscience. Thus, brain banks and biobanks are gaining increasing importance in our field. Now that more and more research institutions and even countries as a whole are getting involved in setting up such infrastructure, there is a definite need to discuss the various practical, societal, ethical, and legal challenges involved and eventually find answers to the most ardent questions. How to set up a biobank or brain bank? How to join already existing banks into a network of banks that benefits the individual researcher? What IT solutions will be required? Can informed consent still be the basis for an individual's participation or will we have to move to a truly broad consent? How can this be regulated by legislatures? How can we involve patients' advocacy group? These and other questions will be addressed by a panel of international experts in biobanking, brain banking, bioethics and international leaders of major research and funding bodies. Thomas G. Schulze (University of Göttingen, Germany & Johns Hopkins University), who coordinates the biobanking efforts at the University of Göttingen will co-chair this study group together with Francine M. Benes, Director of the *Harvard Brain Tissue Center*. Francine Benes and Joel Kleinman (Chief, Neuropathology Section, NIMH) will discuss the logistics of brain banking from their respective institutional backgrounds. Tom R. Insel (NIMH Director) will offer the NIMH's perspective of future brain banking strategies in the US, while Peter G. Falkai (Chairman, Department of Psychiatry, University of Göttingen, Germany) will present experiences from BrainNet Europe. Shawn H.E. Harmon (Lecturer, School of Law, University of Edinburgh) will offer the lawyer's and bioethicist's point of view, drawing from ample experience with the UK and Taiwan Biobank. Camilla Stoltenberg (Deputy Director, Norwegian Institute of Public Health) will share the Scandinavian experience with large epidemiological, population-based health registries and biobanking using these unique resources. Finally, Robert H. Ring (Vice President, Translational Research of "Autism Speaks") will offer the perspective of the largest autism science and advocacy organization in the US and describe the work of the *Autism Tissue Program*.

Disclosure: T. Schulze, Nothing to Disclose; F. Benes, Nothing to Disclose; T. Insel, Nothing to Disclose; J. Kleinman, Nothing to Disclose; C. Stoltenberg, Nothing to Disclose; P. Falkai, **Part 1:** Until the end of 2010, I have been giving paid lectures and was on the advisory boards of the following pharmaceutical companies: Lilly, Servier, AstraZeneca, Janssen-Cilag, Pfizer and Lundbeck, **Part 2:** 20.000 US dollars, **Part 4:** I am currently having a grant from Servier; S. Harmon, Nothing to Disclose; R. Ring, **Part 1:** I was a full time employee of Pfizer Worldwide Research and Development until May 2011 before joing the non-profit foundation Autism Speaks in my current role, **Part 2:** I was a full time employee of Pfizer Worldwide Research and Development until May 2011 before joing the non-profit foundation Autism Speaks in my current role, **Part 3:** I was a full time employee of Pfizer Worldwide Research and Development until May 2011 before joing the non-profit foundation Autism Speaks in my current role.

Study Group

11. The Role of Corticotropin-releasing Factor (CRF) in the Pathophysiology of Mood and Anxiety Disorders: A Tribute to Wylie Vale

Florian Holsboer, Tracy Bale, George F. Koob, Dimitri Grigoriadis, Charles Nemeroff*, Alon Chen, Elizabeth Flandreau

University of Miami Leonard M. Miller School of Medicine, Miami, Florida

Wylie W. Vale, a giant in the fields of neuroendocrinology and neurobiology and a member of the ACNP since 2001, attended the ACNP annual meeting in 2011 and died a few weeks later at his vacation home in Hawaii. In 1981, Vale and colleagues finally elucidated the structure of the long elusive (25 years) corticotropin-releasing factor (CRF or CRH). He went on to characterize other endogenous ligands for the CRF receptor subtypes, the urocortins, and contributed in a major way to the understanding of CRF circuits in neuroendocrine regulation and in the response to stress. His fundamental research in this area laid the foundation for what is now a large body of work which, taken together, supports a preeminent role for these systems in the pathophysiology of mood, anxiety, and addictive disorders. This panel brings together five individuals who have contributed to this work and who collaborated with Vale over the past 30 + years. Charles B. Nemeroff will serve as chair, introduce Vale's contributions to the field and focus on recent findings, including new unpublished data that CRF circuits and CRF-related polymorphisms mediate the increased risk of depression in patients with a history of child abuse and neglect. Both preclinical studies using viral vectors to increase CRF expression and clinical studies will be presented. Tracy Bale, a former postdoctoral fellow of Vale's, will focus on her studies of the critical role of CRF-containing neurons in the dorsal raphe and the important impact of sex on responses of these neurons. Florian Holsboer will discuss the conundrum of the clinical trials data of CRF1 receptor antagonists in the treatment of depression in relationship to the extant knowledge of the pathophysiology of the disorder. Dimitri E. Grigoriadis will examine the pointillistic impact that Vale's work has had on over two decades of pharmaceutical small molecule CRF antagonist drug discovery research. George Koob will serve as the discussant and will integrate the findings of the four presenters with growing data on the role of CRF circuits in the pathophysiology of addictive disorders. All of the participants have actively collaborated and published with the Vale laboratory.

Disclosure: F. Holsboer, Nothing to Disclose; T. Bale, Nothing to Disclose; G. Koob, **Part 1:** Addex, Alkermes, Arkeo, Embera, Psychogenics; D. Grigoriadis, **Part 1:** I am a full time employee of Neurocrine Biosciences Inc., **Part 2:** Salary and Equity associated with full time employment at Neurocrine Biosciences Inc., **Part 3:** I am a full time employee of Neurocrine Biosciences Inc.; C. Nemeroff, **Part 1:** Research/Grants: National Institutes of Health (NIH), Agency for Healthcare Research and Quality (AHRQ), Speakers Bureau: None; Consulting: Xhale, Takeda, SK Pharma, Shire, Roche, Lilly Stockholder: CeNeRx Bio Pharma, Pharma Neuro Boost, Revaax Pharma, Xhale, Nova Del Pharma; Other Financial Interests: CeNeRx Bio Pharma, Pharma Neuro Boost Patents: Method and devices for transdermal delivery of lithium (US 6,375,990B1) Method of assessing antidepressant drug therapy via transport inhibition of monoamine neurotransmitters by *ex vivo* assay (US 7,148,027B2) Scientific Advisory Boards: American Foundation for Suicide Prevention (AFSP), CeNeRx Bio Pharma, National Alliance for Research on Schizophrenia and Depression (NARSAD), Xhale, Pharma Neuro Boost, Anxiety Disorders Association of America (ADAA), Skyland Trail, Astra Zeneca Pharmaceuticals (2009), Board of Directors: AFSP, Mt. Cook Pharma (2010), Nova Del (2011), Skyland Trail, Gratitude America; Income sources or equity of $10,000 or more: Astra Zeneca Pharmaceuticals (2009), Pharma Neuro Boost, CeNeRx Bio Pharma, Nova Del Pharma, Reevax Pharma, American Psychiatric Publishing, Xhale, **Part 2:** Income sources or equity of $10,000 or more: Astra Zeneca Pharmaceuticals (2009), Pharma Neuro Boost, CeNeRx Bio Pharma, Nova Del Pharma, Reevax Pharma, American Psychiatric Publishing, Xhale; A. Chen, Nothing to Disclose; E. Flandreau, Nothing to Disclose.

Study Group

12. NIMH Research Domain Criteria Project: How Will the Criteria Work for Studies of Diagnosis and New Drug Development?

Will Carpenter*, Bruce Cuthbert, James Waltz, Wayne Drevets, Mark Smith

National Institute of Mental Health, Bethesda, Maryland

NIMH initiated the Research Domain Criteria (RDoC) project in 2009 to implement Goal 1.4 of its Strategic Plan, to "Develop, for research purposes, new ways of classifying mental disorders based on dimensions of observable behavior and neurobiological measures." RDoC represents an inherently translational approach to diagnosis, considering psychopathology as extremes on normal dimensions of functioning that are implemented substantially by particular neural circuits (e.g., fear, working memory, reward processes). Five workshops have been held to define specific neural-systems-based dimensional constructs within each of five major domains of functioning: Negative Valence (i.e., motivational systems for aversive situations), Positive Valence, Cognition, Social Processes, and Arousal/Modulatory Systems. NIMH conceived this program primarily to establish a research base that can inform future nosologies incorporating empirically-validated neural systems, moving beyond current heterogeneous diagnostic categories based largely upon signs and symptoms. However, current concerns about the withdrawal of pharmaceutical companies from CNS drug development have prompted a new urgency to move rapidly toward an experimental medicine, "fast-fail" approach to conceptualizing and evaluating therapeutic targets. In this context, a neural-circuit approach becomes integral to the development process, in providing more focused and homogeneous targets that foster therapeutics development by avoiding the heterogeneity of traditional diagnostic indications that can pose significant difficulties in observing a clear signal. Thus, the RDoC project has become a priority at NIMH for research both in pathophysiology and in seeking new treatments (or better matching of patients to extant treatments), and is expected to represent an increasing proportion of the NIMH portfolio for translational research in the coming years. This dimensional and neural systems approach is already reflected in recent NIMH funding announcements for new therapeutics research in mood/anxiety spectrum, psychotic spectrum, and autism spectrum disorders. However, such a new approach raises a number of questions – for example, how the new classification system actually works in practice (for instance, what are the criteria for classification); the relationship of the new system to current nosologies; experimental designs; measures of outcome and symptoms; and interface with regulatory agencies. This study group is designed to provide an opportunity to discuss these issues in depth in an informal setting, as informed by brief presentations that include a

brief outline of the RDoC project; descriptions of funded or planned RDoC research studies; and a perspective from the pharmaceutical industry new compound development arena.
Disclosures: W. Carpenter**, Part 1:** 2010, 3/24/10, Lundbeck 2500.00, Aug 22-24, Lilly 3937.50, Dec 3-4, Bristol Myers Squibb 3000.00, 2011, May 24, Shire Pharmaceuticals 3,000.00, Astra Zeneca phone calls waiting for payment 2012, Genentech 3,000.000; B. Cuthbert, **Part2:** NIMH Salary**;** J. Waltz, Nothing to Disclose; W. Drevets, **Part 1:** Johnson & Johnson, consultant, Esai, Inc., consultant, Myriad/ Rules Based Medicine, consultant, **Part 2:** Johnson & Johnson; M. Smith, **Part 2:** I am currently a full-time employee for AstraZeneca Pharmaceuticals.

Tuesday, December 04, 2012

Mini Panel
13. Rescuing Novel Mechanisms: Minimizing Placebo Response and Optimizing Signal Detection in Proof of Concept Trials

13.1 Decline in Signal Detection: Background and Proposed Strategies
Michael Thase*

University of Pennsylvania, Philadelphia, Pennsylvania

Background: There is strong and consistent evidence that placebo response rates in randomized controlled trials (RCTs) of antidepressants have grown progressively over the past three decades; the increasing placebo response is the single largest factor in explaining the decline in signal detection (i.e., the likelihood of failed and false negative studies). Indeed, more than 50% of industry-sponsored studies conducted during the past decade have failed to detect significant effects in RCTs using antidepressants with established efficacy. This high failure rate greatly complicates the clinical study of novel antidepressants and has contributed to a growing skepticism about the utility of antidepressants.
Methods: This presentation will review and synthesize the existing literature and make recommendations about changes in practice.
Results: The fundamental problem may be that there are simply not enough "truly appropriate and fully eligible" unmedicated depressed patients who are available to - and interested in - participating in the various large scale RCTs that are underway at any given time, especially in the United States. Attempts to "suppress" placebo response rates, including raising entry symptom severity thresholds and various low-intensity strategies to improve rater reliability, have consistently failed. Brief single blind placebo lead-ins, employing more frequent visits and more detailed assessments, and enrolling larger samples (in order to have adequate statistical power to detect smaller effects) have likewise backfired. Some success, albeit inconsistent across studies, has been achieved by using more labor-intensive methods to ensure enrollment of appropriate patients and provide reliable and valid ratings of outcome measures. As studies of adjunctive therapies, which typically employ prospective lead-ins, and studies using relapse prevention designs, which are enriched by only including patients who have benefitted from a particular acute phase therapy, have yielded much higher success rates during the same era, it is proposed that one way to improve signal detection is to conduct RCTs within the context of longer, sequential care platforms.
Conclusions: Improved signal detection in the next generation of studies of antidepressants appears possible if adequate attention is paid to ensuring high quality control and/or a commitment to conducting research collaboratively with patients across several sequential phases of treatment.
Disclosure: M. Thase, **Part 1:** Alkermes, AstraZeneca, Bristol-Myers Squibb Company, Eli Lilly & Co, Dey Pharma LP, Forest Laboratories, H. Lundbeck A/S, Med Avante Inc, Merck & Co Inc, Neuronetics Inc, Otsuka, Ortho-McNeil Pharmaceuticals, Pamlab LLC, Pfizer, PGx Inc, Pharma Neuroboost, Rexahn, Roche Labs, Shire US Inc, Takeda, Transcept Pharmaceuticals, **Part 2:** University of Pennsylvania, **Part 4:** Alkermes, Eli Lilly and Company, Forest Pharmaceuticals, Otsuka Pharmaceuticals, Pharma Neuroboost, Roche Labs.

13.2 Missing Data, Placebo Response and Positive Controls: How They Influence Signal Detection
Craig H. Mallinckrodt*

Eli Lilly and Company, Indianapolis, Indiana

Background: The rising unmet medical need in CNS disorders and the low rates of success in developing innovative treatments for them has led to greater quantitative rigor in understanding factors that influence drug developed. This research has highlighted the influence of proof-of-concept (PoC) studies on R&D productivity. The present research focuses on factors that influence our ability to distinguish between effective and ineffective therapies in PoC trials for CNS disorders.
Methods: Data simulations were used to assess the impact of patient drop-out and the bias that can result from it on the rates of positive PoC studies. A review of recent literature was used to assess the impact of placebo response on assay sensitivity, and including vs. not including a positive control on placebo response and assay sensitivity. An analytic approach was used to demonstrate the conditions under which positive controls are likely to help or hinder decision-making in proof-of-concept trials.
Conclusions: In simulation scenarios patterned after depression clinical trials patient drop-out and the bias it caused reduced power from over 90% to 40%. In a literature review, confirmatory trials of known effective antidepressants had less than a 25% success rate if placebo response was greater than the median, while the success rate was approximately 75% for trials with less than the median placebo response. The percentage of placebo-treated patients meeting responder criteria was approximately 10% greater in studies that included $>$ 1 active medication compared with studies that tested only 1 drug. Positive controls must be powered at 90% or greater in order to benefit decision making.
Disclosure: C. Mallinckrodt, **Part 1:** Employee of Eli Lilly and Co, **Part 2:** Employee of Eli Lilly and Co, **Part 3:** Employee of Eli Lilly and Co.

13.3 First, Do No … Help!?: The Problems with Therapeutic Alliance and Expectation Bias in Clinical Trials
Michael Detke*

Indiana University; Med Avante, Carmel, Indiana

Background: We are taught as clinicians "first, do no harm". The clinical goals to help patients, to advocate for them, and to seek improvement may be very beneficial in clinical practice. But in clinical trials this may increase placebo response. This is of particular concern in CNS clinical trials due to the subjective nature of many (including primary) outcomes. Therapeutic alliance may (inadvertently) improve outcomes, such as through non-specific supportive psychotherapy, or a subject's reporting improvement to please the rater. A related phenomenon, expectation bias, may occur when clinicians expect subjects to improve over time, and subjects may likewise be biased toward expecting to get better. Finally, rater and subject biases may interact. All of these may result in increased placebo response and decreased drug-placebo separation. These threats to identifying efficacy are most damaging in the initial (proof-of-concept) tests of novel mechanisms, which have the potential to open up truly new therapeutic avenues – or doom them.
Methods: We define therapeutic alliance and expectation bias, and review studies of these across multiple disease areas to assess how common they may be and to assess their impact on placebo response and signal detection. We examine the designs of these studies to identify methods for potentially mitigating these effects. We present new data from randomized clinical trials in anxiety and psychosis to examine the impact of these different methods on signal detection.

Results: Studies in several disease areas demonstrate that therapeutic alliance and expectation bias can increase placebo response and decrease signal detection. Blinding raters to study visit number may reduce the expectation of improvement as treatment progresses. To achieve this it is necessary to use different raters across visits. Using multiple raters also reduces the therapeutic alliance between raters and subjects. Several recent studies showed reduced placebo response and/or increased signal detection using different raters across visits and blinding raters to visit number.
Conclusions: Therapeutic alliance and expectation bias appear to be quite common. Raters who are blinded to study visit number and independent from the study sites appear to yield decreased placebo response and/or better signal detection.
Disclosure: M. Detke, **Part 1:** Med Avante, Sonkei, Rhine, Columbia, Roche, NIH Pharmanet/i3/Inventiv (partner), **Part 2:** Med Avante, **Part 3:** Med Avante.

Mini Panel
14. Exploring Therapeutic Use of Psilocybin, A Classic Hallucinogen

14.1 Experimental Studies of Psilocybin in Healthy Volunteers: Persisting Attribution of Positive Changes in Attitudes, Mood and Behavior
Roland Griffiths*

Johns Hopkins University School of Medicine, Baltimore, Maryland

Background: Clinical research with psilocybin in the 1950s and 60s showed that psilocybin often produced widely variable subjective experiences. This presentation will present recent and ongoing research at Johns Hopkins demonstrating that, in well-prepared and interpersonally support participants, psilocybin can occasion, in most volunteers studied, experiences rated by volunteers as among the most personally meaningful of their lives.
Methods: Fifty-four healthy volunteers (mean age = 46 years; 56% female) without histories of hallucinogen use were recruited from the local community for participation in two double-blind studies comparing either psilocybin to methylphenidate, or comparing a range of doses of psilocybin to placebo. To develop rapport and trust during the psilocybin sessions, they met with study monitors for 8 hours of contact time before the first session. Two to five 8-hr sessions were separated by 1-2 month intervals. During sessions, volunteers used eyeshades and were instructed to direct their attention inward. Volunteers completed questionnaires assessing effects immediately after and 1-2 months after sessions, and at 14 months follow-up. Community observers were interviewed before and after sessions about observed changes in the volunteers' behavior.
Results: Psilocybin produced acute perceptual and subjective effects including, strong or extreme anxiety/fear sometime during the session (33% of volunteers at 30 mg/70 kg) and/or mystical-type experience (65% of volunteers at 20 or 30 mg/70 kg). One month after sessions at the two highest doses, volunteers rated the psilocybin experience as having substantial personal and spiritual significance, and attributed to the experience sustained positive changes in attitudes, mood, and behavior. At 14 months, ratings were undiminished and were consistent with changes rated by community observers. Both the acute and persisting effects of psilocybin were generally a monotonically increasing function of dose, with the lowest dose (5 mg/70 kg) showing significant effects. The results of the dose-effect study suggested that an ascending psilocybin dose sequence is somewhat more likely than a descending sequence to produce long-lasting positive changes in attitudes, mood, and behavior.
Conclusions: In conclusion, recently completed and ongoing studies show that, when administered to healthy volunteers under supportive conditions, psilocybin occasioned experiences similar to spontaneously-occurring mystical or insightful experiences and which were evaluated by volunteers and community observers as having produced substantial and sustained positive changes in attitudes, moods, and behavior. The ability to prospectively occasion such experiences permits rigorous scientific investigations about their causes and consequences, insights into underlying pharmacological and brain mechanisms, and the investigation of possible therapeutic applications.
Disclosure: R. Griffiths, **Part 1:** Heffter Research Institute - Member of Board of Directors - 2 grants for clinical trials with psilocybin, Transcept Pharmaceutical – consulting, Alexza Pharmaceuticals - grant for clinical trial, Bristol-Myers Squibb – consulting, Merck and Co – consulting, Vanda Pharmaceuticals - consulting, **Part 4:** Alexza Pharmaceutical - A grant was provided to Johns Hopkins to conduct an abuse liability evaluation with a novel drug delivery system, Heffter Research Institute – Two grants to conduct clinical trials with psilocybin.

14.2 Psilocybin Treatment for Anxiety in Patients with Advanced-stage Cancer
Charles Grob*

Harbor-University of California, Torrance, California

Background: From the late 1950s to the early 1970s clinical research was conducted exploring the use of hallucinogens to treat the existential anxiety, despair and isolation often associated with advanced-stage cancer. These reports described critically ill individuals undergoing psychospiritual epiphanies, frequently with sustained improvement in anxiety, mood and quality of life. While these promising investigations were halted because of political and cultural pressures, after a thirty year hiatus the development of hallucinogen treatment research for patients with anxiety reactive to advanced-stage cancer has resumed.
Methods: This presentation examines the rationale, methodology and results of a pilot investigation using psilocybin to treat the existential anxiety associated with advanced-stage cancer. Psilocybin, the active alkaloid in hallucinogenic mushrooms and a 5-HT2A and 5-HT2C agonist, was administered to 12 screened subjects diagnosed with advanced-stage cancer and anxiety. A double-blind, placebo-controlled methodology was employed, utilizing a moderate dosage of psilocybin, 0.2 mg/kg. Treatment sessions were conducted on the Clinical Research Unit at Harbor-UCLA Medical Center.
Results: Safe physiological and psychological responses were documented during treatment sessions. There were no clinically significant adverse events with psilocybin. The State-Trait Anxiety Inventory trait anxiety subscale demonstrated a significant reduction in anxiety at 1 and 3 months after treatment. The Beck Depression Inventory revealed an improvement of mood that reached significance at 6 months; the Profile of Mood States identified mood improvement after treatment with psilocybin that approached but did not reach significance.
Conclusions: This study establishes the feasibility and safety of administering moderate doses of psilocybin to patients with advanced-stage cancer and anxiety. Some of the data revealed a positive trend toward improved mood and anxiety. These results support the need for more research in this long-neglected field. To that end, two new studies using psilocybin to treat cancer anxiety, at Johns Hopkins and New York University, are currently on-going. Both of these investigations are evaluating higher doses than used in the Harbor-UCLA study, allowing for greater exploration of the role of psychospiritual or transcendent states of consciousness in facilitating therapeutic outcomes.
Disclosure: C. Grob, Nothing to Disclose.

14.3 Effects of Psilocybin in the Treatment of Addictions: A Review and Preliminary Results from Two Ongoing Trials
Michael P. Bogenschutz*

University of New Mexico Health Sciences Center, Albuquerque, New Mexico

Background: Clinical research on classic hallucinogens in treatment of addiction was halted abruptly in the early 1970s, and is

only now resuming. The purpose of this presentation is to 1) review past clinical trials of classic hallucinogens for addiction 2) discuss possible mechanisms of action, and 3) present design and preliminary results of two ongoing pilot studies of psilocybin in the treatment of nicotine and alcohol dependence.
Methods: A review was conducted of published controlled trials of classic hallucinogens for treatment of addictions. In addition, a broader literature review aimed to identify possible psychological and biological mechanisms of action. Current open-label trials are investigating effects of psilocybin on nicotine dependence and alcohol dependence. The Johns Hopkins smoking cessation study includes 3 psilocybin sessions at 4-week intervals in doses of 20-30 mg/70 kg, combined with cognitive behavioral smoking cessation treatment. The UNM alcohol dependence study includes 2 psilocybin sessions one month apart, at doses of 0.3-0.4 mg/kg, combined with motivational enhancement therapy.
Results: Numerous studies in the 1960s and early 1970s investigated the use of LSD in the treatment of alcoholism. A recent meta-analysis of 6 randomized controlled trials of LSD administered in a single high dose for treatment of alcoholism (total n = 536) demonstrated that the overall effect size of LSD treatment was substantial and highly consistent across studies, with an odds ratio for post-treatment improvement of 1.96 (95% CI 1.36-2.84). Trials investigating other classic hallucinogens and other addictions had methodological problems that limit confidence in their findings. The psychedelic model hypothesized that an overwhelming "peak-psychedelic" or mystical experience could bring about lasting change in personality and addictive behavior. Although this mechanism has not been demonstrated directly in addiction trials, the hypothesis is consistent with data including 1) effects of psilocybin in normal participants; 2) qualitative and quantitative research on transformative/mystical experiences leading to abstinence in addicts; and 3) decreases in substance use among members of religions that use classic hallucinogens sacramentally. Although basic research on anti-addictive effects of classic hallucinogens is lacking, 5HT2A receptor-mediated increases in expression of BDNF and GDNF are potentially relevant to changes in substance use behavior.
In the Johns Hopkins smoking cessation study, four participants have been treated to date. Acute effects of psilocybin were similar to those reported previously in normal volunteers, with all four volunteers showing substantial mystical content in one or more of the sessions. Adverse events included transient anxiety which was well managed with interpersonal support. On no session was pharmacologic intervention required, and any anxiety resolved by the end of all sessions. All participants showed biologically confirmed abstinence use to 6 months (4 participants up to the final 1 year follow up). Recruitment began in February 2012 for the UNM alcohol dependence study. An update on study progress and outcomes will be presented.
Conclusions: Past clinical trials of LSD in treatment of alcoholism, several other lines of supportive evidence concerning hallucinogen effects and models of addiction, and the preliminary results of ongoing pilot studies provide a convincing rationale for further investigation of clinical effects of psilocybin on addictive behaviors.
Disclosure: M. Bogenschutz, Nothing to Disclose.

Panel
15. Neuroscience and the Future of Psychiatric Diagnosis: Updates on Development of the Fifth Edition of Diagnostic and Statistical Manual of Mental Disorders

15.1 Autism Spectrum Disorder in DSM5
Edwin H. Cook*

University of Illinois at Chicago, Chicago, Illinois

Background: Autism was first defined by Leo Kanner in 1943, who in 1971 wrote, "The outstanding pathognomonic characteristics were viewed as (a) the children's inability from the beginning of life to relate themselves to people and situations in the ordinary way, and (b) an anxiously obsessive desire for the preservation of sameness." Autism criteria were first included in DSMIII, which required defined infantile autism based on a small number of obligate criteria including the criterion, "pervasive lack of responsiveness to others." DSM-III-R introduced that only 8 of 16 criterion needed to be met, largely because many criteria could not be met at the range of mental ages necessary. DSM-IV provided some minor changes to the overall structure of DSM-III-R and introduced Asperger's disorder, Rett disorder, and Childhood disintegrative disorder.
The DSM5 Neurodevelopmental Disorders Committee has met extensively and reviewed the literature and existing data sets to revise the categories to Autism Spectrum Disorder instead of the separate disorders of Autistic Disorder, Asperger's disorder, and Pervasive Developmental Disorder not otherwise specified. In addition a profile of specifiers of strengths and weaknesses has been added, as well as a specifier for association with known genetic or medical conditions (e.g. Fragile X syndrome).
Methods: A field trial was conducted at two sites that were meant to be representative of psychiatry clinics that did not specialize in the diagnosis of autism spectrum disorder.
Results: The overall prevalence of autism did not change between DSM-IV and DSM5 criteria. An analysis will be presented of patients who changed diagnosis to make sure that the criteria operate as intended.
Conclusions: Several pervasive developmental disorders have been changed to a single autism spectrum disorder with the additional of dimensional specifiers (e.g. language ability, cognitive ability) that are intended to provide a more comprehensive view of each patient than the previous categories such as Asperger's disorder whose criteria were inconsistently applied and did not fully desribe the strengths and challenges of patients with this diagnosis. Analyses to date support that prevalence does not change but further updated analyses both of DSM5 field trials and other analyses in the literature will be reviewed.
Disclosure: E. Cook, **Part 1:** Consultation - Seaside Therapeutics – 2010; **Part 4:** Seaside Therapeutics - support for site in a multi-site clinical trial.

15.2 DSM-5 Schizophrenia Spectrum: Major Changes, Controversies and Linkage with the NIMH Research Domain Criteria
William Carpenter*

University of Maryland School of Medicine, Baltimore, Maryland

Background: Proposals from the Work Group for the Spectrum include Schizophrenia, Schizoaffective, Schizophreniform, Schizotypal Personality, Attenuated Psychosis Syndrome, and Catatonia. Changes include dropping schizophrenia subtypes, reducing emphasis on Schneiderian first rank symptoms, designating catatonia as a specifier, shifting the concept of schizoaffective from an episode disorder to a life of the illness disorder, and joining some of the disorders together in relation to spectrum criteria. Major innovations involve instituting domains of psychopathology as dimension relevant to each case, and adding new disorders catatonia NEC and Attenuated Psychosis Syndrome.
Methods: Work group member discussions, consultation with advisors, specified literature reviews, consideration of harmonization with ICD and criteria for any changes from DSM-IV, consideration of comments and critiques from the public, the profession, DSM-5 leadership and Task Group, and the Scientific Review Committee and the Clinical and Public Health Committee. A method for assessing cognition in a brief time frame was developed by the Gurs and colleagues and the APA obtained property rights. Reliability between clinicians was tested in field trials on some issues.
Results: Literature reviews supported proposed changes. Domains of pathology provide a method for linking behavioral constructs/neural circuits from RDoC, representing a significant paradigm shift for research in psychotic disorders. Substantial controversy

remains on new disorders, i.e., catatonia nec and attenuated psychosis syndrome. Reliability of the psychopathology dimensions was variable and the final list may be reduced from the nine identified for testing [i.e., hallucinations, delusions, disorganization, restricted affect, avolition, psychomotor, cognition, mania and depression]. While satisfactory Kappa were obtained on some disorders [e.g., attenuated psychosis syndrome] the small N and broad confidence intervals make results uninformative.
Conclusions: Proposals being considered and recommended at the time of abstract preparation will require formal approval/disapproval by the APA Board of Trustees.
Disclosure: W. Carpenter, **Part 1:** I have served as a consultant to Lundbeck, Eli Lliy and Company, Bristol-Meyers Squibb, Shire Pharmaceuticals, and AstraZeneca.

15.3 Anxiety Disorders, Obsessive-compulsive and Related Disorders, Trauma- and Stressor-related Disorders and Dissociative Disorders: Changes for DSM-5

Katharine A. Phillips*

Alpert Medical School of Brown University, Providence, Rhode Island

Background: The field's understanding of mental disorders has advanced substantially since DSM-IV was developed in the early 1990s. These advances have guided the work of the Anxiety, Obsessive-Compulsive Spectrum, Post-Traumatic, and Dissociative Disorders Work Group.
Methods: This Work Group has considered a broad range of changes for DSM-5. In addition to changes to diagnostic categories, the Work Group considered options for adding dimensional approaches to DSM (to be used in addition to categorical diagnoses) and how DSM might better reflect advances in neuroscience. Changes are based primarily on available scientific evidence from a large number of DSM-focused literature reviews, field trials, and secondary data analyses. Clinical utility and input from experts and the field have also been considered.
Results: This presentation will summarize some of the most notable changes for the anxiety disorders, obsessive-compulsive and related disorders, trauma- and stressor-related disorders, and dissociative disorders. Changes that will be discussed include modification of diagnostic criteria, subtypes, and specifiers for individual disorders; addition of new disorders; addition of dimensional approaches; enhancement of the developmental sensitivity of DSM; changes to the metastructure; and how DSM-5 will reflect advances in neuroscience.
Conclusions: DSM-5 will reflect the substantial progress that has been made in understanding of the psychopathology and nosology of mental disorders over the last several decades. The goal of these changes is to enhance the reliability, validity, and clinical utility of DSM for both clinicians and researchers.
Disclosure: K. Phillips, **Part 1:** Forest Laboratories (medication only for an NIMH-funded study) Transcept Pharmaceuticals (research funding) Oxford University Press (royalties) Guilford Publications and The Free Press (potential royalties) Elsevier (future honorarium), **Part 2:** NIMH, FDA, Rhode Island Hospital, Alpert Medical School of Brown University, **Part 4:** Forest Laboratories (medication only for an NIMH-funded study)Transcept Pharmaceuticals (research funding).

15.4 A Potential Biomarker for Addiction

Charles P. O'Brien*

University of Pennsylvania, Wynnewood, Pennsylvania

Background: Recent brain imaging evidence demonstrates functional changes in the brains of addicted individuals. These are not brain "lesions" in the clinical neurology sense of the word, but they can be devastating in their effects on behavior. For example down regulation of D2 receptors has been reported in cocaine addicts, alcoholics, opioid addicts and even in obese subjects. No study has reported longitudinal data showing absence of these changes in the pre-addicted state and subsequent development during addiction, but the consistent reports are suggestive of a pathological process caused by drugs or behaviors that cause excess synaptic dopamine resulting in a physiological down-regulation of D2 receptors.
Methods: PET scanning with C-11 raclopride is the ligand that shows decreased binding in individuals with down-regulated D2 receptors. Functional MRI can also be used to demonstrate the effects of drug-related cues on activation of brain regions known to be involved in reward processing. Regional cerebral blood flow as measured by fMRI can detect increased blood flow to specific reward related areas in addicts when presented with drug cues, but not in normal controls. The involuntary nature of these blood flow changes was demonstrated in one study that showed amygdala response to only 35 msec of cue exposure, far briefer than the duration required for conscious awareness.
Results: While there has been consistent findings of functional brain changes in individuals diagnosed with addiction by DSM-IV criteria, there has never been a published attempt to correlate clinical diagnosis with brain changes. DSM-5 may lend itself to these efforts because in this version, Substance Use Disorder is a unidimensional measure from "not present" to "moderate" to "severe." The brain changes also can be independently rated by density of radio-ligand binding or computer scored blood flow changes. Using these ratings, the severity of the brain changes can be compared to the severity of the clinical ratings. This could pave the way for studies of specificity and sensitivity of the biomarker.
Conclusions: Consistent functional changes in brain activity have been demonstrated in patients diagnosed with addiction ("dependence") according to DSM-IV criteria. DSM-5, as a dimensional diagnostic measure, may be more conducive to studies correlating brain imaging changes with clinical diagnosis. This approach could lead to the first biomarker for the state of addiction.
Disclosure: C. O'Brien, Nothing to Disclose.

Panel
16. Developmental Programming of the Brain: Implications for Shared Mechanisms Across Neuropsychiatric Disorders

16.1 Genome-wide Analysis Identifies Loci With Shared Effects on Five Major Psychiatric Disorders

Jordan W. Smoller*

Harvard Medical School, Boston, Massachusetts

Background: Psychiatric nosology is based on descriptive syndromes, largely without reference to etiology. Genetic studies offer a strategy for dissecting the degree of etiologic specificity vs. overlap among psychiatric disorders. Family and twin studies suggest that genetic contributions to psychiatric disorders do not precisely map onto current diagnostic categories. Recent studies of common and rare genetic variations, including copy number variants, have provided support for these findings at a molecular genetic level. In addition, aggregate genomewide association results have also documented moderate overlap between polygenic influences on schizophrenia and bipolar disorder in particular. There is now widespread consensus that identifying the full allelic spectrum of risk variants for common psychiatric disorders will require sample sizes much larger than those that have been reported to date. The international Psychiatric GWAS Consortium (PGC), comprising individual-level genomewide association data (GWAS) provides a unique opportunity to expand our understanding of shared and disorder-specific effects of common risk variants for multiple diagnostic syndromes. To this end, we conducted a GWAS across five psychiatric disorders: autism spectrum disorder, attention-deficit/hyperactivity disorder, bipolar disorder, major depressive disorder and schizophrenia.
Methods: The combined GWAS meta-analysis of the five disorders comprised 61,220 case and controls. To characterize allelic effects on each disorder, we applied a model selection procedure to

identify the best-fitting model of genotype-phenotype relationships. We also examined cross-disorder effects of genome-wide significant loci previously identified for bipolar disorder and schizophrenia. We conducted pathway analyses to understand biological relationships underlying genetic overlap among the five disorders.
Results: Tests of association for SNPs at four loci surpassed the genome-wide significant threshold ($p < 5 \times 10\text{-}8$) in the primary analysis: regions on chromosome 3p21 and 10q24, and SNPs within two L-type voltage-gated calcium channel subunits, CACNA1C (12p13) and CACNB2 (10p12). Model selection analysis supported effects of these loci across multiple disorders. Loci previously associated with bipolar disorder or schizophrenia demonstrated variable levels of diagnostic specificity. Pathway analysis supported a broader role for calcium channel signaling genes across all five disorders.
Conclusions: Our analyses provide the first demonstration that specific SNPs may be significantly associated with a range of childhood- and adult-onset psychiatric disorders. In particular, variation in calcium channel activity genes appears to have pleiotropic effects on psychopathology.
Disclosure: J. Smoller, Nothing to Disclose.

16.2 Shared Fetal Programming of Sex Differences in Stress Response Circuitry and Endocrine Deficits in Schizophrenia and Depression: Shared Mechanisms but Different Disorders

Jill M. Goldstein*

Harvard Medical School, Boston, Massachusetts

Background: A final common pathway suggested by studies of fetal programming in schizophrenia and depression implicates prenatal stress, i.e., developmental disruption of the hypothalamic-pituitary-adrenal (HPA) axis. Stress-arousal circuitry houses some of the most highly sexually dimorphic regions in the brain. We tested the hypothesis that fetal disruption of stress response circuitry results in shared sex-specific adult brain abnormalities and endocrine disruption in schizophrenia and depression.
Methods: In two 50-year cohort studies with mothers followed through pregnancy and sera stored at NIH, ~160 adult offspring (40 DSM-IV recurrent major depressive disorder (MDD) and 40 psychosis cases (half schizophrenia; half bipolar); 80 healthy controls (equally divided by sex, made comparable within sex by group)) were assessed using fMRI of stress response circuitry and hormonal assays timed to pituitary and steroid hormone responses (ACTH, DHEAS, cortisol, E2, progesterone, T). Prenatal assays included cytokines (IL-1β, IL-6, TNF-α, IL-10) and adrenal hormones (hCG, DHEAS). Subjects viewed images of negative valence/high arousal or neutral valence/low arousal stimuli. 3T Siemens MR scanner and SPM8 determined blood oxygen-level dependent (BOLD) signal changes in hippocampus (HIPP); amygdala (AMYG); anterior hypothalamus (aHYP); orbitofrontal, medial prefrontal and anterior cingulate cortices (OFC, mPFC, ACC); and periacqueductal gray (PAG). General linear models related prenatal cytokine and hormone disruptions to BOLD signal changes in case groups by sex.
Results: MDD men and women were hyperactive across stress circuitry regions, and by FWE-correction for multiple tests in aHYP, AMYG, and HIPP. Men showed greater effect sizes (Cohen's d) in BOLD changes than women in aHYP ($d = 1.2$ vs .50), HIPP ($d = 1.6$ vs .75), AMYG ($d = 1.0$ vs .74), and mPFC ($d = 1.1$ vs ns in women). Cortical sex differences were evident with hyperactivity in OFC and ACC in MDD women, and hyperactivity in mPFC in men. Men with psychoses were significantly hyperactive across the circuitry and by FWE-correction in aHYP ($d = .73$), PAG ($d = .40$) and ACC ($d = .29$). In contrast, women were hyperactive in AMYG ($d = .44$) and HIPP ($d = .45$) but hypoactive in cortex (mPFC $d = -.79$; OFC $d = -.44$). Sex effects were similar in schizophrenia and bipolar psychoses. Across diagnoses, BOLD group differences in hyperactivity were associated with high progesterone, low E2 (in women), low androgen free index (in men), and hypercortisolemia in both sexes. Hypoactivity in women with psychosis was related to low DHEAS, low E2, and lower prenatal TNF-α. Hyperactivity was related to higher maternal pro-inflammatory cytokines (particularly IL-6 in men with schizophrenia).
Conclusions: Findings suggest shared fetal risk factors, implicating developmental disruption of HPA in MDD and psychoses. These may, in part, act by disrupting maternal immune responses, resulting in sex-specific effects on offspring's HPA circuitry in adulthood, with brain activity and endocrine deficits that are shared across psychiatric disorders. Thus, fetal programming of stress response circuitry may be important for understanding shared sex-specific vulnerabilities to MDD and psychoses. We hypothesize that genetic susceptibilities interact with sex-specific effects of fetal stress response programming to differentiate these disorders.
Disclosure: J. Goldstein, Nothing to Disclose.

16.3 Long-term, Sex-specific Effects of Developmental Exposure to Excess Glucocorticoids on Gene Expression in the Hypothalamus

Robert J. Handa*

University of Arizona, Phoenix, Arizona

Background: Adverse environmental experiences during early life can predispose some individuals to the development of neuropsychiatric disorders in adulthood. Previous work exploring risk factors for mood disorders indicate that fetal adversity impacts the developing Hypothalamo-Pituitary-Adrenal (HPA) axis, resulting in elevated circulating glucocorticoid (GC) levels, blood pressure and blood glucose levels in adulthood. In rodents, prenatal stress and prenatal exposure to GCs program the HPA axis such that, in adult offspring, stress-reactivity and stress-related behaviors are disrupted. Such findings implicate the hypothalamic paraventricular nucleus (PVN) since it controls both neuroendocrine and autonomic responses to stress.
Methods: Timed-pregnant Sprague-Dawley dams arrived at gestation day 7 (GD-7). Dams were handled daily from gestation day (GD) 14-17 and then injected with the synthetic GC, dexamethasone (0.4 mg/kg; 500 ul volume) or vehicle on GD 18-21. At birth, litters were thinned to 5 males and 5 females Animals were euthanized for evaluation of gene expression on PD 0, 7, 14 and 60. Adult males and females (diestrus) were examined for HPA reactivity, anxiety and depressive like behaviors and autonomic indicators. mRNA levels for 20 different candidate genes including, glucocorticoid receptor (GR), BDNF, VEGF, IGF-1 receptor (IGF-1R) were measured in the paraventricular n. (PVN) using qRT-PCR.
Results: Results show that adult female-, but not male-, offspring from prenatal DEX treated dams exhibited increases in anxiety (open field arena) and depressive like behaviors (forced swim test). Using *in vivo* telemetry, we also detected a persistent decrease in core body temperature throughout the day in DEX-treated adult female offspring only. This decrease was present on all days of the estrous cycle. Of many different candidate genes measured in the PVN, we detected female-specific changes in the expression of BDNF, VEGF, GR and IGF-1R mRNAs on PD7 and PD60. Long-term changes in GR and BDNF mRNA levels were accompanied by altered methylation of their promoters suggesting epigenetic changes controlling gene expression.
Conclusions: Elevated exposure to GCs during late gestation of the rat, a developmental time-point that corresponds to the 2nd trimester of gestation in humans, programs the developing hypothalamus such that neuroendocrine hyper-reactivity to stressors, altered autonomic function and anxiety and depressive-like behaviors are seen in adulthood. We demonstrate that over-exposure to GCs can act on the fetal brain to alter expression of a select group of growth factors in the PVN: the neurotrophin, BDNF, and the angiogenesis factor, VEGF, as well as circulating IGF-1 and its receptor, IGF-1R. Given the diverse actions of PVN neurons, we propose that such changes modify the function of the adult PVN, particularly since this nucleus serves as a common nodal point for control of neuroendocrine responses, autonomic function and metabolism.
Disclosure: R. Handa, Nothing to Disclose.

16.4 Trans-generational Effects of Endocrine Disrupting Compounds on Brain and Behavior

Emilie Rissman*

University of Virginia School of Medicine, Charlottesville, Virginia

Background: Endocrine disrupting compounds (EDCs) are widespread in the environment. Acting on steroid receptors and/or via epigenetic modifications these compounds have been shown to influence development of the reproductive system, including the brain. My laboratory has examined the effects of one of these EDC's, Bisphenol A (BPA) on juvenile and adult behaviors in mice.
Methods: Female C57BL/6J mice were exposed to BPA in their diet starting before mating and lasting throughout gestation. At birth pups from BPA and control fed females were all fostered to dams on a control diet. This was done to limit exposure to BPA to gestation and to insure that maternal diet did influence pup-dam interactions. Starting on post natal day 21 pups were tested for social and other behaviors. Brains were collected at various ages and regions assayed with qPCR for differences in gene expression. Mice were bred to the fourth generation to produce two linages; BPA and control. These mice were also tested for behavior and gene expression assayed in brain.
Results: In general, juvenile mice exposed to concentrations of BPA similar to those found in humans, displayed fewer social interactions compared with control mice, whereas in later generations (F2 and F4), BPA exposure increased social interactions. These behavioral changes were significantly related to varying expression levels of estrogen receptors, oxytocin and vasopressin. Decreased vasopressin mRNA persisted into F4, at which time oxytocin mRNA was reduced but only in males.
Conclusions: It is clear that both genes and environment influence the expression of a number of psychiatric diseases. Our data suggest that one environmental factor may be EDC which have increased exponentially in their use over the last few decades. The heritable effects of EDCs also have important implications for complex neurobehavioral diseases.
Disclosure: E. Rissman, Nothing to Disclose.

Panel
17. One Size Doesn't Fit All: Molecular Mechanisms Underlying Diverse Estradiol Signaling in the Brain

17.1 Roles of ERβ in the CNS

Jan-Ake Gustafsson*

University of Houston, Houston, Texas

Background: Three methodological advances have led to a better understanding of the functions of ERβ in the CNS. These advances are: (1) Development of ERβ −/− mice; (2) development of reliable antibodies and methodology for their use in the brain; (3) and the development of selective ERβ agonists with high CNS penetrability and low toxicity. Studies with ERβ−/− mice, have demonstrated that ERß plays an important role during brain development and in repressing anxiety: it is necessary for the development of calretinin-immunoreactive GABAergic interneurons; and for neuronal migration in the cortex through modulating EGFR expression at middle and later embryonic stages. More recent studies with the use of a selective ERβ agonist have revealed a role of ERβ in repression of anxiety and depression.
Methods: An ERβ ligand with a 90-fold preference for ERβ over ERα (Eli Lilly 3201) was administered in subcutaneous pellets releasing to mice. Controls were treated with blank pellets. Pellets released 0.04 mg of LY3201/day. Three days after treatment had begun brains were examined with Golgi staining.
Results: Robust expression of ERβ but not ERα was observed in the tryptophan hydroxylase-positive (TPH or serotonergic) neurons of the dorsal raphe. Ovariectomy of WT mice and absence of ERβ (ERβ−/− mice), leads to a marked reduction in the number of TPH-positive normal-looking neurons and an increase in spindle shaped TPH-positive cells. These changes were prevented in mice 1-3 weeks (but not 10 weeks) after OVX by the selective ERβ agonist there is a window in time after loss of estrogen when ERβ ligands can restore serotonin signaling. In the LY3201-treated group there was: (1) A clear reduction in spines on the dendritic branches but no morphological alteration and no difference in the number of dendritic spines on dendritic stems; (2) higher expression of glutamic acid decarboxylase (GAD65 + 67) in the layer V of cortexand in the CA1 of hippocampus; (3) clearly more GAD65 + 67 (GABAergic) terminals surrounding the pyramidal neurons and fewer glutamate receptors (NMDAR-positive) neurons in the LY3201 group; (4) increased expression of GFAP (astrocyte marker) but no alterations inexpression of Iba1, a microglia marker or in Olig2 or CNPase,oligodendrocyte markers; (5) activation of astrocytes (more projections and increased expression of glutamine synthetase).
ERβ was not detectable in the nuclei of astrocytes.
Our studies suggest that ERβ agonists could be useful pharmaceuticals in maintaining functional neurons in the dorsal raphe to treat postmenopausal depression and in balancing excitatory and inhibitory input to treat anxiety. These novel functions of ERβ have led to new ideas about the etiology and treatment of CNS diseases.
Conclusions: Overall, LY3201 caused a shift in the balance between excitatory and inhibitory neurotransmission in favor of inhibition and this was due in part to increased synthesis of GABA and increased removal of glutamate from the synaptic cleft by astrocytes. Since treatment with a selective ERβ agonist resulted in changes opposite to those seen with estradiol it is likely that ERα and ERβ play opposing roles in the brain.
Disclosure: J. Gustafsson, **Part 1:** Consultant for Karo Bio AB, **Part 2:** Consultancy fee from Karo Bio AB.

17.2 Development of Ligands for Estrogen Receptor Beta and the Genomic vs. Non-genomic Pathway: Appreciating and Exploiting the Many Dimensions of Activity and Selectivity

John Katzenellenbogen*

University of Illinois at Urbana-Champaign, Urbana, Illinois

Background: The rich and multifaceted pharmacology of estrogen receptor (ER) ligands provides unexpected ways for achieving desirable selective physiological and pharmacological effects, for example, providing the bone, cardiovascular, and neuroprotective effects of estrogens, without stimulating the uterus and breast. While this has been achieved in a number of cases, the mechanistic basis by which selectivity has been achieved is not always clear.
Methods: We have used the analysis of ER crystal structures, organic synthesis, including combinatorial library synthesis, *in vitro* binding, cell-based and *in vivo* assays to prepare and characterize the biological activity of a wide variety of ER ligands.
Results: Through consideration of the structure of the ER ligand binding domain, we decided to take a generic approach to ligand design. Following this paradigm, we prepared a number of ligands of different design having a core structure that was readily constructed by assembly of a core heterocycle or amide-type linkage. A number of these turned out to have high selectivity for one or the other of the estrogen receptor subtypes, ER-alpha (ERα) and ER-beta (ERβ). Surprisingly, when sets of these ligands having nominally the same potency, intrinsic activity, and ER subtype selectivity were studied *in vivo*, they often showed rather divergent activities. In particular, of several ERβ ligands studied in cell and animal models of brain inflammation, one only class showed potent neuroprotective activity. In related studies, we were able to restrict estrogen action through the extra-nuclear signaling pathway by tethering ER ligands to dendrimeric molecules. This pattern of ER pathway selectivity afforded cardiovascular and, in part, bone protection, without stimulation of reproductive tissues. The basis of for this selectivity *in vivo* appears to arise from

three mechanistic levels, restricted subcellular distribution, selective *in vivo* distribution, and cellular inactivation through endocytosis.
Conclusions: The diversity of cellular activities and physiological and pathological processes that are regulated by estrogen action through ERα and ERβ make these receptors both attractive and formidable as targets for therapeutic pharmaceuticals. Success in achieving desired patterns of activity and selectivity have come through design but – in more ways that we might want to admit – also through serendipity.
Disclosure: J. Katzenellenbogen, Nothing to Disclose.

17.3 Acute Estrogen Modulation of Synapses in the Hippocampus

Catherine Woolley*

Northwestern University, Evanston, Illinois

Background: Estrogens influence brain function through multiple mechanisms with time courses ranging from minutes to days. Interest in rapid nongenomic estrogen actions has resurged recently, in parallel with recognition that estrogens are produced as neurosteroids in the brains of both males and females. The hippocampus is among the brain regions now known to contain both the enzymatic machinery to necessary synthesize a key estrogen, 17β-estradiol, as well as extranuclear estrogen receptors (ERs) at or near synapses. This suggests that locally synthesized neurosteroid estradiol could acutely regulate synaptic function in the hippocampus *in vivo*, and may therefore modulate hippocampus-dependent behaviors.
Methods: We have used a combination of electrophysiological recording in hippocampal slices and behavioral studies to investigate mechanisms by which estradiol acutely modulates synaptic function in the hippocampus of adult rats and functional correlates of such modulation.
Results: Electrophysiological studies show that, in females, estradiol rapidly potentiates excitatory synaptic transmission through ERβ activation, and also rapidly suppresses inhibitory synaptic transmission through ERα activation. For both types of synapses, estradiol exerts these effects in females by modulating presynaptic neurotransmitter release probability. In males, estradiol also rapidly potentiates excitatory synaptic transmission through ERβ, but ERα-dependent suppression of inhibitory synaptic transmission is absent in males. In addition, preliminary evidence suggests that ERβ-dependent potentiation of excitatory synapses in males is largely postsynaptic, rather than presynaptic as it is in females. We are now investigating behavioral correlates of acute estradiol modulation of synapses in the hippocampus in both sexes. These studies show that, in females, hippocampal ERβ activation acutely decreases anxiety-like behavior in an open field test with approximately the same time course as its effects on excitatory synaptic transmission in electrophysiological studies. Preliminary analysis of corresponding behavioral tests done in males thus far indicate no acute ERβ modulation of anxiety-like behavior in males.
Conclusions: Together, these contrasts point to both mechanistic and functional sex differences in acute estrogen modulation of synapses in the hippocampus, which may be related to sex differences in the prevalence and/or symptoms of affective disorders.
Disclosure: C. Woolley, Nothing to Disclose.

17.4 Serotonin Transporter Function: Interaction among Ovarian Steroids and Antidepressants

Alan Frazer*

The University of Texas Health Science Center, San Antonio, Texas

Background: The effectiveness on depressed mood of either hormone replacement (HR) or estrogen replacement (ER) therapy during the postmenopausal period is controversial. Using chronoamperometry, we had shown previously that acute systemic administration of either estradiol benzoate (EB) and/or progesterone (P) blocked the ability of SSRIs such as fluvoxamine to inhibit the function of the serotonin transporter (SERT). Also, EB itself, but not P, blocked the function of the SERT. Thus, EB had two effects: (1) a possibly beneficial antidepressant (AD)-like effect in blocking the SERT; (2) a deleterious effect by blocking the inhibitory effect of SSRIs on the SERT. We have now extended these initial observations to understand the mechanisms underlying these effects and to see if the effects observed had behavioral consequences.
Methods: Ovariectomized Sprague-Dawley female rats were used. Estradiol benzoate and/or progesterone were given systemically using a paradigm that produced hormone levels seen during proestrus. Behavioral effects of the hormones themselves or antidepressants were assessed using the forced swim test (FST). Chronoamperometry in the CA3 region of the hippocampus was used to assess the effect of local administration or hormones into this area on the functioning of the serotonin transporter (SERT) and/or on the ability of SSRIs such as fluvoxamine to inhibit the SERT (by measuring the rate of clearance of exogenously administered serotonin). Time course studies, use of hormone-bovine serum albumin (BSA) conjugates and selective hormone receptor agonists and antagonists were used to evaluate the contributions of different types of hormone receptors to the effects observed.
Results: The decreased immobility and increased swimming caused by fluvoxamine in the FST was blocked in rats treated acutely with either EB and/or P. Local application of 17β-estradiol (E2), but not P, slowed the clearance of 5-HT and either hormone blocked the ability of fluvoxamine to inhibit the clearance of 5-HT. The results of the time course studies, and those using hormone receptor agonists and antagonists and hormone-BSA conjugates revealed that the effects of estradiol were mediated by activation of both membrane and nuclear receptors whereas the effect of P is due solely to activation of intracellular P receptors. Further, use of subtype specific receptor agonists indicated that the inhibitory affect of E2 alone on the SERT involved ERβ and GPR30 whereas its blockade of fluvoxamine's effect was mediated by ERα.
Conclusions: Targeting ERβ or GPR30 might be a way to permit beneficial behavioral effects of estrogen without its deleterious effect on the actions of SSRIs.
Disclosure: A. Frazer, **Part 1:** Lundbeck, Takeda, Lilly, Cyberonics, **Part 4:** Lundbeck.

Panel
18. Optimizing Cognitive Interventions for Schizophrenia: Predictive Biomarkers and Pharmacologic Enhancement

18.1 Predictors of Cognitive Improvement after "Neuroplasticity-based" Computerized Cognitive Training in Schizophrenia

Sophia Vinogradov*

University of California, San Francisco, California

Background: Schizophrenia is characterized by dysfunction in the distributed neural systems that subserve auditory processing and verbal memory. In a randomized double-blind controlled trial, we have previously shown that 50 hours of "neuroplasticity-based" cognitive training of auditory and verbal learning processes can drive significant improvement in verbal memory and global cognition in patients. A range of responses to this treatment is observed, with approximately 65% of patients showing an improvement of 0.2 SD or greater on a Global Cognition summary score.
Methods: We report the clinical, pharmacologic, functional neural connectivity, and genetic findings that correlate with the magnitude of the cognitive response to this form of cognitive training.
Results: We find that demographic and clinical factors such as age, IQ, symptom severity, gender, and baseline cognition do not show an association with treatment outcome. However, self-ratings on the Insight into Cognition Scale and the Behavioral Activation Scale do show a positive association with cognitive improvement,

even after controlling for hours of training. Medication-induced anticholinergic burden (serum anticholinergic activity measured via radioreceptor immunoassay) shows a significant negative association with cognitive improvement after training. Reduced resting state functional connectivity in the alpha band in prefrontal and parietal regions (measured via magnetoencephalography) shows a significant correlation with improvement in cognition. Finally, individuals with the A/A genotype of the COMT variant rs165599 show a statistically significant greater improvement in cognition after training than those with the G/G or A/G genotype.
Conclusions: Schizophrenia is characterized by dysfunction in the distributed neural systems that subserve auditory processing and verbal memory. In a randomized double-blind controlled trial, we have previously shown that 50 hours of "neuroplasticity-based" cognitive training of auditory and verbal learning processes can drive significant improvement in verbal memory and global cognition in patients. A range of responses to this treatment is observed, with approximately 65% of patients showing an improvement of 0.2 SD or greater on a Global Cognition summary score.
Disclosure: S. Vinogradov, **Part 1:** I am a consultant to Brain Plasticity, Inc.; Amgen; Genentech; Hoffman-LaRoche.

18.2 Neuroanatomical Predictors of Response to Cognitive Remediation

Matcheri Keshavan*

Harvard Medical School, Boston, Massachusetts

Background: Cognitive deficits strongly predict functional disability in schizophrenia, and can be significantly improved with cognitive remediation approaches. The predictors of response to CR remain poorly characterized. We asked whether a greater neurobiologic reserve, as measured by cortical gray and white matter volumes, will predict a favorable response to Cognitive Enhancement Therapy (CET).
Methods: Early course of schizophrenia or schizoaffective disorder patients were randomly assigned to CET (n = 29) or an Enriched Supportive Therapy (EST) control (n = 21) and treated for two years. Cortical surface area and gray matter volumes (MRI), neurocognition and social cognition data were measured before, and after one and two years of treatment. Moderator analyses examined the impact of pre-treatment cortical surface area and gray matter volume on differential neurocognitive and social-cognitive response to CET.
Results: Pre-treatment, whole brain cortical surface area and gray matter volume significantly moderated the effects of CET on social cognition, but not neurocognition. Greater neurobiologic "reserve" predicted a rapid social-cognitive response to CET in the first year of treatment; patients with less neurobiologic reserve achieved a comparable social-cognitive response by the second year. While several brain regions contributed to this accelerated social-cognitive treatment response, effects were the strongest in the temporal cortex. We also observed that anticholinergic levels at baseline moderated both brain structure and response to CET.
Conclusions: A broad cortical brain reserve is associated with an accelerated social-cognitive response to CET in early schizophrenia; however, the benefits of cognitive rehabilitation may accrue, albeit somewhat later, even in those with less initial cognitive resources.
Disclosure: M. Keshavan, **Part 1:** I have received two grants, one from Sunovion and another from GSK, in the last two years. **Part 4:** I have received two grants, one from Sunovion and another from GSK, in the last two years.

18.3 Combining a Cognitive Enhancer and Cognitive Training: Proof of Principle and Potential Complexities in Real-life

Shitij Kapur*

Institute of Psychiatry, King's College London, London

Background: Improving cognition is the next major challenge in the treatment of schizophrenia. However, several conventional efforts to develop drugs that enhance cognition have failed. Cognitive Remediation (CR) approaches have shown modest success - but are not widely available or adopted. There is growing recognition that a different approach (rather than just different drugs) may be needed. Learning requires the identification of specific circuits and an enhancement of their synaptic strengths. By combining cognitive training/remediation which engages specific and relevant circuits, and pairing it with neurochemical modulators that might enhance synaptic strengths, it may be possible to increase the rate and amount of learning. Such a paradigm might be applied towards enhancing the therapeutic impact of cognitive interventions in schizophrenia patients.
Methods: To explore this principle, we completed a double-blind, randomised trial in healthy individuals (n = 33) using the combination of modafinil (200 mg) or placebo with a selection of cognitive learning tasks (word list learning, new language learning, and attentional training tasks). Subjects received training and drug/placebo concomitantly over 14 days; additional tests examined the generalizability of effects to general test batteries, and whether these effects lasted 2 weeks after exposure.
Results: All subjects improved with training. However, those given modafinil showed significantly improved early learning in word-list recall and steeper learning curves on an implicit language learning task. Moreover, when tested again after a two-week period of no-training and no-modafinil, the group that received modafinil during learning maintained its relative advantage.
Conclusions: Together, these data suggest that combining a cognitive enhancer with training may improve learning; may do so over a short time period; and may confer retention of gains. A similar study in schizophrenia patients is now underway. If this approach is successful in schizophrenia - taking it from the experimental to clinical stage will require addressing a number of practical and regulatory issues, which will be addressed in the talk.
Disclosure: S. Kapur, **Part 1:** Has served as a one-off consultant and/or speaker for AstraZeneca, Bioline, Bristol Meyers Squibb, Eli Lilly, Envivo, Janssen - Johnson and Johnson, NeuroSearch, Otsuka, Pfizer, Sanofi-Aventis, Schering-Plough, Servier, Solvay Wyeth and serves on the Scientific Advisory Boards for Lundbeck and Roche, **Part 2:** GSK, Lundbeck.

18.4 Memory Consolidation Deficits in Schizophrenia and the Combination of D-cycloserine with Cognitive Remediation

Donald Goff*

Nathan Kline Institute, New York, New York

Background: We have demonstrated deficits in memory consolidation in schizophrenia subjects following training on a procedural memory task and following extinction of conditioned fear. The deficit in consolidation of procedural memory was associated with a reduction in sleep spindles (Manoach et al, 2010) and the deficit in recall of fear extinction was associated with a failure to activate the medial ventral prefrontal cortex (Holt et al, 2012). These deficits might be expected to impede the therapeutic impact of cognitive interventions in schizophrenia; conversely, medications that mitigate these deficits might be expected to augment the benefits of cognitive therapies. Consistent with a large animal literature in which D-cycloserine enhances memory consolidation, we demonstrated that a single dose of D-cycloserine selectively improved recall after 7 days for themes on the Logical Memory Test (Goff et al, 2008) and, in a preliminary trial, found that the combination of D-cycloserine with two sessions of a CBT exercise significantly reduced severity of delusions (Gottlieb et al, 2011).
Methods: In this new study, we combined once-weekly D-cycloserine with the "Brain Fitness" cognitive remediation (CR) program in an eight-week placebo-controlled trial in 40 schizophrenia subjects.
Results: D-cycloserine significantly improved learning of the auditory discrimination task compared to placebo, but no improvement was found in performance on the MATRICS

cognitive battery. Improvement on the auditory discrimination task with D-cycloserine was predicted by genotypes for BDNF, COMT and G72.
Conclusions: In summary, memory consolidation is impaired in schizophrenia and, in 2 studies, improved with D-cycloserine. The enhancement by D-cycloserine of learning appears to be task-specific rather than generalizable to other cognitive domains. Amelioration of deficits in memory consolidation and learning could substantially improve outcomes in cognitive behavioral therapy and rehabilitation.
Disclosure: D. Goff, **Part 1:** In the past 2 years Dr. Goff has received honoraria for consulting or speaking from Hoffman-La Roche, Eli Lilly, Takeda, Dainippon Sumitomo, Endo Pharmaceuticals, Janssen, Cypress Bioscience, Bristol Myer Squibb, Abbott Laboratories and served on a DMC for Otsuka, **Part 4:** Dr. Goff received research funding from Pfizer, GlaxoSmithKline, Janssen, Novartis and PamLab.

Panel
19. The Developmental Trajectory of Cannabis Effects on Neurobiological Functioning (In Response to RFP on Changes in the Adolescent Brain during Development and in Neuropsychiatric Disorders)

19.1 Cannabis and the Adolescent Brain: Differentiating Vulnerability from Pathology
Dan I. Lubman*

Turning Point Alcohol and Drug Centre, Monash University, Melbourne, Australia

Background: There is growing evidence that regular cannabis use is associated with alterations in regional brain volumes. While it is long-term heavy use that appears to be most related to structural brain changes, recent studies among adolescent cannabis users suggest that the adolescent brain may be particularly susceptible to damage. Although these changes are frequently attributed to the neurotoxic effects of cannabis, it is possible that some abnormalities predate use and represent markers of vulnerability. To date, no prospective studies have examined whether structural brain abnormalities are present prior to the onset of use in adolescence.
Methods: As part of a larger study examining adolescent emotional development, we examined whether individual differences in brain volume at age 12 were associated with early use of cannabis. Specifically, we examined whether adolescents who had initiated cannabis use early (i.e., prior to age 17) showed premorbid structural abnormalities in the amygdala, hippocampus, orbitofrontal cortex (OFC), and anterior cingulate cortex (ACC). Participants underwent structural magnetic resonance imaging at the baseline assessment (M age = 12.6 years), and were assessed for cannabis use at follow-up four years later (M age = 16.5 years).
Results: Of the final sample of 121 adolescents (48.8% female) who completed these measures at both time points, 28 (23.1%) reported ever using cannabis. Using logistic regression, adolescents who reported initiating cannabis use by the follow-up assessment were found to have smaller baseline volumes of the right lateral OFC ($p = .029$), compared to adolescents who had not initiated cannabis use by this time point. For both analyses, these results remained significant even after controlling for lifetime use of other substances and substance use prior to baseline assessment.
Conclusions: These results suggest that some structural abnormalities observed in cannabis users may exist prior to exposure, with alterations in the OFC potentially influencing risk for early cannabis use. The study also supports recent findings that structural changes in the amygdala and hippocampus are a consequence of chronic cannabis exposure rather than a premorbid vulnerability. Together, these findings highlight the need for further prospective studies that examine the influence of neurobiological factors on adolescent substance use.
Disclosure: D. Lubman, **Part 1:** Honorarium for lectures (Astra Zeneca, Janssen).

19.2 Trajectory of Adolescent THC Exposure on Mesocorticolimbic Molecular, Epigenetic and Structural Modifications: Transgenerational Effects
Yasmin Hurd*

Mount Sinai School of Medicine, New York, New York

Background: Adolescence is a critical phase of brain development sensitive to multiple factors including drug exposure. Marijuana (Cannabis sativa) is the most frequently used illicit drug by teenagers with as much as 20% of 16-year olds in the US reporting use. Despite epidemiological evidence of an association between adolescent cannabis use and subsequent abuse of drugs such as heroin and cocaine, far less is known about the neurobiological basis of such apparent vulnerability. To address such gaps of knowledge, we used rodent models to directly evaluate specific long-term behavioral and neurobiological impact of D9-THC (psychoactive component of cannabis) relevant to addiction vulnerability. We evaluated behaviors relevant to traits relevant to addiction including reward, impulsivity and decision making and also evaluated mesocorticolimbic structures such as the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC) highly implicated in addiction.
Methods: Adolescent rats (postnatal day 28-49) were treated with periodic, moderate dose of THC (1.5 mg/kg every 3rd day) and studied in adulthood for effects on impulsivity (intolerance to delay), decision-making, heroin self-administration and locomotor activity as well as mRNA expression, histone modifications (chromatin immunoprecipitation; ChIP) and dendritic morphology (Lucifer yellow cell loading) in mesocorticolimbic brain areas.
Results: Consistent with previous findings, adolescent THC exposure was associated with enhanced heroin intake behavior in adulthood as well as deficits in inhibitory control. In addition, there were specific molecular alterations. For example, mRNA levels of the proenkephalin (PENK) opioid neuropeptide and dopamine D2 genes, which are coexpressed in the corticostriatopallidal No/Go circuit, were preferentially altered in the NAc of adults with adolescent exposure. No significant disturbance was evident for the prodynorphin opioid neuropeptide and dopamine D1 receptor genes, preferentially expressed in the striatonigral pathway. Moreover, selective knockdown of the PENK gene in the NAc by lentiviral manipulation reduced THC-induced enhancement of heroin self-administration behavior. ChIP analyses revealed significant enduring dysregulation of repressive marks such as H3K9me2 at the promoters of THC-altered genes. Adolescent THC was also associated with structural changes in the complexity of dendritic branching of pyramidal cells in the mPFC. In fact, adolescent THC exposure altered the normal developmental trajectory of cortical branching in both the pyramidal apical and basal trees. Molecular disturbances were also detected in the expression levels of glutamatergic genes as well as those associated with axonal branching such as stathmin-2. Intriguingly, adult offspring of animals mated in adulthood following adolescent THC exposure also showed glutamatergic disturbances by such germline exposure.
Conclusions: Overall, these findings demonstrate that adolescent THC exposure directly influences the development of mesocorticolimbic neuronal systems relevant to impulsivity, reward and goal-directed behavior. Moreover, THC exposure impacts the normal epigenetic landscape of adolescent neurodevelopment via dysregulation of repressive histone H3 methylation that may underlie the long-term behavioral consequences of adolescent THC and could influence the progression to drug abuse and even transgenerationally. Discussion of these findings will also be placed in the framework of new data obtained in human subjects that polymorphisms of genes identified from the rodent model are associated with cannabis dependence and behavioral traits relevant to addiction vulnerability.
Disclosure: Y. Hurd, Nothing to Disclose.

19.3 Δ9-tetrahydrocannabinol Impairs Reversal Learning and Visuo-spatial Associative Memory in Rhesus Macaques

Michael Taffe*

The Scripps Research Institute, La Jolla, California

Background: Human adolescence is an interval in which critical aspects of formal education often coincide with the initiation of recreational drug use. This elevates interest in the specific cognitive effects of drugs such as Δ9-tetrahydrocannabinol (Δ9-THC), the major psychoactive constituent of marijuana. Controlled laboratory studies in nonhuman primates provide mixed evidence for specific effects of Δ9-THC in learning and memory tasks, with a suggestion that frontal-mediated tasks may be most sensitive. Prior studies in monkeys have not shown consistent evidence of memory specific effects of Δ9THC on recognition tasks and it remains unclear to what extent Δ9-THC causes sedative versus specific cognitive effects.
Methods: In this study, adult male rhesus monkeys were trained on tasks which assessreversal learning, extradimensional attentional shifs, spatial delayed-response, spatial working memory, visuo-spatial associative memory and learning as well as motivation for food reward. Subjects were subsequently challenged with 0.1-0.5 mg/kg Δ9THC, i.m., in randomized order and evaluated on the behavioral measures.
Results: Peak plasma levels of Δ9-THC were observed 30 min after 0.2 mg/kg (69 ± 29 ng/ml) and 60 min after 0.5 mg/kg (121 ± 23 ng/ml) was administered; motor slowing on a bimanual motor task persisted for up to 2 hrs after injection. The tasks which assess visuo-spatial associative memory and spatial working memory were impaired by Δ9-THC in a dose and task-difficulty manner. An increase in errors-to-criterion in reversal learning was caused by Δ9-THC in a dose-dependent manner however discrimination learning and extradimensional shifts were not affected by Δ9-THC. Spatial delayed-response performance was impaired by Δ9-THC in a retention-interval dependent manner. It is concluded that Δ9-THC disrupts cognition in a way that is consistent with a direct effect on memory. There was evidence for interference with spatial working memory, visuo-spatial associative memory and incremental learning in the latter task. Reversal learning was affected whereas extra-dimensional shift and discrimination learning was spared.
Conclusions: These results and the lack of specific effect of Δ9-THC in prior visual recognition studies imply a sensitivity of executive functions, spatial memory processing and working memory to endocannabinoid perturbation. Overall the pattern of results suggest a more profound effect of Δ9-THC on tasks mediated by parahippocampal mechanisms and by orbitofrontal versus dorsolateral prefrontal mechanisms.
Disclosure: M. Taffe, Nothing to Disclose.

19.4 Effects of Cannabis Abuse on the Functional Brain Architecture for Visual Learning and Recognition Memory: Psychopharmacological and Developmental Evidence before and after Sustained Abstinence

Frank Haist*

University of California, San Diego, California

Background: Cannabis is the most widely used illicit drug in the U.S., yet little is known about the neurocognitive consequences of excessive cannabis use. The psychoactive component of cannabis, Δ9THC, impairs learning, memory, and executive functions. Here, using functional MRI (FMRI) we evaluated brain activity while cannabis dependent (CD) adults, heavy cannabis using (CU) adolescents, and matched control participants, performed a visual learning and recognition memory task at two times; the day of abstinence initiation (D1) and 28 days after monitored abstinence (D28). The CD adults were divided into two groups to consider functional correlates of precipitated withdrawal, one receiving CB_1 antagonist Rimonabant (RIM) and the other receiving placebo. We examined developmental factors of cannabis abuse and functional recovery after abstinence.
Methods: *Adults*: We tested 24 CD adults (18-30 years) and 15 non-cannabis using subjects. Ten CD adults received the CB_1 antagonist Rimonabant (RIM) and 14 received placebo. *Adolescents*: We tested 11 CU adolescents (15-17 years) and 11 non-cannabis using subjects. *Task*: The FMRI visual paired associate learning and memory task (PAL) was modeled on the CANTAB neuropsychological test. Participants were shown 3, 4, or 6 nonverbal symbols in one of six spatial locations during the ENCODE phase. Participants were next shown symbol pairs and asked to make recognition judgments (RECOG) indicating which symbol had been shown in that location. The task was administered during FMRI scanning at D1 and D28.
Results: CD adults showed significant recognition memory impairment in the hardest condition (6 items). RIM plasma levels at D1 were positively correlated with FMRI signal in the right hippocampus and bilateral entorhinal cortex during ENCODE, suggesting a link between withdrawal and abnormal use of declarative memory. At D1, control subjects showed extrastriate and frontoparietal activity during ENCODE and RECOG indicating reliance on a visuoperceptual working memory strategy. The CD group showed reduced activity in these networks and increased activity in medial temporal lobe regions during RECOG. We observed no reliable changes from D1 to D28 in the adult CD participants. Preliminary findings from CU adolescents are similar to the CD adults at D1, but CU showed some recovery of activity at D28.
Conclusions: CD adults show behavioral memory impairment and functional brain activation changes suggesting altered memory systems related to cannabis use. The brain activity changes show little evidence of recovery after 28 days of abstinence. In contrast, adolescent cannabis users show some recovery of function. There appears to be evidence for a developmental trajectory for recovery of neurocognitive function with heavy cannabis use. The analysis of precipitated withdrawal using RIM showed increased changes in medial temporal lobe memory system functions related to withdrawal.
Supported by NIDA P20 DA024194 and NICHD R01 HD060595
Disclosure: F. Haist, Nothing to Disclose.

Panel
20. Mullti-level Classification of Schizophrenia and Bipolar Disorder: New Evidence and Controversies

20.1 Cognitive Heterogeneity in Bipolar Disorder: Implications for Overlap with Schizophrenia

Katherine E. Burdick*

Mount Sinai School of Medicine, New York, New York

Background: Bipolar disorder (BPD) is diagnostically distinct from schizophrenia (SZ); however, there are several phenotypic similarities. In addition to overlapping affective and psychotic symptoms, recent evidence suggests that neurocognitive deficits in attention, verbal learning, and executive function are core features of both BPD and SZ (Hill et al, 2009). In addition to a partial overlap in clinical and cognitive features, evidence is converging at the molecular level to suggest shared pathophysiology. BPD and SZ are highly heritable disorders once believed to represent distinct disease processes with unique etiologies; however, compelling data from a recent, large-scale, study support a significant comorbidity between BPD and SZ within families, largely explained by additive genetic effects common to both disorders (Lichtenstein et al, 2010). Moreover, consortium-based genome-wide association studies (GWAS) have now identified replicable risk loci in SZ and in BPD (Ripke et al, 2011; Sklar et al, 2011), the majority of which show trans-disorder effects (Williams et al, 2011). Genomic risk factors other than SNP-based allelic variation have also been shown to influence risk for both BPD and SZ, including copy number variants (CNVs; Walsh et al, 2008; Zhang et al, 2009; Malhotra et al, 2011) and aggregate risk scores for multiple

common alleles with very small effect (polygenic score; Purcell et al, 2007). These data suggest partial overlap in the genetic architectures of BPD and SZ but they do not imply that these disorders are a unitary condition. Rather, these findings support the likelihood that genetic variants that influence risk for both illnesses do so via their effects on shared phenotypes (e.g. psychosis, affective symptoms, and cognitive deficits). Here we will focus on neurocognitive dysfunction as a shared trait of critical importance. Although the pattern of cognitive impairment in BPD is qualitatively similar to that seen in patients with SZ, effect sizes suggest that the deficits in SZ are approximately twice as severe as those noted in BPD. An important consideration which we will address in the current session is the frequency of cognitive impairment in BPD and the cognitive heterogeneity that exists relative to the somewhat more uniform presentation seen in SZ. Recent data suggest that ~40-60% of BPD patients are impaired at a level that would be considered clinically-relevant (> 1 SD below average), but a substantial proportion are characterized as cognitively-spared (Martino et al, 2008; Bora et al, 2010). In contrast, the vast majority of patients with SZ display pronounced deficits (Reichenberg et al, 2009) and few, if any, are considered neuropsychologically "normal" (Wilk et al. 2005).
Methods: This presentation will highlight new data derived from a large, well-characterized, sample of more than 80 sibling pairs discordant for BPD which allowed for subgrouping based on neurocognitive performance both in the probands and in their unaffected siblings. Distributions were evaluated and subgroups were data-driven. Neurocognitive measures encompass all domains and subjects were carefully diagnosed (SCID) and clinically characterized using standardized measures.
Results: Results suggest that: a) the distributions of neurocognitive functioning in BPD is bimodal - there exists a subgroup of BPD patients who experience clinically significant cognitive impairment strongly resembling that noted in SZ (cognitively-impaired) and a discrete proportion of patients (~40%) who perform within the normal range (cognitively-spared); b) there are specific clinical features of BPD that contribute to the degree of cognitive impairment and may be useful predictors of cognitive group membership regardless of DSM-IV diagnosis; and c) based on familial subtypes, there are likely to be tractable molecular markers of neurocognitive dysfunction in BPD patients that have yet to be identified.
Conclusions: The characterization of cognitive subgroups in BPD may provide important insight into the phenotypic and genetic overlap between SZ and BPD. Moreover, identifying clinical and molecular predictors of cognitive dysfunction could guide novel interventions and/or prevention strategies for treating these disabling symptoms.
Disclosure: K. Burdick, Nothing to Disclose.

20.2 Cognitive and Functional Deficits in Schizophrenia and Bipolar Disorder Vary by Psychosis Presence and History

Christopher R. Bowie*

Queen's University, Kingston, Ontario, Canada

Background: Deficits in neurocognitive functions are known to be common and functionally disabling in schizophrenia (SZ). A number of recent studies suggest that a similar profile of impairments is observed in bipolar disorder (BPD) yet the magnitude of cognitive deficits is typically modest and functional deficits lack the depth and breadth in comparison to SZ. The considerable conceptual, etiological, and symptom overlap between these chronic mental disorders requires comparisons beyond the diagnostic boundaries.
Methods: In this study, we examined neurocognitive profiles and functional impairments in SZ and BDP as a function of the presence of psychotic symptoms at the time of assessment.
Results: Results suggest that psychosis is at least as strong a predictor of neurocognitive impairment as diagnostic status. Information processing speed and vigilance were more impaired in BPD subjects with current psychosis than SZ subjects. Interestingly, the presence of psychosis had strong associations with real world functional behaviors, but laboratory-based measures of functional competence were more sensitive to diagnostic groups.
Conclusions: These findings suggest that the categorical distinction between diagnostic groups may obfuscate symptom-specific impairments in neurocognition and functioning.
Disclosure: C. Bowie, **Part 1:** I have been a consultant and advisory board member for Abbott Pharmaceuticals from 2010 to present.

20.3 MRI Can Be Used to Differentiate Schizophrenia Patients from Those with Bipolar Disorder: Clinical and Theoretical Implications

Rene Kahn*

The University Medical Center Utrecht, Utrecht, Netherlands

Background: The question of whether brain changes in schizophrenia and bipolar disorder are similar is not only relevant regarding their possible genetic and biological overlap; it also has clinical and diagnostic implications. Here we address the issue of whether brain volumes can be used to differentiate the two disorders. In a recent study we built a structural-MRI-based schizophrenia classification model and tested its predictive capacity in an independent test sample (Nieuwenhuis et al, 2012). Using two large data sets, we confirmed the feasibility to use structural MRI for individualized prediction whether a subject is a schizophrenia patient or a healthy control, with an accuracy of 70.4%. Although scientifically interesting, the clinical use is limited: these classification models become really useful if they can predict a subject's future status, or its current status if this cannot be determined by other means.
Methods: Structural magnetic resonance whole brain images of 66 patients with schizophrenia, 66 with bipolar disorder (41 using lithium), and 66 healthy controls were used to train a linear Support Vector Machine (SVM) (Vapnik, 1999) to separate the three groups. Age was matched between the groups. The input features, used to train the models, were so-called gray matter densities (used in voxel based morphometry (VBM)). To test the reliability of the models, we applied leave-one-out (LOO) cross-validation.
Results: Schizophrenia patients could be correctly classified versus healthy subjects with an accuracy of 86%; they could be differentiated from bipolar patient with the same level of accuracy, i.e. also 86%. The model separating bipolar patients from healthy control subjects performed worse: 75% of the healthy subjects were correctly classified and only 58% of the bipolar patients. There was no significant difference in performance between lithium-users and non-users.
Conclusions: We demonstrated the feasibility to use structural MRI for individualized prediction whether a subject is a schizophrenia or a bipolar patient, with an accuracy of 86%. While the use of MRI to separate schizophrenia patients from healthy subjects has been shown before and has limited clinical value, the accurate separation of schizophrenia patients from bipolar patients could become a diagnostic aid for psychiatrists. The results also indicate that the gray matter pathology differs between schizophrenia and bipolar disorder to such an extent that they can be reliably differentiated using machine learning paradigms. Nieuwenhuis et al 2012, NeuroImage, in press. Vapnik,V.N. 1999. IEEE Trans Neural Netw. 10, 988-999.
Disclosure: R. Kahn, **Part 1:** AstraZeneca, BMS, Lilly, Otsuka, Roch, Sunovion.

20.4 Developmental Trajectories in Schizophrenia and Bipolar Disorder: Evidence for Distinct Etiologies

Michael Davidson*

Tel-Aviv University, Tel Aviv

Background: Cognitive impairment is ubiquitous in patients with schizophrenia and is considered core to the pathophysiology of the illness. Premorbid childhood and adolescence cognitive deficits that precede the appearance of adult schizophrenia have been consistently documented, and have been interpreted as supporting

a neurodevelopmental model of schizophrenia. We investigated two unresolved questions about premorbid cognitive deficits: (a) Are premorbid cognitive deficits specific to schizophrenia or shared by bipolar disorder?; (b) What is the etiology of the relation between premorbid cognition, schizophrenia and bipolar disorder?
Methods: Participants were members of the Israeli Conscripts Cohort – Schizophrenia and Bipolar Study, a population based birth cohort of more than 500,000 individuals with cognitive assessment completed at age 17 who are continuously followed up for psychiatric morbidity using national registers. We compared cognitive functioning in three groups of adolescents: those who developed schizophrenia, bipolar disorder and healthy controls. 7,000 twin pairs were similarly followed and allowed testing hypotheses about the etiological relation between schizophrenia, bipolar disorder and impaired cognition.
Results: Adolescents who developed adult schizophrenia exhibited developmental deficits on tests indexing processing speed, reasoning and knowledge acquisition. These patterns were not observed in adolescents who later developed psychotic bipolar disorder. In contrast, adolescents who later developed non-psychotic bipolar disorder exhibited superior performance. The relationship between adolescent cognitive impairment and schizophrenia was due to a sub-group with familial developmental cognitive impairment. Twin modeling revealed that in this sub-group the co-occurrence of developmental cognitive impairment and schizophrenia was due to shared genetic factors.
Conclusions: Schizophrenia and bipolar disorder present distinguishable developmental etiologies. The origins of the cognitive impairment in schizophrenia involves at least two developmental processes.
Disclosure: M. Davidson, Nothing to Disclose.

Panel
21. Immune Modulation of Neurodevelopment in Schizophrenia and Autism

21.1 Novel Roles for Immune Molecules in Early Postnatal Cortical Development: Implications for Schizophrenia and Autism Spectrum Disorders

Kimberley McAllister*

The University of California, Davis, California

Background: The focus of the panel is on novel findings from clinical, basic neuroscience, and epidemiologic research that suggest important roles of in utero infection and immune disruptions during early development in the risk of schizophrenia and autism.
Methods: Pregnant mice were injected with saline or poly(I:C) on E12.5 and postnatal offspring examined at 5 postnatal ages. Tissue was obtained from these mice at several ages and processed for electron microscopy (EM) or immunocytochemistry (ICC). Cytokines were measured using 23-plex Luminex chips. In addition, neurons were cultured from prefrontal cortex of newborn mice and treated with severalcytokines. Changes in synapse density, dendritic complexity, and surface expression of MHCI were assessed using EM and ICC.
Results: Schizophrenia (SZ) and autism spectrum disorders (ASD) are complex diseases likely caused by a combination of genetic and environmental factors during early development. Recent work points to a central role for immune-related genes and immune responses to environmental stimuli in SZ and ASD, and specifically for maternal infection during early gestation as a risk factor for both disorders. The development of a mouse model of maternal infection with strong face and construct validity for SZ and ASD has strengthened the link between maternal immune activation (MIA) and many of the abnormal behaviors and neuropathologic findings characteristic of these disorders. Results from this MIA mouse model and recent human studies have converged on the hypothesis that MIA causes a chronic immune-dysregulated state in the offspring that alters brain development and behavior. In this talk, I will present recent data showing supporting this hypothesis.
Conclusions: Our results are providing novel insights into how immune molecules regulate cortical development and how alterations in the expression of these immune molecules might contribute to neurodevelopmental disorders, including SZ and ASD. Our research is currently aimed at testing the hypothesis that MHCI proteins mediate the effects of MIA-induced changes in cytokines on brain development and behavior and determining the mechanism used by MHCI to limit cortical synaptogenesis.
Disclosure: K. McAllister, Nothing to Disclose.

21.2 Neuroimmune Changes in a Mouse Model of the Maternal Infection Risk Factor for Schizophrenia and Autism

Paul H. Patterson*

California Institute of Technology, Pasadena, California

Background: Several types of viral and bacterial maternal infections during pregnancy are associated with increased risk for schizophrenia or autism in the offspring. Modeling this risk factor in mice using influenza infection, or activation of the dam's immune system by injection of the synthetic RNA, poly (I:C), or the cytokine interleukin-6 (IL-6) yields offspring with characteristic endophenotypes of these disorders. Maternal immune activation (MIA) by injection of poly (I:C) activates downstream IL-6 signaling pathways in the placenta, leading to changes in its endocrine functions. MIA also activates IL-6 signaling in subpopulations of neurons in the fetal brain. In addition, IL-6 mRNA is induced in the fetus, raising the possibility of a feed-forward mechanism that could lead to permanent changes in immune status as is seen in the brain and peripheral immune system of schizophrenia cases.
Methods: Pregnant mice are injected with saline, poly (I:C), or IL-6, on E12.5, or given an intranasal influenza infusion on E9.5, and the fetuses and postnatal offspring examined at various ages.
Results: Transcriptome profiling of the fetal brain response to either poly (I:C), IL-6 or flu infection reveals an acute, transient up-regulation of 5 members of the crystallin gene family. Furthermore, levels of crystallin gene expression are correlated with the severity of MIA as assessed by placental weight. Crystallins are considered to be neuro-protective, anti-inflammatory factors in other contexts. In support of the possibility of a feed-forward mechanism that could lead to permanent changes, cytokine levels in the MIA postnatal brain undergo changes in levels that are region-and age-specific. Also of interest is our finding that MIA leads to a significant deficit in systemic regulatory T cells and elevated granulocyte levels. In addition, both fetal and adult MIA hematopoietic stem cells exhibit altered lineage potential and differentiation. Furthermore, behaviorally-abnormal, adult MIA offspring that have been irradiated and transplanted with immunologically normal bone marrow no longer exhibit several abnormal behaviors, suggesting that the immune changes in MIA offspring can contribute to these behaviors.
Conclusions: These observations indicate that MIA causes permanent changes in immune status of the offspring. They also suggest a link between cellular immune dysregulation and behavioral abnormalities in a mouse model of a schizophrenia and autism risk factor.
Disclosure: P. Patterson, Nothing to Disclose.

21.3 Elevated Maternal C-Reactive Protein and Autism in a National Birth Cohort

Alan Brown*

Columbia University College of Physicians and Surgeons, New York, New York

Background: Autism is a complex neuropsychiatric syndrome with a largely unknown etiology. Inflammation during pregnancy may represent a common pathway by which infections and other insults cause the disorder.
Methods: We investigated the association between early gestational C-reactive protein (CRP), an established inflammatory biomarker,

prospectively assayed in maternal sera, and childhood autism in the Finnish Prenatal Study of Autism (FiPS-A), a large national birth cohort (N = 1.6 million pregnancies). The FiPS-A is based on a nested case-control design. The sampling frame consisted of all offspring born in Finland from 1987-2005, and subjects were followed up until 2007. All offspring were derived from the Finnish Maternity Cohort, which consists of virtually all pregnancies with archived serum specimens from the first and early second trimesters (one per pregnancy) beginning in 1983. The Finnish Hospital/Outpatient Discharge Registry was used to identify all cases with childhood autism (ICD-10 F84.0). Cases (N = 677) were matched 1:1 to controls from the birth cohort who were without ASD or severe/profound mental retardation on date of birth, sex, birthplace, and residence in Finland. CRP was quantified by a latex immunoassay.
Results: The analysis revealed a significant association between increasing maternal CRP and risk of autism in the offspring (OR = 1.12, 95% CI = 1.02-1.24, p = .02). We observed an 80% increase in risk of childhood autism (OR = 1.80, 95% CI = 1.09-2.97, p = .02) following exposure to elevated maternal CRP, defined *a priori* as a CRP level in the top decile (> 9.55 mg/dl).
Conclusions: Elevated maternal CRP during pregnancy is related to an increased risk of autism in offspring. This exposure may represent a final common pathway by which infections, other inflammatory insults, and the cytokine response, elevate risk for autism and these outcomes. The present investigation may stimulate work on possible molecular mechanisms by which elevated CRP disrupts placental function and alters fetal brain development. These findings may also have important implications for prevention of autism.
Disclosure: A. Brown, Nothing to Disclose.

21.4 Neuroimmune Changes in the Brain of Subjects with Schizophrenia or Autism

Karoly Mirnics*

Vanderbilt University, Nashville, Tennessee

Background: The focus of the panel is on novel findings from clinical, basic neuroscience, and epidemiologic research that suggest important roles of in utero infection and immune disruptions during early development in the risk of schizophrenia and autism. Both schizophrenia and autism show strong heritability, with clear and distinct genetic contributions to the pathophysiology of these syndromes. However, it is also clear that environmental influences contribute to the emergence of these disorders. Importantly, the interplay of genetic and environmental factors results in complex immune system activation in the brain, and neuroimmune dysfunction is potentially related to symptomatology.
Methods: We performed multiple gene expression profiling experiments of subjects with schizophrenia and autism. The experiments were performed on matched postmortem brains and focused on prefrontal and temporal cortex. The transcriptome profiling experiment were carried out using prefabricated and custom-designed oligonucleotide DNA microarrays, normalized by RMA, and analyzed at the level of "most changed genes" and "most changed pathways". The outcomes of these brain transcriptome profiling experiments were verified by qPCR.
Results: These findings suggest that 1) a long-lasting and correlated signature of an early environmental insult during development actively contributes to the pathophysiology of both of these disorders, 2) the neuroimmune disturbances represent an active and ongoing process throughout the lives of patients 3) the neuroimmune changes observed in these two disorders have distinct signatures.
Conclusions: Conceivably, the observed disease-specific immune-related disturbances in the brain are a result of genetic-environmental interactions, in which the underlying genetic component specifies the nature of the immune dysfunction.
Disclosure: K. Mirnics, Nothing to Disclose.

Panel
22. New Perspectives on the Role of Glutamatergic Neurotransmission in Alcoholism and Drug Addiction

22.1 Translational Support for the Glutamate Hypothesis of Addiction

Derik Hermann*

Central Institute of Mental Health, Mannheim, Germany

Background: In addiction, excessive glutamatergic neurotransmission has long been implicated in the acute withdrawal syndrome and as a key signal for dependence-related neuroplasticity. Even if acute withdrawal symptoms have subsided, a destabilization of glutamatergic neurotransmission may contribute to increased stress sensitivity, negative emotional states and consecutive relapse. Identifying patients fitting into this model may hold the potential to increase the response rate to drugs targeting glutamatergic neurotransmission like acamprosate.
Methods: We measured brain glutamate levels during detoxification in alcohol dependent patients (N = 47) and in healthy controls (N = 57) as well as in a rat model of alcoholism by state-of-the-art ^{1}H-magnetic magnetic resonance spectroscopy (MRS) at 3 and 9.4 T, respectively. In a clinical study in alcohol-dependent patients (N = 380) data of withdrawal severity were reanalysed, and correlated to abstinent duration and placebo-controlled acamprosate treatment. Furthermore, MRS was performed in opioid dependent patients (N = 17) and related to addiction history data.
Results: We found significantly increased glutamate levels during acute alcohol withdrawal in corresponding prefrontocortical regions of treatment seeking alcoholic patients and alcohol dependent rats versus respective control subjects. The augmented spectroscopic glutamate signal was likely related to increased glutamatergic neurotransmission, because, enabled by the high field strength of the animal scanner, we detected a profoundly elevated glutamate/glutamine ratio indicating withdrawal-induced impairment of the glutamate-glutamine cycle between neurons and astroglia, a mechanism that contributes importantly to the synaptic glutamate pool. All dependence induced metabolic alterations normalize within a few weeks of abstinence in both humans and rats. In the clinical study, patients requiring pharmacological treatment of alcohol withdrawal symptoms relapsed earlier in comparison to patients not requiring medication. Acamprosate failed to improve abstinence duration in both patients groups in comparison to placebo treatment. In opioid maintenance patients glutamate in the anterior cingulate cortex was positively associated with the number of previous withdrawals.
Conclusions: Our data support the glutamate hypothesis of alcoholism by corroborating elevated glutamate levels during acute withdrawal and an association of markedly withdrawal symptoms to shorter abstinence duration. However, we failed to identify acamprosate responders. The association of the number of previous withdrawals and glutamate indicate a destabilization of the glutamate system driven by the frequency of experiencing withdrawal symptoms.
Disclosure: D. Hermann, Nothing to Disclose.

22.2 The Effects of Chronic, Heavy Alcohol and Cocaine Use on Glutamatergic Gene Expression in Postmortem Human Hippocampus

Mary-Anne Enoch*

National Institutes of Health, Rockville, Maryland

Background: Chronic exposure to heavy alcohol or drug use is known to result in widespread neuronal adaptations. Many of the symptoms of addiction are due to the resulting imbalance in neuronal excitation and inhibition. We sought to detect changes in expression of genes encoding glutamate receptors and transporters that might be specific to alcohol or cocaine exposure or might reflect shared pathways in addiction. We focused on the hippocampus, a brain region that is a constituent of the memory/

conditioning neuronal circuitry of addiction that is considered to be important in reinforcement behaviors in animals and craving and relapse in humans. We have previously performed a similar analysis of GABAergic pathway genes in the same human samples.
Methods: Using RNA-Seq we quantified mRNA transcripts in postmortem total hippocampus from eight alcoholics, eight cocaine addicts and eight controls, all male. An analysis of the 28 genes expressed in the hippocampus that encode glutamate ionotropic (AMPA, kainate, NMDA) and metabotropic receptor subunits, together with glutamate transporters was undertaken.
Results: The alcoholics showed FDR corrected ($p < 0.05$) up-regulation of six genes relative to controls and cocaine addicts: GRIA4 (encoding the AMPA subunit GluA4); GRIK3 (kainate receptor subunit GluR7); GRIN2D (NMDA receptor subunit GluN2D); and GRM1, GRM3 and GRM4 (metabotropic receptor subunits mGluR1, mGluR3 and mGluR4 respectively). Both alcoholics and cocaine addicts showed up-regulation of GRIN2B ($p = 0.008$) that encodes the NMDA receptor subunit GluN2B but the effect was greater in cocaine addicts. The only finding unique to cocaine addicts was down-regulation of GRIN3A that encodes the NMDA receptor subunit GluN3A. Finally, SLC1A3 that encodes the glial high affinity glutamate transporter EAAT1 (GLAST) was down-regulated in the alcoholics.
Conclusions: Our study has shown that, at least in the hippocampus, the effect of chronic, heavy alcohol use is largely to up-regulate genes encoding subunits of all four groups of glutamate receptors whereas the effect of chronic cocaine exposure appears to be more limited. It is possible that the NMDA GluN2B receptor subunit might be implicated in a common pathway in addiction. In contrast, our earlier study showed that there were specific and common effects of both chronic alcohol and cocaine exposure on multiple GABAergic genes and this was predominantly down-regulation of expression. These opposing effects might be expected since glutamate and GABA are respectively the major excitatory and inhibitory neurotransmitters.
Disclosure: M. Enoch, Nothing to Disclose.

22.3 Adaptations of Glutamatergic Transmission in Extended Amygdala in Stress and Reward

Danny Winder*

Vanderbilt University School of Medicine, Nashville, Tennessee

Background: The bed nucleus of the stria terminalis (BNST), a key component of the extended amygdala, plays a critical role in alcohol/drug-induced negative affect and in stress-induced reinstatement of drug-seeking behavior, yet little is known regarding underlying mechanisms. Studies have demonstrated that injection of antagonists of corticotropin-releasing factor (CRF) and noradrenergic receptors into the BNST can disrupt stress-reward related behaviors. For example, evidence suggests that norepinephrine (NE) and CRF work in a serial process within the BNST to initiate stress-induced reinstatement of drug seeking. We have shown previously that NE and CRF work serially to enhance glutamatergic transmission in the BNST. These data suggest that during periods of stress glutamatergic drive is enhanced in the BNST. This heightened glutamatergic activity may lead to homosynaptic and/or heterosynaptic plasticity at specific glutamatergic inputs in the region to promote long-lasting changes in output.
Methods: In work to be presented, whole cell patch clamp and extracellular field potential recordings are utilized to study glutamatergic transmission in the BNST from acutely prepared mouse brain slices. Fluorescent targeting strategies are employed to identify unique populations of neurons for study.
Results: Long-term potentiation (LTP) and long-term depression (LTD) at glutamatergic synapses has been postulated to play key roles in alcohol and drug addiction; yet, to date, little is understood regarding the mechanisms governing these processes in the extended amygdala. Our research indicates that NMDA-receptor-dependent LTP can be elicited in BNST, as can at least two forms of LTD engaged by Gq-linked G-protein coupled receptors (GPCRs), such as metabotropic glutamate receptor 5 (mGlu5) and alpha1-adrenergic receptors (alpha1-ARs). The NMDAR-dependent LTP critically depends upon NMDARs containing the GluN2B subunit. Despite occurring on the same cell types, and being elicited by Gq-linked GPCRs, the two forms of characterized LTD are differentially regulated by stressors and drugs of abuse, and involve distinct molecular mechanisms. LTP and LTD are coordinately regulated at glutamate synapses in the BNST, as chronic intermittent ethanol exposure (CIE), which drives enhanced alcohol intake, drives an enhancement of LTP and a reduction of alpha1-adrenergic receptor induced LTD. Using knockout mouse-validated pharmacological approaches with Ro25-6981 and memantine, we have also performed experiments suggesting that CIE enhances LTP in the BNST via paradoxical extrasynaptic NMDAR involvement.
Conclusions: Altogether, this work begins to delineate the microcircuitries recruited within the extended amygdala to produce stress-induced reinstatement and dependence-induced drinking, and demonstrates that alcohol exposure and withdrawal produces coordinated regulation of specific forms of plasticity.
Disclosure: D. Winder, Nothing to Disclose.

22.4 A Functional Grm2 Stop Codon Increases Alcohol Preference in Alcohol Preferring (P) Rats

David Goldman*

National Institutes of Health, Rockville, Maryland

Background: Alleles altering alcohol preference have been selected in Preferring (P) and Non-Preferring (NP) rat lines. These variants, and the genes in which they reside, remain obscure despite mapping of some of their chromosome locations and progress in the neurobiology of alcohol preference, including the role of glutamate.
Methods: We used genomic sequencing and functional genetic analyses to identify loci modulating alcohol preference. Sequencing of 8 P and 8 NP rats was performed on genomic and gene-centric levels using an Illumina GA2X sequencer. Bioinformatic analysis predicted damaging variations genetically fixed in P and NP rats. Function was confirmed by measurement of RNA and protein (the latter via Western blots with an mGluR2-specific antibody), and by linkage in iP x iNP F2s made by cross-mating inbred P (iP) and NP (iNP) strains. To evaluate global effects of a *Grm2* stop codon, RNA-Seq was performed on hippocampus, followed by gene ontology analysis. To investigate effects of *GRM2* on human alcohol consumption, alcoholism, and Harm Avoidance, 5300 Finns and 320 Plains Indians, were genotyped on the Illumina 550k platform. Additional GRM2 missense variants were individually genotyped. Novel *GRM2* variants detected by exome sequencing were tracked using local haplotypes.
Results: We identified 19,129 SNPs that homozygously segregated between P and NP rats and constructed a genome map highlighting differences between the two lines. Among 235 segregating missense variants, 22 were predicted to be damaging. Two stop codons included one at codon 407 of metabotropic glutamate receptor 2 (*Grm2*) that was homozygous in P rats, and also found iat lower frequency n outbred Wistar rats, the parental strain of P and NP. Loss of mGluR2 protein expression in P rats was complete. The *Grm2* stop codon led to a 32% increase in alcohol consumption and a 28% increase in preference. P rats were also homozygous for a nonsense variant at codon 137 of lipocalin 2 (*Lcn2*). This stop codon was also linked to higher alcohol consumption. Genes carrying segregating SNPs in their coding sequences and UTR's were enriched among genes differentially expressed in the hippocampal transcriptome. In particular, and consistent with presence of the *Grm2* stop codon in P rats, there was over-representation of genes involved in glutamate transmission and synaptic functions. In both Finns and Plains Indians, two *GRM2* SNPs were associated with Harm Avoidance ($p < 0.002$).
Conclusions: A *Grm2* stop codon leads to higher alcohol preference and consumption in P rats, and this stop codon also occurs in outbred Wistar rats. Other loci, including an Lcn2 stop

codon explain additional variation in alcohol preference, and point to genes altering alcohol-related behaviors in humans. Two *GRM2* SNPs were associated with Harm Avoidance (but not alcohol consumption or alcoholism) in humans. Identities of human *GRM2* loci that may be responsible for these associations, and which might be more strongly predictive of behavior, are being pursued by genotyping uncommon *GRM2* missense variants and by exome sequencing.
Disclosure: D. Goldman, Nothing to Disclose.

Panel
23. High Anxiety: Endocannabinoid Regulation of the Stress Response and Emotional Behavior

23.1 PET Reveals Abnormal CB1 Receptor Binding in PTSD

Alexander Neumeister*

New York University, New York, New York

Background: Convergent lines of evidence implicate a defect in CB1 receptor-mediated eCBsignaling in the pathogenesis of PTSD and mediate a PTSD phenotype.
Methods: The recent development of the CB1 receptorselective radiotracer, designated [11C]OMAR nowmakes it possible for the first time to conduct an *in vivo* assessment ofCB1 receptor density in PTSD using positronemission tomography (PET). We determined volume ofdistribution (VT) values, a measure of CB1 receptor density in medication-free PTSDpatients (N = 16/8F, age, ys 30.0 ± 8.5, range 20-44, CAPS 78 ± 11.5), individually-matched healthy control subjects without (N = 16/8F, age, ys 30.6 ± 7.5, range 20-45) and with (N = 7/1F, age, ys 35.3 ± 6.6, range 23-41) trauma exposure.
Results: We found elevated CB1 binding in PTSD relative to the non-traumatizedhealthy control subjects in a amygdala-hippocampal-cortico-striatal PTSD circuit ($p < .0017$). Amygdala CB1 binding was significantly higher in traumatized healthy control-subjects compared to non-traumatized healthy controls ($p < .012$). Independentof diagnosis, we found significantly higher CB1 binding in women relative tomen ($p < .0039$).
Conclusions: Our data show that the maladaptive neurobehavioral trauma response inPTSD is associated with impaired eCB signaling as evidenced by upregulation ofCB1 receptors. Elevated CB1 binding in the amygdala in trauma-exposed healthycontrols further suggests that trauma exposure influences molecular adaptationsin neuronal networks that are dysfunctional in PTSD.
Disclosure: A. Neumeister, Nothing to Disclose.

23.2 Reduced Plasma Endocannabinoid Levels in PTSD

Rachel Yehuda*

Mount Sinai School of Medicine, New York, New York

Background: Endocannabinoid signaling has been identified as an endogenous modulator of adaptation to stress, and is integral to basal and stress-induced glucocorticoid regulation. Converging preclinical evidence has shown that deficits in endocannabinoid signaling are associated with preservation of, and activation is required for extinction of emotional memories. These findings suggest that endocannabinoids maybe associated with vulnerability to the development of post-traumatic stress disorder (PTSD), and perhaps to recovery from episodes of illness.
Methods: Plasma endocannabinoids were measured in a population-based cohort selected for physical proximity to the World Trade Center at the time of the 9/11 attacks. Longitudinal clinical data with respect to the extent of exposure and occurrence of symptoms consistent with PTSD were obtained beginning one month post 9/11. Interview-based clinical diagnostic assessments, including the Clinician Administered PTSD Scale (CAPS), and blood samples were obtained; endocannabinoid values (in particular, of 2-arachidonoylglycerol (2-AG) and anandamide (AEA)) were available for 46 subjects (no PTSD, n = 22; current PTSD, n = 12; and past PTSD, n = 12).
Results: 2-AG was significantly reduced among subjects directly affected by the WTC collapse ($F = 7.42$, $df = 1,43$, $p = .009$) compared to those with less immediate exposure, and also reduced in subjects with past or current PTSD at the time of assessment (mean ± SD, 5.04 ± 2.79) in comparison to those without history of PTSD development (9.41 ± 7.59; $F(1,43) = 7.24$, $p = .009$, controlling for gender). Within the PTSD sample, 2-AG was even further reduced in past (3.83 ± 1.95) compared to current PTSD (6.25 ± 3.05; $F(1,21) = 5.09$, $p = .035$, controlling for gender). There were no significant group differences for AEA levels. Among subjects with PTSD, 2-AG was correlated with 8am plasma cortisol ($r = .436$, n = 24, $p = .033$. CAPS intrusive symptom scores were negatively associated with AEA levels ($r = -.532$, n = 19, $p = .019$), whereas avoidance symptoms were positively associated with 2-AG levels ($r = .618$, n = 19, $p = .005$).
Conclusions: This report shows that PTSD is associated with a reduction in circulating levels of the endocannabinoid 2-AG, with higher levels among persons resistant to PTSD development. It is possible that in PTSD, a disorder with comparatively reduced 2-AG levels, current illness is associated with relative 2-AG elevation, which functions to constrain sympatho-adrenal activation associated with PTSD symptom expression. The positive association between 2-AG and avoidance scores is in keeping with the relative elevation of 2-AG in current vs. past PTSD – while avoidance is practiced in the interest of reducing exposure to traumatic reminders, it is also an indicator of persistent PTSD. In contrast, the negative association between AEA levels and intrusive symptoms in this cohort is consistent with animal data indicating that reductions in AEA promote retention of aversive emotional memories, possibly through disinhibition of amygdala projection neurons. Future work will aim to replicate these findings and extend their relevance to clinical pathophysiology, as well as to neuroendocrine and molecular markers of PTSD.
Disclosure: R. Yehuda, Nothing to Disclose.

23.3 The Endocannabinoid System as a Therapeutic Target for Stress-related Disorders

Daniele Piomelli*

University of California, Irvine, California

Background: The endocannabinoids are a family of lipid messengers that engage the same cell surface receptors targeted by D9-tetrahydrocannabinol, theactive principle in marijuana. They are produced on demand through cleavage of membrane precursors and are employed in the execution of various short-range signaling processes. In the brain, they combine with CB1 cannabinoid receptors on axon terminals to regulate ion channel activity andneurotransmitter release. Their ability to modulate synaptic efficacy has a surprisingly wide range of functional consequences and provides uniquepossibilities for therapeutic intervention.
Methods: Biochemical and behavioral analyses were conducted as described previously (Kathuria et al, 2003; Gobbi et al, 2005; LoVerme et al, 2006; Bortolato et al, 2007; Clapper et al, 2010; Fu et al, 2011).
Results: In the first part of my talk, I will describe the molecular and cellular mechanism responsible for producing and eliminating the endocannabinoids, anandamide and 2-arachidonoyl-sn-glycerol (2-AG), and outline their functions as retrograde synaptic messengers. I will then turn to describe threenew classes of chemicals that interfere with endocannabinoid deactivation: inhibitors of anandamide hydrolysis (FAAH inhibitors), inhibitors of anandamide transport (FLAT inhibitors), and inhibitors of 2-AG hydrolysis (MGL inhibitors). In particular, I will focus on the effects of these agents in animal models of stress coping and human stress-related disorders.
Conclusions: The results suggest that pharmacological strategies aimed at enhancing intrinsic endocannabinoid activity in the brain might be beneficial in the treatment of human stress-related disorders.
Disclosure: D. Piomelli, Nothing to Disclose.

23.4 Stress-induced Regulation of Endocannabinoid Signaling in the Amygdala: Mechanisms and Functional Implications

Matt Hill*

University of Calgary, Calgary, Alberta, Canada

Background: Endocannabinoid signalling is known to be a negative regulator of the physiological, behaviour and neuroendocrine effects of stress. Increasing evidence has suggested that the amygdala may act as a primary seat of action of these effects of endocannabinoid signalling on stress. We have previously established that acute exposure to stress causes a rapid increase in metabolism of the endocannabinoid anandamide (AEA) within the amygdala, and that local inhibition of AEA metabolism within the amygdala can dampen stress-induced activation of the HPA axis. These data suggest that AEA signalling in the amygdala acts as a gatekeeper for the activation of the stress response.
Methods: Using both genetically modified mice (lacking the enzyme, fatty acid amide hydrolase [FAAH], responsible for AEA hydrolysis) and pharmacological studies in rats, we explored the effects of disruption of FAAH activity on the effects of chronic stress (changes in electrophysiological parameters of the amygdala, changes in dendritic arborization and spine densities in the amygdala, changes in anxiety-like behaviors). In addition, we also employed pharmacological studies of blocking CRH signalling to determine the contribution CRH to the effects of chronic stress on FAAH activity and AEA content within the amygdala.
Results: We found that 24 h following the cessation of stress there is a persistent increase in AEA hydrolysis and reduction in AEA tissue content within the amygdala. The functional relevance of this increase in AEA hydrolysis by FAAH following chronic stress is highlighted by the fact that mice deficient in FAAH (FAAH KO) do not develop an increase in anxiety, nor do they exhibit the structural changes in the amygdala (increased dendritic arborisation and spine densities on pyramidal neurons of the basolateral nucleus of the amygdala) which equate to increased anxiety, following chronic stress. Similarly, rats treated orally with a FAAH inhibitor throughout a chronic stress regimen do not exhibit an increase in anxiety, indicating that the increase in FAAH-mediated AEA hydrolysis following chronic stress is necessary for the structural and behavioural indices of anxiety that are augmented by chronic stress. One putative mechanism by which AEA may be regulating amygdalar excitability is through a gating of incoming excitatory inputs to the amygdala. Consistent with this hypothesis, we have found that exposure to stress results in a protracted and sustained elevation in the frequency, but not amplitude, of miniature excitatory post synaptic currents within the lateral amygdala, and this effect is prevented by administration of a FAAH inhibitor prior to stress-induction. The mechanism by which stress modulates FAAH activity and AEA content appears to be through a CRH-mediated pathway as sustained exposure to stress hormones up regulate CRH levels within the amygdala, and the ability of sustained elevations in stress hormone levels to increase FAAH activity and reduce AEA within the amygdala is reversed by a CRHR1 antagonist.
Conclusions: Collectively, these data indicate that under steady state conditions, there is an AEA tone within the amygdala that gates the release of glutamate from excitatory inputs. In response to stress, FAAH activity increases, resulting in a decline in AEA signalling which disinhibits glutamate release and promotes excitation of the amygdala, in turn driving the generation of a stress response. Following chronic stress, AEA signalling in the amygdala is compromised by a sustained increase in FAAH activity, which promotes excitation of the amygdala, resulting in structural remodelling of the amygdala and the generation of a persistent state of anxiety and stress. This increase in FAAH activity appears to be mediated by a glucocorticoid-mediated up regulation of CRH signalling within the amygdala. Accordingly, inhibition of FAAH may be a suitable target for the development of a novel class of agents which act to treat anxiety disorders, such as PTSD.
Disclosure: M. Hill, Nothing to Disclose.

Panel
24. Longitudinal Neuroimaging of Emerging Substance Use: Brain Indicators of Early Risk and Effects of Use

24.1 Individual Differences in Control and Reward and Their Relationship to Substance Use Risk: The IMAGEN Study

Hugh Garavan*

University of Vermont, Burlington, Vermont

Background: A sizeable body of research has identified neurocognitive impairments in cognitive and reward/reinforcement processes in substance abusers. However, separating the impairments that preceded use from those that arose from use is a perennial problem in drug abuse research. This separation can be achieved, in part, with a longitudinal study designed to identify predictors of the transition to use.
Methods: The IMAGEN project is a multi-site neuroimaging study of 2,400 adolescents that includes extensive phenotyping and genotyping in eight sites in Ireland, England, Germany and France. Participants, all fourteen years of age, completed a motor response inhibition STOP task, which provides a measure of prefrontally-mediated cognitive control, and a Monetary Incentive Delay (MID) task, which provides measures of cortical and subcortical activity during reward anticipation and reward delivery.
Results: Analyses of the STOP task reveals reduced activation in orbitofrontal cortex in those adolescents who have experience with alcohol (approximately 60% of the sample). Notably, this effect was not related to the severity of alcohol use and, indeed, was observed in those participants who had no more than 4 drinks in their lifetimes, a result which is indicative of a pre-existing trait that predisposed towards alcohol use at an early age. Conversely, right prefrontal activation showed elevated levels of activity, relative to alcohol-naïve controls, but only in those participants who had experience with alcohol, nicotine and other drugs. Activation in right prefrontal cortex also increased as a function of the extent of drug use, thereby suggesting that the alcohol-related effects in this area arose from use. Ventral striatal activation during reward anticipation on the MID task was reduced in drinkers relative to alcohol-naïve participants. This effect was present in very light drinkers but was also related to the extent of drinking so may reflect a pre-existing trait and/or a dose-related neurotoxic effect. Follow-up data on the participants' drug and alcohol use obtained three years later reveals that some of the effects that discriminated drinkers from non-drinkers at baseline also predict the transition to use in those who reported no drinking at baseline. For example, activity related to reward anticipation in medial prefrontal cortex at baseline was lower in those participants who were relatively heavy drinking three years later compared to those who remained either alcohol-naïve or who were relatively light drinkers three years later. Similarly, orbitofrontal activation on the STOP task at baseline was reduced in those who transitioned to heavier drinking relative to those who remained zero-to-low drinkers.
Conclusions: Combined, these results suggest certain brain differences that precede alcohol use and might therefore be hypothesized to confer risk for alcohol use and other brain differences that are affected in a dose-response manner by use. The longitudinal nature of this research, combined with its large sample size, offers insights into the roles played by both cognitive control and reward-related processes that confer risk for, or arise from, early adolescent alcohol use.
Disclosure: H. Garavan, Nothing to Disclose.

24.2 Longitudinal fMRI Studies of Impulse Control and Incentive Responding: Effects of Risk and Alcohol Use

Mary Heitzeg*

University of Michigan, Ann Arbor, Michigan

Background: The adolescent period is a time when most alcohol use is initiated and also a time when neural alterations in frontal control and subcortical incentive systems are taking place. Evidence from cross-sectional studies is accumulating for an imbalance between subcortical, dopamine-related activation and prefrontal control, which has been proposed to underlie the impulsive, risky decision making associated with adolescence. Adult alcoholics have alterations in this circuitry, but it remains unclear whether these alterations reflect pre-existing traits predisposing to alcohol use or are secondary to alcohol exposure. The focus of the work reported here is to probe the systems hypothesized to underlie the development of risk for alcohol and other substance use disorders as well as determine the impact of alcohol exposure on the development of these systems.
Methods: We studied a high-risk sample, recruited from the 8-22 year old offspring in the Michigan Longitudinal Study of alcoholic families (COAs) and control families recruited from the same neighborhoods. Two cohorts completed a monetary incentive delay (MID) task and a go-no-go task at 2-year intervals in an ongoing longitudinal functional MRI study: child cohort (n = 89 baseline scans at ages 7-12; n = 54 2nd scan; n = 23 3rd scan) and young-adult cohort (n = 120 baseline scans at ages 18-22; n = 97 2nd scan; n = 52 3rd scan). Cumulative lifetime drinking in the young adults was calculated from data collected prospectively during annual assessments since age 11. Linear mixed model analyses were used to investigate: 1) effect of cohort and age on activation during anticipation of reward and loss and inhibition of motor response; 2) within each cohort, interactions between age, risk status (COA versus controls) and lifetime drinking (in young adults) on activation.
Results: During the MID, there was a positive effect of age on nucleus accumbens (NAC) activation to incentive anticipation, which interacted with cohort, wherein the effect was only present in the child cohort. Within the child cohort, age interacted with risk status, with greater increases in NAC activation to incentive anticipation in COAs. Within the young-adult cohort, there was a risk X drinking X age interaction on NAC activation. *Post hoc* analyses revealed an age X drinking effect in the COA group but not the control group, with low-drinking COAs showing increasing activation from late adolescence to early adulthood and a blunting of this effect in high-drinking COAs. During the go-no-go task, there was a positive effect of age on activation in the left postcentral gyrus during response inhibition in the child cohort, but not the young adult cohort. In addition, only in the child cohort, the mean and variance in reaction times to go trials decreased with age and showed a negative correlation with activation in the left postcentral gyrus. These effects interacted with risk status, with activation increasing and reaction time decreasing from childhood to adolescence only in COAs. Overall reaction time was lower in controls; therefore this effect may represent a developmental delay in the COAs. In contrast, as a group, the young adult cohort showed no linear effects of age on activation during impulse control. However, heavier drinking young adults showed linear increases in caudate and orbitofrontal activation during impulse control from late adolescence to early adulthood, but no improvement in performance, suggesting greater effort necessary to maintain performance.
Conclusions: This work demonstrates early differences in the development of incentive and impulse control systems in at-risk youth that may contribute to vulnerability for substance abuse and suggest that alcohol use may impact these developmental trajectories.
Disclosure: M. Heitzeg, Nothing to Disclose.

24.3 Effects of Alcohol Use Initiation on Brain Structure and Behavioral Functions in Adolescents

Monica Luciana*

University of Minnesota, Minneapolis, Minnesota

Background: The use of alcohol in large quantities or for extended periods of time is associated with impairments in attention, memory, and executive functions. Alcohol use also results in neurotoxicity as evidenced by studies of fetal alcohol exposure and chronic use in older adults. Whether alcohol used in smaller amounts has effects on brain development in children or adolescents is less clear, although a growing accumulation of evidence suggests that adolescent heavy drinkers show differences in regional brain volumes, differences in white matter connectivity between cortical regions, differences in functional brain activity while performing working memory tasks, and relative performance impairments on several measures of neurocognitive function. Many participants in such studies are heavy users with pronounced histories of binge drinking and with other evidence of externalizing behavior. This study was designed to prospectively examine the effects of alcohol use initiation on patterns of brain structure and function in a typically developing sample of adolescents without histories of externalizing behavior.
Methods: Adolescents between the ages of 9 and 20 were recruited from the metro community. At intake, participants (n = 188) were free of psychopathology as determined by structured diagnostic interview, including substance use disorders. Most reported no use of alcohol, and other drug use in any amount was an exclusion factor. Participants completed a structural MRI scan, including DTI, and a comprehensive behavioral testing battery. Two years later, participants (90% of the original sample) returned for a similar follow-up assessment. At follow-up, a significant proportion of the original sample had initiated use of alcohol. Alcohol use frequencies and quantities were quantified and associated with brain structural volumes and white matter connectivity.
Results: Increased use of alcohol at the time 2 assessment was associated with increases in the volume of the nucleus accumbens, particularly in the left hemisphere, but not in other areas of the striatum such as the caudate nucleus. This pattern suggests a disruption of the normative process of synaptic pruning that should accompany adolescent development. The integrity of white matter connections in anterior regions of the superior longitudinal fasciculus were also compromised in the context of increased alcohol use (decreased connectivity with prospective increases in use) as were connections between the nucleus accumbens and medial orbitofrontal cortex as determined by probabilistic tractography. These effects on white matter were most pronounced when accelerated drinking occurred at younger ages. Effects on neurocognitive functions are not evident, suggesting increased cognitive reserve in this age range even in the context of disrupted neurodevelopment.
Conclusions: In summary, findings to date suggest disruptions in the normative pattern of neurodevelopment (regional brain volumes and white matter connectivity) in association with the onset of alcohol use in an otherwise typically developing adolescent sample.
Disclosure: M. Luciana, Nothing to Disclose.

24.4 Longitudinal Studies of Alcohol Effects on Academic Grades and MRI Hippocampal Volumes in the BARCS College Sample

Godfrey D. Pearlson*

Yale University School of Medicine, Hartford, Connecticut

Background: Adolescence is a high-risk period for initiating alcohol use and for problem drinking. The biological mechanisms underlying thetransition to problem drinking remain to be fully elucidated. Family history of alcohol use disorders has been found to be a predictor of future dysfunctional alcohol use; this liability may be mediated thro impulsive/disinhibited/sensation-seeking / externalizingbehaviors, variant reward sensitivity, and/or different

physiologic responses to alcohol. The 2000-person, NIAAA-funded Brain and Alcohol Research in College Students (BARCS) study endeavors to unravel these relationships through detailed longitudinal assessment, including genotyping and structural/functional MRI.
Methods: In study #1 we assessed 200 BARCS college freshmen (42.5% male) aged 18 + 0.6, obtaining high school SAT scoresand the first 2 years of college academic grades for all subjects from official school records. Alcohol consumption was assessed using monthly, secureweb-based self-report surveys. Alcohol abstainers were not excluded fromthe study. Drinking patterns examined over 3 academic terms, with multiple measures including past months drinking days N of days subject engaged in heavy (binge) drinking, and maximum number of drinks thatthe subject consumed in a 24-hour period. In study #2 we performed high-quality T1 and T2-weighted MRI scans using Freesurfer to derive regional gray matter volumes atbaseline in 420 subjects and again at 24 months ($N = 45$ to date), which we compared to monthly alcohol use self-reports.
Results: Study #1- Linear regression models significantly predicted a given semester's GPA by combining SAT scores with all previous semester'sGPAs to generate a predicted grade in each semester. We found a significant negative correlation between GPA and number ofdays of binge drinking ($p < 0.05$) and a dose-related decrement in predicted GPA ($p < 0.05$) whose significance diminished with successive semesters. Study #2-left hippocampal volume change at 24 months was proportional to N of alcoholic blackouts; ($r = -0.39$, $p < 0.01$) i.e. more blackouts associated with greater volume decreases.
Conclusions: Study #1 findings support previous reports that SAT scoresare strong predictors of college GPA, mostly during the first college year. Academicperformance during the freshman college year shows the strongest negativecorrelation with alcohol consumption, with subsequent semesters showing asteady decline in the negative trend between GPA and alcohol use, perhaps dueto development of alcohol tolerance or moderation of drinking patterns. Study #2 volumetric data are preliminary (full sample data to be reported at meeting),but significant and consistent with prior reports of hippocampal sensitivity to alcoholicblackouts in teenaged populations.
Disclosure: G. Pearlson, Nothing to Disclose.

Panel
25. Neuropeptide Receptor Ligands in Psychiatric Diseases: New Hopes after Multiple Failures

25.1 Neuropeptides to Treat Affective Disorders: Did Animal Model Fail to be Predictive, or Did Clinical Research Fail to Detect Effects?
Catherine Belzung*

Université de Tours, TOURS, France

Background: In the 20 last years, preclinical evidence using rodent models of psychiatric disorders provided growing piece of evidence suggesting that ligands of peptidergic receptors would be promising tools to treat affective disorders. Particularly, ligands of the CRH1 and of the Vasopressin 1B receptors as well as ligands of hypocretin (also termed as orexin) or tachykinin receptors have been suggested to represent innovative targets for the treatment of affective disorders including depression and anxiety. However, later on clinical trials failed in showing convincing results. How can we interpret these rather disappointing results? Is this related to a failure of animal models of psychiatric disorders to predict the effectiveness of treatments?
Methods: We will provide a meta-analysis of the literature on the effects of these ligands in animal models and analyze the underlying mechanisms of action, particularly focusing on the function of the hypothalamus-pituitary-adrenal axis, and the function of cerebral networks. We will then confront these findings to the available recent clinical data.
Results: This analysis shows that in many case clinical efficacy has been predicted on the basis of inappropriate animal models: for example, in case a treatment is targeting pathological anxiety, investigating the effects of a compound in animal tests for adaptive anxiety (using the openfield or the elevated plus maze), in normal animals, may lead to false positive or false negatives. Further, conclusions have frequently been obtained using only one or two behavioural readouts, which may represent only one aspect of the complex phenotype associated to mood disorders. On the other hand, in case preclinical research showed that a treatment is acting to counteract a pathological mechanism, there is no reason to expect efficacy in patients in which the targeted process is not dysfunctional. For example, if a putative treatment for major depressive disorder is acting by reversing an abnormal function of the hypothalamus-pituitary-adrenal (HPA) axis, there is no reason to test it in patients that have a normal HPA function.
Conclusions: This analysis clearly shows that it might be necessary to refine animal models to render pre-clinical research more relevant. Further, it would also be necessary to try to test different dimensions of the mood pathology, and not just one limited phenotype. Finally, clinical research has to take into account results from the preclinical studies, particularly those investigating the mechanisms of action of the putative treatments, in order to study these compounds in the adequate psychiatric nosological entity. To achieve these goals, translational research has to reinforce the dialog between basic and clinical science.
Disclosure: C. Belzung, Nothing to Disclose.

25.2 Neuropeptides and Major Depression/Depression-like Behavior: Focus on Substance P and Galanin
Tomas Hokfelt*

Karolinska Institutet, Stockholm, Sweden

Background: Neuropeptides and their receptors represent, by far, the largest messenger system in the brain. Its wide distribution, including in areas of interest for mental diseases, has for many decades attracted the attention of academia and, up till recently, of pharmaceutical companies. In this respect substance P, an undecapeptide, has played a 'pioneering' role: Early animal experiments from many laboratories suggested that a substance P antagonist would have analgesic effects. So it came as a surprise, when a blood-brain-barrier penetrating NK1 receptor antagonist, aprepitant by Merck, in 1998 was reported to be as efficacious as an SSRI in a clinical trial on depressed patients, and virtually without side effects. In spite of a failed phase 3 study, work on antagonists, not only for the NK1 but also the NK2 and −3 receptors, has continued. Many companies, including GSK, Pfizer, Eli Lilly, Sanofi and AstraZeneca have generated compounds that often have been active in animal models. Some have gone on to clinical trials, a few have shown initial efficacy but failed in later trials, some because of side effects. In parallel, many other neuropeptide systems have been targeted, including NPY receptors and the corticotropin-releasing hormone receptor. Another candidate is galanin, a 29/30 amino acid peptide, expressed among others in rat noradrenaline neurons in the locus coeruleus and in serotonin neurons in the dorsal raphe nucleus. It acts via three receptor subtypes, GalR1–3, and animal experiments by several groups suggest involvement in depression-like behavior.
Methods: NK1, −2 and 3 receptor antagonists have been generated and tested in animal experiments, and in clinical trials involving patients with various mental disorders. Galanin and galanin ligands have been studied in animal experiments. Galanin and its receptors have been analysed in human postmortem tissue.
Results: Findings with NK1antagonists in MDD and anxiety disorders have in general been inconsistent, one of many possible explanations being existence of subgroups patients. With regard to NK2 antagonists, saredutant showed promising effects in animal models of depression and some (albeit less promising) effects in models of anxiety, but these did not translate to significant clinical activity. The available clinical findings with selective NK3 antagonists in psychiatric diseases, in particular schizophrenia, have not convincingly established that blockade of this neuropeptide

receptor may be sufficient to improve these clinical conditions. Animal experiments suggest that GalR1 and GalR2 receptors are targets for treatment of depressive-like behavior. However, analysis of post mortem tissue (locus coeruleus, dorsal raphe region) shows that GalR3, and not GalR1, should be a more suitable target for developing novel anti-depressants.
Conclusions: Our and others' experiments comparing distribution of neuropeptide receptors in rodents and human postmortem tissue suggest that failure of drugs in clinical trials may be due to species differences.
Disclosure: T. Hokfelt, Nothing to Disclose.

25.3 Hypocretin/orexin, Sleep and Narcolepsy: Immune and Pharmacological Implications

Emmanuel Mignot*

Stanford University School of Medicine, Palo Alto, California

Background: The hypocretin/orexin (Hcrt/Ox) neuropeptide system was discovered in 1998, a finding rapidly followed by the finding that null mutations in one of its receptors, the hypocretin receptor 2 (also called OXR2), causes autosomal recessive canine narcolepsy, a disorder characterized by sleepiness, cataplexy and REM sleep onset. Hcrt/Ox-producing cells are located in the hypothalamus, and project widely in the CNS. Two peptides (Hcrt-1 and 2 or orexin-A and B) with high C-terminal homology are derived from a single prepropeptide precursor and bind two receptors OXR1 and OXR2 with complementary anatomical distribution. In humans, narcolepsy-cataplexy is caused by a 95% loss of 70,000 Hcrt/Ox producing cells. In its severe form, complete Hcrt/Ox deficiency (narcolepsy-cataplexy), it affects approximately 1 for 3,000 people across the world. The prevalence of milder phenotypes (with partial Hcrt/Ox cell loss) is unknown but could be substantially higher (up to 0.3%). Recent work suggests that the cause of the cell loss is autoimmune, with involvement of HLA alleles and specific T-cell receptors, and triggering of an autoimmune process by specific flu strains such as H1N1. Based on this finding, Hcrt/Ox receptor blockers, notably OXR2 or dual orexin receptor antagonists (DORA) have been produced and are being developed as hypnotics. Our current work focuses on discovering the cause of the Hcrt/Ox cell loss and on attempting Hcrt/Ox replacement therapy in long standing narcolepsy.
Methods: One of our primary approaches to understanding the cause of human narcolepsy is genetic (GWAS, exome sequencing) and immunology (animal models, identification of T cells) based.
Results: GWAS analysis in over 1,800 patients has identified 5 susceptibility loci to date, all but one with a clear autoimmune function, HLA, TCRA, P2YR11/DNMT1, TNFSF4 and CTSH. Exome sequencing in one rare family with ataxia, deafness and narcolepsy identified dominant mutations in exon21 of the DNA methylase DNMT1 as the cause of the condition. These results suggest the importance of antigen presentation by a specific HLA allele, DQ0602 of a specific peptide, maybe derived from influenza H1N1, to one or a few specific T cell receptors (TCR), with resulting autoimmune destruction of Hcrt/Ox-containing cells. We are currently using nextGen sequencing to probe TCR repertoire sequence diversity in various subpopulations of T cells in narcoleptics versus controls, and after H1N1 exposure to identify narcolepsy causing T cells. The observed TCR repertoire is vastly more complex that the human genome, with each individual carrying millions of specific TRCA and TCRB variant TCRs. Our works pioneers the use of Next Gen sequencing of the immunome for discovering novel biomarkers of diseases. The fact narcolepsy, unlike most other autoimmune disorders, is genetically associated with a specific genomic TCRA polymorphism, helps us to restrict our search into the TCRA repertoire to 0.8% of its current size. Sequencing millions of clones in patients and controls, we have identified signatures that likely reflect the narcolepsy-associated autoimmune process. The discovery of the culprit T cells may offer the possibility of early detection and blockade of the autoimmune process prior to complete and irreversible Hcrt/Ox cell destruction, a feature of established narcolepsy-cataplexy. For established cases, we believe that developing CNS penetrating Hcrt/Ox agonists will still be needed.
Conclusions: These findings offer pathways toward novel diagnostic tests and therapeutic approaches for the disorder, namely immune suppression therapies close to disease onset for incident cases and or hypocretin/orexin agonism for patients with long standing disease. As CNS Hcrt-1 injection can rescue the narcolepsy phenotype in Hcrt/Ox knock out mice, Hcrt/Ox agonists are also likely to have a future in the treatment of narcolepsy.
Disclosure: E. Mignot, **Part 1:** Novo Nordish – consulting Jazz Pharmaceuticals – consulting GSK - Advisory Board.

25.4 Identifying the Right Patient for Neuropeptide Receptor Ligands - Biomarkers for Central CRH Overexpression

Marcus Ising*

Max-Planck-Institute of Psychiatry, Munich, Germany

Background: Antidepressant drug development during the past two decades followed different strategies. On the one hand, new monoaminergic compounds with a broad mode of action have been introduced to the market, including dual- or triple-acting monoamine reuptake inhibitors and combined monoamine reuptake inhibitors and receptor antagonists. On the other hand, neuropeptide receptor ligands have been developed targeting specific receptors with high selectivity, for instance, selective corticotrophin releasing hormone receptor 1 (CRH1) antagonists. While several new monoaminergic antidepressants could be successfully launched during the past decades, no neuropeptide receptor ligand for depression or anxiety disorders has yet been approved. The most probable reason for this failure of approvals is – besides potential pharmacokinetic issues - the unavailability of appropriate biomarkers to select the right patients for specific treatments like neuropeptide receptor ligands.
Methods: Previous findings suggest that an impaired regulation of the hypothalamus pituitary adrenocortical (HPA) axis is a frequently observed feature in major depression and predicts antidepressant treatment outcome in these patients. Overexpression of hypothalamic neuropeptide including CRH seems to be a major factor contributing to an impaired HPA axis regulation. Another core feature of depression physiology is disinhibited REM sleep connected to elevated cholinergic activity in pons and midbrain, which in turn also interacts with central CRH expression.
Results: The combined dexamethasone (dex)/CRH test as biomarker for HPA axis regulation was conducted before and after treatment in 500 depressed patients participating in the Munich Antidepressant Response Signature (MARS) project. We could confirm that an improved HPA axis regulation indicated by an attenuated cortisol response in the second dex/CRH test predicted beneficial treatment outcome. We further could show that the failure to achieve improvement in HPA axis dysregulations is associated with a specific pattern of sleep impairment symptoms. Studies in CRH over-expressing mice suggest a strong link between CRH and disinhibited REM sleep. When re-analyzing the results of a clinical trial with the CRH1 antagonist R121919 in patients with major depression, we found that the best outcome was obtained in patients showing disinhibited REM sleep at baseline before treatment was initiated.
Conclusions: Neuropeptide receptor ligands like CRH1 antagonists are highly selective treatments that require the availability of biomarkers identifying the right patients who will optimally benefit from such specific interventions. Our findings suggest that the combined dex/CRH test as well as REM sleep disinhibition are promising candidates for such biomarkers reflecting central overexpression of hypothalamic neuropeptides. These biomarkers might be suitable to select appropriate patients for future clinical trials with CRH1 antagonists.
Disclosure: M. Ising, Nothing to Disclose.

Panel
26. Are We at a Turning Point in Psychiatric Genetics?

26.1 How Common and Rare Variants are Beginning to Provide Insights into Biological Mechanisms Underlying Psychiatric Disorders

David A. Collier*

Kings's College, London, United Kingdom

Background: Psychiatric genetics has been successful in identifying primary associations between genetic loci and disorder such as schizophrenia, autism spectrum disorder and bipolar disorder. A number of challenges remain, including the development of methods to discover 'missing' heritability, the refinement of risk loci to specific genes, and improving our understanding of their influence on specific phenotypes such as cognition. Despite this, psychiatric genetics may have reached a turning point, in that although genetic associations can only at present account for a small proportion of genetic risk, they are beginning to throw light on the biological pathways underpinning disease. I will discuss the extent to which genetics has opened a window on the neuronal systems which may be dysregulated within and across diagnostic categories in psychiatry. Pleiotropic genetic risk factors include more than a dozen copy number variants that implicate many brain-expressed genes, including those involved in the synapse (neurexin, neuroligin, SHANK proteins) and neurotransmission, as well as common, low risk variants in transcription factors, ion channels, cell adhesion molecules and microRNA. Is there now enough information on the genetic aetiology of psychiatric disorders to enable us to characterise their underlying pathophysiology and develop potential interventions?
Methods: Genome-wide assocation analysis; copy number variation analysis; exome and genome sequencing; meta and mega analysis; literature review, bioinformatic analysis, genotyping arrays, array CGH.
Results: A dozen or so genetic loci containing common, low-risk polymorphisms identified by GWAS have been associated with schizophrenia and/or bipolar disorder, and a similar number of rare, high-risk copy number mutations have been associated with schizophrenia and autism. While the common, low risk variants identified explain only a small proportion of risk, they point to biological systems that might be involve in aetiology, such as synaptic calcium signalling. The function of many associated genes remains poorly understood, and some do not clerly implictae a specific gene. The many rare, high or moderate risk copy number mutations identified usually contain several genes, making it diffiult to identify underlying biology. However mapping studies or lge CNVs and analysis ofthose that affect single genes also provides insight into specific gene associations and their associated biological pathways, including both pre-synaptic and post synaptic regulatory mechanisms.
Conclusions: Genome-wide association and CNV analysis can provide identifible genetic risk factors and biological pathways that conribute to the risk of developing psychiatric disorder; it is debatable whether these provide sufficient insight into the underlying pathophysiology of disease
Disclosure: D. Collier, **Part 1:** David Collier will become an employee of Eli Lilly on April 23rd 2012.

26.2 Dissecting Complexity in Neuropsychiatric Genetics with Network Inference

Neelroop N. Parikshak*

University of California, Los Angeles, California

Background: As we attempt to bridge genetics with behavior, we must overcome complexity at multiple biological levels. Individual differences in genes and environment interact at the level of molecules, cells, and circuits. Analyzing networks allows us to take high-throughput data from different levels of biology and prioritize which relationships are most important for disease and identify common pathways of phenotypic convergence. Recent work in our lab shows how network inference can be used to move between multiple levels of biology and across experimental systems to identify molecules essential to normal development and neuropsychiatric diseases such as autism and dementia. I will present our general network inference methodology, show new data and analyses supporting this approach, and suggest how we might best collect data to improve our ability to systematically generate testable hypotheses.
Methods: We use RNA profiling from post-mortem human brain tissue to profile the transcriptome using microarrays and RNA sequencing. We then apply weighted gene co-expression network analysis (Zhang & Horvath, 2005) to construct transcriptional networks. These networks allow us to assess relationships of groups or genes to phenotype, test whether these groups of genes are related to known pathways or cell-types, and prioritize molecules for further validation. We also apply the method to other high-throughput data, including epigenetic and neuroimaging data.
Results: Gene networks observed in developing brain are dysregulated in ASD. Networks help group genetic changes into coherent pathways, and our preliminary results validate the functional consequences of disease-related genetic variation. Based on these data, we have developed a framework that enables us to consolidate information from multiple sources to prioritize genes for experimental and therapeutic targeting.
Conclusions: Elucidation of the genetic etiology of neuropsychiatric disorders necessitates collection of comprehensive data at multiple biological levels. This includes the genome, relevant tissue, and comprehensive phenotypic data preferably across time and in the same individuals. Analysis of such data using network approaches permits generation of testable hypotheses which can allow us to leverage the complexity of massive amounts of data to identify important molecular changes in neurpsychiatric disorders.
Disclosure: N. Parikshak, Nothing to Disclose.

26.3 Modeling Schizophrenia Using Induced Pluripotent Stem Cells

Kristen Brennand*

Mount Sinai School of Medicine, San Diego, California

Background: Schizophrenia (SCZD) is a debilitating psychiatric disorder. Though postmortem studies of SCZD brain tissue typically describe defects in mature neurons, such as reduced neuronal size and spine density in the prefrontal cortex and hippocampus, abnormalities of neuronal organization, particularly in the cortex, have also been observed and led to speculation that developmental abnormalities impacting cortical organization may contribute to SCZD.
Methods: We postulated that defects in cortical organization in SCZD may result from abnormal migration of neural cells. To test this hypothesis, we directly reprogrammed fibroblasts from SCZD patients into human induced pluripotent stem cells (hiPSCs) and subsequently differentiated these disorder-specific hiPSCs into neural progenitor cells (NPCs) and neurons.
Results: SCZD hiPSC NPCs and neurons have an increased rate of cellular migration and decreased neuronal connectivity. SCZD NPCs have aberrant expression of a number of cellular adhesion genes, RELN and the migration associated microRNA miR-9.
Conclusions: We now report hiPSC NPC gene expression changes, including RELN and miR-9, and migration phenotypes associated with SCZD.
Disclosure: K. Brennand, Nothing to Disclose.

26.4 Optogenetics and Psychiatric Disease: Focus on Social Behaviors

Karl Deisseroth*

Stanford University, Stanford, California

Background: Achieving circuit-level insight into the nature of psychiatric diseases has long proven elusive. A growing wave of optogenetic research now has allowed the deployment of millisecond-

precision optical excitation or inhibition of specific circuit elements within behaving mammals, including in models of normal and dysfunctional social behavior, to causally probe the impact of defined elements in circuit dynamics and behavior.
Methods: We developed and applied tools to define in mammalian social behavior the real-time causal role not only of a specific brain region and cell type, but also of distinct subpopulations defined by projections to different downstream brain regions, distinct downstream cell types, and distinct receptor signaling pathways in those downstream cell types.
Results: Employing these causal interventions at multiple successive nodes along the candidate circuit pathways in freely-moving mice, we delineated a striking double dissociation of pro-social and pro-anxiety effects in dopaminergic cells with downstream projections to the nucleus accumbens (NAc) and prefrontal cortex (PFC), respectively, thereby resolving functionally distinct (and opposing) pathways interconnecting midbrain, subcortical, and cortical structures that regulate social and anxiety behaviors.
Conclusions: One of the most versatile features of optogenetics (modulation of defined neural projections) illustrated here may continue to allow exciting and productive avenues of research into psychiatric disease.
Disclosure: K. Deisseroth, **Part 1:** Co-founder of Circuit Therapeutics.

Panel
27. The Ups and Downs of AKT Signaling: A Nexus of Risk for Psychiatric Disorders

27. 1 Dissecting the Role of the AKT/PKB Family in Neurodevelopment and Schizophrenia
Amanda Law*

National Institutes of Health, Bethesda, Maryland

Background: The AKT family consists of three highly homologous serine/threonineprotein kinases (AKT-1; 14q32; AKT-2, 19q13.2 and AKT-3, 1q44) that play essential roles in the control of cellmetabolism, growth, and survival. Genetic variation in AKT-1 (14q32) has beenassociated with risk for schizophrenia and prefrontal cortical (PFC) physiologyin humans. Rodent studies suggest a role for AKT-1 in hippocampal and PFCdevelopment. Recent data also demonstrate that dopamine D2 receptors, the main target ofclinically effective antipsychotic drugs modulate AKT phosphorylation andactivity, a proposed mechanism of their therapeutic action. Given that data is absent on the neurologicalrole of AKT-2 and AKT-3 and both genes are highly expressed in the nervoussystem, combined with the absence of knowledge of which AKT isoform(s) are modulatedby antipsychotic drugs, we sought to examine the neurobiological consequences of genetic deletion of individual AKT isoforms. Here we examined the consequencesof AKT-2 genetic deletion on brain function using murine behavior profilesrelated to schizophrenia, including memory, cognition and sensorimotor gating.
Methods: AKT-2 heterozygous mice (floxed-POU34 germline-HETS-B6.Cg-AKT2tm1.1Mbb/J) ($N=12$), AKT-2 knockout mice ($N=5$) and WT littermates ($N=11$), all males, were tested for general health, locomotor activity, temporal order object recognition memory and prepulse inhibition (PPI) ofstartle.
Results: AKT-2 mutant mice demonstrated impairedrecency discrimination memory ($F_{2,29}=14.48$, $p<0.0001$) and PPI ($F_{2,28}=54.32$, $p<0.01$). Furthermore, AKT-2 mutants exhibited significantly decreased locomotoractivity in open field ($F_{2,25}=6.752$, $p=0.004$), with less time spentin the centre ($F_{2,25}=4.036$, $p=0.03$). In addition, mice exhibited inhibited forepaw reaching, increased positional passivity andrearing events ($F_{2,28}=20.98$, $p=0.001$), together suggestive of ananxiety-related phenotype.
Conclusions: Our data demonstrate a novel role for AKT-2in brain and show that its deletion impacts behavioral profiles related to schizophreniaand anxiety. Investigation of the impact of AKT-3 genetic deletion on braindevelopment and function is currently being assessed. Further investigation ofAKT-2 and AKT3's role in schizophrenia and human brain function are warranted.
Disclosure: A. Law, Nothing to Disclose.

27.2 Integrated Approaches to Understand the Actions of GPCRs: The β-arrestin-dependent D2R Signaling Axis
Marc G. Caron*

Duke University Medical Center, Durham, North Carolina

Background: In the brain dopamine (DA) is an important modulator of fast neurotransmission and is implicated in the behavioral control of locomotion, cognition, affect, and reward. Dysregulation in these systems has been implicated in the manifestation of several neurological conditions. These actions of DA are mediated through activation of D1 and D2-like G protein-coupled receptors (GPCR). Like other GPCRs, D2Rs display functional selectivity by engaging Akt/GSK3 signaling in a G protein-independent way through the ability of b-arrestin2 to scaffold an Akt/PP2A/GSK3 complex. These biochemical interactions are targets of antipsychotics and lithium (Beaulieu et al, 2008; O'Brien et al, 2011).
Methods: To further validate the biochemical, cellular and behavioral functions of this signaling pathway we are combining several *in vivo* genetic approaches to either delete components of this signal transduction pathway in a cell specific fashion or to reconstitute engineered D2R that can signal selectively through G protein- or arrestin2-dependent mechanisms into medium spiny neurons (MSN) of the striatum which lack endogenous D2Rs.
Results: In order to selectively inactivate GSK3b in striatal MSNs we have crossed GSK3bflox mice with D1RCre or D2RCre/A2ARCre mice, which respectively inactivate GSK3b in D1R- and D2R- expressing MSNs of the striatal complex. These mice were then analyzed in behaviors commonly used to test antipsychotic efficacy or behaviors that are sensitive to lithium treatment. Inactivation of GSK3b in D2R but not D1R expressing MSNs recapitulates the effects of antipsychotics on behavior in the animals. Thus, D2RGSK3b-/- but not D1RGSK3b-/- mice showed a reduced locomotor response to amphetamine, rearing response to apomorphine, a lack of pre-pulse inhibition disruption by amphetamine or apomorphine, and increased spontaneous alternations in the Y-maze, reminiscent of the actions of antipsychotics. However, haloperidol induced catalepsy and place preference to amphetamine was unchanged in either the D2RGSK3β-/- or D1RGSK3β-/- mice compared to control mice. Interestingly, stabilization of b-catenin, a downstream target of GSK3β which phenocopies deletion of GSK3b, in D2R expressing MSNs did not affect any of the behaviors tested. Moreover, D2RGSK3β-/- or D1RGSK3β-/- mice showed similar responses to littermate controls in the tail suspension and dark-light emergence test, behaviors, which we have previously demonstrated to be b-arrestin2 and GSK3b dependent and sensitive to lithium treatment. To complement this approach we have also engineered D2R mutants that retain membrane expression and agonist dependency but are functionally selective for G protein- or b-arrestin2-dependent signaling. *In vivo* viral reconstitution of these selective D2Rs into mice lacking D2R in MSNs should recapitulate the above properties, demonstrate the physiological role of functional selectivity, and facilitate the identification of downstream targets of the functionally selective D2Rs.
Conclusions: Taken together these results suggest that deletion of GSK3b but not stabilization of β-catenin in D2R MSNs mimics antipsychotic action without affecting signaling pathways involved in catalepsy or mood stabilization suggesting that these behaviors might be mediated through other neuronal pathways. These genetic approaches coupled with the feasibility of identifying functionally selective ligands for each pathway (Allen et al, 2011) may provide new avenues for more effective and selective therapies for modulation of GPCR signaling.
Disclosure: M. Caron, **Part 1:** Lundbeck, Omeros, Roche, Forest Laboratories, **Part 2:** Omeros, **Part 3:** Lundbeck, Roche, Forest Laboratories.

27.3 DISC1 Regulation of Neural Development through AKT-mTOR-CYFIP1 Signaling

Guo-li Ming*

Johns Hopkins University, Baltimore, Maryland

Background: A large body of evidence supports a neurodevelopmental contribution to the pathophysiology of mental disorders, including schizophrenia and autism. Rapid progress in human genetic association and sequencing analyses has lead to the identification of a large number of risk genes. The function of many these risk genes in neuronal development are unknown. DISC1 (Disrupted-in-Schizophrenia 1) is a susceptibility gene for schizophrenia, major depression and autism. Using adult neurogenesis as a model system, we have previously shown that DISC1 plays a multifaceted role in neural development of newborn neurons in the adult hippocampus via AKT-mTOR signaling. The signal transduction mechanisms along the mTOR pathway underlying DISC1 regulation of neurodevelopment is not well understood.
Methods: To identify the missing link between AKT and mTOR in DISC1-dependent regulation of neuronal development, we assessed the functional role of Rheb1, a direct target of ASD risk gene TSC1/2 and a upstream activator of mTOR in newborn neurons during adult hippocampal neurogenesis. We further examined the 4E-BP signaling pathway downstream of mTOR. We used retroviral approach for single cell genetic manipulation and labelling of newborn neurons in the adult hippocampus *in vivo*.
Results: we show that developmental defects of newborn neurons during adult hippocampal neurogenesis from dysfunction of DISC1 are recapitulated by hyper-activation of Rheb1, an upstream activator of mTOR, and are rescued by Rheb1 genetic deletion. CYFIP1, another schizophrenia and autism susceptibility gene, encodes an eIF4E binding protein that mediates mTOR signaling via inhibition of eIF4E, which itself also has been implicated in autism. Suppression of CYFIP1 in newborn neurons recapitulates and further enhances DISC1-induced neuronal developmental defects, whereas eIF4E suppression largely rescues DISC1- and CYFIP1-induced defects.
Conclusions: Our genetic and functional studies delineate a signal transduction cascade involving DISC1-Rheb1-mTOR-CYFIP1-eIF4E in regulating neuronal development and support the notion that multiple susceptibility genes may functionally converge onto a common pathway in contributing to the etiology of certain psychiatric disorders.
Disclosure: G. Ming, Nothing to Disclose.

27.4 Studying AKT1 Signaling in Human Brain

Daniel R. Weinberger*

Lieber Institute for Brain Development, Baltimore, Maryland

Background: AKT signaling modulates the effects of multiple neuropsychiatric risk associated genes and therapeutic targets, including DRD2, DISC1, BDNF, NKCC1, and GSK3b. While the interactions of AKT and these other proteins in cell and animal models have been widely studied, the effects of AKT and these proteins on the function of neural systems implicated in neuropsychiatric illness and its treatment are largely unexplored. We have used genetic association of neuroimaging derived network phenotypes to elucidate these effects in the living brain and genetic prediction of drug effects in patients based on AKT1 and related genotypes.
Methods: We studied tan AKT1 SNP (rs 1130233) that affects protein expression and its interaction with functional SNPs in several genes of neuropsychiatric interest: DISC1 (rs1000731), NCKK1 (rs10089), BDNF (val/met), COMT (val/met), GSK3b (rs12630592), DRD2 (rs1076560). The GSK3b SNP significantly affects mRNA expression in human prefrontal cortex in the "BrainCloud" database (www.libd.org/braincloud). All other SNPs were previously functionally validated. Imaging of episodic memory and of working memory was performed in normal subjects (n = 93-480, depending on specific tasks and genotypes) using SPM8 and functional network analytic modeling, including PPI and DCM. The effect of treatment on cognition was determined in 111 patients with schizophrenia receiving antipsychotic drugs with or without other agents impacting on AKT (i.e. Li, valproate).
Results: Significant three way interactions were found and independently replicated for AKT1/COMT/BDNF in risk for schizohprenia (OR1.5) and hippocampal engagement and episodic memory (Tan et al, 2011). Adding GSK3b to these earlier models did not enhance the associations. NCKK1 and DISC1 show replicated interactions on risk for schizophrenia (Kim et al, 2012) and strongly significant and independently replicated effects on hippocampal engagement and connectivity with prefrontal cortex during episodic memory. GSK3b does not enhance these interactions, but does show significant effects in combination with the DISC1 functional polymorphism (ser/cys) on prefrontal engagement during WM. Using dynamic causal models to define cortical and subcortical networks selectively engaged separetely during WM maintenance and manipulation operations, we identified differential effects of functional polymorphisms in the DRD2, AKT1 and COMT genes on prefrontal-parietal and prefrontal-striatal circuits engaged during maintenance and manipulation, respectively. The DRD2 and AKT1 polymorphisms sigificantly altered dose-response effects of antipsychotic drugs and of LI and valproate on cognition in schizophrenia. GSk3b variation enhanced these effects.
Conclusions: We demonstrate higher order effects of genetic variation in AKT1 on genes implicated in neurodevelomental processes linked to neuropsychiatric disorders and the cognitive effects of drugs used in their treatment.
Disclosure: D. Weinberger, Nothing to Disclose.

Wednesday, December 05, 2012

Mini Panel
28. Behavioral Paradigms to Improve Signal Detection in Trials of Cognition Enhancing Drugs

28.1 The Phosphodiesterase 4 Inhibitor, HT-0712, Facilitates Cognitive Rehabilitation Following Traumatic Brain Injury

Tim Tully*

Dart Neuroscience, San Diego, California

Background: Rehabilitation therapy can improve functional recovery after brain injury. This functional recovery is thought to result from neural plasticity in surviving neurons. Activation of the CREB pathway is an essential step for experience-dependent changes in neural plasticity. Previously, we reported that the phosphodiesterase-4 (PDE4) inhibitors, Rolipram and HT-0712, facilitate CREB signaling and enhance memory formation in mice and facilitate motor rehabilitation after ischemic stroke in rats.
Methods: One week after injury, one-day memory after novel object recognition (NOR) training remained impaired. These animals then were dosed with HT-0712 or vehicle alone 20 minutes before each of five cognitive rehabilitation NOR sessions, one every two days during which different object pairs were used each time.
Results: After cognitive rehabilitation was completed and drug was no longer administered, one-day memory after new NOR training was higher for the drug + rehabilitation group than for the rehabilitation alone group one day, one week and eight weeks later. Cognitive rehabilitation of NOR also generalized to another hippocampus-dependent task, trace fear conditioning, again only in the drug + rehabilitation group.
Conclusions: These results demonstrate that drug-augmented cognitive rehabilitation after traumatic brain injury can produce a lasting facilitation of long-term memory formation, with generalization to another domain-specific tasks.
Disclosure: T. Tully, Nothing to Disclose.

28.2 Cognitive Remediation with D-cycloserine Added to Cue Exposure Therapy

A. Eden Evins*

Harvard Medical School, Boston, Massachusetts

Background: Following our initial findings of modest effects ofdaily dosing of D-cycloserine (DCS), a partial agonist of the NMDA receptor, 50 mg/day for negative symptoms and cognitive dysfunction in schizophrenia and evidence of rapid tolerance to repeated dosesof DCS, as well as evidence that DCSenhances extinction learning in animal models, wehave worked to further our understanding of the effect of intermittentdosing of DCS in enhancing memory and efficacy of exposure-basedtreatments.
Methods: Randomized, double-blind, placebo controlled trialsof single dose 50 mg DCS or matching placebo added to CET were conducted inadults with social anxiety, panic, and during early abstinence in adult smokerspre-treated with varenicline or nicotine replacement therapy (NRT) in order totest whether DCS strengthens consolidation of extinction memories.
Results: Isolated 50 mg doses of DCS enhanced CET for social anxiety disorder and CBT for panicdisorder and enhanced the training of attentional biases away fromthreat stimuli, but did not augment non-emotional and non-extinction memory tasks in ahealthy sample and had only limited beneficial effects for verbal memoryenhancement and negative symptoms of schizophrenia. Single-dose DCSenhances efficacy of exposure therapy in anxiety disorders, presumably byenhancing the memory of successful exposure sessions. In cue reactivesmokers, DCS enhances effects of CET. Inrecently abstinent nicotine dependent smokers, the effect of DCS on cravingappears to be moderated by varenicline, as varenicline ameliorated cuereactivity prior to the first CET session. Additional data on effects of CETalone and combined with DCS on laboratory-based assessments of physiologicalreactivity in response to smoking cues rates of lapse and relapse in the first6 weeks of abstinence will be presented.
Conclusions: Intermittent or isolated dosesof DCS 50 mg when given in conjunction with cue exposure therapy appears toenhance emotional extinction learning.
Disclosure: A. Evins, **Part 1:** Pfizer, GSK, Envivo Pharmaceuticals, **Part 4:** Pfizer, GSK, Envivo Pharmaceuticals.

28.3 The Effects of Modafinil and Cognitive Training on Cognitive Performance

Avi Reichenberg*

King's College, London, United Kingdom

Background: Pharmacological compounds and non-pharmacological interventions, such as cognitive remediation techniques, can improve cognition in neuropsychiatric patients and healthy volunteers with modest effect sizes. The present study examines the hypothesis that combining pharmacological and non-pharmacological approaches may bring greater cognitive enhancement than either approach alone.
Methods: Healthy volunteers and schizophrenia patients were included in a randomised, double-blind, placebo-controlled study. Participants received daily cognitive training for 10 days and either 200 mg modafinil daily or placebo. Baseline neuropsychological and functional assessments (MCCB and UPSA) were administered before and after intervention.
Results: Results of the trial in the healthy volunteers group ($N = 33$; 15 Modafinil, 18 Placebo) showed improvement over time in all cognitive training tasks, as well as between pre- and post training MCCB and UPSA. Treatment with Modafinil was associated with improved performance, compared to placebo ($p < 0.05$) in training tasks involving verbal learning, and in particular on an implicit language learning task. Growth modelling analysis demonstrated that on the latter task Modafinil induced greater performance gains, and that overall performance remained superior through the duration of the training and post-training periods. Modafinil did not effect performance on training tasks of working memory and attention processes. Data collection in schizophrenia patients ($N = 40$) is currently on going.
Conclusions: Pharmacological augmentation of a restricted cognitive training protocol shows beneficial effects beyond cognitive training. Cognitive tasks sensitive to pharmacological compounds can be characterized and implemented in early phases of drug development.
Disclosure: A. Reichenberg, Nothing to Disclose.

Mini Panel
29. Pathology Driven Biomarker Development for Major Depressive Disorder: Bridging Central to Peripheral Markers

29.1 Targeting Monoamine Oxidase A as a Biomarker for Major Depressive Disorder

Jeffrey Meyer*

Centre for Addiction and Mental Health; University of Toronto, Toronto, Canada

Background: Monoamine oxidase A (MAO-A) is an enzyme found on the outer mitochondrial membrane of neurons, astrocytes and glia that metabolizes monoamines, facilitates apoptosis and oxidation. In order to develop measures of MAO-A density as biomarkers, we intend to demonstrate that the biomarker abnormality is replicable, that it has a relationship to a useful clinical aspect of illness and that there is a low cost method of assessing its concentration.
Methods: [^{11}C] harmine positron emission tomography was applied to measure regional MAO-A V_T, an index of MAO-A levels, during major depressive episodes ($n = 45$) as well as during a number of high risk states for major depressive episodes including recovery from major depressive episodes ($n = 18$), acute alcohol withdrawal in alcohol dependent subjects ($n = 16$), acute cigarette withdrawal ($n = 22$), during postpartum blues ($n = 15$), and in health ($n = 45$). Primary regions of interest were prefrontal and anterior cingulate cortex, but other regions including hippocampus, dorsal striatum, ventral striatum, thalamus, and midbrain were evaluated. MAO-A level was measured in plasma applying a method of enzyme degredation with subsequent mass spectroscopy in 6 subjects.
Results: MAO-A V_T, is elevated in the prefrontal and anterior cingulate cortex in a third replication sample of depressed subjects. Across every high risk state for a major depressive episode, despite different mechanisms of risk, prefrontal and anterior MAO-A V_T was highly elevated. Stable and consistent MAO-A levels were measurable in plasma.
Conclusions: Indexes of elevated MAO-A density have promise as a biomarker in major depressive disorder for three reasons: First, elevated MAO-A binding is a replicable finding in the prefrontal and anterior cingulate cortex in major depressive disorder. Second, this finding is also prominent in high risk states for onset of major depressive disorder, suggesting utility in predicting course of illness. Third it is possible to measure MAO-A in plasma. Future work will need to evaluate the specific utility of the plasma measurement of MAO-A for predicting course of illness.
Disclosure: J. Meyer, **Part 1:** The author has been a consultant for companies that make antidepressants, including Eli Lilly, SK Life Sciences, Lundbeck, Takeda, Bristol-Myers Squibb, and GlaxoSmithKline, and has received operating grants from some of these companies. It is likely that he will in the future receive consulting contractsor operating grants from companies that make MAO-A inhibitors. He has applied for a patent to use MAO-A measures to predict the course of illness and to treat major depressive disorder, **Part 2:** The author has been a consultant for companies that make antidepressants,including Eli Lilly, SK Life Sciences, Lundbeck,

Takeda, Bristol-Myers Squibb,and GlaxoSmithKline, and has received operating grants from some of these companies, **Part 3:** The author has been a consultant for companies that make antidepressants, including Eli Lilly, SK Life Sciences, Lundbeck, Takeda, Bristol-Myers Squibb, and GlaxoSmithKline, and has received operating grants from some of these companies.

29.2 Imaging the 18 kDa Translocator Protein (TSPO) *in Vivo*

Eugenii A. Rabiner*

Imanova Centre for Imaging Sciences, London, United Kingdom

Background: *In vivo* imaging of the 18 kDa Translocator Protein (TSPO, formerly known as the peripheral benzodiazepine receptor – PBR) has been complicated by the limitations of the first generation TSPO PET ligand – [11C]PK11195. [11C]PK11195 has poor signal-to-noise, and poor quantification, disallowing the estimation of global changes in TSPO signal, and making the estimation of gradual changes in TSPO density, due to disease progression, or pharmacological treatment, very difficult. The 2nd generation TSPO PET ligands (e.g. [11C]PBR28) promise accurate quantification of TSPO density, however have been troubled by differences in the affinity of these ligands for the TSPO across the population, making comparisons across groups difficult. We have identified three binding classes for these compounds across the population, named high, mixed and low affinity binders (HAB, MAB & LAB). We demonstrated that almost all TSPO ligands have differential affinity for the TSPO, with the HAB: LAB ratio ranging from 5 to 50-fold. The same phenomenon was seen for a novel putative anxiolytic, XBD173, the failure of which in clinical trials could plausibly be attributed to inadequate TSPO occupancy in ~50% of the population.
Methods: We used evaluated the TSPO genotype in 41 healthy volunteers of known TSPO binding status (27 HAB, 12 MAB, 2 LAB). PET studies conducted with two TSPO PET ligands of differing affinity ratio for the binding classes ([11C]PBR28 and [18F]PBR111) to evaluate the levels of specific TSPO binding across the brain regions of healthy volunteers and patients with neuropsychiatric conditions. The relationship of the TSPO binding status to physiological parameters of healthy volunteers was assessed.
Results: The rs6971 SNP, coding for the Ala147Thr substitution, fully explained the characteristics of the different binding classes. The binding of the PET ligands *in vivo* was predicted by the genotype, while physiological responses differed between individuals of differing genotypes.
Conclusions: Our data provides the explanation for the heterogeneity observed in the binding of 2nd generation TSPO ligands and provides a method for their successful use in future studies. The rs6971 SNP provides an important parameter to include in the design of future studies of novel pharmaceuticals, and the evaluation of normal physiological responses.
Disclosure: E. Rabiner, **Part 1:** Until October 2011, was a full time employee of Glaxo Smith Kline. I am currently a consultant for Glaxo Smith Kline, Biotie and Takeda, **Part 2:** Full time employee of GlaxoSmithKline up to October 2011.

29.3 Protein Kinase A: Biomarker for Major Depression

Yogesh Dwivedi*

University of Illinois, Chicago, Illinois

Background: Emerging evidence indicates that mood disorders are associated with altered neuronal plasticity. Adaptive responses in intracellular molecules, together with the modulation of functional responses mediated by the phosphorylation of critical proteins, and ultimately gene expression, controlled by intracellular signaling cascades, all participate in a major way in synaptic and structural plasticity. Protein kinase A (PKA) occupies a central position in the adenylyl cyclase-cyclic AMP signaling system and responds after binding with cAMP. It participates directly in many physiological functions regulating gene transcription, cell survival, and neural plasticity. In addition, by phosphorylating the components of other signaling cascades, it provides the means for cross-talk between the AC-cAMP and other signaling systems. Given the central role of PKA in neural plasticity, using multiple clinical and pre-clinical approaches, we thoroughly investigated the role of PKA in depression and whether PKA can be used as a biomarker.
Methods: cAMP binding to regulatory subunits, catalytic subunit-specific activation of PKA and expression of various PKA regulatory (RIα, RI β, RIIα, RIIβ) and catalytic (Cα, Cβ) were studied in the PFC and hippocampus of antidepressant-free depressed subjects (n = 40) and matched normal controls (n = 40). These measures were also examined in rat model of depression (learned helpless, non-learned helpless and tested control rats) as well as platelets of depressed (n = 20) and comparison normal controls (n = 20) and bipolar manic patients (n = 20). Depression-like behavior was also studied in PKARIIβ mutant mice. PKA-mediated functional analyses were also examined in peripheral tissues and human brain of depressed subjects.
Results: cAMP binding to regulatory subunit of PKA was decreased in depressed subjects, which was associated with decreased Cβ-associated cAMP-stimulated catalytic activity and decreased expression of selective RIIβ and Cβ subunits. In the animal model, whereas single stress paradigm increased levels of RIα, and RIIα, and decreased expression of RIIβ and Cβ, repeated stress paradigm showed only decreased expression of RIIβ and Cβ subunits. These changes were replicated in platelets of depressed subjects, which was found to be opposite in mania patients. Functional analysis in depressed subjects revealed that PKA substrate Rap1 was less activated and that a series of low molecular weight proteins were less phosphorylated in presence of cAMP. PKARIIβ mutant mice showed increased escape latency in the behavioral paradigm for learned helplessness.
Conclusions: These data show hypoactivation of PKA in depressed subjects, which appears to be related to less expression of RIIβ and Cβ subunits. This hypoactivation can be successfully replicated in peripheral tissues of depressed subjects. As with depressed subjects, repeated stress paradigm also showed changes only in RIIβ and Cβ subunits. It appears that normalization of single stress paradigm-induced increased expression of RIα and RIIα may be an adaptive response to chronic stress. Functional analysis further confirms hypoactivation of PKA in depressed subjects and that PKA gene deletion may induce depressive behavior. Overall, these studies suggest that PKA may serve as strong predictor of depressive behavior.
Disclosure: Y. Dwivedi, Nothing to Disclose.

Panel
30. Molecular and Cellular Mechanisms Underlying Resilience in Mood and Other Social-psychological Stress-related Disorders: New Avenue for Novel Therapeutics?

30.1 The Lasting Legacy of Early Social Stress on the Epigenome

Dietmar Spengler*

Max-Planck-Institute of Psychiatry, Munich, Germany

Background: The exceptionally strong impact of early experience on brainarchitecture makes the early years of life a period of both great opportunityand great vulnerability for brain development. Childhood adversity, includingexposure to sexual and physical abuse, is a global burden with reportedprevalence rates ranging from 11 to 35%. Despite strong evidence for a linkbetween early life adversity and psychopathology, insight into the molecularmechanisms underlying the long-lasting consequences for mental health has remainedlimited. The DNA modification hypothesis of memory storage poses that thephysical basis of lasting memories could lie in the enzymatic modification ofthe DNA of nerve cells whereby the modification consists of methylation ordemethylation. Thus, brain activity induced by exposure to detrimental levelsof stress early in life might elicit long-lasting

epigenetic changes in braincells that instruct our bodies' response to adversity throughout lifespan. In aparallel fashion, positive mastery experiences might initiate lastingepigenetic changes that bring about the expression of genes in brain cells thatare essential for successful learning and stress management.
Methods: Periodic infant-mother separation in mice (3 hours per day from postnatal day (PND) 1–10) has been used to model early life adversity. Mice were followed up for 1 yr by endocrine and behavioral tests. Key regulatory genes of the hypothalamic-pituitary-adrenal (HPA) axis were analyzed for changes in gene expression, DNA methylation and underlying molecular mechanisms.
Results: Social DNA memories mirror the complexity of the stress response as well as sex differences in brain epigenetics and comprise risk-prone and defensive responses over time.
Conclusions: Epigenetic mechanisms can mediate the effects from early social life on the epigenome and produce persistent memories hard coded by DNA methylation. The hypothalamic-pituitary adrenal axis canalizes differences in the quality of early social life which can leave their footprints at key regulators sites of this versatile system. Early life adversity is a global burden and timely therapeutic interventions should aim to attenuate early social stress derived DNA marking and their life-long consequences for mental health.
Disclosure: D. Spengler, Nothing to Disclose.

30.2 Blockade of the Inflammasome in Brain Produces Antidepressant Effects and Resilience to Stress

Ronald S. Duman*

Yale University School of Medicine, New Haven, Connecticut

Background: Elevated immune and inflammation responses can be caused by psychological and environmental stress and play a key role in the pathophysiology of depression. The inflammasome is a key molecular scaffold found in macrophages and microglia that underlies the processing and release of inflammatory cytokines. Here we present new data on the role of the inflammasome in the formation of cytokines in brain and behavioral responses to stress, the mechanisms underlying stress-activation of the inflammasome, and potential targets for blocking these effects.
Methods: The effects of acute and chronic stress on pattern recognition receptors (i.e., NRLP3) that make up the inflammasome, activate caspase-1 and thereby drive inflammation in the prefrontal cortex (PFC) and hippocampus of rat are being studied. Levels of ATP, an upstream activator of the inflammasome, as well as the downstream cytokine IL-1b, are being determined by microdialysis. The effects of stress and manipulation of the inflammasome, including blockade of the ATP-P2X7 receptor), on depression related behaviors (i.e., forced swim, novelty suppressed feeding, and sucrose preference) are being determined.
Results: The results demonstrate that acute immobilization stress rapidly increases levels of NLRP3 and activated caspase-1 in the PFC and hippocampus, and these effects are blocked by pretreatment with a P2X7 receptor antagonist. In addition, immobilization stress rapidly increases levels of ATP with a time course consistent with activation of the inflammasome and release of IL-1b. Finally, blockade of inflammasome activation by treatment with the P2X7 receptor antagonist reverses the effects of chronic unpredictable stress on anhedonic and anxiogenic behaviors.
Conclusions: The results demonstrate that acute and chronic stressors activate the inflammasome and thereby increase cytokine levels in limbic brain regions that control mood and depression. Stress also increases extracellular ATP, an activator of the inflammasome, and blockade of the P2X7 receptor blocks activation of the inflammasome, resulting in enhanced resilience to depression related behaviors. Studies are currently underway to identify the mechanisms for regulation of ATP and other factors that control inflammasome activation in brain, as well as determinants that contribute to vulnerability.
Disclosure: R. Duman, **Part 1:** Lundbeck, Lilly, Taisho, Forest, J&J, Bristol Myers Squibb, Pfizer, **Part 4:** Lundbeck, Lilly, Forest, J&J.

30.3 Plausible Roles of Bcl-2 Family Proteins in Cellular and Behavioral Resilience to Mood Disorders

Guang Chen*

Janssen Pharmaceutical Companies of Johnson and Johnson, La Jolla, California

Background: Bcl-2 (B cell lymphoma protein 2) family proteins are key modulators of apoptosis and cell survival. These proteins modulate mitochondrial and ER function as well as calcium homeostasis. They are involved in neurodevelopment, adult neurogenesis, axonal and dendritic remodeling, AMPA receptor trafficking, and synaptic plasticity. Studies have also demonstrated that these proteins modulate glucocorticiod receptor translation to nuclei and to mitochondria. Human postmortem brain studies show reduced levels of Bcl-2 (an anti-apoptotic protein) and increased levels of Bad and Bax (pro-apoptotic proteins) in cerebral cortical tissue from patients with bipolar disorder. Genetic and biomarker data suggest that a Bcl-2 SNP is associated with increased risk for bipolar disorder, as well as lower mRNA and protein levels and abnormal ER stress response and calcium regulation in lymphoblast cells. We also found that chronic treatment with mood stabilizers up-regulated Bcl-2 and BAG1 (Bcl-2 associated athanogene) mRNA and proteins in animal brain and promoted Bcl-2 function. Together, these data suggest that Bcl-2 family proteins are likely to play key roles in behavioral resilience to mood disorders. Targeting the function of Bcl-2 family proteins in the brain is a potential way to develop novel mood stabilizers.
Methods: Clinical studies were conducted in patients with mood disorders who received lithium treatment to assess whether the up-regulation of Bcl-2 proteins by mood stabilizers also occurs in humans. To investigate the role of Bcl-2 proteins in behavioral resilience, behavioral studies were conducted with Bcl-2, BAG1, and BI-1 mutant mice treated with Bcl-2 and Bid inhibitors. Signal transduction and gene expression regulation studies are carried out to explore the mechanisms via which mood stabilizers regulate Bcl-2. Postmortem human brain studies were conducted to examine whether there is a dysfunction in the Bcl-2 expression regulatory pathway. A bioinformatics search was performed to identify CNS-specific ways to regulate Bcl-2 function.
Results: Chronic, but not acute, lithium treatment upregulated Bcl-2 levels in the lymphocytes of patients with mood disorders. BAG1 and BI-1 over-expression protected mice from developing behavioral deficits in the learned helplessness and monoamine depletion paradigms of depression. Bcl-2 HET KO and mice treated with a Bcl-2 inhibitor appeared normal without manipulation; however, both were more susceptible to developing behavioral deficits in the helplessness model and were super-sensitive to amphetamine challenge models of mania. Both ERK and PI3K pathways were at least partially involved in the upregulation of Bcl-2 by mood stabilizers. Reductions in components of the ERK pathway were found in postmortem brain tissue from patients with mood disorders. The bioinformatics search revealed potential targets and pathways that may up-regulate Bcl-2 function, such as neuropeptides and brain-specific phosphatase.
Conclusions: Clinical, postmortem human brain, pharmacological, and behavioral evidence all support the notion that Bcl-2 family proteins play a robust role in behavioral resilience in mood disorders. Bcl-2 proteins are widely expressed throughout the body and modulate several essential cellular functions, including cell turnover. One way to avoid the mechanistic side effects of Bcl-2 potentiation is to selectively regulate its function in the brain. Although confirmation studies are needed, informatics analysis indicates that selective regulation seems possible.
Disclosure: G. Chen, **Part 1:** Janssen Pharmaceutical Companies of Johnson and Johnson, **Part 2:** Janssen Pharmaceutical Companies of Johnson and Johnson, **Part 3:** Janssen Pharmaceutical Companies of Johnson and Johnson, **Part 4:** Janssen Pharmaceutical Companies of Johnson and Johnson.

30.4 Mechanisms Underlying the Resilience to Severe Social Stress and the Role of Ventral Tegmental Area (VTA)

Ming-Hu Han*

Mount Sinai School of Medicine, New York, New York

Background: Prolonged stressful events are a major cause of major depressive disorder in some individuals (susceptibility), but not in others (unsusceptibility or resilience). Primarily, studies in the field of depression have explored the pathogenic mechanisms in the brain. Until recently, sufficient attention has not been paid to the understanding of resilience, possibly because resilient individuals are psychophysiologically perfectly stable following prolonged stress. Notably, most individuals successfully employ active coping skills in response to stress, such as optimism, rationalization, wishful thinking, relaxation, and humor. These skills have been linked to the function of the VTA dopamine (DA) system in the brain's reward circuit, and only recently, work in this field has begun to understand the neurobiological basis for these psychosocial coping skills. Consistent with these studies, using a chronic social defeat stress model of depression, we surprisingly found that over three-fold more genes in the VTA were regulated in resilient mice than in the susceptible subpopulation (Cell 2007), demonstrating active brain mechanisms of the resilience phenotype. This provides a possible new avenue to explore therapeutic strategies for depression treatment by understanding the naturally occurring active resilience mechanisms. Here, in our continued work, we hypothesize that additional molecular changes in response to stress maintain a physiological and behavioral stable output. The idea that the more things change, the more they stay stable, lends itself to a novel neurophysiological concept for resilience.
Methods: Based on behavioral tests, susceptible and resilient mice were segregated following a chronic (10-day) social defeat stress paradigm, a highly validated mouse model of depression. The molecular and cellular causal mechanisms that underlie the resilient phenotype were investigated in TH-Cre and TH-GFP mice by the combined use of optogenetic approaches and electrophysiological techniques.
Results: We previously found abnormal hyperactivity in the VTA DA neurons of susceptible mice, but NOT of the resilient subgroup – the cell firing is the same as control mice. Recently, our optogenetic studies demonstrated that the hyperactivation of these neurons is a direct pathogenic mechanism to promote susceptibility. Further, increased hyperpolarization-activated cation current (Ih) was found to underlie this hyperactivity. In an effort to understand the resilience phenotype, we were interested in exploring how the firing activity of these DA neurons in resilient mice stays the same as in control mice. Unexpectedly, we found that the Ih current was increased significantly more in the resilient subgroup compared to susceptible and, more importantly, observed a compensatory upregulation of potassium (K+) channels ONLY in resilient mice. It appears that the Ih channels are more responsive or sensitive to defeat stress in resilient mice, which in turn may induce a K+ channel upregulation to compensate for the Ih-induced hyperactivity. Consistent with this idea, we found that chronic *in vivo* Ih potentiation induced a similar compensatory upregulation of K+ channel function and converted susceptible mice to resilient, an interesting treatment efficacy. Moreover, we showed that K+ channel potentiators act as more efficient antidepressants. These findings demonstrate that resilient mice functionally utilize additional channels, such as K+ channels, to homeostatically counteract the pathophysiological hyperactivity of VTA DA neurons and pharmacological potentiation of this naturally occurring resilience mechanism, functions as an antidepressant. Our evidence of homeostatic resilience ion mechanisms provides a novel direction for exploring channel specific drug targets for treatment of depression.
Conclusions: Based on our novel understanding of resilience, we demonstrate that the pathogenic hyperactivation is actually a failure of homeostatic plasticity in the dopamine system. Additionally, we show that resilient mice employ homeostatic modulation via K+ channels to successfully cope with stress and avoid developing depressive behaviors. More importantly, we found that mimicking the naturally occurring resilient phenotype by enhancing K+ channel function shows an antidepressant effect.
Disclosure: M. Han, Nothing to Disclose.

Panel
31. Reward/Motivation Deficits in Attention Deficit Hyperactivity Disorder (ADHD) and the Effects of Medication

31.1 Functional Connectivity of Reward Circuits in the Rat and Human Brain

Elliot A. Stein*

National Institutes of Health, Baltimore, Maryland

Background: Abnormalities in the brain dopamine reward circuit are increasingly implicated in the neurobiology of ADHD. This presentation focuses on the neurocircuitry underlying the disruption of the mesocorticolimbic (MCL) reward circuits and the effects of acute psychostimulant administration in this circuit. Extended access (LgA) cocaine self administration (SA) in rats induces behaviors that are thought to capture important criteria of human addiction and were used as a model system to study induced circuit neuroadaptations and to compare these with that seen in a human cross sectional study.
Methods: To understand the neuroadaptations associated with LgA and to distinguish MCL circuits that accompany addiction from general learning processes associated with goal-directed behavior, two groups of rats were trained to self-administer either cocaine (iv) or sucrose (orally) followed by an identically enforced abstinence period. A third group of sedentary animals controlled for handling effects. Resting-state functional connectivity (rsFC) was employed as a measure of intrinsic neurobiological interactions between brain regions. Imaging data were collected under anesthesia after 20 days of LgA SA followed by 30 days of enforced abstinence. rsFC were also obtained from human cocaine dependent and matched healthy control subjects.
Results: Decreased rsFC was seen in the cocaine-SA compared with both sucrose-SA and housing control groups between prelimbic cortex (PrL)- entopeduncular nucleus and nucleus accumbens core (AcbC)-dorsomedial prefrontal cortex (dmPFC). Circuits reflecting general learning were seen within the dorsal striatum and between PrL and dorsal striatum. Moreover, individual differences in cocaine SA escalation predicted connectivity strength in the Acb-dmPFC circuit. Acute cocaine administration increased circuits between and within NAc shell and core. SImilar MCL circuit reductions were seen in human cocaine dependent subjects.
Conclusions: These preclinical data provide evidence of fronto-striatal plasticity across the addiction trajectory, are consistent with Acb-PFC hypoactivity seen in abstinent human drug addicts as well as in persons with ADHD and suggest potential circuit level biomarkers that may inform therapeutic interventions. They further suggest that data from cross sectional human studies reflect the consequence of rather than a predispositional predecessor to their dependence. Insofar as ADHD is treated using stimulant medications similar to cocaine, and rsFC has identified similar circuit impairments in ADHD, these data also speak to shared circuit mechanisms.
Disclosure: E. Stein, Nothing to Disclose.

31.2 Reward Circuitry, Risky Behaviors and ADHD

Francisco Xavier Castellanos*

New York University Langone Medical Center, New York, New York

Background: Effective treatments for attention-deficit/hyperactivity disorder (ADHD) include either enhancing catecholaminergic neurotransmission (e.g., stimulants) or positive reinforcement effectiveness. Nevertheless, identifying differential behavioral

deficits in reward-related processing has been challenging. In contrast, a range of functional neuroimaging approaches are beginning to yield convergent results implicating ventral striatum-related circuits in the pathophysiology of ADHD. Based on a placebo-controlled study of levodopa modulation of striatal circuitry in healthy young adults (Di Martino et al, 2008; Kelly et al, 2009), and the relationships between striatal intrinsic functional connectivity and expected benefit from risky behaviors in healthy adults (Cox et al, 2010), we now examine striatal intrinsic functional connectivity in adults with DSM-IV ADHD in relation to the same measure of expected benefit from risky behaviors we previously obtained in healthy adults.

Methods: We analyzed resting state BOLD EPI data (3T; 6 min, TR = 2000 ms; 3x3x3 mm) from 41 adults with ADHD (age 31.7 +/− 10.2 y; 25 males; 14 pred. Inattentive, 25 Combined type; 2 H/I type). Participants were assessed with the Cognitive Appraisal of Risky Events (CARE; Fromme, Katz & Rivet, 1997) regarding their likert ratings of the expected negative and positive consequences of illicit drug use, aggressive/illegal behavior, risky sexual behavior, and drinking, along with an estimate of the frequency of such behaviors in the preceding 6 months. As in a prior analysis of healthy participants (Cox et al, 2010), we averaged ratings over the 4 risky behavioral domains. Resting state functional connectivity (RSFC) analyses used the same ventral striatal seeds (Di Martino et al, 2008; Cox et al, 2010). Imaging analyses were corrected for whole-brain comparisons via Gaussian random fields theory ($Z > 2.3$; $p < .05$, corrected).

Results: Expected benefit (positive expectations) from risky behaviors were significantly positively related to RSFC between right ventral striatum and right superior parietal cortex and significantly negatively related to dorsomedial and anteriormedial prefrontal cortex (PFC). For negative expectations from risky behaviors (risk aversion) RSFC between right ventral striatum was positively related to left dorsolateral PFC, and negatively to visual cortex for both right and left ventral striatum. For left ventral striatum, negative expectations were related positively to precuneus RSFC.

Conclusions: Intrinsic functional connectivity appears to yield robust indices that are related to phenotypic trait measures. In a sample of 21 healthy adults scanned twice on average 11 months apart, we found an inverted (negative) relationship between expected benefit and ventral striatum-parieto-occipical connectivity. Here, in 41 adults with ADHD, we found a significant positive relationship in this circuit, such that risk seeking was greater in those with greater correlation/connectivity between ventral striatum and parieto-occipital cortex. Risk aversion was greater in participants with ADHD with stronger connectivity between ventral striatum and anterior default network nodes, suggesting that greater self-reflection may play a beneficial role in ADHD.

Disclosure: F. Castellanos, Nothing to Disclose.

31.3 Dopamine Reward Circuitry in Attention Deficit Disorder

Nora D. Volkow*

National Institutes of Health, Rockville, Maryland

Background: There is increasing evidence of deficits in reward and motivation in ADHD. However the neurobiological substrates underlying this dysfunction have not been properly characterized. Here we have used positron emission tomography (PET) and appropriate radiotracers to evaluate the DA reward pathway in never medicated adults with ADHD and its association with clinical symptoms and to assess the effects of acute and chronic treatment with methylphenidate (MP).

Methods: PET studies to evaluate the involvement of the DA reward pathway measured DA D2 receptors or D2R (using [11C]raclopride) and DA transporters or DAT (using [11C]cocaine) and DA release (measured as changes in [11C]raclopride binding when challenged with iv MP) were done in 53 never medicated adults with ADHD and 44 healthy controls. PET studies done to study neuroadaptations in the brain DA system (D2R, DAT and DA release) with chronic MP was a prospective study done in 20 never medicated adults with ADHD and 12 controls (followed over 12 month but without treatment intervention). ADHD participants were tested prior to initiation of treatment and 12 months following treatment with MP (Concerta).

Results: Never medicated ADHD adults when compared with healthy controls show significant decreases in DAT and D2R availability in the ventral striatum (nucleus accumbens area) and these were associated with symptoms of inattention and reduced motivation. When challenged with iv MP ADHD participants showed a significant reduction in MP-induced changes in [11C]racopride binding (reduced DA release) in VS. Twelve month treatment with MP led to a significant increase in DAT, a significant decrease in D2R and a blunting of DA release with MP in VS. The upregulation of DAT with chronic MP treatment in the ADHD participants predicted the attenuation of DA release after chronic MP treatment. We also showed that the DA release with MP in VS predicted the response to treatment (improvement in symptoms of inattention but not hyperactivity).

Conclusions: Our results in adults with ADHD provide evidence that the DA reward pathway is disrupted with ADHD and contributes to both inattention and disrupted motivation and that stimulant medications exert their therapeutic effects in ADHD in part by compensating for signaling deficits in the DA reward pathway. We also provide evidence of neuroadaptations in the DA reward pathway with chronic stimulant medication.

Disclosure: N. Volkow, Nothing to Disclose.

31.4 Altered Sensitivity to Reinforcement in Individuals with ADHD: Implications for the Development of Aberrant Health Behaviors

Scott H. Kollins*

Duke University School of Medicine, Durham, North Carolina

Background: Compared to their non-diagnosed peers, individuals with ADHD demonstrate differential sensitivity to reinforcing stimuli. A direct link between altered reinforcement processes and risk for substance abuse has not been demonstrated. This presentation will present data on the differential reinforcing effects of cigarette smoking in adults with and without ADHD, and the potential moderating effects of several genes associated with dopaminergic activity in the brain.

Methods: In 2 studies, adult smokers between 18-45 years were recruited. Half of the subjects were diagnosed withadult ADHD. In Study 1, subjects completed 2 sessions: 1 following smoking asusual, and one following 24-hour abstinence. During each session, smokingreinforcement was assessed with a 90-min Progressive Ratio (PR) task. In Study2, subjects completed 3 sessions in which they were administered 0, 10, and 40 mg methylphenidate under double-blind conditions and subsequently completed thePR task. Blood samples were obtained for genetic analysis of the DRD2, DAT1, DRD4, and COMT genes.

Results: In Study 1, smokers worked more for cigarette puffs when abstinent, and smokers with ADHD worked more for puffs, regardless of condition. Smokers with ADHD worked relatively more for cigarette puffs during abstinence, as compared to non-ADHD smokers, suggesting that smoking reinforcement is greater during abstinence for this high-risk group of smokers. Across groups, there were several dopamine-related genes that were associated with abstinence-induced smoking reinforcement.

Conclusions: Several conclusions can be drawn from these data. First, differential sensitivity to reinforcing stimuli may underlie adverse smoking outcomes in adults with ADHD. Second, our results show that, even with relatively small sample sizes, variation in specific dopamine-related genes is association with smoking reinforcement.

Disclosure: S. Kollins, **Part 1:** Research Support: Rhodes Pharmaceuticals, Shire Pharmaceuticals, Otsuka Pharmaceuticals, Addrenex/Shionogi Pharmaceuticals Consultant/Advisory Board: Shire Pharmaceuticals, Otsuka Pharmaceuticals, Addrenex/Shionogi Pharmaceuticals, WebMD/Medscape, **Part 2:** Shire Pharmaceuticals, Otsuka Pharmaceuticals, **Part 4:** Shire Pharmaceuticals.

Panel
32. Opioid and Cannabinoid Mechanisms in Alcohol Addiction: Recent Evidence from Functional Brain Imaging

32.1 The Effects of Alcohol Consumption on Endogenous Opioid Release in the Human Orbitofrontal Cortex and Nucleus Accumbens

Jennifer Mitchell*

The University of California, San Francisco, California

Background: Understanding how alcohol produces reward, motivates further consumption, and eventually leads to addiction is necessary to design treatments for alcohol abuse, dependence, and relapse. Although ethanol modulates a variety of molecular targets, including several neurotransmitter receptors, the neural mechanisms that underlie its rewarding actions and lead to excessive consumption are unknown. Studies in animals suggest that release of endogenous opioids by ethanol promotes further consumption. To examine this issue in humans and to determine where in the brain endogenous opioids act to promote alcohol consumption, we measured displacement of a radiolabeled m opioid receptor agonist, [11C]carfentanil, before and immediately after alcohol consumption in both heavy drinkers and control subjects.
Methods: Heavy social drinkers (n = 13; 10-20 drinks per week) and matched healthy control subjects (n = 12; fewer than 7 drinks per week) were recruited based on alcohol consumption and were matched for gender, age, and ethnicity.
PET data were acquired for a total of 90 min per imaging session. A 10-min positron transmission image was acquired before injection using a rotating 68Ge source. Each scan entailed the injection of 10 to 15 mCi of [11C]carfentanil. After the first scan (pre-alcohol), subjects were removed from the scanner for 30 min to stretch and use the restroom. Subjects were then given 5 min to consume a standardized drink of alcohol [males = weight in kilograms × 1.2658 × 0.421 = milliliters ethanol (EtOH); females = weight in kilograms × 1.2658 × 0.356 = milliliters EtOH] mixed in a 2:1 volume ratio with juice and were then immediately repositioned in the scanner for a second imaging sequence identical to the first.
Results: Drinking alcohol induced opioid release in the nucleus accumbens and orbitofrontal cortex, areas of the brain implicated in reward valuation. Opioid release in the orbitofrontal cortex and nucleus accumbens was significantly positively correlated. Furthermore, changes in orbitofrontal cortex binding correlated significantly with problem alcohol use and subjective high in heavy drinkers, suggesting that differences in endogenous opioid function in these regions contribute to excessive alcohol consumption. Additionally, subjects with the COMT Val158 allele were found to have increased opioid release in the right NAc and decreased release in bilateral medial OFC following alcohol consumption, consistent with previous reports suggesting that the Val158 polymorphism may be a risk factor for alcohol abuse and dependence.
Conclusions: These data are consistent with previous studies implicating endogenous opioids and MOR activation in alcohol reward and suggest that MOR activation in the OFC contributes to ethanol reward processing. Furthermore, our finding that changes in OFC MOR binding correlate significantly with problem alcohol use and subjective feelings of well-being after alcohol consumption in heavy drinkers but not in controls suggests that dysfunction of the OFC contributes to excessive consumption of alcohol. Our data also suggest that genetic regulation of dopamine levels can affect alcohol reward in part by changing endogenous opioid release in reward-related brain regions.
Disclosure: J. Mitchell, **Part 1:** I am a founding member of a company that will eventually provide *ex vivo* GPCR screening.

32.2 Influence of Mu-Opioid Receptor (OPRM1) A118G Polymorphism on Pharmacological Effects of Alcohol: A Translational Approach

Vijay A. Ramchandani*

National Institutes of Health, Bethesda, Maryland

Background: Mesolimbic dopamine release is a key signal for the reinforcing effects of drugs of abuse, including alcohol, and endogenous opioids are thought to exert their effects by modulating this release. A functional polymorphism (A118G) in the mu-opioid receptor (OPRM1) gene has been associated with greater subjective responses to alcohol, increased risk for alcoholism, and the response to naltrexone treatment. Additionally, alcohol preference and alcohol-induced stimulation has been observed in rhesus macaques carrying the functional equivalent of the OPRM1 A118G variant. This differential response to alcohol observed as a function of the OPRM1 A118G polymorphism may reflect differential activation of striatal dopamine release following acute alcohol administration. Thus, the objective of this study was to examine the influence of the OPRM1 A118G polymorphism on (1) subjective and neuroendocrine responses and on striatal DA release, measured by 11C-raclopride displacement using PET, following intravenous (IV) alcohol administration in humans, and (2) alcohol response and effects of naltrexone in mice.
Methods: Healthy male social drinkers were screened to obtain 2 groups: 1) homozygous for the major 118A allele (118AA); and 2) carrying 1 or 2 copies of the variant 118G allele (118GX). Subjects underwent 2 PET scan sessions with 11C-raclopride while they received pharmacokinetically-controlled IV infusions of alcohol (6%v/v) to a target level of 0.08g% or saline, in counter-balanced order. Serial blood samples for cortisol and ACTH levels and self-ratings of subjective effects were obtained. 11C-raclopride binding potentials following alcohol and saline were compared in pre-defined striatal regions: anterior and posterior ventral striatum, caudate and putamen. To confirm the causal role of OPRM1 A118G variation, 2 humanized mouse lines carrying the respective human sequence variant were generated. Brain microdialysis was performed following alcohol challenge in these mice to evaluate differences in dopamine release. Also, the effect of naltrexone on alcohol self-administration was compared between the two mice lines.
Results: Subject groups did not differ in peak scores for "high" and "intoxication", however the 118GX subjects showed higher scores for "liking effects" and "wanting more" than the 118AA subjects. The 118GX group showed significantly higher cortisol and ACTH levels at baseline and greater alcohol-induced decreases in cortisol and ACTH levels compared to the 118AA group. The 118GX group, but not the 118AA group, showed significant decreases in 11C-raclopride binding potential in striatal regions, indicating greater dopamine release, following alcohol compared to placebo. Brain microdialysis in mice showed a four-fold greater peak dopamine response to an alcohol challenge in h/mOPRM1-118GG than in h/mOPRM1-118AA mice. The h/mOPRM1-118GG mice showed a preferential dose-related decrease in alcohol self-administration following naltrexone compared with h/mOPRM1-118AA mice.
Conclusions: Social drinkers carrying the 118G variant allele had greater striatal dopamine release following alcohol. Mice carrying the human sequence 118G variant allele showed greater brain dopamine release following alcohol challenge, and a selective reduction in alcohol self-administration following naltrexone. Taken together, these data indicate that OPRM1 A118G polymorphism is a determinant of dopamine response to alcohol, a mechanism by which it likely modulates alcohol reward. These data add to the growing body of literature highlighting the role of the opioid system in the pharmacological effects of alcohol and as a target for alcoholism treatment. The striatal dopamine response to alcohol may also be useful as an endophenotype to examine the role of other genetic determinants of alcohol response and alcoholism risk.
Disclosure: V. Ramchandani, Nothing to Disclose.

32.3 CNR1 Variation Is Associated with BOLD Response to Alcohol Cues and Alcohol Dependence Symptom Count

Kent Hutchison*

University of Colorado, Boulder, Colorado

Background: At a neurobiological level, the etiology of alcohol dependence is related to changes in the neuronal systems involved in the anticipation of reward and executive control. Genetic and epigenetic variations that are associated with individual differences in these mechanisms may be important in terms of predicting risk for the development of dependence and risk of relapse after treatment. Previous studies have linked variation in the cannabinoid receptor gene (CNR1) with craving for alcohol, risk for alcohol dependence, and related phenotypes like impulsivity. The objective of the present study was to test CNR1 variants for an association with BOLD response to alcohol cues and alcohol dependence.
Methods: A sample of 326 individuals with an Alcohol Use Disorder completed an alcohol cue exposure fMRI task. For each subject, 13 CNR1 SNPs were assayed using the Illumina 1 M Duo array and tested for an association with BOLD response to alcohol cues. As a replication, the top two findings were tested for an association with alcohol dependence symptom counts in a large population based, publicly available sample (SAGE).
Results: Of the 13 SNPs tested for an association with responses during the alcohol cue fMRI task, two (rs10485171 and rs1406977) were significantly associated with BOLD response in the striatum and prefrontal cortex ($p < .01$). Rs10485171 was significantly associated with symptom count both in the overall sample ($p < .05$) as well as a subsample of Caucasians only ($p < .05$), whereas rs1406977 was not.
Conclusions: Consistent with previous studies, these analyses suggest that CNR1 variation may be linked with individual differences in the neuronal systems that underlie the attribution of incentive salience to alcohol and risk for alcohol dependence.
Disclosure: K. Hutchison, Nothing to Disclose.

32.4 Reduced Cannabinoid CB1 Receptor Binding in Alcohol Dependence Measured with Positron Emission Tomography

Markus Heilig*

National Institutes of Health, Bethesda, Maryland

Background: Stimulation of CB1 receptors increases alcohol intake across a variety of rodent models, whereas the opposite is observed with genetic or pharmacological CB1 receptor blockade. In rats and mice, chronic alcohol exposure increases the concentration of endogenous cannabinoids in most brain regions and decreases the density of CB1 receptors, a change that is reversible upon abstinence. Together, these animal studies suggest that reinforcing properties of alcohol are in part mediated through the endocannabinoid system, but no human data are available to address this issue. Furthermore, a common single nucleotide polymorphism (SNP), rs2023239, in the gene encoding CB1 receptors (CNR1) tags a haplotype associated with substance use disorders including alcohol dependence. A recent study of patients with alcohol dependence linked the rs2023239 C allele with greater subjective reward from alcohol, greater midbrain and prefrontal cortex activation in response to alcohol cues, and higher density of CB1 receptors in post-mortem samples of prefrontal cortex. Whether this SNP also moderates CB1 receptor density *in vivo* is unknown.
Methods: We measured CB1 receptors in alcohol dependent patients in early and protracted abstinence, and in comparison with control subjects without alcohol use disorders, using positron emission tomography (PET) and [18F]FMPEP-d2, a radioligand for CB1 receptors. We scanned 18 male inpatients with alcohol dependence twice, within 3–7 days of admission from ongoing drinking, and after 2–4 weeks of supervised abstinence. Imaging data were compared with those from 19 age-matched healthy male control subjects. Data were also analyzed for potential influence of a common functional variation (rs2023239) in the CB1 receptor gene (CNR1) that may moderate CB1 receptor density.
Results: On the first scan, CB1 receptor binding was 20–30% lower in patients with alcohol dependence than in control subjects in all brain regions and was negatively correlated with years of alcohol abuse. After 2–4 weeks of abstinence, CB1 receptor binding remained similarly reduced in these patients. Irrespective of diagnostic status, C allele carriers at rs2023239 had higher CB1 receptor binding compared to non-carriers.
Conclusions: In conclusion, we found that CB1 receptor binding is decreased in patients with alcohol dependence and that this downregulation persists several weeks into abstinence. We also found that the C allele of the rs2023239 locus is associated with higher CB1 receptor binding *in vivo*, similar to what has been found on post mortem analysis. Our findings suggest that CB1 receptor plays a different role in early vs. late phases of alcohol dependence. A potential implication of our findings is that enhanced, rather than blocked CB1 signaling may be beneficial in late stage, treatment seeking alcohol dependent patients.
Disclosure: M. Heilig, Nothing to Disclose.

Panel
33. Beta-amyloid Neuropathology in Cognitively Normal Individuals: Preclinical Alzheimer's Disease or Cognitive Resilience?

33.1 The Detectability of Ab Amyloid by PET Imaging in Mouse Models of Alzheimer's Disease with Different Rate of Plaque Accumulation

Alena Savonenko*

Johns Hopkins University School of Medicine, Baltimore, Maryland

Background: Formation of senile plaques composed of Ab peptides, a pathological hallmark of Alzheimer's disease (AD), precedes the onset of symptoms by years. Non-invasive detection methods for Ab plaques in the human brain would allow for a presymptomatic diagnosis and early preventive treatments. However, a number of *in vivo* imaging studies documented the presence of amyloid pathology in cognitively normal individuals. The phenomenon of 'cognitively-silent' plaques challenges the concept of plaques as main toxic species and has a number of analogies in mouse models of AD. Using the same PET tracers in human and mouse studies would allow for the correlation of imaging data with the morphological and immunohistochemical data from animal models under highly controlled conditions. One of the most successful noninvasive techniques so far to visualize Ab plaques in patients with AD is PET imaging with C11-PIB tracer. However, in addition to C11-PIB's short half-life, the limitations of this tracer include a low sensitivity to Ab plaques in brains of AD mouse models.
Methods: In this study, we tested a reliability of microPET in mice using F18- Florbetapir tracer. We hypothesize that by virtue of its longer half-life, F18- Florbetapir tracer can provide more favorable conditions to meet the requirement of high specific activity to Ab that is difficult to achieve with C11-PIB tracer.
Results: In the case of 11C-PIB tracer, Maedo and colleagues (2007) had suggested that this tracer is particularly sensitive to such Ab subtypes as N-terminally truncated and modified Ab. This type of amino-terminal modifications is a major component of human plaques and is likely related to proteolytic activities of a number of proteases taking place over prolonged time. In AD patients, the percentage of truncated and modified amino-terminus has been shown to increase at the expense of full length Ab and is directly correlated with AD progression. In APP transgenic mice, the occurrence of N-terminally truncated Abspecies was demonstrated to be decreased in comparison with human AD cases. These findings imply that the detectability of Ab amyloid by 11C-PIB PET can differ in Alzheimer's disease (AD) and Tg mouse brains due to

differential presence of modified Ab species. In addition, such findings suggest that 11C-PIB signal in human brains may predominantly come from N-terminally modified "aged" plaques. Thus, in this study, we tested a hypothesis that mouse models of AD with fast rate of amyloid plaque deposition yield lower F18 Florbetapir PET signal than models with slow accumulation. To this end, we compared two AD models, TetOffAPP Line 107 and PrP APPPS1 Line 85, with high and low rates, respectively. PrP APPPS1 Line 85 mice had significantly higher signal in Cortex, hippocampus and other forebrain structures supporting our hypothesis.
Conclusions: These studies validate the use of F18 Florbetapir for the visualization of mouse amyloid by PET imaging in living brains of AD mice. These findings imply that similar to 11C-PIB tracer, the detectability of Ab amyloid by F18 Florbetapir can be modified by the "age" of the plaques likely due to differential presence of modified Ab species in old vs new plaques.
Disclosure: A. Savonenko, Nothing to Disclose.

33.2 Preclinical AD: Evidence for Amyloid-associated Alterations in Brain Function and Structure

Reisa Sperling*

Brigham and Women's Hospital, Boston, Massachusetts

Background: The pathophysiological process of Alzheimer's disease (AD) is thought to begin years, if not decades, prior to the onset of clinical dementia.
Methods: Converging data from PET amyloid imaging and cerebrospinal fluid studies suggest that approximately one-third of clinically normal older individuals harbor a substantial burden of cerebral amyloid-beta. Recent multi-modality imaging studies, using PET amyloid imaging and functional MRI, have demonstrated that amyloid deposition in key nodes of the default network is associated with aberrant default network fMRI activity during the encoding of new memories, as well as disrupted default network connectivity at rest.
Results: These findings provide support for the hypothesis that amyloid pathology is linked to synaptic dysfunction in the networks supporting memory processes, detectable prior to the emergence of significant cognitive impairment. Several studies have reported evidence of cortical thinning and accelerated rates of atrophy in amyloid-positive normals. A small number of studies have also found an association between higher amyloid burden and lower cognitive performance, even among the range of clinically normal older individuals, and an increased likelihood of cognitive decline over time. The National Institute on Aging and Alzheimer's Association guidelines on preclinical AD provide a staging framework: Stage 1: Asymptomatic cerebral amyloidosis; Stage 2: Amyloidosis + Synaptic Dysfunction and/or Neurodegeneration; and Stage 3: Amyloidosis + Neurodegeneration + Subtle cognitive decline.
Conclusions: Longitudinal studies are ongoing to determine if these amyloid positive older individuals are in the preclinical stages of AD, and are at increased risk for cognitive decline and development of AD dementia. A number of secondary prevention trials are being planned in both genetic at-risk and amyloid-positive older populations, including the "A4" trial: Anti-Amyloid Treatment in Asymptomatic AD.
Disclosure: R. Sperling, **Part 1:** Consulting for Bayer, Biogen-IDEC, Bristol-Myers-Squibb, Eisai, Eli Lilly, Janssen, Pfizer, Roche, **Part 2:** Pfizer, Janssen, Bristol-Myers-Squibb, Medivation.

33.3 Neuroimaging Predictors of Cognitive Impairment and Resilience: Insights from the Baltimore Longitudinal Study of Aging

Susan M. Resnick*

National Institutes of Health, Baltimore, Maryland

Background: Since 1994, we have examined changes in brain structure and function as predictors of cognitive decline and resilience through serial neuroimaging studies in the Baltimore Longitudinal Study of Aging (BLSA).
Methods: PET imaging of fibrillar amyloid-β (Aβ) using 11-C-Pittsburgh Compound B (PiB) was added in 2005, and more than 200 PET-PiB studies have been performed for 100 individuals (age 55 and older), with up to 6 serial evaluations.
Results: Consistent with autopsy data from the BLSA and other studies, approximately 30% of cognitively normal BLSA participants have detectable brain amyloid on *in vivo* imaging. Individuals with higher levels of Aβ on imaging are older, are more likely to carry the apolipoprotein E epsilon 4 allele, have greater longitudinal memory decline, and show greater longitudinal increases in amyloid deposition. Our observation of associations between higher levels of Aβ on *in vivo* PET imaging and longitudinal declines in memory in cognitively normal individuals contrasts with stable antemortem cognitive performance in cognitively normal individuals with substantial Alzheimer's disease (AD) pathology at autopsy. We suggest that the discrepancy between *in vivo* imaging and autopsy studies reflects the heterogeneity of participants examined through *in vivo* imaging. These individuals are younger and have not fully passed through the risk period for cognitive impairment and AD. Initial results of longitudinal imaging studies of Aβ have shown remarkable convergence across centers and radioligands, with significant increases in Aβ over time in individuals with detectable levels at baseline.
Conclusions: Some individuals with elevated Aβ on imaging are likely in a preclinical stage of AD, while others may demonstrate cognitive resilience over their lifetimes. Continued longitudinal imaging and cognitive follow-up of individuals with elevated Aβ will distinguish between individuals who will eventually develop cognitive impairment versus those who will remain stable. Further, amyloid imaging studies in combination with genetic information and other neuroimaging and cerebrospinal fluid biomarkers may identify factors that promote maintenance of cognitive health in addition to predicting cognitive impairment, leading to new avenues for interventions that delay onset or modify progression of AD.
Disclosure: S. Resnick, **Part 1:** Spouse: Research funding from Amgen, Avid, Biotie, GE, Intracellular, Johnson and Johnson, Lilly, Lundbeck, Merck, Otsuka, Roche, Sanofi-Aventis; Spouse: Consultant Amgen, **Part 2:** Spouse: Research funding Amgen, Avid, Biotie, GE, Intracellular, Johnson and Johnson, Lilly, Lundbeck, Merck, Otsuka, Roche, Sanofi-Aventis.

33.4 Resilient Brain Aging: Neuropathological, Cellular and Biochemical Features of Pathological Alzheimer's Disease with Normal Cognition

Steven E. Arnold*

University of Pennsylvania, Philadelphia, Pennsylvania

Background: The neuritic plaques and neurofibrillary tangles of Alzheimer's disease (AD) correlate with cognitive impairment and severity of dementia, but it has long been recognized that the relationships are imperfect and that some people exhibit normal cognition despite high levels of AD pathology. We investigated the cellular, synaptic and biochemical composition of the cerebral cortex in subgroups with similar levels of AD pathology but very different levels of cognitive functioning.
Methods: We used clinical x pathological stratification in the Religious Orders Study to select 10 cases for each of three groups: 1) pathological AD with normal cognition ("AD-Resilient"), 2) pathological AD with AD-typical dementia ("AD-Dementia)" and 3) pathologically normal with normal cognition ("Normal Comparison"). Immunostaining, photomicroscopy and image analysis were used to measure densities of neurons (NeuN), astrocytes (GFAP), presynaptic terminals (SY38) and dendritic spines (synaptopodin) as well as Aβ plaques and PHFtau neurofibrillary tangles in midfrontal cortex. A broad cellular proteome was measured with an antibody microarray.

Results: The AD-Resilient group, defined by AD pathology, had PHFtau and Ab lesion densities comparable to the AD-Dementia group but preserved densities of presynaptic terminals and dendritic spines similar to the Normal Comparison group, and increased densities of astrocytes compared to both the AD-Dementia and Normal Comparison groups. Candidate protein abnormalities associated with diagnostic groups were identified in the discovery proteomic analysis.
Conclusions: These data characterize cellular and synaptic features and identify novel biochemical targets that may be associated with resilient cognitive brain aging in the setting of pathological AD.
Disclosure: S. Arnold, Nothing to Disclose.

Panel
34. Inhibition of Phosphodiesterases to Treat Psychiatric Disorders: Advances through Innovation in Preclinical Models and Feedback from the Clinic

34.1 The Phosphodiesterase Isoform 4A5 (PDE4A5) is the Critical Mediator of Hippocampus-dependent Cognitive Impairments Induced by Sleep Loss
Ted Abel*

University of Pennsylvania, Philadelphia, Pennsylvania

Background: In a previous study by our laboratory, we showed that 5 hours of sleep deprivation led to elevated protein levels of PDE4A5 in the hippocampus and an increase in PDE4 activity leading to an attenuation of the cAMP pathway. However, it has not been determined whether the elevated hippocampal PDE4a5 levels and activity are the key mediator of the memory deficits observed after brief sleep deprivation.
Methods: We used Adeno-Associated Viruses (AAV) in combination with a CaMKII alpha promoter fragment to express the following transgenes in excitatory neurons of the hippocampus: 1) a catalytically inactive dominant negative form of PDE4A5 (PDE4A5DN) or 2) wild-type PDE4A5 (PDE4A5WT). Mice bilaterally injected with enhanced green fluorescent protein (eGFP) virus into the hippocampus served as controls. Four weeks after the bilateral injection of AAV, mice were trained in hippocampus-dependent or hippocampus-independent learning paradigms. From a different cohort of mice, tissue from the hippocampus and other brain regions was collected for biochemical and electrophysiological studies.
Results: We found that overexpression of the PDE4a5DN selectively in the hippocampus reversed the sleep loss-induced memory deficits in the object-location memory task. Overexpression of the PDE4A5DN did not affect memory formation in the absence of sleep deprivation. Overexpression of PDE4a5WT increased hippocampal PDE4 activity and reduced cAMP levels in the hippocampus, but not in the cerebellum and prefrontal cortex. Furthermore, overexpression of PDE4A5WT impaired forskolin-mediated potentiation at the CA1 Schaffer collateral pathway and reduced AMPA receptor phosphorylation at the GluR1 Serine 845 site, two phenomena also observed after sleep deprivation. Behaviorally, we found that overexpression of hippocampal PDE4A5 protein impaired the formation of long-term memories in the contextual fear conditioning task and object-location memory task. In contrast, long-term memory formation in hippocampus-independent tasks was not affected.
Conclusions: Together, these findings suggest that PDE4A5 in the hippocampus is the critical mediator of the memory and plasticity deficits observed after 5 hours of sleep deprivation. Studies are currently underway to determine which downstream targets of the cAMP pathway are affected by the sleep deprivation-induced increase in PDE4A5 activity, but not by sleep deprivation under conditions of PDE4A5DN expression. Together, these studies may define new therapeutic strategies to ameliorate the cognitive deficits observed after sleep loss.
Disclosure: T. Abel, Nothing to Disclose.

34.2 Alcohol Drinking and Seeking Behaviors: Role of Phosphodiesterase-4 (PDE4)
Han-Ting Zhang*

West Virginia University Health Sciences Center, Morgantown, West Virginia

Background: Alcohol dependence is a complex psychiatric disorder, which is mediated by cyclic AMP (cAMP) signaling. Phosphodiesterase-4 (PDE4), an enzyme that specifically hydrolyzes cAMP, plays a critical role in the regulation of intracellular cAMP levels in the brain. However, it is not clear how PDE4 regulates behaviors related to alcohol dependence.
Methods: The effects of rolipram, a prototypical, selective PDE4 inhibitor, on ethanol consumption was evaluated using the two-bottle choice paradigm in high-alcohol preferring (HAP) mice and alcohol preferring fawn-hooded (FH/Wjd) rats. In addition, the mouse ethanol drink-in-dark test was conducted as verification. Further, ethanol self-administration also was evaluated in FH/Wjd rats treated with rolipram. Finally, mice deficient in PDE4A, PDE4B, or PDE4D were examined for ethanol intake in order to identify the involvement of individual PDE4 subtypes.
Results: Rolirpam treatment decreased ethanol (7-12%) intake and preference in the two-bottle choice test in both mice and rats, without altering sucrose consumption. It also decreased ethanol intake in the mouse drink-in-dark test. In addition, rolipram decreased operant self-administration of ethanol (5%), but not sucrose (10%). Mice deficient in PDE4A or PDE4B displayed robust decreases in ethanol (9%) intake and preference in the two bottle choice test compared to wild-type controls. They also exhibited decreases in ethanol intake in the drinking-in-dark paradigm. In contrast, mice deficient in PDE4D showed only a slight decrease in ethanol intake and preference.
Conclusions: Inhibition of PDE4 produced promising reduction of ethanol intake and preference in various alcohol drinking models; this appears to be involved by PDE4A and PDE4B subtypes. Overall, the results suggest that PDE4 is a novel target for drugs that reduce ethanol drinking and seeking behaviors; selective inhibitors of PDE4, in particular PDE4A and/or PDE4B, may be used for treatment of alcohol dependence.
Disclosure: H. Zhang, **Part 1:** Asubio Pharmaceuticals Cordex Biosolutions Lundbeck Pharmaceuticals, **Part 4:** Lundbeck Pharmaceuticals.

34.3 Phosphodiesterases Differentially Determine the Spatial and Temporal Modalities of cAMP Signal Integration in the Cortex and Striatum.
Pierre Vincent*

National Scientific Research Center, Université Pierre et Marie Curie, Paris, France

Background: The PDE4 inhibitor rolipram has a procognitive and antidepressant effect, while PDE10 inhibitors are currently tested in clinics for their antipsychotic effect. We used these inhibitors to reveal the physiological functions of PDE4 and 10 in the brain.
Methods: The recent development of genetically encoded biosensors allow real-time imaging of cAMP/PKA signaling events, with Epac-based sensors to monitor changes in cAMP concentration and AKAR sensors for protein kinase A activity. Wide-field fluorescence microscopy or two-photon microscopy provide high temporal or spatial resolution, allowing for the resolution of cAMP/PKA events at a time scale of a few seconds in specific cellular domains such as dendrites, somatic cytosol or nucleus. In addition, electrophysiological recordings of the slow-AHP potassium current in the cortex reports PKA activation at the membrane.
Results: In pyramidal cortical neurons, a tonic cAMP production or cAMP produced in response to the stimulation of β-adrenergic

receptors directly affects PKA-sensitive membrane channels, thereby controlling neuronal excitability. cAMP is under a tight control by PDE4 which maintains subcellular compartmentation of cAMP at the membrane and cAMP levels in the bulk cytosol at a sub-micromolar level. PDE4 inhibition by rolipram boosts the cytosolic response and increases neuronal excitability. Medium spiny neurons in the striatum lack this PDE4 activity thus allowing their intracellular cAMP to reach much higher levels. In addition, striatal neurons produce cAMP faster and express DARPP-32 which exterts a positive feedback through phosphatase inhibition. These molecular properties result in faster, larger and longer-lasting cAMP/PKA signals, conferring on this brain structure the amazing ability to respond to sub-second dopamine stimulations. Finally, PDE10 inhibition in the striatum produces a strong PKA activation exclusively in the neurons of the indirect pathway.
Conclusions: Our results show that PDE4 is critical in the cortex for cAMP signal compartmentation at the membrane, and PDE4 inhibition by rolipram facilitates cAMP signal propagation throughout the cell. In the striatum, lack of PDE4 activity (in addition to other molecular specificities) allows this structure to efficiently respond to sub-second dopamine stimuli, a feature which bears important implications in the understanding of reward-based learning theories. PDE10 inhibition in the striatum increases PKA activity specifically in spiny neurons of the indirect pathway. PDE10 inhibition thus has a similar net effect as the inhibition of D2 receptors, which may contribute to the antipsychotic activity of PDE10 inhibitors.
Disclosure: P. Vincent, Nothing to Disclose.

34.4 Inhibition of Phosphodiesterase10A for the Treatment of Schizophrenia: Preclinical Rationale and Clinical Evaluation

Christopher J. Schmidt*

Pfizer Inc., Groton, Connecticut

Background: PDE10A is a striatally expressed, dual substrate phosphodiesterase. Preclinical studies indicate that PDE10A inhibition reproduces the biochemical, electrophysiological and behavioral effects of D2 receptor blockade suggesting that inhibitors of this enzyme may be effective antipsychotic agents. We will describe the preclinical characterization of the selective PDE10A inhibitor PF-2545920 and its initial clinical evaluation in patients with schizophrenia.
Methods: PDE10A localization was assessed using immunohistochemistry. The effects of acute enzyme inhibition on neurochemical, electrophysiological and behavioral measures of striatal function was assessed in both rodents and nonhuman primates. Clinical evaluation of the PDE10A inhibitor PF-2545920 included both phase 1 safety and phase 2 efficacy studies. Antipsychotic activity was assessed in a DB, placebo controlled 4 week trial in treatment responsive patients with acute exacerbation. Two doses of study drug were evaluated based upon preclinical predictions and a single dose arm of risperidone was included as an active comparator.
Results: PDE10A is expressed in both striatopallidal and striatonigral medium spiny neurons (MSNs) of all examined species including humans. In addition, postmortem studies indicate that striatal PDE10A expression is unchanged in schizophrenia. In rodents, PDE10A inhibitors produce dose dependent increases in both striatal cGMP and cAMP in parallel with increased phosphorylation of the PP1 inhibitor DARPP32, the transcription factor CREB and the NR1 and GluR1 subunits of the NMDA and AMPA receptors, respectively. Phosphorylation of ERK, MSK1 and H3 histone are also increased. Consistent with the activation of several transcriptional regulators, acute enzyme inhibition has a dramatic effect on striatal gene transcription including increases in preprotachykinin and preproenkephalin. Like D2 antagonists, PDE10A inhibitors disrupt conditioned avoidance responding, reduce PCP or amphetamine stimulated locomotion and improve D2 agonist-disrupted prepulse inhibition and amphetamine-disrupted auditory gating. In electrophysiological studies, PDE10A inhibition increased the sensitivity of MSNs to cortical stimulation. Both rodent and primates studies predict that the overall EPS liability of PDE10A inhibitors is less than that of atypical antipsychotic agents. Based on these preclinical results, PF-2545920, was advanced into clinical trials. The compound was well tolerated at predicted efficacious exposures in both single and multidose safety studies in healthy volunteers. In the phase 2 study, neither dose of PF-2545920 differed from placebo on either the total or PANSS subscales while risperidone produced a decrease in total PANSS consistent with historical data.
Conclusions: The strength of the preclinical data supporting the clinical assessment of PDE10A inhibition and the clinical failure of PF-2545920 pose an important challenge to the future of novel antipsychotic drug development. Typical issues such as patient and dose selection as well as tolerance to the effects of enzyme inhibition will be addressed as will the potential to identify key features of antipsychotic efficacy based upon a detailed comparison of the circuitry effects of PDE10A inhibition and D2 receptor blockade.
Disclosure: C. Schmidt, **Part 1:** I am employed by Pfizer, Inc, **Part 2:** Pfizer, Inc, **Part 3:** Pfizer, Inc.

Panel
35. Dendritic Spine Plasticity in Depression and Addiction

35.1 Molecular Basis of Structural Plasticity of Nucleus Accumbens Neurons Induced by Drugs of Abuse

Eric J. Nestler*

Mount Sinai School of Medicine, New York, New York

Background: Addictive drugs cause persistent restructuring of several neuronal cell types in limbic regions of brain, including medium spiny neurons (MSNs) of the nucleus accumbens (NAc), which are thought to contribute to the long-term behavioral plasticity driving addiction. Although several types of structural changes are well documented, only recently has insight been obtained into the detailed underlying molecular mechanisms involved.
Methods: Structural plasticity of NAc MSNs is studied with several methods, including direct injection of dyes into individual neurons or viral-mediated overexpression of fluorescent proteins, followed by unbiased and semi-automated analysis of dendritic arborizations, including dendritic spine number, type, and size, by use of NeuronStudio. Molecular mechanisms of spine regulation are interrogated by use of genetic mutant mice and viral-mediated gene transfer.
Results: Our findings to date demonstrate the involvement of several drug-regulated transcription factors in the ability of cocaine to induce dendritic arborizations and spine number in NAc MSNs. These include DeltaFosB, CREB, NFkappaB, and MEF2, among others. Not surprisingly, these transcription factors work in concert with a range of chromatin regulatory proteins, including specific histone deacetylases and methyltransferases and DNA methyltransferases, to control the expression of a range of target genes which then proximally control dendritic structure. Among these gene targets are several whose protein products regulate the actin cytoskeleton and the formation, stabilization, and retraction of dendritic branches and spines. Current efforts are focused on analyzing these regulatory processes in distinct subtypes of NAc MSNs, such as D1-type and D2-type neurons, as well as to expand the analysis of cocaine action to that of several other drugs of abuse.
Conclusions: This work is gradually and progressively delineating the precise molecular steps by which cocaine, and other drugs of abuse, alter the structure of NAc MSNs. In parallel, it is allowing increasingly direct links to be established between such structural plasticity and functional synaptic plasticity and the behavioral abnormalities that define a state of addiction.
Disclosure: E. Nestler, **Part 1:** Psycho Genics, SAB chair Merck, Consultant Berg Pharma, Consultant, **Part 2:** Psycho Genics, SAB chair Merck, Consultant Berg Pharma, Consultant.

35.2 Epigenetic Regulation of Synaptic Remodeling in Depression
Scott Russo*

Mount Sinai School of Medicine, New York, New York

Background: Depression is thought to occur via long-lasting changes in synaptic structure of brain reward nerve cells resulting in social avoidance and anhedonia. We hypothesize that epigenetic mediated changes in RhoGTPases that control actin binding and cytoskeletal reorganization may mediate these changes.
Methods: We used a combination of quantitative chromatin immunoprecipitations and transcriptional profiling along with neuranatomical assessments of excitatory synapses to study the mechanisms and functional relevance of these synaptic adaptations in depression.
Results: Transcriptional profiling of nucleus accumbens (NAc) for 18 RhoGTPase related genes, known regulators of synaptic structure, following chronic stress in rodent depression models, revealed a selective and long-term reduction in Rac1 transcription. This was marked by a repressive chromatin state surrounding its proximal promoter. There was a similar repressive chromatin state surrounding the Rac1 promoter in human postmortem NAc from depressed subjects that corresponded with reduced Rac1 transcription. Using targeted viral gene transfer and Rac1 conditional mice we show this adaptation is necessary and sufficient for depression behavior and the formation of immature stubby excitatory spines by redistributing synaptic cofilin, an actin severing protein downstream of Rac1.
Conclusions: In summary, we have strong evidence that implicates epigenetic regulation of Rac1 as a novel disease mechanism in depression. Our animal models reveal a critical functional role of Rac1 in depression behavior and the restructuring of the actin cytoskeleton during chronic stress exposure, highlighting this as a potential therapeutic target for depression in humans.
Disclosure: S. Russo, Nothing to Disclose.

35.3 Subcellular Synaptic Connectivity in the Nucleus Accumbens
Adam Carter*

New York University, New York, New York

Background: Medium spiny neurons (MSNs) in the Nucleus Accumbens (NAc) process diverse long-range excitatory inputs to shape basal ganglia output. MSNs are segregated into the direct and indirect pathways, which have opposing effects on rewarding behaviors. However, the mechanisms by which these two populations of neurons are activated by different inputs remain unknown.
Methods: We use a combination of electrophysiology, two-photon calcium imaging and optogenetics to study functional connections made by different inputs onto MSNs in acute slices of the NAc.
Results: We use optogenetics to show that different inputs make distinct connections onto direct and indirect MSNs. We use two-photon microscopy to show that different inputs contact unique spines and dendrites. We use two-photon uncaging to show that this targeting strongly impacts synaptic responses. Finally, we use a combination of modeling and experiments to show how subcellular connectivity shapes the output of the NAc.
Conclusions: Our findings indicate that selective targeting onto spines and dendrites governs signal processing via the two output pathways of the NAc.
Disclosure: A. Carter, Nothing to Disclose.

35.4 Opposing Effects of Fear Conditioning and Extinction on Dendritic Spine Remodelling in the Mouse Cortex
Wenbiao Gan*

New York University, New York, New York

Background: Fear conditioning is a classic paradigm for studying emotional associative learning in which a conditioned neutral stimulus (e.g. auditory cue) is paired with the presentation of an unconditioned aversive stimulus (e.g. footshock) to elicit a conditioned response (e.g. freezing response to an auditory cue in the absence of a footshock). Repeated exposure to a conditioned stimulus diminishes the expression of the conditioned response, a process called fear extinction. It is generally believed that fear extinction is a new learning that inhibits, rather than erases, the previously acquired fear memory. While this view has gained much support from behavioral and electrophysiological studies, the hypothesis that extinction causes the partial erasure of fear memories remains viable. To investigate how neural circuits are modified by fear learning and extinction, we examined the formation and elimination of postsynaptic dendritic spines in the mouse frontal association cortex and primary motor cortex, two cortical regions that are involved in the consolidation of fear and extinction memories.
Methods: Using transcranial two-photon microscopy and transgenic mice expressing Yellow Fluorescent Protein in Layer V pyramidal neurons, we imaged dendritic spine dynamics in response to fear conditioning and extinction over days in the dorsal medial region of the frontal association cortex as well as in the primary motor cortex.
Results: In both cortical regions, we found that fear conditioning in which an auditory cue is paired with foot shock increases the rate of spine elimination. In contrast, fear extinction by repeated presentation of the same auditory cue alone increases the rate of spine formation. The degree of spine remodeling induced by fear conditioning and extinction strongly correlates with the expression and extinction of conditioned fear responses, respectively. Notably, fear conditioning- and extinction-induced dendritic spine changes occur on the same dendritic branches and are reversible: extinction causes the formation of dendritic spines within a 2 μm distance from spines previously eliminated after fear conditioning. Reconditioning preferentially induces elimination of those dendritic spines that were reformed during extinction.
Conclusions: Our findings indicate that fear conditioning, extinction and reconditioning lead to opposing changes of dendritic spines at the same or nearly identical locations within complex neural networks. They also suggest that fear memory traces in the cortex are partially erased after extinction.
Disclosure: W. Gan, Nothing to Disclose.

Panel
36. Circadian Rhythms and Mood Disorders: Clock Genes and New Treatment Implications

36.1 Circadian Clock Genes in Control and Major Depressive Disorder Brain Tissue: Potential Role in Mode of Action of Ketamine
William Bunney*

University of California Irvine School of Medicine, Irvine, California

Background: Depression has been associated with circadian abnormalities including temperature, hormones, sleep and mood although there has been no direct link between the illness and circadian machinery. Core clock genes are essential for the coordination of circadian rhythms. It is hypothesized that the expression of circadian genes are dysregulated in major depressive disorder patients (MDD) compared to controls matched for time-of-death (TOD) and that ketamine's (K), rapid treatment for MDD interacts with core clock genes.
Methods: Study 1: Test the effects of K on clock genes in neuronal cell culture. A reporter constituted by the mPer1 gene promoter fused with the luciferase gene was expressed in NG108-15 neuronal cells. E-boxes which drive CLOCK/BMAL1 transcription of per and cry genes were mutated to determine a site of action. Study 2: Test if clock genes in controls show a significant circadian pattern in brain tissue based on 24hr TOD and if MDD patients have altered circadian patterns. Run microarrays and fit data to a sinusoidal curve as a function of time.

Results: Study 1: Ketamine influences CLOCK/BMAL1 function in cell culture, and blocks BMAL1 transcription of per1. E-box elements were critical for K's actions. K induced repression of CLOCK/BMAL1 is decreased by a GSK3B inhibitor. Study 2: Clock genes showed a significant circadian 24hr rhythm in controls and MDD patients showed loss of circadian patterns.
Conclusions: Study 1: Ketamine's inhibition of CLOCK/BMAL1 could potentially provide a mechanism by which 'resetting' clock gene machinery could normalize dysregulated rhythms and treat mood disorders. Study 2: Clock genes in controls show significant circadian patterns based on TOD over 24 hrs while MDD patients had a disruption.
Disclosure: W. Bunney, Nothing to Disclose.

36.2 Patients' Self-reported Chronotype and Social Rhythm Changes after Treatment of Major Depression

Ellen Frank*

University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Background: E. Corruble[1], E. Frank[2], F. Gressier[1], P. Courtet[3], G. Vaiva[4], F. Baylé[5], PM Llorca[6], D. Kupfer[2], P. Gorwood[5]. [1]Bicêtre Hospital, Department of Psychiatry, Le Kremlin Bicêtre, France. [2]University of Pittsburgh School of Medicine, Department of Psychiatry, Pittsburgh, USA. [3]Lapeyronie Hospital, Department of Psychiatry, Montpellier, France. [4]Michel Fontan Hospital, Department of Psychiatry, Lille, France. [5]Sainte Anne Hospital, Department of Psychiatry, Paris, France. [6]University Hospital, Department of Psychiatry, Clermont Ferrand, France. Chronotype or the tendency to morningness or eveningness and regularity of social rhythms appear to be important factors in mood disorders. Previous research had suggested that chronotype was a stable characteristic of individuals. In contrast, regularity of social routines would be expected to stabilize as depression improves. This study examined both chronotype and social rhythm regularity before and after treatment for depression.
Methods: We assessed social circadian rhythms before and after 8 weeks of treatment with agomelatine, using the Composite Scale of Morningness (CSM) and the Social Rhythm Metric (SRM) in a group of 721 adult outpatients with a unipolar major depressive episode (MDE) (DSM-IV).
Results: Women comprised 74.2% of the final study population, and the average age was 47.5 years (SD = 11.9). 57.4% of patients were responders and 30.7% were remitters after 8 weeks of treatment. At baseline, 24.6% of patients reported being an Evening type (CSM $\leqslant$ 29), 26.2% a Morning type (CSM $\geqslant$ 39) and 49.2% an Intermediate type. In bivariate analyses, Evening types were younger, had a more severe MDE and less regular social rhythms. Logistic regression showed that the unique explaining variable of Morningness/Eveningness at baseline was age. In bivariate analyses, disturbance of social rhythm regularity was higher in younger people, in women, and in those with a higher number of previous MDEs. In multivariate analyses, low social rhythm regularity was explained by age and the number of previous MDEs. An increase in the proportion of participants endorsing Morning type and a decrease in the proportions of participants endorsing Evening type were shown after 8 weeks of agomelatine treatment ($p < 0.0001$). 67% of patients did not change CSM type between baseline and follow-up; however, among those whose chronotype changed, significantly more changed to an 'earlier' type (24.3%) than to a 'later' type (8.7%). The increase in morningness scores over time under treatment, was greater in responders than non-responders ($p < 0.0001$) and in remitters than non-remitters ($p < 0.0001$). A significant increase in social rhythm regularity was also observed under treatment, but it did not differ between responders and non-responders.
Conclusions: Although previously thought to be a trait characteristic, we found that under agomelatine treatment nearly one-quarter of patients' shifted to an earlier chronotype. Furthermore, an increase in morningness was positively associated with the probability of response and remission. As anticipated, social rhythm regularity increased over the 8 weeks of treatment which is in line with the resynchronizing effect of agomelatine.
Disclosure: E. Frank, **Part 1:** Servier International (Consultant) Vanda Pharmaceuticals (Consultant) Guilford Press and American Psychological Association Publishing (Royalties), **Part 2:** Servier International (Consultant) Spouse: Consultant to the American Psychiatric Assocation.

36.3 Use of the Clock Mutant Mice to Identify New Mood Stabilizing Agents

Colleen A. McClung*

University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Background: Bipolar disorder is a devastating illness with few treatment options. The design of new medications has been difficult due to a lack of appropriate animal models, and due to the fact that the biological basis of the disease is poorly understood. Altered circadian rhythms are extremely prominent in subjects with bipolar disorder, and mice with a mutation in a central component of the circadian system (CLOCK) have a complex behavioral profile that is strikingly similar to human mania. In turn, the behavioral phenotypes of these mice are reversed with chronic lithium treatment. Thus these mice provide us with a useful tool to design and test new compounds which might be efficacious as mood stabilizing agents.
Methods: Mice with a mutation in the Clock gene (ClockD19), as well as wild type littermates, were subjected to a variety of behavioral tests to measure locomotion, anxiety-related behavior, and depression-related behavior with and without drug treatment. Molecular measures of gene expression, electrophysiology, and chromatin modifications were also conducted.
Results: We have found that certain classes of compounds are efficacious in reversing specific components of the overall manic-like phenotype of the ClockD19 mice. For example, specific inhibitors of GSK3b reduce the hyperactivity of these mice but have no effect on anxiety or depression-related behavior while both lithium and valproate normalize anxiety and depression-related behavior but have no effect on locomotion. Moreover, a drug that is known to modulate circadian rhythms CK01, a casein kinasee/d inhibitor, strongly influences anxiety-related behavior but has less robust effects on depression-related behavior. These drugs also lead to interesting molecular and cellular changes specifically in the ClockD19 mice, which may underlie their mechanism of action.
Conclusions: The results that we have obtained with the use of these mice will help determine which type of compounds will potentially be therapeutically active in treating specific components of bipolar disorder, and help identify which protein targets should be the focus of future drug development.
Disclosure: C. McClung, **Part 1:** I have received honoraria from Johnson & Johnson, Servier, and Pfizer, **Part 4:** We received research funding from Glaxo Smith Kline and Pfizer.

36.4 The Circadian Transcriptional Network in Mammals

Joseph S. Takahashi*

University of Texas Southwestern Medical Center, Dallas, Texas

Background: The circadian clock mechanism in animals involves an autoregulatory transcriptional feedback loop in which CLOCK and BMAL1 activate the transcription of the Period and Cryptochrome genes. The PERIOD and CRYPTOCHROME proteins then feedback and repress their own transcription by interaction with CLOCK and BMAL1.
Methods: We have studied the biochemistry of the CLOCK:BMAL1 transcriptional activator complex as well as the genomic targets

of CLOCK and BMAL1 using ChIP-seq methods. We describe the dynamics of the core circadian clock transcriptional system. In addition, we have used cell-based circadian rhythms to screen for small molecules that perturb the clock system.
Results: CLOCK and BMAL1 interact with the regulatory regions of thousands of genes. The gene network and dynamics of the system will be discussed as well as the effects of compounds that can modulate circadian rhythms.
Conclusions: A mechanistic description of the core circadian clock mechanism should promote our understanding of how the circadian clock system influences complex behavior and behavioral disorders such as mood disorders.
Disclosure: J. Takahashi, **Part 1:** Co-founder and SAB member of Reset Therapeutics, Inc., **Part 2:** Co-founder and SAB member of Reset Therapeutics, Inc.

Panel
37. Neuronal Circuit Regulation of Ventral Tegmental Area Neurons

37.1 Subcellular Segregation of Dopamine and Glutamate Signaling by a Subset of Ventral Tegmental Area Neurons

Marisela Morales*

National Institutes of Health, Baltimore, Maryland

Background: We previously found that in addition to the classical dopamine neurons expressing tyrosine hydroxylase (TH), the Ventral Tegmental Area (VTA) contains neurons expressing vesicular glutamate transporter 2 (VGluT2) mRNA. VGluT2 selectively transports glutamate into synaptic vesicles, thus, neurons containing VGluT2 use glutamate as neuronal signaling neurotransmitter. There are 2 classes of VGluT2 neurons: the VGluT2-only neurons (containing VGluT2 mRNA without TH) and dual TH-VGluT2 neurons (co-expressing TH and VGluT2 mRNA). Both types of VGluT2 neurons target the medial prefrontal cortex (mPFC) and the nucleus accumbens (nAcc), brain structures involved in drug abuse. These findings indicate that neurons of the VTA innervating the mPFC or the nAcc have the capability to (a) release dopamine, (b) release glutamate or (c) co-release dopamine and glutamate. Co-release of dopamine and glutamate could constitute a novel mechanism of neuronal signaling.
Methods: First, we performed *in vivo* tagging of VTA neurons in the rat by either region specific injections of tract tracing molecules or viral vectors. Findings from these approaches showed expression of TH in axon terminals lacking VGluT2, and expression of VGluT2 in axon terminals lacking TH. We next induced expression of the light activated opsin channelrhodopsin-2 tethered to m-Cherry (ChR2-mCherry) in TH or VGluT2 neurons following viral infection into the VTA of TH::Cre mouse or VGluT2::Cre mouse. Finally, by combination of *ex vivo* electrophysiology and optogenetics (light induced activation of fibers from VTA neurons expressing ChR2-mCherry in TH or VGluT2 neurons), we found that the above identified axon terminals from VTA VGluT2 neurons innervating the mPFC or nAcc evoked excitatory postsynaptic currents.
Results: (a) All axon terminals from VTA tagged neurons making asymmetric synapses contained VGluT2 protein but lacked TH, and those making symmetric synapses contained TH but lacked VGluT2. (b)There was a lack of coexistence of TH and VGluT2 within the same axon terminal. (c) All axon terminals from TH-VGluT2 neurons that made asymmetric synapses contained VGluT2, but lacked TH. These findings indicate that indeed TH-VGluT2 neurons have the capability to synthesize VGluT2 protein, and that this protein gets incorporated into vesicles located in axon terminals for the accumulation and synaptic release of glutamate. (d) Afferents from both TH-VGluT2 and VGluT2 neurons evokes excitatory postsynaptic current in the mPFC or NAcc.
Conclusions: We provide ultrastructural, optogenetic and electrophysiological evidence indicating that the VTA has a unique set of neurons with the unanticipated capability to co-release two different signaling molecules from two distinct subcellular compartments: glutamate from axon terminals, and dopamine mostly from dendrites and axons. Further studies are necessary to determine the role of this novel dual signaling mechanism in brain function.
Disclosure: M. Morales, Nothing to Disclose.

37.2 Anatomically-specific Ventral Tegmental Area Afferents Control Reward and Aversion

Garret Stuber*

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Background: The Ventral Tegmental Area (VTA) is a heterogeneous brain structure containing neuronal populations that are essential for the expression of behaviors related to addiction and other neuropsychiatric illnesses. The VTA contains a mixture of dopaminergic (DAergic), GABAergic, and glutamatergic neurons, which are thought to act in concert to orchestrate and regulate behavior. The activity of VTA neurons is controlled by diverse anatomically specific excitatory and inhibitory inputs from regions such as the lateral habenula (LHb), bed nucleus of the stria terminalis (BNST) and nucleus accumbens (NAc). While neuroanatomical studies have demonstrated the presence of projections from these regions to the VTA no studies to date have selectively manipulated the activity of VTA afferents to determine 1) how distinct inputs are functionally connected to different types of postsynaptic VTA neurons and 2) how modulating the activity of these inputs affects behavior.
Methods: We selectively expressed the light gated cation channel, channelrhodopsin-2 (ChR2) fused to enhanced yellow fluorescent protein (EYFP) in distinct populations of neurons that project to the VTA in wild type and transgenic mice. Following trafficking of ChR2 to presynaptic fibers that innervate the VTA, we performed whole cell recordings from TH+ (dopamine) or TH- (non-dopamine) neurons in the VTA to determine the specific afferents that form functional connections with distinct neuronal populations in the VTA. In separate mice expressing ChR2-EYFP in VTA afferents, we implanted optical fibers directly above the VTA in order to selectively activate these anatomically distinct inputs in awake and behaving mice during reward or aversive-related behavioral tasks.
Results: Following targeted expression of ChR2-EYFP into projection neurons of the LHb, BNST, or NAc, presynaptic ChR2-EYFP positive fibers were observed in the VTA. Optical stimulation of LHb inputs to the VTA and neighboring Rostral Medial Tegmental Nucleus (RMTg) resulted evoked glutamate-mediated excitatory postsynaptic currents almost exclusively onto non-DA neurons. Optical stimulation of this pathway *in vivo* resulted in passive behavioral avoidance of a distinct environment previously associated with pathway activation. In addition, mice readily learned to perform an operant response to actively avoid optogenetic stimulation of this pathway, demonstrating that it can support negative reinforcement. Furthermore, optical stimulation of LHb-to-VTA/RMTg inputs suppressed positive reinforcement when the stimulation was selectively paired with the operant response to obtain a sucrose reward. Additional data on role of BNST and NAc inputs in controlling reward and aversive behaviors will also be presented.
Conclusions: These data suggest that selective activation of VTA afferents from the LHb promotes aversive behaviors. Consistent with this, recent reports have demonstrated that direct activation of VTA GABA neurons (which are activated by LHb glutamate inputs) can reduce reward consumption or produce a conditioned place aversion. This is likely due to VTA GABA neuron's ability to potently suppress the activity of neighboring VTA DA neurons. Taken together, these studies demonstrate that afferent-specific modulation of VTA inputs selectively alters the activity of distinct genetically defined VTA neuronal populations, which can profoundly influence both reward and aversive-related behaviors.
Disclosure: G. Stuber, Nothing to Disclose.

37.3 Plasticity and Function of Distinct Subtypes of Dopamine Neurons

Robert Malenka*

Stanford University School of Medicine, Palo Alto, California

Background: Midbrain dopamine (DA) neurons of the ventral tegmental area (VTA), which play critical roles in learning and motivated behaviors, are not homogeneous but differ in their molecular properties and the brain areas to which they project. However, the functional roles of the distinct subtypes of DA neurons are poorly understood.
Methods: We examined whether the modulation of excitatory synapses on DA neurons by rewarding or aversive stimuli depends on the respective brain area to which these DA neurons project. Specific DA neuron target areas were injected with fluorescent "retrobeads" *in vivo* so that the distinct subpopulations of DA neuron subpopulations could be visually identified in slices from which targeted whole cell patch clamp recordings were made. Animals were subjected to a rewarding experience (cocaine administration) or an aversive experience (injection of formaline in a hindpaw) prior to preparation of slices. Optogenetic manipulations of specific DA cell VTA subpopulations and specific inputs to the VTA are currently being performed as well as viral mediated tracing of the circuits in which distinct DA cell subpopulations participate.
Results: The DA neurons projecting to different target sites (medial prefrontal cortex, medial and lateral shell of the nucleus accumbens, dorsal striatum) exhibited different basal properties. *In vivo* administration of cocaine selectively modified excitatory synapses (assayed by AMPAR/NMDAR ratios) on DA cells projecting to nucleus accumbens medial shell while an aversive stimulus selectively modified synapses on DA cells projecting to medial prefrontal cortex. In contrast, synapses on DA neurons projecting to NAc lateral shell were modified by both rewarding and aversive stimuli, which presumably reflects saliency. The behavioral consequences of optogenetic activation of different subtypes of DA neurons as well as the consequences of activating different inputs are currently being assayed.
Conclusions: These results suggest that the mesocorticolimbic DA system is comprised of anatomically distinct circuits each modified by distinct aspects of motivationally relevant stimuli. The results of the optogenetic manipulations that will be presented will reveal differences in the functional roles of the distinct circuits in which the different DA cell subpopulations participate.
Disclosure: R. Malenka, Nothing to Disclose.

37.4 Dopamine Neurons Modulate the Neural Encoding and Expression of Depression-related Behavior

Kay M. Tye*

Massachusetts Institute of Technology, Cambridge, Massachusetts

Background: Major depression is characterized by diverse debilitating symptoms including hopelessness and anhedonia. Among many neural populations hypothesized to be relevant, dopamine neurons have been considered as potentially involved in the pathophysiology of these diverse symptoms, in part because certain antidepressant treatments including medications and brain stimulation therapies may (along with other effects) target aspects of the complex CNS dopamine system. But until now, it has not been possible to directly test this hypothesis, even in animal models, since existing therapeutic interventions do not provide specificity for dopamine neurons.
Methods: Here, we directly investigated the causal contributions of defined dopamine neurons to multidimensional depression-like phenotypes induced by chronic mild stress, by integrating behavioral, optogenetic and electrophysiological methods in freely-moving rodents.
Results: We found that bidirectional control (inhibition or excitation) of specified midbrain dopamine neurons immediately and bidirectionally modulated (induced or relieved) multiple unrelated depression symptoms caused by chronic stress.
Conclusions: In probing circuit implementation of these effects, we observed that optogenetic recruitment of these dopamine neurons potently altered the neural encoding of depression-related behaviors in the downstream nucleus accumbens of freely-moving rodents, suggesting that depression symptoms could involve alterations in the neural encoding of action in limbic circuitry.
Disclosure: K. Tye, Nothing to Disclose.

Panel
38. Applying Translational Research and Imaging to Treatment Strategies in Alcoholism

38.1 Translational Neuroimaging Studies of Alcoholism: From Rats to Man

Adolf Pfefferbaum*

SRI International, Menlo Park, California

Background: Quantitative neuroimaging has revealed consistent depictions of neuroanatomical systems affected in alcoholism. Multiple factors, namely, age at onset of hazardous drinking, drinking pattern, and nutrition, contribute to alcoholism-related brain structural and biochemical abnormalities but are, by nature, uncontrollable in human study. Thus, animal models are essential for disentangling the selective factors responsible for disordered brain structure and function.
Methods: Using *in vivo* MR imaging tools, we have distinguished unique neuroradiological signatures of rats given kainic-acid induced seizures, binge and chronic alcohol exposure paradigms, and frequent concomitants of alcoholism, including thiamine deficiency and liver disease that cause or exacerbate alcoholism-related markers of neuropathology.
Results: The differential changes to brain structure, biochemistry, and connectivity described by each animal model provide insight into the neural mechanisms underlying alcoholism's effects on brain structure and function as well as the scope and limits of potential recovery of neural nodes, circuits, and functions.
Conclusions: Translational studies across species, by providing a full depiction of the consequences of chronic alcoholism from human studies, and components of targeted aspects of alcoholism from animal studies, enable a more thorough interrogation of the complex and heterogeneous effects of alcohol on the brain.
Disclosure: A. Pfefferbaum, Nothing to Disclose.

38.2 Brain Mechanisms of Behavioral Changes Promoting Relapse in Alcohol Dependent Individuals; A Translational Approach

Theodora Duka*

University of Sussex, Brighton, United Kingdom

Background: Alcoholic patients who have undergone multiple withdrawals show impaired cognitive control and altered processing of emotional signals.
Methods: Participants were abstaining multiply detoxified (MDTx, $n = 12$) or singly detoxified alcoholic patients (SDTx, $n = 17$) and social drinker controls (SD, $n = 31$). They were tested in an incentive conflict task to investigate ability to abstain from responding during presentations of incentive cues. Functional magnetic resonance imaging was performed during presentation of a task in which subjects had to recognise the emotion in morphs containing a component of fearful facial emotional expression.
Results: MDTx were severely impaired on the incentive conflict task and had reduced gray matter volume in ventromedial prefrontal cortex and superior frontal gyrus compared to SDTx and SD. Animals having undergone repeated withdrawals showed also impairment in an homologous task. MDTx were also less able

than SDTx and SD to recognise fearful expressions, and showed decreased activation in prefrontal areas, including orbitofrontal cortex and insula. MDTx also showed a decreased connectivity between insula and prefrontal areas, and between amygdala and globus pallidus compared to SDTx and SD. In contrast, increased connectivity was found between insula and the colliculus neuronal cluster, and between amygdala and BNST. The strength of connectivity between insula and areas involved in regulation of emotion (inferior frontal cortex and frontal pole) was negatively correlated with the number of detoxification and dependency score in explicit condition.
Conclusions: Repeated episodes of detoxification from alcohol are associated with altered function in control of behaviour and fear perception pathways, and in cortical modulation of emotions. Such changes may confer inability in conflict resolution, increased sensitivity to emotional stress and impaired social competence, contributing to relapse.
Disclosure: T. Duka, Nothing to Disclose.

38.3 Altered Prefrontal Structure and Function Predicts Heavy Drinking and Alcohol Relapse: Are There Clues for Novel Treatment Strategies?

Rajita Sinha*

Yale University School of Medicine, New Haven, Connecticut

Background: Growing evidence from basic science and translational neuroimaging research indicates neuroadaptations associated with chronic alcohol abuse. However, evidence of whether chronic alcohol-related changes are predictive of motivation to consume alcohol and relapse risk is rare.
Methods: Novel neuroimaging results from two separate studies will be shown. In the first study, structural and functional magnetic resonance imaging (MRI and fMRI) data from 4-week abstinent recovering alcohol dependent individuals compared to controls will be presented assessing structural gray matter volume and neural responses to brief script-driven guided imagery of stress, alcohol cues and neutral relaxing states, and to examine whether neural changes in structure and function are predictive of future relapse using a prospective clinical outcome design. The second study will assess changes in neural function in well-matched non-smoking, light versus binge/heavy drinking individuals during stress, cue and neutral relaxing states and to assess neural changes that correlate with motivation for alcohol in a alcohol taste test.
Results: Highly consistent and complementary results from the two studies were found showing lower gray matter volume in medial prefrontal and anterior cingulate regions with heavy and chronic alcohol intake. Furthermore, disrupted ventromedial prefrontal (VmPFC) control under stress and with high alcohol craving was observed with fMRI. These changes in VmPFC function was predictive of future alcohol relapse in recovering alcoholics and also associated with motivation to consume alcohol in non-dependent binge/heavy drinkers.
Conclusions: The results suggest that the medial prefrontal networks are vulnerable to the effects of chronic alcohol intake and are also involved in the pathophysiology of loss of control drinking and relapse risk. Findings will be discussed in the context of translational data on the pathophysiology of alcoholism and potential novel therapeutics for the treatment of alcoholism. (Supported by UL1-DE019586, R01-AA013892; PL1-DA24859).
Disclosure: R. Sinha, Nothing to Disclose.

38.4 Striatal-limbic Suppression during Anticipatory Anxiety in Alcohol-dependent Men

Bryon Adinoff*

University of Texas Southwestern Medical Center, Dallas, Texas

Background: Trauma and adversity are associated with a dramatic increase in the risk of alcohol use disorders, presumably due to neuroplastic changes induced by trauma/adversity. This study was designed to assess the relative contributions of childhood trauma/adversity and alcohol use to the neurobiologic response to stress in abstinent alcohol-dependent and healthy control participants.
Methods: Fifteen, alcohol-dependent men, abstinent for 3-5 weeks, and 15 age-, sex-, and race-matched healthy controls were studied. Anticipatory anxiety was induced by a conditioned stimulus (CS) paired with an uncertain physically painful unconditioned stressor (high threat US) compared to a low pain US (low threat). Pain thresholds were personalized for each participant. Neural responsiveness was assessed with fMRI ($Z = 2.3$, corrected cluster p threshold $= 0.01$). Childhood trauma and adversity were assessed by the Childhood Adversity Interview, a semi-structured rater-administered interview.
Results: Both groups experienced significant, and similar, levels of anticipatory anxiety in response to the high and low threat CS. During high relative to low threat US, control participants showed marked increases in the BOLD response of striatal-limbic regions, including the caudate, putamen, bilateral insula, thalamus, lateral orbitofrontal cortex, superior frontal cortex and anterior cingulate. In contrast, alcohol-dependent participants only showed superior frontal cortex activation. Striatal-limbic activation in the alcohol-dependent group was negatively correlated with childhood trauma/adversity, particularly in the left ($r = -.73$, $p = .002$) and right ($r = -0.65$, $p = .008$) dorsal striatum. Childhood loss and separation, rather than trauma, primarily contributed to this relationship. In contrast, recent alcohol use was associated with an increase in the striatal response to anticipatory anxiety.
Conclusions: Childhood adversity in alcohol-dependent participants is associated with a blunted striatal-limbic response to anticipatory anxiety. These findings suggest persistent neural effects induced by childhood losses in alcohol-dependent participants. These findings were not accounted for by alcohol use.
Disclosure: B. Adinoff, **Part 1:** Auxilium Pharmaceuticals, Shook, Hardy & Bacon LLP (medical malpractice consultant, tobacco companies), **Part 2:** Department of Veterans Affairs, UT Southwestern Medical Center.

Panel

39. Lesson from Animal Studies of Genetic Risk Factors for Psychiatric Disorders of Neurodevelopmental Origin: How Can We Move Forward with Our Research for Novel Treatment Interventions?

39.1 Insulin-like Growth Factor 1 Therapy in Rett Syndrome: From Animal Studies to Clinic

Daniela Tropea*

Trinity College Dublin, Dublin, Ireland

Background: Rett syndrome (RTT) is a devastating neurodevelopmental disorder that affects one in ten thousand girls, and has no cure. The majority of RTT patients display mutations in the gene that codes for the Methyl-CpG binding protein 2 (MeCP2). Clinical observations and neurobiological analysis of mouse models suggest that defects in the expression of MeCP2 protein compromise the development of the central nervous system, especially synaptic and circuit maturation. Thus, agents that promote brain development and synaptic function, such as Insulin-like growth factor 1 (IGF1), are good candidates for ameliorating the symptoms of RTT. IGF1 and its active peptide, (1-3)IGF1, cross the Blood Brain Barrier, and (1-3) IGF1 ameliorates the symptoms of RTT in a mouse model of the disease, therefore they are ideal treatments for neurodevelopmental disorders, including RTT.
Methods: Cellular Studies: We used primary neuronal cultures from mice cerebral cortex to examine the intracellular pathways activated by medium application of IGF1 or GPE. We dissected prefrontal cortex from P0-P2 pups and cultured dissociated nerve cells for 7-10 days *in vitro* (DIV). Two days before processing, we added rhIGF1 or GPE (100 ng/ml) to the culture medium. Cells

were fixed and immunostained for markers of phosphoIGF1 receptor (pIGFR), MAPK and PI3K signaling pathways (anti phosphoMAPK, anti phosphoAKT). We measured the immunostaining for the selected markers to compare the effects of IGF1 and (1-3) IGF on brain cells. Clinical study: We performed a pilot study to establish whether there are major risks associated with IGF1 administration in RTT patients. Six young girls with classic RTT received IGF1 subcutaneous injections twice a day for six months, and they were regularly monitored by their primary care physicians and by the unit for RTT in Versilia Hospital (Italy).
Results: Animal studies show that IGF and (1-3)IGF administration ameliorate Rett Symptoms in a mouse model of the disease. We examine the effects of IGF1 and (1-3)IGF1 application on primary cultural cultures and we report four main findings: first, both molecules induce the phosphorylation of IGF1 receptor; second, the two drugs activates different intracellular pathways. Third, both IGF1 and (1-3)IGF1 increase the expression of synapsin 1 and PSD95. Fourth, we show that treatment with (1-3)IGF1 increases the expression of endogenous IGF1, therefore interfering with IGF1 signalling. In order to proceed on Rett patients, it is necessary to test the safety of IGF1 administration in RTT. A safety assessment of IGF1 treatment in Rett patients has been performed in a pilot study in Viareggio (Italy) under the supervision of Dr. Giorgio Pini. Six young girls (between four and eleven years) were treated with IGF1 for six months. IGF1 was administered twice a day subcutaneously. Ematic parameters, EEG, cardiac activity, respiration, neurological condition, bone density, were evaluated every three months for one year. The study revealed no major risk for IGF1 administration.
Conclusions: The cellular studies show that both IGF and (1-3)IGF promote the expression of synapse markers and are good candidate for curing the disorders affecting synapse function. The clinical data show that IGF1 administration is safe and well tolerated by the tested patients, and can be administered to RTT patient with constant monitoring of the risk parameters.
Disclosure: D. Tropea, Nothing to Disclose.

39.2 Modeling Lissencephaly- From Pathogenesis to Therapies

Anthony Wynshaw-Boris*

The University of California, San Francisco, California

Background: Type I lissencephaly ("smooth brain") is a devastating brain abnormality where children have an absence or paucity of gyri and sulci, associated with severe mental retardation, seizures and often early death. The major cause of lissencephaly is haploinsufficiency of the gene *LIS1*, and *LIS1* was the first genetic defect identified in any organism with neuronal migration defects. Studies in model organisms, particularly *Aspergillus nidulans*, a bread mold, as well as those in the mouse, have uncovered an evolutionarily conserved pathway that involves LIS1 and cytoplasmic dynein in a complex with other proteins that positively regulates its conserved function in nuclear migration.
Methods: To further investigate the role of LIS1 in neuronal migration and other aspects of brain development, we have produced and studied mice with various levels of *Lis1*. These mice displayed neuronal migration defects proportional to the gene dose of *Lis1*. Complete loss of murine *Lis1* results in peri-implantation lethality, and genetic studies in model organisms suggest a role for *Lis1* in cell division. We demonstrated an essential role for *Lis1* in neuroepithelial stem cells in mice using a genetic approach to produce mice with a graded reduction of *Lis1* as well as the complete loss of *Lis1* using a conditional *Lis1* allele and transgenic Cre lines. Our genetic and cell biological studies in mice suggest that LIS1 plays an important role throughout the development of the brain and participates in the regulation of neurogenesis and neuronal migration.
Results: We have used this information to develop a therapeutic strategy in mice. LIS1 protein is degraded by calpain, and the *in vitro* and *in vivo* treatment of *Lis1* mutant mice with calpain inhibitors have resulted in marked phenotypic improvement. We are currently testing whether calpain inhibitors can improve cellular phenotypes of human patient derived neural progenitors and neurons by reprogramming fibroblasts from lissencephaly patients into induced pluripotent stem cells. We have successfully reprogrammed these cells from lissencephaly patients and controls, and determining whether they display defective neurogenesis and neuronal migration.
Conclusions: We will continue to examine the effects of calpain inhibitors in these human patient-derived cells with the ultimate goal of using such therapies to improve the severe phenotypes of human patients with lissencephaly.
Disclosure: A. Wynshaw-Boris, Nothing to Disclose.

39.3 Regulatory Role of DISC1 for Excitatory Action of GABA Signaling in Prefrontal Cortex Development and Function

Atsushi Kamiya*

Johns Hopkins University School of Medicine, Baltimore, Maryland

Background: GABA signaling is a major inhibitory mechanism for neuronal communication in the CNS. Accumulating evidence from genetic and pharmacological studies as well as studies using post-mortem brain from patients with schizophrenia suggests that disturbance of GABA function in inhibitory neurotransmission is associated with pathophysiologies of schizophrenia. In addition to the inhibitory function of GABAergic neurotransmission, it is noteworthy that the excitatory action of GABA regulates multiple cellular processes during brain development. Given that many genetic risk factors for schizophrenia play various roles in brain development, exploring convergence of excitatory GABA action and risk genes-mediated pathways may shed light on not only understanding the molecular mechanisms underlying brain development, but also decipher how genetic disturbances affect brain maturation and functioning relevant to schizophrenia, which may in turn identify novel therapeutic targets for schizophrenia. In this project, we are examining the potential mechanism of Disrupted-In-Schizophrenia-1 (DISC1), one of the genetic risk factors for major mental disorders, for excitatory GABA signaling during prefrontal cortex development with particular focus on its role for dendritic development. The specific effect of DISC1 on behaviors associated with the prefrontal cortex is also examined by using mice in which DISC1 is selectively suppressed in post-migratory neurons in the prefrontal cortex by using *in utero* electroporation.
Methods: DISC1 is a complex molecule; it has multiple functions in neurodevelopmental processes, including cell proliferation, neuronal migration, axon growth, and dendritic development. In order to examine the role of the DISC1 pathway in specific post-migratory periods, we utilize *in utero* electroporation with the Cre/loxP-mediated inducible gene expression system. This method specifies DISC1's role for dendritic development in the developing prefrontal cortex, independent from the secondary effects of DISC1 in dendrites caused by disturbed cell proliferation and migration. To explore the possibility that DISC1 may regulate dendritic development via the modulation of GABA-mediated calcium signaling, we perform a calcium imaging assay in the developing primary cortical neurons transfected with DISC1 shRNA and a genetically-encoded calcium indicator. The underlying molecular mechanisms are further examined by biochemical experiments.
Results: Our *in vivo* data suggests that DISC1 regulates dendrite development independently from its effect on proliferation and migration in the prefrontal cortex. This is an important baseline for further investigation to address the underlying mechanisms of the role of DISC1 for dendritic development. In fact, another set of data suggests that DISC1 may regulate dendritic development via the control of excitatory action of GABA-mediated signaling in the developing neurons. Based on our data from biochemical experiments, we conclude that this effect is, at least in part, mediated by the regulatory role of DISC1 for the control of $GABA_A$ receptor function.
Conclusions: We are currently examining further detailed mechanisms of how DISC1 regulates the function of GABAA

receptor. We are also characterizing behavioral outcome associated with schizophrenia, including cognitive functions in mice in which DISC1 is selectively suppressed in post-migratory neurons in the prefrontal cortex by *in utero* electroporation with inducible knockdown system. Understanding the involvement of DISC1 in GABA-mediated signaling for prefrontal cortex development and function may offer hope for novel treatment interventions for psychiatric disorders, such as schizophrenia.
Disclosure: A. Kamiya, Nothing to Disclose.

39.4 Pathological Mechanisms of Aberrant Neuregulin Signaling Revealed by Temporal Control of Expression

Lin Mei*

Georgia Health Sciences University, Augusta, Georgia

Background: Schizophrenia is a common and disabling mental illness that affects 1% of the population worldwide and accounts for 3% of the total economic burden of human disease. The lack of a pathognomonic lesion makes it one of the least understood mental disorder although postmortem and brain imaging studies suggest impaired neural connectivity as an overarching pathological mechanism. Schizophrenia is believed to be a neural developmental disorder. Impaired neural development could lead to synaptic dysfunction of various neurotransmitters including glutamate, GABA, dopamine, and acetylcholine. Schizophrenia has a strong genetic component and linkage and association studies have identified several loci and susceptibility genes that may confer risk to schizophrenia including neuregulin 1 (NRG1), a trophic factor, and its receptor ErbB4. Although NRG1 and ErbB4 expression or signaling is abnormal in schizophrenic patients, however, underlying pathological mechanisms remain unclear.
Methods: Both type I and IV isoforms of NRG1 are elevated in the forebrain of schizophrenia patients; however, the copy number of type I mRNAs is 10-100 times more than that of type IV in human brains. Therefore, to mimic increased type I NRG1 levels in schizophrenic patients, we generated transgenic mice that carry the type I NRG1b gene under the control of the tetracycline-responsive promoter element (TRE) tetO (thereafter referred to as TRE-Nrg1, or control, mice). Since the majority of NRG1 in the brain is produced in excitatory neurons, TRE-Nrg1 mice were crossed to CamK2a-tTA mice that express the tTA under the control of the CamK2a promoter in excitatory neurons. Resulting bitransgenic CamK2a-tTA;TRE-Nrg1 mice (thereafter referred to as ctoNrg1 mice) produced HA-Nrg1 in excitatory neurons in the absence of doxycycline (Dox). HA-NRG1 expression could be turned off after mice were fed with Dox.
Results: ctoNRG1 mice exhibited schizophrenia-related phenotypes including hyperactivity, impaired PPI, and cognitive deficits, providing evidence that NRG1 gain-of-function may cause schizophrenia-related behavioral deficits. Remarkably, after the level of NRG1 was reduced at adult age, behavioral deficits were completely ameliorated in ctoNrg1 mice, in support of the causal effect of NRG1 overexpression. Electrophysiological studies indicate that NRG1 gain-of-function impaired neurotransmission at glutamatergic as well as GABAergic synapses, and the synaptic dysfunction was eradicated by shutting down the expression of the NRG1 transgene, providing cellular mechanisms for schizophrenia-related behaviors.
Conclusions: These observations indicate that the continuous presence of high levels of NRG1 is necessary for synaptic dysfunction and behavioral deficits. The findings that reduction of NRG1 levels to normal eliminates synaptic dysfunction and associated schizophrenia-related phenotypes in mice that had been symptomatic indicate that the damage by NRG1 gain-of-function is not irrevocable; rather it is reversible, suggesting that relevant schizophrenic patients may benefit from therapeutic intervention to restore NRG1 levels and function.
Disclosure: L. Mei, **Part 1:** SAB member, Mind-NRG.

Panel
40. Glucocorticoid Receptors as Pharmacologic Targets in Psychiatry

40.1 Corticosteroid-dependent Plasticity Mediates Compulsive Alcohol Drinking in Rats

George F. Koob*

The Scripps Research Institute, La Jolla, California

Background: Glucorticoids have multiple functions in the central nervous system subsumed under the constructs of arousal, activation, and responses to stressors. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis is associated with depression, chronic stress, posttraumatic stress disorder, and alcoholism. Alcoholism is characterized by a compulsion to seek and ingest alcohol, loss of control over intake, and emergence of a negative emotional state during withdrawal. Previous work with animal models of compulsive drinking have shown a pronounced dysregulation of the HPA axis in human alcoholics and a pronounced sensitization of the extrahypothalamic brain stress systems, such as those mediated by corticotropin-releasing factor (CRF).
Methods: We hypothesized that dysregulation of the HPA axis and activation of the brain CRF systems are linked by alterations in glucocorticoid receptors (GRs) to drive compulsive alcohol drinking.
Results: Our results showed that rats exposed to alcohol vapor to the point of dependence displayed increased alcohol intake, compulsive drinking measured by progressive-ratio responding, and persistent alcohol consumption despite punishment compared with control rats that were not exposed to alcohol vapor. Acute alcohol withdrawal was accompanied by downregulated GR mRNA in various stress/reward-related brain regions (i.e., prefrontal cortex, nucleus accumbens [NAc], and bed nucleus of the stria terminalis [BNST]), whereas protracted alcohol abstinence was accompanied by upregulated GR mRNA in the NAc core, ventral BNST, and central nucleus of the amygdala. Chronic GR antagonism with mifepristone (RU38486) prevented the escalation of alcohol intake and compulsive responding induced by chronic, intermittent alcohol vapor exposure. Chronic treatment with mifepristone also blocked escalated alcohol drinking and compulsive responding during protracted abstinence.
Conclusions: Thus, the GR system appears to be involved in the development of alcohol dependence and may represent a potential pharmacological target for the treatment of alcoholism.
Disclosure: G. Koob, **Part 1:** Addex, Alkermes, Arkeo, Embera, Psychogenics.

40.2 Working Memory is Modulated by Glucocorticoid Receptor and Dopaminergic Genes

Wissam El-Hage*

Université de Tours, Tours, France

Background: Different genes are known to modulate frontal cortical activity during working memory tasks. In an fMRI study we examined the potential interaction between the glucocorticoid receptor (GR) gene (NR3C1) and the Val158Met Catechol-O-Methyl-Transferase (COMT) polymorphisms and the BOLD response within brain networks involved in working memory.
Methods: We recruited a sample of 90 right-handed white Caucasian healthy individuals. We have used fMRI at 3T to examine the BOLD response during an n-back task with varying cognitive load (1, 2, 3-back). We assessed the association between the polymorphisms of COMT (Met158Val) and the three single-nucleotide polymorphisms of the GR gene (BclI C/G rs41423247, 9β A/G rs6198 and rs1866388 A/G) with brain activations during working memory performance. Multiple regression and haplotype analysis were performed.

Results: We found that variations for GR alleles, as well as for the Val158Met variation for COMT, have significant independent and interactive effects (greater activation in right dorsolateral prefrontal cortex) on f-MRI activation on working memory in healthy individuals. These differences in activations remained significant after controlling for whole brain perfusion (arterial spin labelling).
Conclusions: Brain activity in frontal regions during working memory task is differently modulated by BclI and COMT minor alleles. The interactive effect of these polymorphisms is associated with higher activation, suggesting lower efficiency, of DLPFC during WM.
Disclosure: W. El-Hage, **Part 1:** WEH acted as a speaker or consultant for AstraZeneca, Bristol-Myers Squibb, Eli Lilly & Co., Janssen, Lundbeck, and Servier France.

40.3 A Human Laboratory Study of Mifepristone Treatment for Alcohol Dependence

Barbara J. Mason*

The Scripps Research Institute, La Jolla, California

Background: A focus for medication development in alcoholism is the protracted abstinence phase, whereby a drug may prolong abstinence by normalizing dysregulated motivational systems specifically associated with alcohol dependence. This phase of heightened relapse vulnerability following acute withdrawal is driven by dysregulation in stress systems in the CNS, including impaired glucocorticoid receptor feedback and hypothalamic-pituitary-adrenal (HPA) axis activity. Clinically, this state involves craving, sleep disturbance and negative affective states, all of which have been identified as risk factors for relapse. A key rationale for the study of modulators of brain emotional systems in alcohol dependence treatment is that medications that normalize the dysregulation of central stress systems may protect against relapse during protracted abstinence. This project uses the human laboratory model of risk factors for relapse in alcohol dependence that we previously developed and validated to evaluate the therapeutic potential of mifepristone, a potent glucocorticoid antagonist. The primary hypotheses under test are that alcohol dependent subjects treated with mifepristone will have significantly less physiological reactivity and craving for alcohol following cue exposure in the laboratory, and report fewer symptoms of protracted abstinence (e.g., craving, anxiety, mood and sleep disturbance, and relapse to drinking) under naturalistic conditions, compared to those treated with placebo.
Methods: Subjects are medically healthy, male or female paid volunteers, 18-65 years of age, who meet DSM-IV criteria for current alcohol dependence and are not seeking treatment. Subjects are randomly assigned to double-blind dosing for 1 week with mifepristone 600 mg/d or placebo. Subjects are verified as abstinent and not in acute withdrawal at the time of the lab session. Human laboratory cue reactivity and stress exposure procedures are conducted on the last day of dosing. A 3 (affective stimuli: positive, neutral, negative) x 2 (beverage cue: alcohol, water) within-subjects, block-factorial design (6 repeated measures) is employed for the cue reactivity manipulation. All six mood-beverage cue combinations are presented to each subject. A previously recorded personalized stress script is played over headphones during stress induction. Subjects return 1-week after the lab session and drug discontinuation to assess clinical status, and at 1-month to assess long term drug effects on drinking, craving, mood and sleep.
Results: Preliminary analyses found subjects (mean age 44.8 yrs) had 18.5 yrs of heavy drinking, consuming an average of 9.4 drinks on 6.2 days per week, prior to screening. Alcohol-cued craving was attenuated in the mifepristone condition compared to placebo. Mifepristone has been well tolerated in the alcohol dependent sample, with no serious or unexpected adverse events or evidence of abuse potential.
Conclusions: A positive signal on human laboratory and naturalistic follow-up measures in this non treatment-seeking, alcohol-dependent sample may lend support to the potential utility of mifepristone in the treatment of alcohol dependence.
Disclosure: B. Mason, **Part 1:** SAB: Addex Pharmaceuticals, Lohocla Research Corporation; Consultant: Johnson & Johnson Pharmaceutical Research & Development, LLC, Lilly USA, LLC; Speaker: Merck KGaA.

40.4 The Role of Glucocorticoid Receptors in Bipolar Disorder

Allan H. Young*

Imperial College London, London, United Kingdom

Background: Previous research has suggested that glucocorticoid receptors may be abnormal in bipolar disorder. Our previously published trial of the putative glucocorticoid receptor antagonist mifipristone, found evidence of benefits of brief administration of this agent on mood and cognitive measures in bipolar depressed patients.
Methods: A randomised, placebo controlled trial of add on therapy with mifipristone in bipolar depressed patients. Outcome effects were tracked over time.
Results: Significant benefits were evident on measures of cognitive function. The effects on depression scores did not seperate from placebo.
Conclusions: These data confirm the benefits of mifipristone on cognition in bipolar depression but do not find an antidepressant effect. The effects of mifipristone on cognitive measures were not immediately evident but manifested in a delayed fashion.
Disclosure: A. Young, **Part 1:** Professional involvement with all leading companies in the field of mood disorders in the last 2 years including: AstraZeneca, Janssen, GSK, Otsuka, BMS, Eli Lilly, Servier, Sanofi, and Brain Cells Inc.

Panel
41. Anxiety Disorders: New Evidence for Structural and Functional Connectivity Abnormalities

41.1 Structural and Functional Alterations Predict Individual Differences in Behavioral Inhibition

Andrew S. Fox*

University of Wisconsin-Madison, Madison, Wisconsin

Background: Children with extreme anxious temperament (AT) are particularly sensitive to new social experiences and have increased risk to develop anxiety and depression. The young rhesus monkey is optimal for studying the origin of human AT because it shares with humans the genetic, neural, and phenotypic underpinnings of complex social and emotional functioning. AT often manifests as early life behavioral inhibition (BI). We have validated a rhesus monkey model of childhood BI that shares these features with human children and allows for understanding of the neural circuit underlying BI.
Methods: To assess responses to a mild threat, we exposed 592 young rhesus monkeys (mean Age: 1.9 years; range: .74 to 4.2 years) to a human intruder who made no eye contact (NEC) with the animal. BI and brain metabolism were assessed during NEC, and brain structure was assessed on a separate day. Brain metabolism was assessed in response to NEC using FDG-PET, and brain structure was assessed using diffusion tensor imaging (DTI).
Results: Analyses revealed individual differences in BI to be associated with alterations in metabolism within a distributed brain network that includes the amygdala, hippocampus, bed nucleus of stria terminalis (BNST), as well as insular and orbitofrontal cortices. Moreover, we identified structural variation within core components of this network that were also associated with increased BI. Interestingly, these results include differences in fractional anisotropy within the stria terminalis, a principle tract of the extended amygdala

that contains fibers connecting those amygdala and BNST regions where metabolism was predictive of BI.
Conclusions: These results provide novel evidence for the functional components of an integrated circuit that underlies BI. Additionally, that BI is associated with altered structural connectivity between key components of the BI circuit. These findings will be important in furthering our understanding of AT and the development of anxiety and depression.
Disclosure: A. Fox, Nothing to Disclose.

41.2 Intrinsic Connectivity Abnormalities in Social Anxiety

Jennifer Blackford*

Vanderbilt University, Nashville, Tennessee

Background: Social anxiety disorder is a common, chronic, and disabling disorder affecting 1 in 10 Americans. Social inhibition is a key component of social anxiety and reflects an underlying trait difference, observable early in childhood. While the amygdala has been identified as a key neural substrate, little is known about the underlying neurocircuitry. Intrinsic connectivity methods provide a unique insight into fundamental structural and functional brain circuitry. Here we examine the relationship between social inhibition and functional connectivity of the amygdala.
Methods: Intrinsic connectivity ('resting state') fMRI data were collected in 40 young adults with either high social inhibition or low social inhibition. Three amygdala subnuclei seeds (centromedial, basolateral, superficial) were created based on a standard probabilistic atlas. Connectivity was estimated between each of the three amygdala subnuclei and the whole brain.
Results: Individuals with low social inhibition showed amygdala connectivity patterns similar to previously published studies in healthy controls. However, the high social inhibition group had several connectivity abnormalities ($p < .05$). First, connectivity was reduced between the centromedial amygdala seed and a region in the dorsal anterior cingulate cortex that has been implicated in a previous functional study by this lab. Also, connectivity was reduced between the superficial amygdala seed and the rostral anterior cingulate cortex.
Conclusions: These novel findings demonstrate altered amygdala connectivity with multiple anterior cingulate regions involved in emotion regulation. Given the known structural connections between these regions, reduced connectivity may reflect reduced ability of cingulate regions to effectively inhibit amygdala responses. Thus, reduced amygdala-anterior cingulate connectivity may be one mechanism by which social inhibition contributes to social anxiety disorder. These findings may have implications for the development of new treatments targeting amygdala-anterior cingulate connectivity.
Disclosure: J. Blackford, Nothing to Disclose.

41.3 Frontolimbic Connectivity in Generalized Anxiety Disorder

Jack B. Nitschke*

University of Wisconsin-Madison, Madison, Wisconsin

Background: Emotion regulation deficits figure prominently in generalized anxiety disorder (GAD), as well as other anxiety and mood disorders. Research examining emotion regulation and top-down modulation has implicated reduced coupling of the amygdala with prefrontal and anterior cingulate cortex (ACC), suggesting altered frontolimbic white matter connectivity in GAD.
Methods: Diffusion tensor imaging (DTI) and functional magnetic resonance imaging (fMRI) scans were obtained from 49 GAD patients and 39 healthy volunteers, including a subset of 21 patients without comorbid Axis I diagnoses and 21 healthy volunteers matched for age, sex, and education. The DTIdata were analyzed using tractography methods to assess structural connectivity, and the fMRI data were analyzed using psychophysiological interactions (PPI) methods to assess functional connectivity.
Results: Lower mean FA values in bilateral uncinate fasciculus indicated reduced frontolimbic structural connectivity in GAD. This reduction in uncinate fasciculus integrity was most pronounced for patients without comorbidity and was not observed in other white matter tracts. Across all subjects, higher FA values were associated with more negative functional coupling between the pregenual ACC and amygdala during the anticipation of aversion.
Conclusions: Decreased frontolimbic structural connectivity suggests a neural basis for emotion regulation deficits in GAD. The functional significance of these structural differences is underscored by decreased functional connectivity between the ACC and amygdala in subjects with reduced structural integrity of the uncinate fasciculus. Unique Data These data for connectivity in GAD have not yet been published but are currently in press in Archives of General Psychiatry.
Disclosure: J. Nitschke, Nothing to Disclose.

41.4 Targeting the Medial Prefrontal Cortex in the Treatment of Pediatric Anxiety

Danny Pine*

National Institutes of Health, Bethesda, Maryland

Background: Considerable research shows anxiety disorders to be developmental conditions. Most importantly, the majority of adult anxiety disorders begin as anxiety disorders in childhood. This demonstrates the need for more research on treatment in pediatric anxiety. Two areas of research remain particularly under developed. First, minimal research identifies targets in pediatric anxiety disorders that can be used in basic neuroscience research to develop novel treatments. Second, minimal research examines the long-term effects on the primate brain of currently effective treatments. The current presentation addresses both of these issues.
Methods: Two studies will be presented. First, data from a fear-conditioning study in humans will be presented. This study involved 150 individuals, with approximately half suffering from anxiety disorders and half being healthy. Moreover, half were adults and half were adolescents. Subjects were studied in the psychophysiological laboratory with a novel fear conditioning and extinction protocol (see Lau et al. PNAS 2011). Two weeks later, 98 were studied in the fMRI scanner during an extinction recall episode. Second, data from a long-term treatment study in monkeys will be presented. This study involved 32 monkeys, half of whom were exposed to early life stress and half of whom were raised under standard conditions. Half of these 32 monkeys were treated with fluoxetine from ages 3 to 4 years, and the other half were treated with placebo. All 32 underwent a series of imaging and behavioral experiments, approximately one year later, one year after all treatment had ended.
Results: The study in humans demonstrates no differences among anxious and healthy adolescents or adults in levels of fear conditioning assessed with startle or skin conductance. However, adolescent and adult patients did report higher levels of fear than their age-matched healthy comparison groups. Adolescent patients also showed a particularly high rate of study discontinuation. At extinction recall, both adolescent and adult anxiety disorder patients manifested reduced engagement of the ventral medial prefrontal cortex, with both patient groups differing from their respective age-matched comparison groups. The study in non-human primates demonstrated persistent effects of long-term fluoxetine exposure on behavior and brain structure, specifically in the medial prefrontal cortex. These effects on the brain were documented with MRI assessment of brain structure and PET assessment of serotonin neurochemistry, approximately one year after treatment had ended.
Conclusions: Studies in humans implicate the medial prefrontal cortex in anxiety disorders. Studies in non-human primates suggest that long-terms fluoxetine treatment influences medial prefrontal cortex development.
Disclosure: D. Pine, Nothing to Disclose.

Panel
42. Army STARRS Suicide Research: From Bench to Battlefield

42.1 The Army STARRS Study Plan
Robert Ursano*

Uniformed Services University School of Medicine, Bethesda, Maryland

Background: For the US Army the rate of completed sucides increased substantially during the conflicts in Iraq and Afghanistan. Concern for Army soldiers as well as the national security brought the US Army and NIMH together to address this national issue. In fact suicide is a problem for the entire nation: The number of suicides in the United States far exceeds the number of homicides and approaches the number of fatalities from Motor Vehicle Collisions. The number of suicide attempts per year is about 1 million (in adults over age 18).
Methods: The Army Study to Assess Risk and Resilience in Servicemembers is a comprehensive Framingham like study to identify risk and resilience factors for suicide behaviors. The goal of Army STARRS is develop actionable findings to assist the Army in addressing suicide risk in soldiers. The study is funded at present for 5 years at $65 million dollars. The study includes 6 components: the Historical Data Study; New Soldier Study; All Army Study, Pre/Post Deployment Study; SHOS-A, and SHOS-B. Each component addresses different phases of suicide risk. Data collection includes assessments of risk and resilience factors, potential endophenotypes, blood collection (at joining the army and pre and post deployment) and available historical data. Overall the historical dat includes over 1.6 million soldiers and over i billion lines of data. The direct assessments will include nearly 100,000 soldiers in the various components of the study and nearly 40,000 soldiers providing blood samples.
Results: The components of the Study are addressing specific hypotheses on suicide risk with the goal of identifying concentrated risk to assist in direct health care and interventions. Genetic risk and resilience factors as well as gene x environment interactions are being identified.
Conclusions: The long-term value of the Army STARRS study is substantial. It provides the opportunity to move psychiatric care and the science of suicide behavior to both "risk calculation" in clinical care as in the Framingham studies and greatly advance the genetic determination of risk and resilience endophenotypes.
Disclosure: R. Ursano, Nothing to Disclose.

42.2 Executive Functioning and Suicidal Behavior among Soldiers: Results from the Army STARRS Study
Matthew Nock*

Harvard University, Cambridge, Massachusetts

Background: Suicide is the second leading cause of death among 25-34 year olds in the U.S. The rate of suicide among U.S. Army soldiers is even higher, following a marked increase in recent years. Given that people considering suicide may be unwilling or unable to report on their level of risk, a key challenge for suicide research is the identification of objective, behavioral or biological risk markers. Prior research suggests that deficits in executive functioning, such as problems with impulsiveness, cognitive flexibility, attention, and working memory may increase the risk of suicidal behavior (Keilp et al, 2001; Harkavy-Friedman et al, 2006). In an effort to test the potential utility of performance-based measures of executive functioning as behavioral risk markers for suicidal thoughts and behaviors, the current study examines: (a) the extent to which soldiers who have experienced suicide ideation and suicide attempts show deficits in executive functioning, and (b) the extent to which computer-based measures of executive functioning can improve the statistical prediction of recent suicidal behavior.
Methods: Data are from the New Soldier Study component of Army STARRS. In this study, new Army recruits ($N > 25{,}000$) complete a comprehensive, self-administered, computerized battery of eight neurocognitive tests that include measures of executive functioning, such as the: Go-No-Go test, continuous performance test, conditional exclusion test, and n-back test. Soldiers also complete a comprehensive, computer-administered survey that assesses history of suicidal behavior, mental disorders, and other putative risk and resilience factors. This presentation reports on the associations between executive functioning and suicidal thoughts and behaviors.
Results: Results reveal that: (a) these tests, modified in the current study for mass self-administration, show good psychometric properties, and (b) overall, poor performance on measures of executive functioning is associated with recent (past 30 days) suicide ideation and attempts ($rs = -.32$ to $-.04$), $R2 = .12$-$.19$, but not with lifetime suicide ideation and attempts ($rs = -.04$ to $.01$), $R2 = .01$-$.02$. This presentation also will include the results of tests of the incremental predictive validity of deficits in executive functioning.
Conclusions: Executive functioning deficits are associated with recent suicidal behavior among U.S. Army soldiers. The cross-sectional, bivariate nature of these analyses does not allow for any strong inferences about the association between executive functioning and suicidal behavior. Additional analyses, from this study and others, are needed to better understand the nature of this association. Nevertheless, these preliminary data support future efforts aimed at identifying behavioral markers for suicidal thoughts and behaviors.
Disclosure: M. Nock, Nothing to Disclose.

42.3 A Beating of Minds: Suicide and Traumatic Brain Injury
Murray B. Stein*

University of California, San Diego, California

Background: Suicide is a major problem for the US military. Studies suggest that suicidal thoughts, attempts, and completed suicides all increase in concert with deployments. As part of the Army STARRS goal of delineating risk and resiliency markers for suicide (and other deployment-related mental health problems), we examined associations between various putative risk factors – including self-reported history consistent with various levels of severity of traumatic brain injury (TBI) – and suicidality in a representative sample of over 3,000 Army personnel in the All-Army Survey (AAS). The AAS is designed to be a representative snapshot survey of all Army personnel exclusive of those in training.
Methods: The analysis of correlates reported here focuses on predictors of current, past 30-day, and past 12-month suicidality. The latter was broken down into: ideation, plan(s), attempt(s), and other forms of self-destructive behavior. We report conditional prevalence as well as odds-ratios (ORs) based on multiple logistic regression analyses.
Results: 13.5% of AAS respondents reported lifetime histories of suicidal ideation and 6.4% reported lifetime histories of self-destructive behaviors. 3.4% of AAS respondents reported lifetime histories of suicide plans (3.4%) and 2.9% reported lifetime histories of suicide attempts. in bivariate analyses, various levels of probable TBI were associated in a dose-response relationship (OR ranging from 1.7 to 4.0) with increased 12-month likelihood of suicide attempt(s) and/or self-destructive behaviors. At the extreme, the 7.1% of AAS respondents who experienced four or more types of head, neck, or blast injuries are 3.0-4.7 times as likely as those who experienced none to have 12-month suicidality.
Conclusions: One potentially important implication of these results that needs to be sorted out more exactly in future AAS analyses is that much of the recent suicidality associated with head, neck, or blast injuries among Army personnel appears to be associated with injuries that occurred prior to entering the Army, although perhaps exacerbated by subsequent repeat injuries that occurred while in the Army. If this is the case, it could have important intervention implications.

Disclosure: M. Stein, **Part 1:** Up To Date: Co-Editor-in-Chief for Psychiatry Content Depression and Anxiety (journal): Deputy Editor, **Part 2:** University of California San DiegoVA San Diego Healthcare System Up To Date Depression and Anxiety (journal): Wiley Press.

42.4 TBI and Medical Illness as Predictors of Suicide Risk in US Army Soldiers

Michael Schoenbaum*

National Institutes of Health, Bethesda, Maryland

Background: The Army Study to Assess Risk and Resilience in Servicemembers (Army STARRS; www.armystarrs.org) is the largest study of mental health risk and resilience ever conducted among military personnel. The study has several major components, including a unique historical database of administrative data on the characteristics, experiences and exposures of the 1.6 million individuals who served on active duty in the US Army between 2004 and 2009. This includes soldiers with adverse outcomes, particularly suicide or other manners of death, as well as those with positive outcomes. We examine the association between traumatic brain injury and other types of medical illness and injury and subsequent risk of suicide, as part of a broader effort to identify predictors of risk and resilience in soldiers.
Methods: We conduct case-control analyses, using discrete time survival models, to estimate the association of indicators of traumatic brain injury and other types of medical illness and injury and subsequent risk of suicide. We focus particularly on "concentration of risk," i.e., the extent to which we can use data on TBI, other medical illness/injury, and other covariates to identify subgroups of soldiers with especially elevated suicide risk. Such empirical information would enable the Army to target suicide prevention/intervention efforts to high-risk groups.
Results: We report the independent associations between indicators of TBI and of other medical illness and injury, based on health care claims and encounter data from the military health system, on subsequent risk for suicide death. We also report the extent to which these predictors, along other other measures of soldiers' characteristics and experiences, support the development of predictive algorithms for suicide risk.
Conclusions: Predictive risk algorithms based on medical and other Army and Department of Defense administrative data can be used to identify subgroups of soldiers with particularly elevated suicide risk. These findings may help the Army to focus prevention/intervention programs, and may suggest hypotheses to be tested in future research using primary survey, neurocognitive and biological data.
Disclosure: M. Schoenbaum, Nothing to Disclose.

Thursday, December 06, 2012

Mini Panel
43. Beyond Ketamine, Can Selective Targeting of the NMDA Receptor Produce Antidepressant Response without Psychotomimetic Effects: Clinical Results with Three Novel Compounds

43.1 Beyond Ketamine: Next generation NMDA Antagonists Show Rapid Antidepressant Effects, without Psychotomimetic Effects

Nancy Diazgranados*

Medpsych Associates, Lutherville, Maryland

Background: The rapid onset of antidepressant effects with ketamine has led to exploring compounds that also modulate the N-methyl-D-aspartate (NMDA) receptor complex with the hope of developing drugs with rapid antidepressant effects, but without ketamine's adverse properties. Two studies were conducted in treatment-resistant major depressive disorder (TRD) determine whether they produced rapid antidepressant effects: study 1 was with a low-trapping NMDA channel blocker (AZD6765) and study 2 was with a selective NR2B antagonist (MK-0657).
Methods: Two randomized, crossover, placebo-controlled studies were conducted in treatment-resistant major depressive disorder (TRD) at NIMH. In study 1, after a two week drug-free period, 22 subjects received an intravenous infusion of either AZD6765 (150 mg) or placebo on two test days one week apart. The MADRS and HAM-D were used to rate subjects at baseline, at 60, 80, 110, and 230 minutes post-infusion, and on Days 1, 2, 3, and 7 post-infusion. In study 2, TRD subjects underwent a one-week drug-free period and were subsequently randomized to receive either MK-0657 monotherapy (4-8 mg/day) or placebo for 12 days.
Results: Study 1: Depressive symptoms as measured by MADRS, significantly improved in subjects receiving AZD6765 compared to placebo (effect size $d = 0.40$); the greatest improvement over placebo was at 80 and 110 minutes. On the HAM-D scale, the drug difference was significant ($d = 0.49$) with the largest differences at the same time points in addition to day 2. Thirty-two percent of subjects responded to AZD6765 and 15% to placebo at some point during the trial. There were no differences in psychotomimetic or dissociative symptoms at anytime between groups. AZD6765 was well-tolerated. In study 2, significant antidepressant effects were observed as early as Day 5 in patients receiving MK-0657 compared to placebo as assessed by the HAM-D and Beck Depression Inventory; however, no improvement was noted when symptoms were assessed with the MADRS, the primary efficacy measure. No serious or dissociative adverse effects were observed in patients receiving this oral formulation of MK-0657.
Conclusions: In patients with treatment-resistant major depressive disorder, rapid but short-lived antidepressant effects resulted from a single intravenous dose of a low trapping NMDA channel blocker. An oral selective NR2B antagonist showed onset of antidepressant effects by day 5. Both compounds at the dose tested, were not associated with psychotomimetic or dissociative side effects or significant blood pressure changes. This suggests that the acute antidepressant response of drugs that target the NMDA receptor complex is not dependant or necessarily associated with psychotomimetic effects. Furthermore, the potential of un-blinding of the studies due to medication side effects that has been questioned with the ketamine studies was not present with this two compounds.
Disclosure: N. Diazgranados, Nothing to Disclose.

43.2 A Phase 2, Randomized, Double Blind, Single Intravenous Dose Study of GLYX-13, an NMDA Receptor Glycine Site Functional Partial Agonist, in Subjects with Major Depressive Disorder with Inadequate Response to Antidepressant Medication

Ronald M. Burch*

Naurex, Inc., Morris, Connecticut

Background: Previous studies of the NMDA receptor antagonists ketamine and CP-100,606 have demonstrated rapid and robust antidepressant effects following single IV administration. Both of these agents, like other NMDA receptor antagonists, are also associated with acute psychotomimetic effects. Unlike full antagonists, glycine site partial agonists such as D-cycloserine, have not been associated with acute psychotomimetic effects. GLYX-13 is a glycine site functional partial agonist at the NMDA receptor. In animals it does not cause any behavioral effects referable to psychotomimetic effects at doses 100 times greater than doses that elicit pharmacologic effects in antidepressant models such as Porsolt assay.
Methods: Subjects who had $<25\%$ reduction in depressive symptoms during the current episode assessed using the Antidepressant Treatment Response Questionnaire (ATRQ) were admitted to the clinic prior to dosing and remained in the clinic until the 24 h evaluations were completed. Hamilton Depression

Rating Score (HDRS-17) was completed prior to dosing. Bech-6 scores were completed at 2, 4, 8, and 12 hours, HDRS-17 at 24 hours. Brief psychiatric rating scale (BPRS+) was completed at 30, 45, 60, and 90 minutes and at 2, 4, 8, 12, and 24 hours after dosing. Continuous ECG and pulse oximetry were monitored for 4 hours after dosing. Columbia suicide severity rating scale (C-SSRS) was administered at 24 hours. Subjects returned to the clinic at Days 3, 7, 14, 21, and 28 following dosing for HDRS-17, BPRS+ and C-SSRS ratings.
Results: Following IV administration, GLYX-13 is cleared rapidly with plasma half-life of 10 minutes or less. At doses of 1, 5, 10, or 30 mg/kg, GLYX-13 administration was not associated with psychotomimetic effects assessed using the BPRS+, nor with any other adverse events different from placebo. Of the 115 subjects randomized, 3 did not complete the study, none due to intolerance to study drug.
Conclusions: GLYX-13 was well-tolerated following single IV administration without psychotomimetic effects or other notable adverse events. Efficacy data will be presented.
Disclosure: R. Burch, **Part 1:** I am an employee of Naurex, Inc, the Sponsor of the clinical study reported herein, **Part 2:** Naurex, Inc, **Part 3:** Naurex, Inc.

43.3 Randomized Trial of AZD6765, an N-methyl-D-aspartate (NMDA) Channel Blocker, as Adjunct Treatment for Major Depression

Mark A. Smith*

AstraZeneca Pharmaceuticals, Wilmington, Delaware

Background: The NMDA channel blocker, ketamine, induces rapid antidepressant effects in treatment-resistant patients. However, ketamine's clinical utility is limited by its acute psychotomimetic or dissociative effects. This raises the question whether it is possible to develop compounds that target the NMDA receptors without these adverse effects. Here we report on the results from a Phase IIb study to evaluate the antidepressant properties of a novel, low-trapping NMDA channel blocker (AZD6765). The purpose of the study was to determine the effect of adjunct AZD6765 on symptom improvement in patients with major depressive disorder who had a history of inadequate response to antidepressants and had severe symptoms despite current treatment.
Methods: We conducted a Phase IIb (NCT00781742), multicenter, double-blind, randomized, placebo-controlled, parallel group, outpatient study. Patients were randomly assigned to receive a 1 h intravenous infusion of AZD6765 at doses of 100 mg or 150 mg, or placebo (saline), 3 times per week for 3 weeks with a follow-up to 8 weeks. The primary endpoint was change in the Montgomery Asberg Depression Rating Scale (MADRS) total score. Secondary endpoints included an assessment of efficacy at Day 3, response, remission, QIDS-SR16, HAM-A and CGI at each assessment, and safety and tolerability assessments from baseline to Week 8.
Results: MADRS total score change at Week 3 was significant for AZD6765 100 mg, −5.5 (adjusted $P = 0.006$) and 150 mg, −4.8 (adjusted $P = 0.019$) compared with placebo. During the first week of treatment, we observed a transient antidepressant effect at the end of each infusion. Although no statistically significant differences for either dose were observed at Day 3, there was a statistically significant efficacy at 2 weeks for the 100-mg dose ($\Delta = -4.2$; $P = 0.011$). Persistence of efficacy after stopping infusions was seen for several weeks, which was greater for the 100-mg dose. There was no statistical difference between 100 mg and 150 mg, but the 100 mg-dose showed slightly greater improvements in several secondary endpoints including response rates, CGI-I, QIDS-SR16, and HAM-A. Both doses were generally well tolerated, with the most common adverse events being mild and transient dizziness and blood pressure increases. AZD6765 did not produce psychotomimetic or dissociative effects as measured by CADSS.
Conclusions: These data indicate that AZD6765, a low-trapping NMDA channel blocker provides antidepressant effects in patients who have previously had an inadequate response to multiple antidepressants, and can do so without psychotomimetic or dissociative symptoms. Cumulative efficacy was seen over the 3-week treatment period and persisted for several weeks after the last infusion. In summary, we demonstrate that AZD6765 was well tolerated and effective at reducing depressive symptoms and represents a promising approach for severely depressed, treatment-resistant patients.
Disclosure: M. Smith, Nothing to Disclose.

Mini Panel
44. Beyond the NMDA Receptor-alternative Glutamatergic Targets for Antidepressant Treatment

44.1 Altered Affective Behavior in Kainate Receptor Knockout Mice

Anis Contractor*

Northwestern University, Chicago, Illinois

Background: Kainate receptors are glutamate gated ion channels with several diverse cellular functions in modulating and mediating synaptic transmission and cellular excitability. Recent studies genetic studies have identified kainate receptor genes as susceptibility genes for several neuropsychiatric disorders including schizophrenia, bipolar and mood disorders. Studies in knockout mice have also found that affective behavior is altered when individual kainate receptor genes are constitutively disrupted. In this study we generated and analysed a novel kainate receptor knockout mice in which the GluK4 receptor subunit is ablated.
Methods: Kainate receptor knockout mice ($GluK4^{-/-}$) were generated using standard homologous recombination techniques in mouse embryonic stem cells. Mice were characterized to determine that there was loss of the protein product of the gene. Behavioral analysis of mice was performed to determine if there were any changes in anxiety-like behavior or affective behavior. For anxiety, mice were tested in the elevated zero maze, marble burying task, and the novelty-induced suppression of feeding task. For affective behaviors mice were tested in the forced swim test, tail suspension test, and the sucrose preference test. In addition, *in vitro* electrophysiological studies were performed to determine how knockout of this subunit affected synaptic transmission and plasticity in the hippocampus, a region where this receptor subunit is highly expressed.
Results: We found that $GluK4^{-/-}$ mice had reduced anxiety like behavior in three separate behavioral tests. Knockout mice spent more time exploring the open areas of the elevated zero maze, they buried less marbles and spent less time digging in an anxiogenic environment, and they had a reduced latency to bite in the novelty-induced suppression of feeding test. Knockout mice also demonstrated reduced learned helplessness in the forced swim test and the tail suspension test. Also, knockout animals have an elevated preference for sucrose (a test for hedonic behavior). Analysis of synaptic transmission in the CA3 region of the hippocampus demonstrated that there was a specific deficit in mossy fiber LTP, an important form of plasticity in an area of the hippocampus known to be linked to anxiety and depression.
Conclusions: Taken together our studies demonstrate important phenotypes in $GluK4^{-/-}$ mice which support the interpretation that these mice have reduced anxiety and anti-depressant like behavior. Additionally important deficits in plasticity in the CA3 region of the hippocampus are further correlative evidence that kainate receptor function in the hippocampus supports affective behavior.
Disclosure: A. Contractor, Nothing to Disclose.

44.2 RNA Editing of an AMPA Receptor Subunit is Altered in Major Depression and Suicide

Monsheel Sodhi*

University of Illinois, Chicago, Illinois

Background: Major depression is a common and severely debilitating mental illness which is partially heritable and poorly understood. One brain region of interest, the dorsolateral prefrontal cortex (DLPFC), is

important for the regulation of executive function. Consistent data from several previous studies indicate that cortical 5-HT_{2C} RNA editing is increased in major depression and in suicide victims (Niswender et al, 2001; Gurevich et al, 2002; Iwamoto et al, 2003; Dracheva et al, 2008). This form of RNA editing is a post-transcriptional process which is catalyzed by enzymes called Adenosine Deaminases Acting on RNAs or 'ADARs'. RNA editing alters the physiological functions of AMPA and kainate glutamate receptors, and 16% of known microRNAs, in addition to the 5-HT_{2C} receptor (Wulff, 2011). Our previous studies show that the increased level of 5-HT_{2C} RNA editing in mood disorders and suicide may be a consequence of altered activity of the RNA editing enzyme, ADAR1, in these subjects (Simmons et al, 2010). Moreover, recent studies indicate that the glutamatergic system may play a role in the pathophysiology of mood disorders, since ketamine, an antagonist of the NMDA glutamate receptor, has rapid antidepressant effects in patients. In addition to NMDA receptor antagonists, drugs which potentiate AMPA receptor activity have been found to produce antidepressant effects in animal models (reviewed by Zaratec et al, 2010). Since the RNA editing process alters AMPA receptor function, we have tested the hypothesis that there is a generalized change in RNA editing which influences glutamatergic signaling in major depression and suicide.

Methods: Postmortem subjects diagnosed with major depression and a comparison group of subjects without psychiatric illness were included in this study. RNA was extracted from the DLPFC of 74 major depression cases (including 48 suicide cases) and 34 control subjects. All cases included in these analyses tested negative for the presence of antidepressants on toxicology screens. Quantitative polymerase chain reaction (QPCR) was conducted for the ADAR enzymes. QPCR data were normalized to the expression of housekeeping genes and analyzed using the relative standard curve method. Subsequently, levels of RNA editing and alternative splicing of the AMPA receptor subunit, GluR2, were measured using QPCR-restriction fragment length polymorphism (QPCR-RFLP) in these subjects.

Results: Our data indicate that GluR2 RNA editing at the Q/R site was 100% in the human DLPFC. The two GluR2 isoforms produced by R/G site editing were both detectable after RT-PCR followed by sequencing, and subsequently using a QPCR-RFLP method. The GluR2 RNA edited isoform (GluR2-G) has increased expression in major depression cases ($F = 9.38$, $df = 2, 106$, $p = 0.003$) and major depressive suicide victims ($n = 48$) compared with controls ($F = 13.2$, $df = 2, 80$, $p < 0.001$). ADAR1 but not ADAR2 expression showed a gender by diagnosis interaction ($F = 5.37$, $df = 1, 138$, $p = 0.02$). Expression of the GluR2 edited isoform was not correlated with ADAR1 expression. When control subjects were tested alone, ADAR2 expression was correlated with expression of the GluR2 edited isoform (Pearson coefficient $= 0.67$, $n = 34$, $p < 0.00002$).

Conclusions: Our results indicate that the GluR2 subunit of the AMPA receptor, which gates the AMPA receptor ion channel, has greater levels of editing in major depression and major depressive suicides. A faster time to desensitization of the AMPA receptor containing the edited GluR2 isoform compared with the unedited GluR2 isoform has been observed (Krampfl et al, 2002). These data support previous observations of the efficacy of AMPA receptor potentiators in rodent models of depression. The mechanisms by which glutamatergic drugs produce antidepressant effects are uncertain, but our data suggest that reduced AMPA receptor activity may occur in major depression and that the AMPA receptor GluR2 subunit may be a novel target for antidepressant drug development.

Disclosure: M. Sodhi, Nothing to Disclose.

44.3 Selective mGlu5 NAMs for the Treatment of MDD

Carrie K. Jones*

Vanderbilt University Medical Center, Nashville, Tennessee

Background: The metabotropic glutamate receptor subtype 5 (mGlu5) is a member of the family C G-protein-coupled receptors and a close downstream signaling partner of the N-methyl-D-aspartate subtype of glutamate receptor (NMDAR). Antagonism of mGlu5 results in inhibition of NMDAR signaling suggesting that mGlu5 antagonists may provide an alternative approach to NMDAR antagonists for the treatment of Major Depressive Disorder (MDD). Recently, our group has developed a novel series of selective mGlu5 negative allosteric modulators (NAMs), as represented by VU0409106, that offer an excellent opportunity to validate whether selective antagonism of mGlu5 will provide antidepressant-like effects in rodent models of depression comparable to NMDAR antagonists.

Methods: The ability of mGlu5 NAM VU0409106 to produce rapid and sustained antidepressant-like effects was assessed by evaluating changes in antidepressant-like activity in the forced swim test in rats and in the expression and phosphorylation of signaling molecules in the BDNF-TrkB receptor and associated signaling pathways, as previously correlated with the rapid response of ketamine treatment. The effects of VU0409106 were also evaluated in preclinical models of anhedonia, including the chronic mild stress and chronic social stress paradigms. Finally, the potential for VU0409106 to produce the adverse effects associated with excessive NMDAR antagonism observed with ketamine were assessed to establish the therapeutic index for the mechanism of this ligand.

Results: The mGlu5 NAM VU0409106 produced time- and dose-related antidepressant-like activity that was comparable to the effects observed with ketamine. In addition, selective inhibition of mGlu5 increased phosphorylation of several signaling molecules in the BDNF-TrkB receptor and associated signaling pathways, including Akt and GSK3B, and the magnitude of these effects were comparable to those observed with ketamine. Finally, the efficacy of VU0409106 was observed in a dose range that did not induce psychotomimetic-like activity or performance deficits in memory tasks as observed with ketamine.

Conclusions: Collectively, these preliminary findings suggest that selective mGlu5 NAMs such as VU0409106 may provide a rapid and sustained therapeutic strategy for the treatment of symptoms associated with MDD.

Disclosure: C. Jones, Nothing to Disclose.

Panel
45. Hippocampus and Addiction: New Neurons, New Circuits and New Responses

45.1 Role of the Dorsal Hippocampus in the Reconsolidation and Utilization of Associative Memories that Maintain Drug Context-induced Cocaine Seeking

Rita A. Fuchs*

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Background: This panel presentation will review recently published and new findings regarding the role of the dorsal hippocampus (DH) in the expression of cocaine-seeking behavior in an animal model of environmentally-induced drug relapse and in the reconsolidation of contextual associative memories that are thought to maintain cocaine-seeking behavior.

Methods: Rats were trained to self-administer cocaine in a distinct context, followed by extinction training in a different context. In the expression studies, rats were re-exposed to the cocaine-paired or extinction context for tests of cocaine seeking. Prior to testing, the rats received bilateral tetrodotoxin; D1, NMDA, or mGluR1 antagonist; or Src tyrosine kinase inhibitor (PP2) microinfusions into the DH or GABA agonist cocktail (B/M) unilaterally into the DH plus into the ipsilateral or contralateral basolateral amygdala (BLA). In the memory reconsolidation studies, rats were briefly re-exposed to the cocaine-paired context to destabilize cocaine memories or were exposed to a novel context. Rats then received bilateral tetrodotoxin, protein synthesis inhibitor (anysomycin), or PP2 microinfusions into the DH or B/M unilaterally into the DH

plus anysomycin into the ipsilateral or contralateral BLA. The effects were examined on drug seeking in the extinction and cocaine-paired contexts after 0 or 21 days of abstinence.
Results: In the expression studies, bilateral tetrodotoxin (Fuchs et al. 2005), SCH23390 (D1 antagonist; Xie et al, in prep.), AP5 (NMDA antagonist; Xie et al, in prep.), PP2 (Xie et al, in prep), or JNJ16259685 (mGluR1 antagonist; Xie et al, 2011) administration into the DH or unilateral B/M administration into the DH plus into contralateral, but not the ipsilateral, BLA (Fuchs et al, 2007) disrupted drug context-induced cocaine-seeking behavior ($p<0.05$) without altering motor performance. In the memory reconsolidation studies, bilateral tetrodotoxin and PP2, but not anisomycin, administration into the DH after cocaine-memory reactivation, but not after novel context exposure, disrupted subsequent drug context-induce cocaine-seeking behavior ($p<0.05$; Ramirez et al, 2009, Wells et al, in prep). Similarly, unilateral administration of B/M into the DH plus anisomycin into the contralateral, but not ipsilateral, BLA inhibited the same behavior in a memory reactivation-dependent fashion ($p<0.05$; Wells et al, 2011). Interestingly, some recovery of cocaine-seeking behavior was observed following 21 days of abstinence, but DH-BLA disconnection at the time of memory reconsolidation attenuated the ability of the cocaine-paired context to maintain cocaine-seeking behavior and disrupted the development of incubation, the time-dependent increase in the magnitude of cocaine seeking ($p<0.05$; Wells et al, 2011).
Conclusions: The present findings and our previous research indicate that, the DH is a unique among the elements of the corticolimbic relapse circuitry in that it selectively controls the reinstatement of cocaine-seeking behavior in response to drug-paired contextual stimuli as opposed to other triggers, such as explicit drug-paired stimuli, aversive stimuli, or cocaine itself. Within the DH, dopamine and glutamate mediate the expression of drug context-induced cocaine-seeking behavior via the stimulation of D1, NMDA, and mGlu1 receptors. In particular, drug context-induced cocaine-seeking behavior depends on Src tyrosine kinase-dependent enhancement of NMDA receptor function. In addition to directly contributing to the expression of cocaine-seeking behavior, the DH also promotes cocaine-seeking behavior via the maintenance of cocaine-related contextual associative memories through reconsolidation via a Src-tyrosine kinase dependent process. Finally, our research reveals that the DH interacts with the BLA in order to regulate the expression of drug context-induced cocaine-seeking behavior, the reconsolidation of cocaine-related contextual associative memories, and the development of the incubation phenomenon.
Disclosure: R. Fuchs, Nothing to Disclose.

45.2 Individual Differences in Substance Abuse Liability: Implicating the Hippocampus

Huda Akil*

University of Michigan, Ann Arbor, Michigan

Background: Animal models have taught us a great deal about the neural consequences of chronic exposure to drugs of abuse, especially within the reward pathway. However, we know less about the biological differences that propel some individuals to seek drugs and become addicts while others do not. Our working hypothesis is that the hippocampus is a major player in determining the initial propensity to substance abuse and is likely to be an important structure in mediating its lasting impact on brain and behavior. We suggest that the hippocampus regulates those aspects of substance abuse that transcend reward and involve reactivity to environmental stimuli. This includes novelty- seeking behavior that bias initial drug taking, but also subsumes neuroplasticity mechanisms that alter salience and can mediate conversion to addiction. For example, we and others have shown the impact of drugs of abuse on hippocampal neurogenesis, especially during withdrawal. Work by Esch's laboratory has shown that these changes are indeed critical to relapse, and we have demonstrated that animals with different genetic propensities for drug seeking and addiction show significant differences in hippocampal neurogenesis during drug withdrawal. This talk will discuss two animal models we utilize, one in mice and the other in rats, both of which focus on altering vulnerability to drug-seeking and drug responsiveness. We will demonstrate that organizing molecules that impact the hippocampus especially during early development have lasting effects on the propensity to seek cocaine. These molecules also alter the response of the hippocampus to the long-term impact of the drugs following addictive behavior.
Methods: We rely on two models: a) two Lines of rats that have been selectively bred for differences in novelty-seeking behavior and show significant differences in the propensity to self-administer psychostimulants and convert to addiction- bred High Responders and Low Responders; b) a mouse model whereby we overexpress the glucocorticoid receptor, GR, specifically in the forebrain, and we can manipulate its induction during development. We have shown that the GR overexpressing mice (GRov) exhibit great sensitization to cocaine. We use these models to study predisposing variables that are antecedents to drug-seeking behavior (e.g. self-administration and sensitization) as well as the consequences of long term drug exposure on behavior (e.g. conversion to addiction, relapse) and associated cellular and molecular changes (e.g. alterations in neurogenesis, gene expression, or changes in specific neuroplasticity genes).
Results: Bred HR (bHR) animals are significantly more prone to cocaine self-administration and show greater conversion to addiction. They also show significant difference in hippocampal gene expression from bLR and these differences occur in early development and surpass the differences observed in the nucleus accumbens. Amongst the differences between bHR and bLR is the differential expression of the Fibroblast Growth Factor2 (FGF2), which is basally higher in the hippocampus of bHRs. Remarkably, giving neonatal animals FGF2 only once, in early life induces a bHR phenotype, enhancing the propensity to self-administer cocaine, and increasing cocaine sensitization primarily in bLRs. This demonstrates that we have identified a clear molecular antecedent of drug-seeking behavior. Similarly, in our mouse model shows a remarkable impact of hippocampal development on responsiveness to cocaine. Thus, a time-limited developmental manipulation, whereby we induce GR only prior to weaning and then allow the animals to grow to adulthood under normal GR conditions, has its greatest impact on the hippocampus and leads to a lifelong change in propensity to sensitize to cocaine. Here again, the changes in the hippocampus are more extensive and more sustained than what we observe in the Nucleus Accumbens. GR overexpression that takes place after this critical period (i.e. post weaning) and continues for the rest of the animal's life, is unable to alter the reactivity to cocaine. Thus, manipulation during a critical period of hippocampal development post natally is necessary and sufficient to alter vulnerability to drugs.
Conclusions: Native differences in hippocampal structure and function are associated with differential vulnerability to drug-seeking behavior, drug reactivity and long-term addiction. Moreover, specific molecular manipulations during early development that alter hippocampal structure and function alter reponsiveness to drugs of abuse. Finally, chronic exposure to cocaine leads to distinctly different effects on hippocampal function, neurogenesis and gene expression that are associated with differences in addictive behavior. Together, these data suggest that both the antecedent of substance abuse, as well as the long-term consequences of substance about are modulated by the hippocampus.
Disclosure: H. Akil, Nothing to Disclose.

45.3 Ventral Hippocampal Regulation of Medial VTA Dopamine System and Its Role in Addiction

Anthony A. Grace*

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: There is substantial evidence that the dopamine system is hyper-responsive in addiction. Several studies have

shown that dopamine neurons undergo changes during the initial stages of psychostimulant administration; however, how this is translated into a long-term hyper-responsive state is unknown. Given that this sensitization is a context-dependent phenomenon, we examined the role of the ventral hippocampus, a context-related structure, on dopamine neuron activity states.
Methods: Rats were administered amphetamine for 5 days, and withdrawn from amphetamine for 5 days before testing. Single unit recordings were made in the VTA using the cells/track method to assess the number of dopamine neurons firing (population activity), firing rate and firing pattern. Behavioral response to amphetamine was tested by administering 1.5 mg/kg and locomotor activity measured. The ventral hippocampus was inactivated by TTX infusion.
Results: Acute amphetamine administration decreased the number of dopamine neurons firing spontaneously without altering average firing rate or pattern. Moreover, this occurred primarily in the reward-related medial VTA region. This was accompanied by a sensitized locomotor response. Inactivation of the ventral hippocampus reversed both the increase in dopamine neuron activity and the behavioral sensitization to amphetamine. Interestingly, acute stressors such as restraint stress also caused an increase in dopamine neuron population activity and a sensitized behavioral response to amphetamine; however, dopamine neuron activity was increased throughout the medial-lateral extent of the VTA. Amphetamine sensitization was also accompanied by LTP in the hippocampal-accumbens pathway; however, unlike in control rats, this LTP was not reversed by high frequency stimulation of the PFC.
Conclusions: These data suggest that the ventral hippocampus mediates the sustained increase in dopamine neuron excitability and sensitized behavioral response to amphetamine. Moreover, the plasticity in the hippocampus-acccumbens pathway is altered in a manner that the prefrontal cortex cannot reset the system to baseline.
Disclosure: A. Grace, **Part 1:** Johnson & Johnson, Lundbeck, Pfizer, GSK, Puretech Ventures, Merck, Takeda, Dainippon Sumitomo, Otsuka, Lilly, Roche, **Part 2:** Johnson & Johnson, **Part 3:** Johnson & Johnson, **Part 4:** Lundbeck, GSK, Lilly.

45.4 Linking Context with Reward: Hippocampal and Septal Circuit Projections to Ventral Tegmental Area Play Critical Roles in Cocaine Relapse

Gary Aston-Jones*

Medical University of South Carolina, Charleston, South Carolina

Background: Relapse to drug-taking is strongly influenced by the learned associations of contextual stimuli with drug experiences. However, the neural pathways that mediate such context-drug reward relationships are not well understood. The purpose of this panel is to describe recent results showing unrecognized and important roles for hippocampus (HC) in addiction behaviors.
Methods: We used transynaptic retrograde tracing, *in vivo* neurophysiology, cocaine self-administration and reinstatement of cocaine seeking in rats to examine brain circuits linking HC with VTA.
Results: We identified a novel circuit from CA3 of dorsal HC to ventral tegmental area (VTA) that uses lateral septum (LS) as a relay. Theta frequency stimulation of CA3 excited VTA dopamine (DA) neurons and inhibited non-DA neurons. DA neuron excitation was likely mediated by disinhibition because local antagonism of GABA receptors in VTA blocked responses to CA3 stimulation. Inactivating components of the CA3–LS–VTA pathway blocked evoked neurophysiological responses in VTA, and also blocked reinstatement of cocaine-seeking by contextual stimuli in self-administering rats. This trans-synaptic link between hippocampus and VTA may be an important substrate by which environmental context regulates goal-directed behavior. Additional experiments found that the relay in this HC-VTA circuit, LS, is also an important regulator of orexin neurons in lateral hypothalamus (LH). Interruption of this projection using an orexin morpholino antisense and contralateral disconnection approach showed that the LS connection to LH orexin neurons is critical for context-associated cocaine seeking in a place preference paradigm.
Conclusions: Together, these results show complimentary novel circuits for regulating VTA DA neurons that involve the LS, and reveal an important but previously unrecognized role for LS in addition behavior.
Disclosure: G. Aston-Jones, Nothing to Disclose.

Panel
46. Neural Networks across Development in Health, Anxiety/Depression and Treatment Implications

46.1 Overcoming the Detrimental Effects of Motion on Resting State fMRI

Michael Milham*

Child Mind Institute, New York, New York

Background: Resting-state fMRI (R-fMRI) has emerged as a mainstream technique for characterizing and mapping brain developmental and maturation processes, as well as the impact of psychiatric illness. The enthusiasm regarding the applicability of R-fMRI approaches for attaining normative indices of brain development and clinically meaningful markers of aberrant development, has recently been countered with growing fears regarding artifacts in the R-fMRI signal. In particular, findings that movements as small as 0.2-0.5 mm can artifactually contribute to findings has raised concerns about the viability of R-fMRI approaches in hyperkinetic populations, including young children. The present work examines the impact of various subject-level (i.e., time-series) and group-level correction approaches on the test-retest reliability of R-fMRI approaches, as well as on age-related effects.
Methods: For commonly used R-fMRI metrics, we compared the impact of two subject-level correction approaches (volume censoring [aka scrubbing], 24-paramter autoregressive modeling of the impact of motion [Friston et al, 1996]), and group-level correction (i.e., covarying for mean frame-wise displacement across subjects) on 1) test-retest reliability (NYU test-retest dataset available via http://fcon_1000.projects.nitrc.org), and 2) development (typically developing controls, ages 7-18, from the ADHD-200 dataset available via http://fcon_1000.projects.nitrc.org). R-fMRI metrics include seed-based correlation, regional homogeneity and fractional amplitude of low-frequency fluctuations (fALFF).
Results: Short- and long-term test-retest reliability are decreased by all correction approaches, though all remain in the moderate (ICC $>$ 0.5) to high (ICC $>$ 0.9) range. Least affected by correction is the frequency-based fALFF measure. Importantly, autoregressive modeling of motion gave comparable results to volume censoring approaches, without destroying the time-series temporal structure that would preclude frequency-based analysis (e.g., fALFF). Commonly observed age-related effects persist for all measures and analyses, albeit reduced.
Conclusions: R-fMRI remains an appropriate approach for examining the developing brain and the impact of psychiatric illness. Determination of the optimal approach for correction of motion-related artifacts remains a challenge, though the recommendation that volume censoring approaches are the only solution may be premature.
Disclosure: M. Milham, Nothing to Disclose.

46.2 Altered Intrinsic Connectivity and Error-processing Function of Salience Network in Pediatric Obsessive Compulsive Disorder

Kate D. Fitzgerald*

University of Michigan, Ann Arbor, Michigan

Background: Altered development of striatum - anterior cingulate cortex (ACC) intrinsic connectivity occurs in pediatric OCD, but the functional significance of this finding remains unknown.

The ACC has been posited to drive modulation of behavior in response to errors – a process that develops dramatically over the course of childhood and adolescence, when OCD often first presents. In OCD, early and exaggerated development of ACC response to errors could drive the onset of illness, given the tendency of young patients to feel that certain situations are 'not right'. The ACC, in addition to the insula, represents a key node within a salience network that activates in response to cognitively relevant events (e.g., errors), but remains connected even at rest. Given prior evidence that error-related ACC hyperactivation occurs in pediatric patients, we hypothesized that this functional abnormality may associate with aberrant resting state connectivity of the ACC, outside of the salience network.

Methods: Functional magnetic resonance imaging (fMRI) data was collected in 45 OCD (14.3 years, range 8-19) and 36 healthy (mean 14.1 years, range 8-19) youth during an error-eliciting interference task. Resting state connectivity was collected in the majority of these subjects (34 OCD, 31 HC). Functionally defined seeds (OCD > healthy youth) in the ACC and striatum were used to assess resting state connectivity within and between groups.

Results: No performance differences were observed between OCD and healthy youth, however, patients exhibited greater error-related activation of dorsal ACC and caudate compared to healthy controls. Both groups activated bilateral anterior insula, but with no significant difference between them. Greater error-related dorsal ACC and caudate activation associated with better performance (i.e., lower error rates) in both groups. In the resting state, patients exhibited excessive connectivity of 1) dorsal ACC with temporal-occipital cortex, postcentral gyrus and precuneus and 2) caudate with temporal-occipital cortex, ventral medial frontal cortex and parahippocampus. Both patient and healthy groups exhibited positive connectivity of dorsal ACC with anterior insula, but also of caudate with these regions. Among patients, greater dorsal ACC - postcentral gyrus and caudate-parahippocampus connectivity associated with less severe OCD. There were no regions in which healthy youth exhibited greater response to errors or greater connectivity than patients.

Conclusions: Hyperactive dorsal ACC and caudate response to errors occurs in the context of altered intrinsic connectivity of the salience network in pediatric OCD. In addition to dorsal ACC, the caudate was positively connected with ACC and insula in patient and healthy groups, suggesting that the salience network may include the caudate in youth. Greater functional engagement of dorsal ACC and caudate by errors and more extended patterns of intrinsic connectivity (dorsal ACC – postcentral gyrus, caudate-parahippocampus) associated with more positive outcomes – better performance, lower symptom severity – suggesting these alterations may be adaptive in patients. Longitudinal studies of the salience network in youth at risk for OCD are needed to determine whether hyperactive function and/or altered connectivity reduce risk of converting to illness.

Disclosure: K. Fitzgerald, Nothing to Disclose.

46.3 What Can Amygdala Functional Connectivity Tell Us about the Development of Anxiety Disorders?

Amy K. Roy*

Fordham University, New York, New York

Background: Anxiety disorders are chronic conditions that often onset during adolescence and persist through adulthood. Dysfunction of the amygdala is implicated in anxiety disorders across the lifespan. Beyond task-based studies, resting-state functional connectivity (RSFC) work demonstrates alterations in broader amygdala-based circuitry in anxiety disorders. To date, few studies have examined the integrity of these amygdala-based networks in pediatric anxiety disorders or in individuals at risk for developing anxiety (i.e., temperament of behavioral inhibition). However, such investigations are essential to the development of etiological models.

Methods: Two new RSFC studies will be discussed. The first compares amygdala RSFC between adolescents with Generalized Anxiety Disorder (GAD, n = 15) and healthy comparisons (HC, n = 20). The second examines amygdala RSFC in young adults characterized in early childhood as behaviorally inhibited (BI; n = 17) and behaviorally non-inhibited (BN; n = 19). For both studies, 6-minute resting state scans were acquired with eyes open. Amygdala regions-of-interest included three subdivisions (basolateral, centromedial, and superficial), based on those used in a previous study of amygdala RSFC in adults (Roy et al, 2009).

Results: Adolescents with GAD exhibited disruptions in amygdala-based networks that include regions in medial prefrontal cortex, insula, and cerebellum. Adolescents with GAD also presented reduced differentiation of the basolateral and centromedial networks. Preliminary findings of the second study reveal reduced amygdala RSFC with rostral anterior cingulate cortex and ventrolateral prefrontal cortex in BI relative to BN.

Conclusions: Together, these findings suggest that adoelscents with early-onset GAD, as well as young adults at temperamental risk for anxiety disorder, exhibit alterations in amygdala-based circuits. These circuits are involved in key cognitive processes, i.e., emotion regulation, threat detection, interoception, and self-monitoring, all implicated in adult anxiety. Thus, the present findings provide important leads towards formulating pathophysiological models of the development of anxiety disorders.

Disclosure: A. Roy, Nothing to Disclose.

46.4 From Correlation to Causation in Resting-state fMRI: Network Dynamics in Psychopathology and with Concurrent TMS/fMRI

Amit Etkin*

Stanford University, Stanford, California

Background: Anxiety and mood disorders are a diverse group of clinical states, characterized by dysfunction in both cognitive and emotional domains. Prior work has implicated abnormalities in a number of brain regions, as well as in large-scale distributed neural networks. Despite an extensive neuroimaging literature in this domain, several key questions remain largely unknown. Specifically, 1) how do neural abnormalities map onto common and disorder-specific clinical presentations, and 2) how can imaging findings, which are only correlative by nature, be translated into a causal neurobiological understanding of psychopathology. Post-traumatic stress disorder (PTSD) and generalized anxiety disorder (GAD), for example, along with the closely-related major depressive disorder (MDD), share elevated anxiety and depressive symptoms, but differ with respect to fear-relevant memory dysregulation. Work in experimental animals and healthy humans has identified distinct roles for the rodent dorsal (human posterior) hippocampus in memory, including emotional memory, through its connectivity with memory circuits such as the default-mode network in humans. By contrast, the rodent ventral hippocampus has been implicated in anxiety through connectivity with limbic-prefrontal circuits. We therefore examined hippocampal sub-region-specific network function in these three disorders using a translational framework, in order to map neural circuit abnormalities on aspects of common vs. disorder-specific clinical presentations. Additionally, we probed causal neural circuit manipulations by non-invasive brain stimulation with transcranial magnetic stimulation (TMS) while neuroimaging concurrently with fMRI in a separate group of healthy subjects, in order to understand the causal regulatory mechanisms driving activity within the default-mode network.

Methods: Network-sensitive functional MRI-based resting-state intrinsic connectivity methods, along with task-based assessment of posterior hippocampal/default-mode network function, were used in cohorts of outpatients with PTSD, GAD, or MDD, and two cohorts of healthy subjects. Separately, concurrent TMS/fMRI was performed on a cohort of healthy subjects targeting two sites in the lateral prefrontal cortex that correspond to different nodes within

the "task-positive network" – a set of regions that are generically activated in attention-demanding tasks, mediating salience monitoring and executive/attentional control. TMS was done to both activate and inhibit these network nodes.
Results: Posterior/anterior hippocampal connectivity was dissociable and consistent with predictions from animal work. Posterior hippocampal/default-mode network function, across resting-state and task measures, was found to be selectively perturbed in PTSD relative to each of the other groups. Anterior hippocampal connectivity, by contrast, was blunted similarly across all patient groups. In the TMS/fMRI experiment, we found that the default-mode network is under demonstrable causal control by the task-positive network, and in particular a node in the posterior middle frontal.
Conclusions: These findings provide new insights into the neural circuit-level dysfunctions that account for similar and different features of major anxiety and mood disorders, through a translational framework built on animal work and carefully-selected clinical disorders. In doing so, our results also help translate animal studies to the diverse range of human disorders. Moreover, our data provide the first evidence for a causal regulatory interaction between large-scale neural networks in the human brain. Together, these findings further a causal neural circuit-level understanding of anxiety and mood-related psychopathology.
Disclosure: A. Etkin, **Part 1:** Grant from Brain Resource, Inc. for taking part in the International Study to Predict Optimzed Treatment in Depression (iSPOT-D).

Panel
47. Harnessing Cortical Plasticity for Therapeutic Purposes

47.1 Shaping Brain Circuits with Auditory Experience

Etienne de Villers-Sidani*

McGill University, Montreal, Quebec, Canada

Background: The rodent auditory cortex has provided a particularly useful model for studying cortical plasticity phenomenology and mechanisms, both in infant and in adult animal models. Much of our initial understanding of the neurological processes underlying learning-induced changes in the cortex stems from the early exploitation of this model. More recent studies have provided a rich and elaborate demonstration of the "rules" governing representational plasticity induced during the critical period, and in the longer post-critical-period "adult" plasticity epoch. These studies have also contributed importantly to the application of these "rules" to the development of practical training tools designed to improve the functional capacities of the auditory, language and reading capacities of both children with developmental impairments, and adults with acquired impairments in the auditory/aural speed and related cognitive domains. In this presentation, I will first present recent data obtained in the rat primary auditory cortex (A1) that provides further insight into the role of sensory experience in shaping functional and structural aspects of adult auditory networks. I will then discuss the results of an ongoing parallel rodent-human study aimed at examining how neuroplasticity-based perceptual training strategies can recover auditory cortical impairments commonly found with natural aging.
Methods: The data that will be presented in the first portion of this presentation was obtained in rodents trained on various auditory discrimination tasks specifically designed to improve the suppression of noisy signals in auditory pathways. Another subset of rats were chronically exposed to different types of noisy stimuli with the aim of reducing signal-to-noise ratio in the auditory cortex. The impact of these experimental manipulations on functional and structural aspects of auditory circuits was then examined using awake or anesthetized intra-cortical recordings, immuno-histochemistry and behavior. An almost identical version of one of the training strategies used was also applied to human subjects from which scalp auditory related potentials were recorded.
Results: 1. Abnormal processing of "oddball sounds" or sounds that slightly deviate from an auditory background is a common feature of human aging and many neuropsychiatric disorders including schizophrenia. The same phenomenon occurs in aged rats. We have found that the inability of aged A1 neurons to faithfully extract oddball sounds relates to their inability to dynamically suppress distracting background sounds. Furthermore, this impairment seems to be contributed to by a dysfunction of a specific subclass of cortical inhibitory inter-neurons, the parvalbumin positive cell.
2. The presence of chronically degraded (noisy) signals in auditory circuits seems sufficient to cause several of the auditory processing documented in the aged.
3. Intensive training on a specifically designed auditory oddball discrimination type task can reverse several functional and structural age-related impairments observed in the aged cortex. That particular task however failed to improve active suppression of background distractors.
4. A newly designed auditory training strategy engaging more strongly "top-down" modulation in A1 was successful at improving auditory distractor suppression. It had however a more restricted impact on other age-related impairments. Aged human subjects trained on an identical task also showed similar improvements in auditory processing.
Conclusions: Key central auditory cortical impairments can be effectively reversed using specifically designed perceptual training strategies, which have a significant impact on several functional and molecular aspects of cortical networks. A1 changes similar to those seen in natural aging can be obtained by chronically introducing noise in auditory circuits. This supports the notion that these changes might not all be due to irreversible degenerative processes but might rather represent progressive "negative" plastic adjustments caused by long-standing alterations in sensory signal characteristics or intrinsic cortical dynamics. Using animal models in conjunction with human studies can accelerate the development of effective neuroplasticity-based training strategies by allowing the precise characterization of the "rules" that govern cortical network plasticity. Future directions should include more studies examining how training strategies could be combined together or with other types of interventions known to induce brain plasticity (i.e. neuromodulator manipulations, magnetic stimulation, etc) to augment their effect.
Disclosure: E. de Villers-Sidani, Nothing to Disclose.

47.2 Directing Cortical Plasticity to Understand and Treat Neurological Disease

Michael P. Kilgard*

University of Texas, Richardson, Texas

Background: A large body of evidence suggests that neural plasticity contributes to learning and to disease. This talk will review a new perspective on neural plasticity and how plasticity might be targeted to reset dysfunctional circuits in patients.
Methods: Neural recordings in humans and animals will be discussed. Neural plasticity is generated by a number of techniques, including behavioral training, deep brain stimulation and vagus nerve stimulation (VNS).
Results: Recent studies suggest that map plasticity is typically a transient phase that improves learning by increasing the pool of task relevant responses. A new model is proposed in which map expansion provides a form of replication with variation that supports a Darwinian mechanism to select the most behaviorally useful circuits.
Conclusions: Precisely targeted neural plasticity provides a new avenue for the treatment of neurological and psychiatric disease and a powerful tool to test the neural mechanisms of learning and memory.
Disclosure: M. Kilgard, **Part 1:** I am a consultant for and have a financial interest in MicroTransponder, Inc., **Part 2:** I am a consultant for MicroTransponder, Inc., **Part 3:** I am a consultant for MicroTransponder, Inc., **Part 4:** I have a sub-contract from MicroTransponder, Inc. to conduct research that is supported in part by the NIH.

47.3 Long-lasting Enhancement of Visual Perceptual Learning in Healthy Humans by the Cholinesterase Inhibitor Donepezil
Michael A. Silver*

University of California, Berkeley, California

Background: The neurotransmitter acetylcholine (ACh) has been shown to play a critical role in cognitive processes such as attention and learning. Previous research in animal models has shown that plasticity in sensory systems often depends on the task relevance of the stimulus, but experimentally increasing ACh release in cortex can replace task relevance in inducing experience-dependent plasticity. Perceptual learning (PL) is a specific and persistent improvement in performance of a perceptual task with training. To test the role of ACh in PL of visual discrimination, we pharmacologically enhanced cholinergic transmission in the brains of healthy human participants by administering the cholinesterase inhibitor donepezil (trade name: Aricept), a commonly prescribed treatment for Alzheimer's disease.
Methods: We conducted a double-blind placebo-controlled crossover study, in which each subject completed one course of training under donepezil and one course of training under placebo. The training task was discrimination of the direction of motion of visual stimuli, and motion direction discrimination thresholds were obtained for each of eight directions of motion and two visual field locations before and after each course of training. Only one direction of motion and visual field location was used during each course of training.
Results: Relative to placebo, donepezil doubled the improvement in perceptual performance following PL. The specificity of the learning for the trained direction of motion and visual field location was also enhanced by donepezil. Finally, a subset of subjects was tested 5-15 months following the end of training and drug administration. These subjects still exhibited the improvements in perceptual performance associated with training under donepezil even though they had not practiced the task or taken donepezil for several months before testing.
Conclusions: We found that five days of perceptual training under donepezil produced an improvement in perceptual performance that persisted for at least several months and was significantly greater than the improvement resulting from training under placebo. This long-lasting facilitation of PL by donepezil is relevant for clinical disorders for which perceptual learning is used as a treatment, including amblyopia and dyslexia.
Disclosure: M. Silver, Nothing to Disclose.

47.4 Noninvasive Brain Stimulation to Enhance the Effects of Computerized Cognitive Training
Alvaro Pascual-Leone*

Harvard Medical School, Boston, Massachusetts

Background: Computerized training can improve cognitive function and ameliorate symptoms in patients with a variety of neuropsychiatric disorders. However, these benefits are variable across patients, can be relatively small in some instances, require prolonged treatment sessions, and demand long courses of daily sessions often followed by a need for longer-term maintenance. Noninvasive brain stimulation with transcranial magnetic stimulation (TMS) or transcranial direct current stimulation (tDCS) can modulate activity in specific brain circuits, promote changes in brain plasticity, and induce cognitive enhancement and behavioral changes. TMS and tDCS might be combined with computerized cognitive training to make it more effective.
Methods: TMS and tDCS are electromagnetic methods to noninvasive induce current in the human brain and probe or modulate cortical reactivity. Integrated with neuroimaging and electroencephalography, TMS and tDCS can be used to characterize brain circuit dynamics and cortical plasticity in health and disease. Applied to specific brain circuits TMS and tDCS can prime brain structures, making them more amenable to modulation by computerized cognitive training.
Results: A number of studies to date have combined diverse protocols of TMS and tDCS with various cognitive training paradigms across diverse cognitive domains and various neuropsychiatric disorders. These studies provide proof-of-principle evidence of the potential of such approaches.
Conclusions: Further work is needed, but TMS- and particularly tDCS-induced modulation of specific brain circuits appears to be a promising strategy to maximize the benefit of computerized cognitive training.
Disclosure: A. Pascual-Leone, **Part 1:** Neosync - Member of Scientific Advisory Board Starlab - Member of Scientific Board Neuronix - Member of Medical and Scientific Advisory Board Johnson & Johnson, Codman - Member of Medical Advisory Board on Neurological and Neurosurgical Technology Nexstim - Advisory Board Member Novavision - Chair, Advisory Board Member Institut Guttman, Spain - Member, Board of Scientific Advisors, **Part 4:** Investigator initiated grants from Neuronix, Nexstim, Neuronetics.

Panel
48. Balancing Benefits, Risks and Cost for New Treatments in Vulnerable Populations: Lessons from Child Psychiatry on the Need for a New Standard of Diverse Methodologies

48.1 One Year Follow-up Longitudinal Study with a Large Sample of Antipsychotic-naïve Children and Adolescents
Celso Arango*

CIBERSAM (Spanish Mental Health Network), Madrid, Spain

Background: In recent years, the use of antipsychotics in children and adolescents has been multiplied by 6 in the US and has doubled in Europe. The use of antipsychotics is mainly off-label in non psychotic conditions and the treatments have been extended to longer periods of time. However, evidence of long term safety of antipsychotics in children and adolescents is scarce, as most studies have follow-up shorter than 12 weeks.
Methods: Unpublished data from a 1-year, non-randomized, cohort study assessing weight, metabolic and hormonal changes as well as the incidence and moderators of extrapyramidal side effects, tardive dyskinesia and global cognition in antipsychotic naïve (45%) and quasi-naïve (55%) children and adolescents will be presented. 266 children and adolescents (mean age 14.40 years, SD 2.9) with different DSM-IV axis I disorder and treated with different second generation antipsychotics were recruited in this naturalistic study. We will also present data from a 6 months follow-up study just finished that included 555 drug naive adults and youth that directly compare risks across these age groups. We have collected DNA from all subjects and will present the predictive value for weight gain for a number of candidate genes.
Results: Unpublished data from a 1-year, non-randomized, cohort study assessing weight, metabolic and hormonal changes as well as the incidence and moderators of extrapyramidal side effects, tardive dyskinesia and global cognition in drug naïve (45%) and quasi-naïve (55%) children and adolescents will be presented. 266 children and adolescents (mean age 14.40 years, SD 2.9) with different DSM-IV axis I disorder were recruited in this naturalistic study. Total Dyskinesia Score ($<.001$) and Total Parkinson Score ($<.001$) increased after follow-up. When comparing the most prescribed antipsychotics, treatment with risperidone was associated with higher increases in Total Dyskinesia Score compared to quetiapine ($<.001$) and higher increase in Total Parkinson compared to olanzapine (.003) and quetiapine ($<.001$). UKU Total Cognitive Score decreased after follow-up with no differences detected among antipsychotics. Presenting psychotic symptoms and length of time of exposure were associated with the presence of tardive dyskinesia at follow-up. In this same study baseline BMI z score, adiponectin level and waist circumference predicted weight gain at 6 months. Weight gain significantly increased between the different

visits (baseline, 1, 3, 6 and 12 months) in olanzapine treated patients, but not in patients treated with other antipsychotics (e.g. quetiapine) in whcih all the weight gain occurred during the first 3 months of treatment. Preliminary data from the latest study supports that pediatric patients are more vulnerable to weight gain and some of the metabolic changes associated.
Conclusions: Second generation antipsychotics increased weight gain, altered metabolic and hormonal parameters and produced neurological side-effects in a naïve and quasinaíve paediatric population and therefore should be carefully monitored. The trajectory of this side effect is different for the different antipsychotics during the first year of treatment. These results are clinically relevant as they may inform on when to expect a "plateau" in the side effects. We will also present predictors of side effects in this population based on our unpublished studies.
Disclosure: C. Arango, **Part 1:** Dr. Arango has been a consultant to or has received honoraria or grants from Astra Zeneca, Bristol-Myers Squibb, Janssen Cilag, Lundbeck, Merck, Otsuka, Pfizer, Roche, Servier and Schering Plough, **Part 4:** Dr. Arango has received grants from Roche and Lundbeck.

48.2 The Value of Randomized Clinical Trials: Data from the Metabolic Effects of Antipsychotics in Children (MEAC) Study

Ginger E. Nicol*

Washington University, Saint Louis, Missouri

Background: Rates of antipsychotic prescription in children have increased, largely driven by off-label use for disruptive behavior. Antipsychotics have well characterized effects on the development of obesity and cardiometabolic risks in adults, but this aspect of safety and tolerability has been less well studied in treatment naïve individuals, in particular for off-label uses such as the treatment of disruptive behavior in children, where non-randomized trials have shown clinical efficacy. The randomized, NIMH-funded Metabolic Effects of Antipsychotics in Children study (MEAC, PI Newcomer, MH072912) characterized the metabolic effects of 12 weeks of antipsychotic treatment in a population where antipsychotics are commonly used to treat disruptive behavior across a range of diagnoses, using gold-standard metabolic techniques.
Methods: Antipsychotic-naive youth ages 6-18 with clinically significant aggression/irritability in the setting of one or more DSM-IV diagnoses indicating a disruptive behavior disorder were randomized to 12 weeks of treatment with aripiprazole, olanzapine or risperidone. Baseline and 12 week measures included body composition analysis with Dual Energy X-ray Absorptiometry (DEXA), a single stage hyperinsulinemic-euglycemic glucose clamp using stable isotopomer tracing, anthropomorphic assessment and plasma measures. Primary endpoints were change in whole body and abdominal adiposity, and whole-body and tissue-specific insulin sensitivity. ANCOVA was used to test effects of time and treatment condition on adiposity and insulin sensitivity. Regression analyses were performed to test the predictive effect of baseline DEXA-measured total % body fat on baseline insulin-stimulated changes in glucose and lipid metabolism during treatment.
Results: MEAC participants had a baseline prevalence of overweight or obesity of 34% (13% overweight, 21% obese) that was similar to the 32% of overweight (15%) or obese (17%) youth in the general population according to 2008 NHANES data. During 12 weeks of initial antipsychotic exposure, differential effects of treatment were observed on measures of adiposity and other endpoints. Specifically, time by treatment condition effects were detected on DEXA %fat ($F[2,123] = 8.81$, $p < 0.0001$). Pooling treatment groups to test the relationship of baseline and change in adiposity to baseline and change in insulin sensitivity, respectively, the magnitude antipsychotic treatment-induced increases in adiposity over 12 weeks of treatment were associated with the magnitude of adverse changes in SI at both adipose ($F[1,95] = 4.973$, $p = 0.028$) and hepatic ($F[1,95] = 2.839$, $p = 0.095$) tissues. Importantly, treatment resulted in marked improvement in Aberrant Behavior Checklist irritability/aggression subscale scores, with a mean decrease of 16.59 points ($p < 0.0001$).
Conclusions: Adverse metabolic effects of antipsychotic are rapidly detectable within 12 weeks of treatment, but importantly occur within the context of significant clinical benefit for disruptive behavior. Randomized clinical trials like the MEAC study, which incorporate adaptive or practical design elements to enhance generalizability to real-world prescribing practices, can be valuable in understanding safety and tolerability issues, developing strategies for effective risk mitigation, and in assessment of costs and benefits of treatment.tial risks and benefits during use of antipsychotic treatment in pediatric populations.
Disclosure: G. Nicol, **Part 1:** Dr. Nicol has received research funding from the National Institute of Mental Health (NIMH), NARSAD, the Dana Brown Charitable Trust Foundation, the Sidney R. Baer, Jr. Foundation, and the CHADS Coalition for Mental Health. She also receives grant support from Pfizer, Inc. for an investigator-initiated clinical trial. She receives royalties from Jones & Barlett Learning for development of a pediatric metabolic monitoring form and has consulted to MedScape. She does not participate in speakers' bureaus, **Part 4:** Investigator initiated clinical trial grant support: Pfizer, Inc.

48.3 Mixed Methods in Health Services Research to Understand Real-world Acceptance of Safety Recommendations for Pscyhopharmacologic Treatments

Elaine H. Morrato*

University of Colorado, Aurora, Colorado

Background: Health system stakeholders want to understand the adoption of behaviors intended to mitigate drug safety risks in the real-world clinical setting. RCTs are controlled experiments and do not reflect real-world use patterns. Post-marketing pharmacoepidemiology studies can estimate adoption rates but do not provide insight into why a prescriber may or may not adopt certain risk mitigation behaviors. Identifying prescriber opinions on patient, practice, and system barriers allows stakeholders to target quality improvement strategies for maximum efficiency and effectiveness. This presentation will present new data concerning the adoption of metabolic monitoring practices with the use of second-generation antipsychotics to illustrate the utility of using mixed methods (survey plus medical records) to identify barriers to screening.
Methods: A survey of antipsychotic prescribers was conducted (November 2011 – January 2012) in 24 Community Mental Health Centers (CMHCs) in Missouri to evaluate metabolic monitoring attitudes and behaviors and to identify barriers to screening. An 84% response rate was achieved ($N = 170$ completed surveys). The Missouri Department of Mental Health tracks metabolic monitoring rates clinically at each CMHC via data prospectively collected using an electronic clinical tracking form ($N = 20{,}887$ clients with an antipsychotic claim in 2010). This panel presentation will discuss the survey findings and then compare self-reported (survey) with observed (clinical tracking form) screening rates in order to estimate the gap between intentions to screen and obtaining screening results.
Results: Most survey respondents (59%) treated adult patients only; 35%, all ages; and 6%, children only. Baseline screening for adult patients starting SGA medication was reported as: 80% would definitely measure weight; 67%, blood pressure; 57% glucose; and 57%, lipids. Relative to baseline rates, annual follow-up rates for measuring weight and blood pressure were similar; rates of glucose and lipid lab ordering were higher (78%). Respondents treating children reported similar rates of metabolic screening and lab ordering. The top barriers to screening were "patients forget to get fasting blood work" and "patients cannot get their blood drawn at my office/clinic". Based on data in the clinical tracking form, 99% of patients receiving an antipsychotic had their vitals (weight and blood pressure) assessed at least once

in the year; 95% had record of a glucose test; 43%, a record of a lipid test. The presentation will present analysis comparing testing rates across CMHCs and barriers unique to centers with lower rates of lipid testing.
Conclusions: This study illustrates the utility of using surveys with clinical records to understand risk management behaviors in real-world clinical practice. The case example of metabolic monitoring with SGA treatment shows how mixed methods were used to de-couple prescriber intended behavior from whether or not the patient received the monitoring and to identify which barriers accounted for differences between the two. This provides value-added information to stakeholders designing interventions to improve drug treatment in clinical practice.
Disclosure: E. Morrato, **Part 1:** I have received research grant funding through my university from Janssen Pharmaceuticals, Inc., **Part 4:** I have received research grant funding through my university from Janssen Pharmaceuticals, Inc (2012).

48.4 Economic Evaluation in Child Psychiatry—An Example from the Metabolic Effects in Antipsychotic Treatment of Children Study

Steven M. Kymes*

Washington University School of Medicine, St. Louis, Missouri

Background: Economic evaluation is the science of rigorously measuring the balance between the cost of an intervention and its benefits. While the fungibility of currency leads us to most often describe "costs" in monetary units, there is no requirement that costs be operationalized as such and can be characterized in any manner that represents a loss of economic well being from the perspective of policy makers—including adverse outcomes. The most common method of economic evaluation, decision analytic modeling, does not provide the same level of evidence as epidemiological studies, but it provides investigators and policymakers a tool by which to test the feasibility of hypotheses and identify key events or parameters which would influence the decision to adopt the new health program. We will present a cost-effectiveness study from the Metabolic Effects of Antipsychotics in Children (MEAC) study in which we describe the incremental cost-effectiveness of treatment of behavioral disorders in both the monetary cost and weight gained. Such information is important to payors, patients, and providers making informed treatment decisions.
Methods: We constructed a decision analytic model using data from the MEAC, which included a subset of the population ($n = 87$) for whom we had data on clinical effectiveness and school suspensions during 12 weeks of initial antipsychotic treatment with aripiprazole, risperidone or olanzapine. The efficacy to treatment was considered in terms of clinical benefits; adverse outcomes were considered in terms of becoming obese (BMI percentile > 95 for age and gender). Monetary costs of treatment were obtained from Missouri Medicaid. A Monte Carlo microsimulation model was created, running 1,000 hypothetical patients through 4 cycles of 12-week data from the to simulate 1 year of antipsychotic treatment. The MEAC reported no important differences in primary outcome; however there are important differences in adverse outcomes (weight gain) and acquisition cost. We report effectiveness as: 1) School suspension avoided and 2) reduction in ABC score. We report the incremental cost-effectiveness ratio (ICER) as dollar cost and pounds per outcome avoided (or gained).
Results: See Table for details. For the subset of the MEAC population that had data on school suspensions, olanzapine and risperidone were more effective in preventing of school suspensions. However, risperidone was the least costly treatment and thus dominated the other two treatments. In modeling data from this subset of the MEAC, we found a very small difference in ABC score (0.82 points) favoring aripiprazole over risperadine, however the higher cost of aripiprazole gives this an ICER of $5,323/point gained. Aripiprazole had the least weight gain. Compared to aripiprazole, risperidone had the fewest school suspensions, with each additional suspension avoided costing 11 lbs gained per year. Aripiprazole dominated the other two treatments on ABC score where cost is considered in lbs.
Conclusions: We have demonstrated how economic evaluation might be used to evaluate the influence of multiple outcomes and costs on treatment decisions.
Disclosure: S. Kymes, **Part 1:** I am a consultant and receive (or have received) research funding for Pfizer, Bayer and Genentech, **Part 2:** Consulting fees from Bayer in excess of $10,000 in 2011 and 2012, **Part 4:** As noted above, Pfizer and Genenech in 2010 and 2011.

Panel
49. Metabotropic Glutamate Receptors (mGluRs) and Addiction

49.1 Estradiol Influences Dopamine and GABA Release in the Striatum via mGluR5

Jill B. Becker*

University of Michigan, Ann Arbor, Michigan

Background: There are sex differences in drug abuse. Approximately 30% of the 1.8 million Americans who use cocaine are women, and the sex differences in patterns of cocaine use and addiction are well documented. Women begin using cocaine at an earlier age than men, after first use they take less time to become addicted, they enter treatment at a younger age than men, and when they present for treatment they have a more severe habit than men. Sex differences in cocaine self-administration behavior are also found in rodents. We have found that in females estradiol enhances stimulated dopamine (DA) detected in dialysate as well the acute behavioral response to cocaine, behavioral sensitization to cocaine, and cocaine self-administration. Female rats exhibit greater behavioral sensitization to cocaine, acquire cocaine self-administration more rapidly than males, and estradiol enhances these sex differences.
Methods: Experiments were conducted with male and female rats. Methods employed include cocaine self administration and *in vivo* microdialysis for determination of DA and GABA in the striatum. Over-expression of the estradiol receptor (ER)-alpha or control protein was performed by introduction of adenoassociated viral vector (AAV) with the cDNA of the desired protein into striatum.
Results: In microdialysis experiments, estradiol rapidly potentiated stimulated DA release in the striatum of female but not male rats. Furthermore, estradiol rapidly attenuated the K + - evoked increase of GABA in dialysate. We hypothesized that these rapid effects of estradiol in the striatum are mediated by ER-alpha located on the membrane of medium spiny GABAergic neurons. We subsequently found that overexpression of ER-alpha in the striatum using an AAV significantly enhanced the effect of estradiol on GABA release, suggesting the rapid effects of estradiol in striatum are mediated, at least in part, by ER-alpha. In recent unpublished studies to be presented we have shown that an mGluR5 receptor antagonist inhibits the effect of estradiol to enhance dopamine release and inhibit the release of GABA. These effects of the mGluR5 antagonist to prevent the effect of estradiol are found when applied systemically or directly into the striatum.
Conclusions: We conclude that effects of estradiol on drug taking behavior are likely to be modulated by mGluR5. Any pharmacotherapies for drug addiction based on glutamatergic agents need to take into consideration sex differences and hormonal influences on metabotropic glutamate receptors.
Disclosure: J. Becker, Nothing to Disclose.

49.2 Estrogen Receptors Located at the Surface Membrane Activate Metabotropic Glutamate Receptor Signaling

Paul G. Mermelstein*

University of Minnesota, Minneapolis, Minnesota

Background: In comparison to men, women are at an increased risk to abuse drugs. Across the spectrum of addiction, women show heightened intake of addictive substances, with greater

craving, leading to an increased likelihood of addiction and relapse. These responses peak during the follicularphase of the menstrual cycle when estrogen levels are at their highest. These findings have been recapitulated in the female laboratory rat, where estradiol heightens multiple measures of drug responsiveness and abuse. Remarkably, the mechanisms by which estradiol mediates enhanced vulnerability to drug addiction are completely unknown. We propose a novel molecular mechanism mediating the actions of estradiol on nucleus accumbens neurons. Specifically we find that estradiol stimulation of estrogen receptor α (ERα) localized to the surface membrane of nucleus accumbens neurons activates metabotropic glutamate receptor 5 (mGluR5) signaling.
Methods: Intact and cultured nucleus accumbens neurons from male and female ratsAnatomical Spine analysis via neuronal labeling.
Measurement of transcription factor activation.
Western Blot.
Results: Activation of ERα/mGluR5 signaling by estradiol has been found to affect activity dependent gene expression and nucleus accumbens spine structure only in neurons from females.
Conclusions: ER/mGluR signaling in females may account for women being at greater risk for addiction then men. Collectively, this work will ultimately provide a foundation for developing novel therapeutic approaches targeted to treating drug addiction in women.
Disclosure: P. Mermelstein, Nothing to Disclose.

49.3 Unique Roles for Ventral and Dorsal Striatum mGluR5 in Extinction Learning and Relapse to Cocaine Seeking

Lori A. Knackstedt*

Medical University of South Carolina, Charleston, South Carolina

Background: Surface expression of mGluR5 is decreased in the nucleus accumbens (NA) core following cocaine self-administration only in animals which underwent extinction training and not abstinence without extinction. Homer proteins regulate mGluR5 intracellular signaling and trafficking to the membrane. The expression of Homer 1b/c is increased in the NA core by cocaine self-administration only when animals undergo extinction training. Intra-accumbens infusion of a peptide designed to interfere with mGluR5-Homer interactions attenuates cue- and cocaine-primed reinstatement. Here we investigated the ability of mGluR5 antagonism in the ventral and dorsal striatum to attenuate cocaine relapse following both extinction and abstinence.
Methods: Animals underwent 2 weeks of cocaine self-administration followed by abstinence without extinction training for 2-3 weeks. Animals were reintroduced to the operant chamber for a context-induced relapse test which also served as Day 1 of extinction training. Extinction training continued until lever pressing was reduced to 25% of self-administration levels, at which time cue-primed reinstatement tests were conducted. Context- and cue-induced relapse tests were conducted in the presence of the specific mGluR5 antagonist MTEP or vehicle infused into either the NA core or dorsal striatum (dSTR). A subset of animals were not tested for relapse and were used for electrophysiological and western blotting studies.
Results: MTEP infused into the NA core attenuated reinstatement following extinction training but had no effect on context-induced drug-seeking after abstinence. Antagonizing dSTR mGluR5 had no effect on cocaine-seeking following either extinction training or abstinence. Intra-dSTR MTEP infused only on Day 1 of extinction prevented the reduction in lever-pressing seen in vehicle-infused animals on Day 2 and 3 of extinction. In contrast, intra-dSTR MTEP did not affect extinction learning in food self-administering animals.
Conclusions: Taken together, this data indicates that modulating the activity and surface expression of mGluR5 in the NA core attenuates cocaine relapse after extinction training but not abstinence without extinction. Dorsal striatum mGluR5 plays a role in extinction learning but not cocaine relapse.
Disclosure: L. Knackstedt, Nothing to Disclose.

49.4 Restoration of Infralimbic mGluR2 Deficit Rescues Control over Drug Seeking in Alcohol Dependence

Wolfgang H. Sommer*

Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany

Background: Emerging evidence from both animal models and human neuroimaging studies point to a critical role of the medial prefrontal cortex (mPFC) in the control of addictive behaviours. The underlying neurobiology of this altered mPFC function is poorly understood, and in particular for alcohol addiction data are lacking.
Methods: We applied five independent methods of profiling gene expression and functionality of the mPFC in alcohol dependent rats after period of protracted abstinence vs. non-dependent controls. Furthermore, mGluR2 transcript levels were compared in human postmortem samples from anterior cingulate cortex samples alcohol dependent vs. control subjects.
Results: Microarray-based transcriptome analysis pointed to mPFC projection neurons as a major site of neuroadaptations in alcohol dependence, which by *in-situ* hybridization with selected candidate genes was further narrowed down to the infralimbic cortex as the most affected target region. Laser capture microscopy-aided microdissection of infralimbic projection neurons followed by quantitative RT-PCR identified a pronounced downrgulation of metabotropic glutamate receptor subtype 2 (mGluR2) associated with a blunted response to mGluR2/3 agonist treatment at the terminals of the infralimbic projections in the nucleus accumbens shell. On a behavioral level we demonstrate that restoring mGluR2 in this pathway by viral gene transfer into the IL restores control over alcohol-seeking behavior in the reinstatement test. In human postmortem brains we found a reduction in mGluR2 expression in the anterior cingulate cortex of alcoholics.
Conclusions: Together these data identify an anatomically and cell type specific blockade in mGluR2 expression as a major sequel to alcohol dependence and a key pathophysiological mechanism for the increased propensity to relapse during abstinence. The translational value of these findings is at least in part supported by comparable findings in human postmortem brain samples.
Disclosure: W. Sommer, Nothing to Disclose.

Panel
50. Affective Neuroscience of Young Monkeys to Developing Population: Translational Studies of Brain Function Informing Interventions

50.1 Identification of Novel Targets in the Developing Primate Amygdala for the Early Treatment of Childhood Anxiety

Ned H. Kalin*

University of Wisconsin School of Medicine and Public Healtlh, Madison, Wisconsin

Background: We have characterized a phenotype in young rhesus monkeys that models early anxious temperament (AT) in children. Since AT is an early risk for the later development of anxiety and depressive disorders, the primate model allows us to explore new avenues for early treatment and possible prevention of major psychiatric illnesses.
Methods: Using functional imaging, we identified the central nucleus of the amygdala (CeA) as critical component of the AT circuit. Based on this, we performed transcriptome wide analyses of CeA tissue in monkeys phenotyped for AT and brain metabolic activity. Microarray and deep RNA sequencing strategies were used to identify transcripts predictive of AT and increased CeA metabolic activity.
Results: In addition to some systems traditionally identified with stress and anxiety, results have identified alterations in the expression of novel genes in the young primate CeA that are

associated with increased dispositional anxiety and increased CeA metabolism. Of particular interest is the finding that high AT individuals have decreased CeA expression of a number of neuroplasticity/neurotrophic genes. Also, the altered expression of these genes appears to be regionally specific.
Conclusions: Using a valid, well characterized, nonhuman primate model of anxious temperament, we have identified alterations in CeA neuroplasticity systems that may underlie AT and provide new targets for early intervention strategies. These findings in the developing primate support a new neurodevelopmental hypothesis to explain the early expression and maintenance of AT as a significant risk for the later development of anxiety and depressive disorders. Moreover, the findings suggest that early focused interventions aimed at enhancing the activity of CeA neuroplasticity processes.
Disclosure: N. Kalin, **Part 1:** Neuronetics CeNeRx Bio Pharma Corcept Therapeutics CME Outfitters Elsevier, **Part 2:** Elsevier, **Part 4:** APIRE/Janssen Resident Psychiatric Mentor Grant National Institute of Mental Health The Stanley Medical Research Institute.

50.2 Translational Imaging Studies of Natural Products as Treatments for Pediatric Depression

Perry Renshaw*

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

Background: Pharmacological treatment for pediatric depression remains a critical need: available drugs are ineffective for many patients, and have unacceptable side effects. We use multinuclear magnetic resonance spectroscopy (MRS) to: 1) identify rational neurochemical treatment targets; 2) match them with natural products to augment standard treatment; and 3) measure changes in brain chemistry associated with augmentation. Data is presented on pilot studies of creatine monohydrate (creatine) for adolescent females with SSRI-resistant major depressive disorder (MDD) (N = 10), and uridine treatment for depressed adolescents with bipolar disorder (BD) (N = 24). MRS studies of adult MDD show alterations in cerebral bioenergetics: decreased beta-nucleoside triphosphate (b-NTP; primarily adenosine triphosphate, or ATP) and increased phosphocreatine (PCr) (Moore et al, 1997; Iosifescu et al, 2008; Forester et al, 2009). Creatine supplementation may be capable of modifying brain high-energy phosphate metabolism (Lyoo et al, 2003). Female, but not male, rats fed a creatine-enriched diet display antidepressant-like behavior on the Porsolt Forced Swim Test (Allen et al, 2010); this effect is independent of SSRI administration (Allen et al, in press). Compared with placebo, adult females (N = 54) with MDD show an earlier response, and a higher remission rate, when adjunctive creatine 5 g daily is added to an SSRI (Yoon et al, in review). MRS studies implicate mitochondrial dysfunction in BD (Stork and Renshaw, 2005). As noted by Manji and others, this view is supported by evidence from multiple disciplines (Quiroz et al, 2008). MRS findings in adult BD include increased gray matter glutamate (Dager et al, 2004), while patients responding to lithium show decreased glutamate (Friedman et al, 2004). One class of compounds with potential to impact both mitochondrial and glutamatergic function is the pyrimidines, including triacetyluridine (TAU), cytidine and uridine. Adults with BD augmented with TAU show increased pH (Jensen et al, 2008), while adjunctive cytidine reduces cerebral glutamate (Yoon et al, 2009).
Methods: Standard clinical trial designs were used in conjunction with phosphorus-31 MRS assessments.
Results: In adolescent girls with SSRI-resistant MDD, we are testing adjunctive creatine 4 g daily. Eight weeks of creatine is associated with a decrease in mean Children's Depression Rating Scale-Revised (CDRS-R) score from 69.0 (SD = 9.6) to 30.6 (SD = 8.5). Repeated MRS scans showed increased frontal lobe PCr ($p = 0.02$). Baseline CDRS-R score was positively correlated with pH ($p = 0.04$), and negatively correlated with b-NTP ($p = 0.03$). We are piloting uridine as a treatment for adolescent BD depression. Following six weeks of uridine 500 mg twice daily, patients' mean CDRS-R scores decreased from 63.6 (SD = 6.1) to 32.3 (SD = 6.3). Compared with healthy controls, uridine-treated adolescents showed alterations in PCr ($p = 0.01$) and β-NTP ($p = 0.007$).
Conclusions: In adolescent depression, translational MRS imaging results have utility in the identification of hypothesis-driven natural treatments, and as secondary outcome measures in clinical trials.
Disclosure: P. Renshaw, **Part 1:** I am a Consultant and stockholder for Ridge Diagnostics, I am a Consultant for Kyowa Hakko Kirin. I have received royalties on a patent describing the use of uridine to treat bipolar depression from Repligen.

50.3 Brain Functional Mechanisms of Treating Pediatric Mania

Mani Pavuluri*

University of Illinois, Chicago, Illinois

Background: To present the treatment mechanisms impacting the interplay of affective and cognitive circuitry changes with recovery in pediatric mania.
Methods: Conventional fMRI task-based activity studies coupled with hemodynamic response function analyses and network connectivity analyses both at rest and during an emotional face processing task, a stop signal task, and an affective N back memory task are examined in pediatric mania relative to healthy controls, before and after treatment with lithium; and contrasted with outcomes on antipsychotic (risperidone) and antiepileptic (divalproex and lamotrigine) medications (Total N = 150; Age = 10 -18 years). All subjects completed neurocognitive lab tasks corresponding to the fMRI probes and the clinical measures of mood and cognition.
Results: Regardless of the type of medications, there are common patterns that emerged in recovery from pediatric mania. Patients show overactivity during negative emotional processing in the insula and -parahippocampal-amygdalar complex. Impulse control is improved by increased activity in the circuitry between and within the ventrolateral prefrontal cortex (VLPFC) and striatum. Greater amygdala engagement correlated with better mood stability and executive function. Normalization of the PFC preceded the reduced amygdala activity during the course of successful treatment. Greater amygdala activity at baseline predicted worse outcome, and greater PFC activity predicted better outcome. Negative emotional stimuli are better able to probe the brain signatures of affective instability and their reversal than positive stimuli. However, each medication has a specific differential impact on brain function in mania: Lithium led to changes in perigenual activity and increased medial and dorsolateral PFC (DLPFC) activity during emotion processing. In contrast, the antiepileptics divalproex and lamotrigine led to increased VLPFC-DLPFC-temporal activity; and the antipsychotic risperidone led to increased subgenual activity and better engagement of insula.
Conclusions: The neural signatures of affective and cognitive systems in mania altered with various medications, influencing the mechanistic brain function underlying these patients' reactivity to negative emotions and enhancing cognitive potential.
Disclosure: M. Pavuluri, **Part 1:** AstraZeneca speaker, **Part 2:** Abbott Laboratories and Jannsen Pharmaceuticals supplied placebo and active drug for NIH Funded Divalproex vs. Risperdal Double-Blind Placebo Controlled Trial.

50.4 Amygdala Activation and Prefrontal Cortex Functional Connectivity: Potential Targets for Treatment of Autism Spectrum Disorders

Christopher S. Monk*

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Background: Amygdala habituation, the initial amygdala response and subsequent decrease in responsiveness to the repeated presentation of stimuli, is an essential process of the nervous

system. Habituation is important for maintaining adaptive levels of arousal to predictable social stimuli and reduced habituation is associated with heightened anxiety. Input from ventral areas of the prefrontal cortex, including the subgenual region, regulates amygdala activity. Prior work demonstrated abnormal amygdala function in youth with autism spectrum disorders (ASD). However, amygdala habituation has not been examined in a pediatric sample of ASD. Moreover, it is unknown how habituation relates to amygdala-prefrontal cortex connectivity in ASD.
Methods: Functional MRI (fMRI) data were acquired from 32 children and adolescents with ASD and 56 typically developing controls as they viewed emotional (fearful, happy, sad) and neutral faces that were briefly presented (250 ms). To monitor attention, participants pressed a button to identify the gender of each face. Habituation was tested by comparing amygdala activation to faces during the first half versus the second half of the session. Amygdala-prefrontal cortex connectivity was examined with psychophysiological interaction analyses.
Results: There was an overall interaction of group x emotion x time, $F(3, 688) = 9.90$, $p < .001$ (this and subsequent p values are reported using a correction of the bilateral amygdala region of interest). Relative to controls, youth with ASD evidenced reduced amygdala habituation to sad faces in the left amygdala, $t(86) = 3.78$, $p < .01$, right amygdala, $t(86) = 3.73$, $p < .01$, and to neutral faces in the right amygdala, $t(86) = 4.41$, $p < .01$. Moreover, reduced amygdala habituation correlated with autism severity as measured by the Social Responsiveness Scale, $t(30) = 3.31$, $p < .05$ in the ASD sample. Finally, relative to controls, the ASD group showed weaker connectivity between the amygdala and subgenual prefrontal cortex, $t(86) = 3.34$, $p < .05$ corrected for Brodmann's area 25, and connectivity predicted amygdala habituation across both groups, $r = .21$, $p < .05$.
Conclusions: Sustained amygdala activation to faces suggests that repeated social stimuli may be overly arousing for individuals with ASD, which could contribute to social impairments. Abnormal modulation of the amygdala by the subgenual prefrontal cortex may play a role in reduced habituation. These findings suggest that treatment approaches that target amygdala habituation and amygdala-subgenual prefrontal cortex connectivity may help to reduce symptoms of ASD.
Disclosure: C. Monk, Nothing to Disclose.

Panel
51. Functional and Structural Alterations in the Insula are Central to the Pathophysiology of Both Anorexia Nervosa and Obesity

51.1 The Role of the Insular Cortex in Flavor Preference Formation
Dana Small*

Yale University, New Haven, Connecticut

Background: Hedonic responses to pure tastes are innate, thuscircumventing the need to learn that sweet signifies calories or bitter toxins. By contrast, hedonic responses to flavors, which are the unitary perceptionsthat result from the integration of taste, retronasal smell and oralsomatosensation, are learned via associative processes. In this way we can learn to like the foods that are available. . Data will be presented showingthat the insular cortex plays a key role in integrating oral sensations to formunitary flavor perceptions and in associating these flavor preferences withtheir post-ingestive consequences. We will also present data showing howdietary experiences change the oral sensory – calorie associations and association learning.
Methods: Three studies were performed in which perceptualand neural responses to flavors were measured using rating scales andfunctional magnetic resonance imaging. All studies ($n = 24$; $n = 14$; $n = 24$) employed a learning paradigm in which subjects rated the pleasantness of novelflavors before and after the flavors were consumed in conjunction withmaltodextrin, which is tasteless and odorless carbohydrate that breaks downinto glucose. fMRI scanning wasperformed post-exposure while subjects sampled the flavors with no caloriesadded. Data were pre- and post-processed-with SPM8 using random effects analyses.
Results: In healthy weight subjects, in all three studies, ratedpleasantness increased significantly pre to post test ($p < .05$) for the flavorpaired with calories during exposure. In contrast, overweight/obese individualsshowed no change in rated pleasantness (group * change in pleasantness interaction $F = 7.3$; $p = .04$). Analysis of fMRI data revealed a network of regions whereresponse was greater to flavors that had been paired with calories vs. thosenot paired with calories (hypothalamus, nucleus accumbens, insula, amygdala, hippocampus, midbrain). The only region in this network where responsecorrelated with change in pleasantness was the insular cortex ($p = .03$). WhereasOW/OB individuals showed no hedonic conditioning they did have enhancedresponse in the hypothalamus ($p = .02$) and striatum ($p = .05$) to the calorie-pairedflavor vs. the no calorie paired flavor.
Conclusions: Our findings suggest that the insular cortex plays acritical role in associating flavors with their biological utility as an energysource by transforming calorie-flavor associations into hedonic responses toflavors. Our results also indicate that obesity is associated with impairedhedonic but enhanced caloric conditioning, since positive associations were observed in regions that reflected caloric associations but no shift inpleasantness. We postulate that this results from a failure of insular circuitsto transform the calorie signal into a hedonic response.
Disclosure: D. Small, Nothing to Disclose.

51.2 Pain and Pending Pictures: Increased Insula Response in Anorexia Nervosa
Alan Simmons*

The University of California, San Diego, California

Background: Recent evidence raises the possibility that symptoms of anorexia nervosa (AN) could be related to impaired interoception. This has been effectively probed using pain and food image anticipation. When coupled these complimentary approaches help probe the interoceptive mechanisms on AN.
Methods: Functional Magnetic Resonance Imaging (fMRI) was used to assess neural substrates of pain processing and food image anticipation in healthy control women (Pain study $n = 10$; Food image study $n = 12$) and individuals recovered from AN (RAN; $n = 12$; $n = 14$) in order to avoid the confounding effects of malnutrition. In the food image anticipatory task they viewed cued standardized images of food and non-food items. In the pain task they received cued pain stimuli of varying intensity.
Results: The between group contrast for the activation difference between food anticipation and image anticipation revealed only one region of significant interaction in the right ventral anterior insula [$F(1, 25) = 29.30$, $p < 0.001$]. There was greater insula activation in RAN versus CW while anticipating images of food. In contrast, there was a deactivation of the insula in RAN while anticipating images of neutral objects. Insula activation in CW was significantly correlated ($r = 0.59$) to more positive pleasantness while no such relationship existed in RAN ($r = 0.06$). In the pain task, RAN compared to CW showed greater activation within right anterior insula (rAI;$t(20) = 5.10$), dorsolateral prefrontal cortex (dlPFC; $t(20) = 3.37$) and cingulate ($t(20) = 4.67$) during pain anticipation, and greater activation within dlPFC and decreased activation within posterior insula during painful stimulation. Greater anticipatory rAI activation correlated positively with alexithymic feelings in RAN subjects (RAN: $rho = 0.602$, $p < 0.05$; CW: $rho = -0.460$, $p = 0.18$).
Conclusions: RAN showed a mismatch between anticipatory responses and self-report. These studies highlight two important premises in our interoceptive model of anorexia. First, RAN individuals are more interoceptively responsive than matched control women. Second, RAN individuals use control mechanisms that center on disconnection from experience and anticipated experience, perhaps to reduce and be detached from bodily signals.
Disclosure: A. Simmons, Nothing to Disclose.

51.3 Regional Gray Matter Volumes in the Insula Distinguish Anorexia Nervosa and Obesity

Guido KW. Frank*

University of Colorado Anschutz Medical Campus, Aurora, Colorado

Background: Little is understood about brain pathways that drive malnutrition in anorexia nervosa (AN) or excessive eating in obesity (OB). Animal (Avena et al, 2008; Carr et al, 2003) and human (Frank et al, 2012) studies suggest opposite neural response patterns associated with under- and overfeeding. The insula is an important relay station that could reduce or enhance taste perception prior to stimulating reward pathways or higher order processing of food stimuli. In this study we wanted to test whether we would find structural insula abnormalities in AN and OB that could be related to altered brain function and behavior in those disorders.
Methods: We recruited 19 restricting-type AN women, 24 women recovered from AN (AN-R), and 24 matched healthy control women (CW), and in a second study 19 OB and 24 matched CW. All subjects underwent magnetic resonance imaging (MRI) to obtain structural images (T1, SPGR field of view 22 cm, flip angle 10°, slice thickness 1.2 mm, scan matrix 256x256, TR 10, TE 3, voxel size 1.2 mm^3). Brain images were analyzed using voxel-based morphometry (VBM) in SPM8. Whole-brain analysis for group comparison included age, depression scores, total intracranial volume (TIV), and medication use as covariates. The significance for brain result maps was set at a $p<0.001$ uncorrected with an extent threshold of >50 voxels, separate for gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). Results were small volume corrected for anatomical region, and regional GM values were extracted for *Post hoc* comparisons.
Results: Total GM, WM and CSF corrected for TIV were not different across groups. GM in CW versus AN and AN-R: There were significant GM group differences in the right insula ($x=30$, $y=14$, $y=-12$, $Z=3.50$, $P_{FWEcorrected}<0.050$ and $x=42$, $y=9$, $z=4$, $Z=3.81$, $P_{FWEcorrected}<0.022$), and in the left insula ($x=-29$, $y=12$, $z=-17$, $Z=3.59$, $P_{FWEcorrected}<0.044$). *Post hoc* tests indicated that right insula volume was significantly increased in AN and AN-R compared to CW, and left insula GM volume increased in AN versus CW. GM in CW versus OB: There was significantly reduced GM in OB compared to CW in the right ($x=36$, $y=-6$, $y=-12$, $Z=4.48$, $P_{FWEcorrected}<0.001$), and in the left insula ($x=-47$, $y=-1$, $z=-5$, $Z=4.49$, $P_{FWE\ corrected}<0.001$).
Conclusions: This study indicates that insular volume is increased in AN during the ill state as well as after recovery, but reduced in OB. The mechanism for such alterations is uncertain, but they could be adaptive changes in response to high or low weight. Increased insula volume in AN after recovery suggests either long lasting remnants form the illness or a premorbid trait that could contribute to illness development. The insula serves important functions in taste perception and reward system activation, but also interoceptive awareness, and interoceptive awareness has been associated with insula GM volume (Critchley et al, 2004). AN is associated with extreme body awareness while OB appears to have reduced body awareness (Smith et al, 2012). Thus, altered insula structure could directly affect taste reward processing, as well as altered body awareness in AN and OB.
Disclosure: G. Frank, Nothing to Disclose.

51.4 Circuits Connecting Somatic/Visceral-related Insular Areas with Eating/Reward Areas in the Ventromedial Prefrontal Cortex

Joseph L. Price*

Washington University School of Medicine, St Louis, Missouri

Background: Neuroanatomical studies in monkeys have defined two distinct but interconnected systems within the ventromedial prefrontal cortex (PFC), (1) a "medial network" centered in the medial PFC that is involved in visceromotor modulation and is strongly implicated in mood disorders, and (2) an "orbital network" in the central part of the orbital cortex that is involved in assessment of sensory objects, especially food, and is important for reward processing. Both networks are also connected to other brain structures, including insular areas, forming two complementary brain systems. Other observations show that the anterior and middle insula process somatic sensory and visceral sensory activity, and suggest that these constitute "body sense", which may carry pleasurable vs aversive signals. Connections between the orbital network and this insular system may provide the basis for reward judgements generated in the orbital cortex.
Methods: Neuroanatomical connections were mapped in monkeys by making small injections of anterograde and retrograde axonal tracers into restricted areas of the PFC and related structures. After sacrifice, the brains were sectioned and the distribution of the tracers was mapped.
Results: In addition to the data summarized above, the related areas of the insula have been mapped in greater detail. The medial network is connected to specific agranular insular areas, but the orbital network is interconnected with a larger region, most notably in the dysgranular insula and frontal operculum. Other experiments have focused on the lateral aspect of the PFC, and have shown that it can also be subdivided into three networks or systems, (1) a dorsal system that is closely related to the medial prefrontal network, (2) a caudal system that is connected with visuomotor and attention areas, and (3) a ventrolateral system, that is closely related to the orbital prefrontal network. Of particular interest in the present discussion, the ventrolateral system, like the orbital network, is strongly interconnected with the dysgranular insula and frontal operculum. It is likely that this cortical region is also involved in object assessment and reward processing.
Conclusions: There is an extended system of interconnected cortical areas in the orbital and ventrolateral prefrontal cortex, parts of the agranular insula, and the dysgranular insula that appears to be involved in the assessment of reward or aversion in relation to food and other stimuli. Within this system, the dysgranular insula may provide "body sense" information that could provide the basis for affective judgements. This is coupled with multimodal sensory information in the orbital and ventrolateral PFC to link affective signals to a particular sensory object. Dysfunction in the medial/dorsal prefrontal network has been implicated in mood disorders. Eating disorders may be similarly linked to the circuit linking the orbital/ventrolateral PFC and the dysgranular insula. Interconnections between these systems may explain the close relation between mood and eating disorders.
Disclosure: J. Price, Nothing to Disclose.

Panel
52. Neuroimaging Predictors of Treatment Effects in High-risk and Bipolar Individuals across the Lifespan

52.1 Neurobiological and Genetic Risk Factors for Antidepressant-induced Mania in Youth at Risk for Bipolar Disorder

Kiki Chang*

Stanford University School of Medicine, Stanford, California

Background: At least 29 published case reports describe pediatricpatients with apparent antidepressant-induced mania (AIM) (Goldsmith et al, inpress). In 21% of such patientsrepresented in these studies, there was a family history of bipolar disorder (BD). We sought to determinepossible risk factors for AIM in a cohort of children at high-risk for bipolardisorder – those with a biological parent with BD - who themselves had not yetexperienced a full spontaneous manic episode. We hypothesized that thefollowing variables would be associated with history of AIM: increased symptoms of subthresholdmania, presence of the 5-HTTPLR s-allele, and decreased amygdalar volume.
Methods: Subjects were 9 – 18 years, with a biological parent with BD I or II, and diagnosis of either Major Depressive Disorder (MDD), or ADHD. Subjects with ADHD had a YMRS score $>$ 11 or CDRS score $>$ 29. Parents were asked to describe anyadverse reactions that had arisen within three months of increasing the dose orintroducing a new antidepressant. AIM was defined as an

expansive, euphoric, or irritable mood, plus three other cardinal symptoms of mania (four if the mood was onlyirritable), lasting at least one day. MRI images were collected on a 3T GE Signa scanner, and amygdala regions were hand traced. DNA was extracted from 200 µl of frozen blood. Oligonucleotide primers flanking the5-HTT-linked polymorphic region and a 100 bp marker was used to measure the PCR product size for l and s allele. We performed x2 analyses for effect of 5-HTTLPR genotype on AIM status. ANCOVA was used determine effect of amygdala volume on AIM status, covarying for total brain volume and age.

Results: We included 100 youth at high-risk for BD. 30 had MDD, 18 had ADHD and no MDD, and 52 had both MDD andADHD.). Mean age was 13.7 +/− 3.0 years, and 63% were male. 52% had a past exposureto at least one SSRI. 29 out of those 52 (55.8%) had a history of AIM. In the group of 52, there was nosignificant difference in gender (chi square = .15; p = .93) or age (t = 1.4;p = .16) between the − AIM group. Presence of the 5−HTTLPR s-allelewas not significantly associated with AIM+ group (chi squared = 5.2, p = 0.08). We had morphometric MRI data for 32 of the 52 subjects with antidepressant exposure. Of those subjects, 19 had experiencedAIM, and 15 had not. Those with AIM had significantly smaller amygdalar volumes than those without AIM (4.43 +/−.39 vs. 5.01 +/−.74. t = 2.9; p = .01).

Conclusions: We found 56% of our sample to have ahistory of AIM, indicating the common occurrence of this outcome in youth whohave a parent with BD. In this cohort, presence of the5-HTTLPR s-allele was not significantly associated with a history of AIM. As our p value was 0.08, this may bedue to type II error – indeed, meta-analysis of studies examining the 5-HTTLPRgenotype and AIM suggest a role of the s-allele for creating risk for AIM (Daray et al, 2010). We also found that youth with a historyof AIM had decreased amygdalar volumes compared with those without AIM. Inprevious studies, the s-allele has been associated with increased amygdalaractivation in healthy adults (Hariri et al, 2002; von dem Hagen et al, 2011). Furthermore, increased amygdalar activation in youth with BD has beencorrelated with decreased amygdalar volume (Blumberg et al, 2009). Decreased amygdalar volume hasconsistently been found in youth with BD (Pfiefer et al, 2008) and thus it ispossible that a reduced amygdalar volume could somehow mechanistically beinvolved with increasing risk for AIM. This finding might be considered a riskfactor for AIM; however, it may also be that AIM may directly or indirectlylead to a smaller amygdala. Nonetheless, it is intriguing to consider thatSSRIs may directly affect the amygdala, particularly in s-allele carriers withalready compromised amygdala volume, leading to a manic episode. These results are preliminary inthat data was collected retrospectively. Nonetheless, our work suggests that certain characteristics may beassociated with AIM in populations already at high-risk for mania.

Disclosure: K. Chang, **Part 1:** Dr. Chang is a consultant for GSK, Merck, BMS, and Lilly. He receives research funding from GSK and Merck, **Part 2:** GSK, Merck.

52.2 Neurofunctional Effects of Ziprasidone in Manic Adolescents with Bipolar Disorder

Melissa DelBello*

University of Cincinnati, Cincinnati, Ohio

Background: Recent findings suggest that adolescents with bipolar disorder exhibit abnormalities in ventral prefrontal and amygdala development. However, most of the patients in these studies were treated with a variety of medications and were in variable mood states making it difficult to interpret whether the neurobiological alterations are due to medication effects or are related to the underlying illness. With these considerations in mind, we conducted a study examining the neurobiological effects of and predictors of response to ziprasidone for adolescents with mania associated with bipolar disorder.

Methods: Adolescents with a manic episode associated with BP (n = 23) were recruited to participate in a 28-day double-blind, placebo-controlled study of ziprasidone. A fMRI scan was acquired from each subject during performance of a task of sustained attention, a Continuous Performance Task-Identical Pairs (CPT-IP) test, using a 4.0 Tesla (4T) Varian Unity INOVA MRI scanner. Patients were scanned at baseline, prior to the administration of study medication and on day 28 of treatment or at study termination. Age and sex matched healthy control subjects (n = 10) were scanned at a single time point. The following regions of interest (ROIs) were defined using Analysis of Functional Images (AFNI); left and right Brodmann areas (BA) 10, 11, and 47 and left and right amygdala. The average percent change in activation for these ROIs was compared between treatment groups (corrected p < 0.006).

Results: Baseline activation in each ROI was compared between responders and non-responders. Treatment with ziprasidone was associated with greater increases over time in right BA 11 and 47 activation. Patients who subsequently responded to ziprasidone showed significantly greater deactivation in the right BA 47 at baseline than those who did not respond to ziprasidone. Similarly, among the BP adolescents who were treated with ziprasidone, baseline activation in right BA 47 was negatively correlated with improvement in manic symptoms.

Conclusions: The increases in right BA 11 and 47 activation observed during sustained attention tasks following ziprasidone treatment and the association identified between lower baseline BA 47 activation and ziprasidone treatment response suggests that ziprasidone may correct ventral prefrontal dysfunction in manic adolescents with BP.

Disclosure: M. DelBello, **Part 1:** Eli Lilly, Amylin, AstraZeneca, Pfizer, Bristol Myers Squibb, Janssen, Johnson and Johnson, Somerset, Shire, Novartis, **Part 2:** Bristol Myers Squibb, Merck, **Part 3:** Bristol Myers Squibb, **Part 4:** Eli Lilly, Amylin, AstraZeneca, Pfizer, Bristol Myers Squibb, Janssen, Johnson and Johnson, Somerset.

52.3 Neurophysiological Effects of Bipolar Medications across Mood State

Caleb Adler*

University of Cincinnati College of Medicine, Cincinnati, Ohio

Background: The impact of medication on neuronal activity and neurochemistry in patients with bipolar disorder remain poorly understood. Studies are complicated by the potential effects of individual mood state and medication class, making comparisons difficult. To address this question, we studied manic and depressed bipolar I patients receiving lithium (Li) monotherapy. The former group was also compared with manic patients receiving quetiapine monotherapy.

Methods: Patients with bipolar I mania or depression (18-55) participated in an 8 week open-label study. Cohorts of both manic and depressed patients received Li, while a separate group of manic patients received quetiapine. Diagnoses were confirmed using the SCID-IV. Medications were dosed clinically throughout. Functional magnetic resonance imaging (fMRI) and magnetic resonance spectroscopy (1H-MRS) was acquired for all subjects prior to medication and after 8 weeks of treatment. fMRI data were obtained during a continuous performance task incorporating an inhibition component, as well as emotional and neutral distractors consisting of selected IAPS pictures (CPT-END). Activity was extracted for specific regions of interest including ventrolateral prefrontal cortex (VLPFC), caudate, lenticulate, thalamus, nuclease accumbens (NA) and amygdala. 1H-MRS data were acquired from 2 cm3 voxels placed in the left and right VLPFC and anterior cingulate (ACG). All subjects were scanned using a 4.0 Tesla Varian Unity INOVA MRI scanner.

Results: Li treatment across mood states was associated with decreased activity in portions of the VLPFC and subcortical structures, as well as neurochemical changes in VLPFC. Manic and depressed patients receiving Li showed significant differences in activity change over 8 weeks, and significantly differed in some neurochemical effects. Manic patients receiving quetiapine showed decreasing NA activity, and differed from manic patients receiving Li on prefrontal and amygdala activation; quetiapine was also associated with differential neurochemical effects in the ACG.

Conclusions: Taken together, these data suggest that Li and quetiapine differ in their effects on brain activity and neurochemical concentrations in regions suggested to be involved in the pathophysiology of bipolar disorder. Furthermore, at least some effects of Li vary across mood state.
Disclosure: C. Adler, **Part 1:** I have participated in multi-site trials including AstraZeneca, Eli Lilly, Pfizer, Otsuka, Forest, Sunovion, Novartis, Glaxo Smith Kline, and Amylin. I have received research support from AstraZeneca. I have been a speaker and consultant for Merck., **Part 2:** Merck, **Part 3:** Merck, **Part 4:** AstraZeneca.

52.4 White Matter Correlates of Antipsychotic and Lithium Response in Bipolar Disorder: A Meta-analysis and Meta-regression of Diffusion Tensor Imaging findings

Sophia Frangou*

King's College, London, United Kingdom

Background: Bipolar Disorder (BD) is reliably associated with abnormalities in white matter (WM) microstructure reflected in reduction in fractional anisotropy (FA) measures obtained from diffusion tensor imaging (DTI) studies. Impaired WM integrity in BD is thought to provide the anatomical basis for the observed abnormalities in the functional integration of emotional processing brain regions. Psychotropic medication can potentially influence WM microstructural modifications but this has not been systematically examined. The aim of the present study was to use meta-analytic techniques to identify statistically consistent FA changes in BD associated with treatment response.
Methods: We employed the Cochrane review technique to search the major databases for relevant studies up to December 2011. We then applied whole brain signed differential mapping to combine primary data from 11 studies that jointly included FA data from 322 patients with BD who had shown evidence of clinical response to antipsychotics or lithium. Meta-regression analyses were further used to examine the effect of age, duration of illness and sex.
Results: An effect of diagnosis was noted in 6 clusters of reduced FA located bilaterally in the inferior medial occipitotemporal WM and in the WM of the ventral anterior cingulate (ACC) extending to the genu of the corpus callosum, the left dorsal ACC and in the right posterior limb of the internal capsule. Antipsychotic treatment was associated with further FA reductions the WM of the left ACC and right insula. Lithium treatment was also associated with further reduction FA in the WM around the right insula but "normalised" FA measures in the WM of the dorsal ACC. None of the moderator variables was significant.
Conclusions: The effect of psychotropic medication on WM microstructure is complex. Not all WM changes observed can be conceptually associated with clinical improvement. In the case of antipsychotics, our data raise the possibility that their long-term use may have a negative impact on WM microstructure. At the same time we identified a significant link between lithium treatment and "normalization" of FA measures within the dorsal ACC. Our results suggest that the role of medication in BD should be considered not just in terms of symptomatic response but also in connection to biological measures reflecting disease-related processes that may impact on long-term prognosis.
Disclosure: S. Frangou, Nothing to Disclose.

Panel
53. Sink or Swim: Take Your Raft and Fyns Down the STEPs to Navigate NMDA Receptor Pools in Neuropsychiatric Disorders

53.1 Genetic Manipulation of Striatal Enriched Phosphatase (STEP) Rescues Behavioral Abnormalities and Seizures in a Mouse Model of Fragile X

Janice R. Naegele*

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Background: Fragile X syndrome (FXS), the most common inherited form of intellectual disability and prevailing known genetic basis of autism, is caused by an expansion in the Fmr1 gene that prevents transcription and translation of fragile X mental retardation protein (FMRP). FMRP binds to and controls translation of mRNAs downstream of metabotropic glutamate receptor (mGluR) activation. Recent work demonstrates that FMRP interacts with the transcript encoding striatal-enriched protein tyrosine phosphatase (STEP) (Ptpn5). STEP opposes synaptic strengthening and promotes synaptic weakening by dephosphorylating its substrates, including ERK1/2, p38, Fyn, Pyk2, and subunits of NMDA and AMPA receptors. We have shown that basal levels of STEP are elevated and mGluR-dependent STEP synthesis is absent in Fmr1KO mice. We hypothesized that the weakened synaptic strength and behavioral abnormalities reported in FXS may be linked to excess levels of STEP. To test this hypothesis, we reduced or eliminated STEP genetically in Fmr1KO mice and assessed mice in a battery of behavioral tests.
Methods: Two cohorts of age-matched, sexually na¿ve mice were used for testing: cohort 1 for audiogenic seizures (AGS) and c-Fos analysis, and cohort 2 for the remainder of the behavioral battery. All mice in cohort 2 were tested in the following order with a minimum of a one week inter-test interval: open field, marble burying, elevated plus maze, social choice test, social dominance tube test, and light/dark box. Mice carrying a targeted null mutation of the Ptpn5 gene (B6N.129-Ptpn5tm1Pjlo, referred to as STEPKO mice, C57BL/6N background backcrossed for 9-12 generations) (Venkitaramani et al, 2009, Venkitaramani et al, 2011) were crossed with mice carrying a targeted null mutation of the Fmr1 gene (B6N.129-Fmr1tm1Cgr, referred to as Fmr1KO mice. Mice used in the current study were generated by mating STEP heterogygous /Fmr1 heterozygous females and STEP heterozygous/Fmr1KO male mice on the C57Bl/6 inbred strain background to obtain littermates that were WT, heterozygous or null for STEP on the Fmr1 WT or KO genotype.
Results: In addition to attenuating audiogenic seizures and seizure-induced c-Fos activation in the periaqueductal gray, genetically reducing STEP in Fmr1KO mice reversed characteristic social abnormalities, including approach, investigation, and anxiety. Loss of STEP also corrected select non-social anxiety-related behaviors in Fmr1KO mice, such as light side exploration in the light/dark box.
Conclusions: Our findings indicate that genetically reducing STEP significantly diminishes seizures and restores select social and non-social anxiety-related behaviors in Fmr1KO mice, suggesting that strategies to inhibit STEP activity may be effective for treating patients with FXS.
Disclosure: J. Naegele, Nothing to Disclose.

53.2 Neuroprotective Role of STEP, a Brain-enriched Tyrosine Phosphatase, in Focal Cerebral Ischemia

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Background: The striatal enriched tyrosine phosphatase, STEP is an important regulator of glutamatergic transmission in the brain. STEP is expressed in neurons of the striatum, neo-cortex and hippocampus. Stimulation of NR1/NR2B containing NMDARs leads to dephosphorylation and subsequent activation of STEP. Substrates of STEP include extracellular regulated kinase 1 and 2 (ERK 1/2), p38 stress-activated kinase (p38 MAPK) and the tyrosine kinase Fyn that are involved in ischemic brain damage. However the physiological function of STEP *in vivo* is still poorly understood. The present study examined the temporal profile of STEP activation and subsequent degradation following cerebral ischemia and reperfusion, and evaluated the relationship with p38 MAPK activation. The study also determined the efficacy of a cell-permeable STEP peptide in attenuating ischemic brain injury. In addition we determined whether genetic deletion of STEP exacerbates ischemic brain damage.

Methods: To determine the temporal profile of activation of endogenous p38 MAPK and STEP as well as to evaluate the efficacy of a STEP peptide in ischemic brain damage adult male rats were subjected to middle cerebral artery occlusion (MCAO) followed by reperfusion for specified time periods. Additional studies determined whether genetic deletion of STEP exacerbates ischemic brain damage in STEP knockout mice. In some experiments primary neuronal cultures were exposed to oxygen glucose deprivation to evaluate the effect of the TAT-STEP peptide *in vitro*.
Results: A role of endogenous STEP in neuroprotection is evident from activation of STEP during an ischemic insult that appears to inhibit the p38 MAPK signaling pathway. However, degradation of active STEP during reperfusion limits efficacy of STEP and results in up-regulation of the p38 MAPK pathway. Based on these observations, we developed a peptide mimetic that constitutively binds to p38 MAPK and is resistant to proteasomal degradation. Transduction of neurons with this peptide was sufficient to block the up-regulation of p38 MAPK during recovery and prevent neuronal injury in an *in vitro* model of ischemic injury. Intravenous injection of the peptide, even after an ischemic insult, was able to attenuate ischemic brain damage *in vivo*. Conversely, mice with a targeted deletion of STEP gene exhibit a dramatic increase in brain injury when exposed to a mild ischemic insult.
Conclusions: In conclusion, it appears that an ischemic insult not only triggers a multitude of cytotoxic pathways in the brain, but it also triggers some endogenous protective responses capable of limiting injury. Here we identify STEP as one such protein that is activated following an excitotoxic/ischemic insult and may act to provide initial neuroprotection against neuronal vulnerability to injury. However prolonged insult may lead to loss of endogenous STEP and its neuroprotective efforts resulting in secondary activation of the cytotoxic pathways. Accordingly, intravenous delivery of the TAT-STEP peptide even after the ischemic insult attenuates the progression of brain damage and might provide a promising new tool for treatment of stroke.
Disclosure: S. Paul, Nothing to Disclose.

53.3 Fyn, Tyrosine Phosphatases and Alcohol Drinking Behaviors

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The University of California, Emeryville, California

Background: Drugs of abuse, including alcohol, can promote maladaptive synaptic plasticity mechanisms. We previously observed that repeated systemic administration of non-intoxicating doses of alcohol and excessive alcohol intake results in a long-term facilitation (LTF) of the NMDA receptor (NMDAR) specifically in the dorsomedial striatum (DMS), a brain region associated with goal-directed behaviors. We further showed that alcohol-mediated LTF depends on long-lasting activation of Fyn kinase and the phosphorylation of its substrate, the NR2B subunit of the NMDAR. Finally, we found that inhibition of NR2B-NMDARs or Fyn in the DMS decreases operant self-administration for alcohol but not sucrose, suggesting a selective reduction in the motivation to seek and consume alcohol. Importantly, similar treatments also blocked relapse of alcohol seeking. Our data suggest that the long-lasting upregulation of NR2B-NMDARs activity in the DMS by alcohol contributes to the maladaptive synaptic changes that lead to the development of excessive alcohol intake and increased propensity to relapse. The tyrosine phosphatases PTPalpha and STEP positively and negatively regulate the activity of Fyn. Here, we set out to determine the contribution of the STEP and PTPalpha in the DMS of rodents on the NMDAR activity and alcohol drinking behaviors.
Methods: siRNA and viral-mediated gene delivery was used to specifically knockdown genes in the DMS. Two-bottle choice paradigm was used to measure alcohol Saccharine and Quinine. Biochemical methods include lipid rafts and synaptic fractionation, and western blot analysis. Patch clamp recording was used to measure the activity of NMDAR.
Results: We found that repeated systemic administration or binge drinking of alcohol results in an increase in the localization of active PTPalpha in the same site where Fyn resides. We further provide data to suggest that PTPalpha and STEPs activities are modulated in response to alcohol exposure leading to Fyn-mediated facilitation of NMDAR activity. Finally, inhibition of PTPalpha in the DMS of rats reduces excessive alcohol intake and seeking.
Conclusions: Our results suggest that the long-term activation of Fyn kinase in response to alcohol exposure in the DMS results from the activation of PTPalpha and the inhibition of STEP.
Disclosure: D. Ron, Nothing to Disclose.

53.4 The Role of GluN2B Receptors and STEP in the ERK Shutoff Induced by Cocaine Self Administration in Rats

Jacqueline F. McGinty*

Medical University of South Carolina, Charleston, South Carolina

Background: ERK dephosphorylation (or "shutoff") in the PFC occurs within 2 hr of the end of repeated, daily cocaine SA. Reversing the ERK shutoff with a single infusion of brain-derived neurotrophic factor (BDNF) into the PFC normalizes glutamate transmission in the nucleus accumbens and suppresses cocaine-seeking in abstinent animals for as long as three weeks. However, BDNF is not a therapeutically useful medication because it is a neuropeptide that does not effectively cross the blood-brain barrier. Therefore, it is necessary to characterize the molecular mechanisms underlying the cocaine SA-induced ERK shutoff in order to identify alternative targets for medication development.
Methods: Rats were trained to self-administer cocaine or received a yoked saline infusion on a standard 2 hr daily schedule for a minimum of 10 days. Two hr after the end of the last session, they were euthanized and Western blots were performed on medial prefrontal cortical tissue. The phosphorylation state of AMPA (p-Ser845-GluA1) and GluN2B (p-Y1472-GluN2B) receptors and the ERK phosphatases, STEP61 and p-Y307-PP2A, were measured by Western blot in dmPFC whole tissue lysates and synaptic fractions.
Results: Two hr after the end of cocaine self administration, there was a decrease in both GluN2B and p-Y1472-GluN2B in whole cell lysates in cocaine SA vs. yoked-saline controls. In addition, phospho-STEP61-ir was decreased in the dmPFC at 2 hr, but not immediately (0 min), after the end of cocaine SA, suggesting that STEP is activated during early withdrawal and may play a role in the cocaine SA-induced ERK shutoff. In contrast, phospho-Y307-PP2A was elevated 2 hr after the end of cocaine SA, suggesting that it is inactivated and does not mediate the cocaine SA-induced ERK shutoff.
Conclusions: These data suggest that early withdrawal from repeated cocaine exposure resulted in a reduction in the total number of GluN2B receptors as well as fewer cell surface receptors because phosphorylation of the Y1472 site is critical for surface expression (Goebel-Goody et al, 2008). Further, STEP activation (dephosphorylation) concurrently with PP2A deactivation (phoshorylation) supports the hypothesis that STEP mediates the cocaine-induced ERK shutoff.
Disclosure: J. McGinty, Nothing to Disclose.

Panel

54. Non-invasive Brain Modulation to Enhance Inhibitory Control and Drive in Psychiatric Disorders: a Translational Approach with a Focus on Dopaminergic Modulation of Fronto-striatal Pathways

54.1 The Effects of Modafinil and Methylphenidate in Neuropsychiatric Disorders

Barbara J. Sahakian*

University of Cambridge, Cambridge, United Kingdom

Background: Neuropsychiatric disorders including schozophrenia are associated with a range of impairments including cognitive and motivational ones. These cognitive changes include deficits in

working memory and cognitive flexibility and problems of impulsivity and risky decision making. Studies of modafinil and methylprenidate in a range of neuropsychiatric disorders will be presented.
Methods: CANTAB computerised tests were used in double blind, placebo controlled studies of patients with neuropsychiatric disorders.
Results: Improvements can be seen in a range of psychological processes, including spatial working memory, cognitive flexibility, risky decision making in patients with neuropsychiatric disorders, including first episode psychosis and schizophrenia and frontal dementia.
Conclusions: Impaired cognition is a major problem in neuropsychiatric disorder which impacts on functional outcome, quality of life and wellbeing. Cognitive enhancing drugs, such as modafinil and methylphenidate, can improve motivation, working memory, cognitive flexibility and impusivity and risky decision making in acute studies. The impact of these drugs in long term sudies and combined with other treatments such as cognitive training remains to be determined.
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 holds a grant funded by Johnson and Johnson. 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 was on Panel LS5 for the European Research Council. As an Associate Editor, she also receives an honorarium from the journal Psychological Medicine. Prof. Robbins has consulted for Cambridge Cognition, Lundbeck, Pfizer, and Lilly, and has received research grants from GlaxoSmithKline, Lundbeck and Lilly, **Part 2:** Professor Sahakian holds a grant funded by Johnson and Johnson.

54.2 Oral Methylphenidate Improves Inhibitory Control and Resting-state Functional Connectivity in Cocaine Addiction: An fMRI Study

Rita Goldstein*

Brookhaven National Lab, Upton, New York

Background: Deficits in the dopaminergically modulated striato-thalamo-prefrontal circuit drive self control and motivation compromises in cocaine addiction. Methylphenidate (MPH), a dopaminergic agonist, has been successfully used to enhance both inhibitory control and salience attribution in children with attention deficit hyperactivity disorder (ADHD); indeed MPH increases activity in the brain regions that modulate these behavioral, cognitive and emotional functions. However, in clinical treatment studies in cocaine addiction, MPH has not shown significant promise, as attributed to its detrimental effects on craving. We hypothesized that oral MPH at a low dose and in combination with inhibitory control motivationally salient tasks will have a beneficial impact in individuals with cocaine use disorders (CUD).
Methods: Individuals with CUD and matched healthy controls received a single oral dose of 20 mg MPH or placebo in a double-blind and counterbalanced order over two consecutive sessions (separated by >1 week). During peak MPH effects (60-90 min post administration), subjects performed a classical inhibitory control task (the color-word Stroop) and its emotional variant (where drug or neutral words replaced the color words and where subjects gained or did not gain monetary reward) during functional magnetic resonance imaging (fMRI). Four resting-state scans were also acquired (during MPH and placebo, before and during peak effects).
Results: Results showed that this oral dose of MPH did not increase self-reported cocaine craving in the CUD. Importantly, MPH enhanced performance on both the classical Stroop task (as measured with reduced errors and enhanced post-error slowing) and its emotional variant (as measured with reduced errors of commission, a common measure of impulsivity, especially during the drug-related context). Whole-brain Statistical Parametric Mapping analyses of the classical Stroop fMRI data revealed that in the CUD as compared to controls MPH normalized error-processing in the dorsolateral prefrontal cortex, a region known to be crucially involved in cognitive control implementation. During the emotional Stroop task, hypoactivations in the midcingulate cortex and hyper-deactivations in the rostroventromedial anterior cingulate cortex/medial orbitofrontal cortex, regions implicated in cognitive control detection and value processing, were also normalized. In addition, MPH increased connectivity of several cortical-cortical and cortical-subcortical pathways that were previously shown to have disrupted connectivity in cocaine addiction; higher connectivity was associated with less frequent cocaine use. Finally, MPH also reduced the connectivity of the ventral striatum (location of the nucleus accumbens) with the dorsal striatum (putamen/globus pallidus), a pathway classically involved in habit formation and compulsive drug-seeking; lower connectivity between these regions was associated with less severe dependence.
Conclusions: These results show that oral MPH at 20 mg (a dose commonly prescribed to children with ADHD) enhanced responses to motivationally salient tasks of inhibitory control – at the behavioral and brain levels - during both a neutral and an emotionally valenced task in CUD; craving was not increased. These results suggest that oral MPH in combination with prefrontally-mediated executive function tasks may improve inhibitory control (reducing impulsivity) and enhance reward processing (and motivation) in CUD. The directionality of the resting-state connectivity results, together with correlations with severity of drug use, suggests that MPH could facilitate control of behavior in cocaine addiction by improving interactions within striatal (decreasing connectivity between ventral and dorsal regions) and cortical (increasing intra-cortical and cortical-subcortical connectivity) pathways. These results are highly relevant to understanding the mechanisms that could enhance advantageous decision making (through salience attribution to advantageous reinforcers) and self control during high risk situations (e.g., when non-drug reinforcement is insufficiently salient or during an overpowering immediate drug-related context) in drug addiction. Novel cognitive rehabilitation interventions in cocaine addiction, where MPH is combined with learning exercises, could improve treatment outcome in this treatment resistant disorder.
Disclosure: R. Goldstein, Nothing to Disclose.

54.3 Noninvasive Nonpharmacological Approach to Modulate Cognition and Decision Making in Addiction

Felipe Fregni*

Harvard Medical School, Boston, Massachusetts

Background: Weighing the risk against benefits of a decision is a critical balance that, if altered, can have significant real-life consequences. Decision making is mediated by a complex neural network including the dorsolateral prefrontal cortex (DLPFC). By modulating the activity in this area, it may have clinically significant implications, specifically for patients with altered decision making capabilities. Using a form of non-invasive brain stimulation, namely transcranial direct current stimulation (tDCS) we may be able to change the activity of this area, and therefore modify decision-making behaviors.
Methods: In our research with healthy subjects, we use simultaneous tDCS stimulation of the DLPFC along with decision making tasks. Specifically, bilateral tDCS stimulation of the DLPFC - with either anodal tDCS of the right DLPFC/cathodal stimulation of the left DLPFC, or vice versa, compared with sham tDCS. We have also looked at the effects of bilateral stimulation vs. unilateral DLPFC stimulation.
Results: We have found that individuals who received anodal stimulation of the the right DLPFC/cathodal left DLPFC chose more often the "safer" prospect or had more caution in their decision making. We also found that there was no change in decision making when participants received unilateral tDCS stimulation – we observed this effect only with bilateral stimulation.

Conclusions: Our results support the notion that the DLPFC has an involvement in decision making processes. In addition, our data supports that there may be an interhemispheric interaction – or balance of activity – between the right and left DLPFC that is critical in decision making activities. Thus, this type of stimulation may be beneficial for those who have high risk taking behaviors (ex. addition, gambling), and may be translated into therapeutic interventions for such patients.
Disclosure: F. Fregni, Nothing to Disclose.

54.4 Dopaminergic Modulation in a Preclinical Model of Risky Decision Making

Barry Setlow*

University of Florida, Gainesville, Florida

Background: Elevated risk-taking is a feature of several neuropsychiatric conditions, and in some cases (e.g., addiction), such maladaptive behavior may perpetuate and exacerbate these conditions. To model risky decision making behavior in animals, we have developed a task in which rats are given discrete choices between a small "safe" food reward and a large food reward associated with varying degrees of risk of punishment. Rats in this task are sensitive to the degree of risk of punishment, choosing the large reward when the risk of punishment is low, and switching to the small but safe reward as the risk of punishment goes up. Importantly, there is considerable and stable variability among individual rats' performance in this task, such that rats can be reliably classified according to their degree of preference for risk-taking. This task was used to characterize relationships between risky decision-making and cocaine self-administration, and to determine the roles of dopaminergic signaling in risky decision making.
Methods: A first set of experiments was used to determine the relevance of the risky decision making task to addiction-related behavior. Rats characterized in the risky decision making task were subsequently allowed to self-administer cocaine, followed by re-testing in the risky decision making task. A second set of experiments was used to determine the relationship between risky decision making and dopamine receptor mRNA expression in prefrontal cortex (PFC) and striatum. A final set of experiments was used to determine the effects of systemic modulation of dopamine signaling on risky decision making.
Results: Rats characterized as "risk-taking" subsequently acquired cocaine self-administration more rapidly than their risk-averse counterparts, supporting the predictive validity of the risky decision making task. Following chronic cocaine self-administration, rats displayed significantly and persistently elevated preference for risky rewards, consistent with findings in human cocaine users. In drug-naïve rats, high levels of risk-taking were associated with elevated D1 receptor mRNA in ventral striatum and reduced D2 receptor mRNA in dorsal striatum. In subregions of PFC, D2 receptor mRNA expression was related to flexibility in modulating choice behavior according to the degree of risk, such that both low expression in medial PFC and high expression in orbital PFC were associated with inflexible patterns of choice behavior. Finally, behavioral pharmacological experiments showed that acute systemic administration of either amphetamine or the D2-like agonist bromocriptine attenuated risk-taking, whereas drugs acting on D1-like receptors had no effect.
Conclusions: High levels of risk-taking in the risky decision making task were associated with cocaine self-administration and reductions in striatal D2 receptors, consistent with findings in humans cocaine users. Pharmacological activation of D2-like receptors reduced risk-taking, indicating the potential of this approach for ameliorating elevated risk-taking in addiction and other disorders.
Disclosure: B. Setlow, Nothing to Disclose.

Panel
55. Neuronal Mechanisms for Behavioral and Psychiatric Vulnerability in Adolescents

55.1 Neuronal Processing Differences in the Orbitofrontal Cortex and Striatum of Adolescents and Adults during Motivated Behavior

Bita Moghaddam*

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: Adolescents often respond differently than adults to the same salient motivating contexts, like peer interactions and pleasurable stimuli. Delineating the neural processing differences of adolescents is critical to understanding this phenomenon, as well as the bases of behavioral and psychiatric vulnerabilities, such as drug abuse, mood disorders, and schizophrenia. We believe that age-related changes in the ways salient stimuli are processed in key brain regions could underlie the unique predilections and vulnerabilities of adolescence. Because motivated behavior is the central issue, it is critical that age-related comparisons of brain activity be undertaken during motivational contexts.
Methods: We compared single-unit activity and local field potentials (LFPs) in the orbital frontal cortex (OFC) nucleus accumbens (NAc) and dorsal striatum (DS) of adolescent and adult rats during a reward-motivated instrumental task.
Results: We observed striking age-related differences in the neural encoding of salient events in the OFC and DS. In the OFC, we observed diminished inhibitory response of neurons to salient events, which was accompanied by detrimental impact on coordinated large-scale activity. In the DS, a region generally associated more with habit formation than reward processing, we observed that adolescents, but not adults, had a large proportion of neurons that activated in anticipation of reward. In contrast, similar response patterns were observed in NAc of the two age groups.
Conclusions: The OFC data indicated that there is reduced efficiency in the processing of cortical neural activity and related behaviors in adolescents. The DS data demonstrates that in adolescents, a region critically involved in learning and habit formation is highly responsive to reward. It thus suggests a mechanism for how rewards might shape adolescent behavior differently, and for their increased vulnerabilities to affective disorders.
Disclosure: B. Moghaddam, Nothing to Disclose.

55.2 Reward Network in Adolescents: Longitudinal Data and Functional Connectivity

Monique Ernst*

National Institutes of Health, Bethesda, Maryland

Background: Youth vulnerability for addiction has been attributed to normative functional changes in the reward-related neurocircuitry, with a particular emphasis on dopamine function. The triadic neural systems model complements this core mechanism by describing neural modulators that can explain the large inter-individual variability in behavior.
Methods: Following a brief introduction on these mechanisms, new data will be presented that clarify (1) age-related changes in striatal response to incentives using prospective data and (2) commonalities and differences between adults and adolescents of the functional connectivity of core structures engaged in response to incentives using DCM analysis.
Results: The first study reports striatal activation to incentives that increases from adolescence to early adulthood in 23 individuals tested twice at 3 y interval with the monetary incentive delay task. This result will be discussed in the context of task designs and cognitive processes captured during reward paradigms. The second study reports that functional connectivity among nucleus accumbens, thalamus and insula during incentive anticipation was

quite similar between 30 adults and 24 adolescents, although significant directional links showed unique patterns in each group.
Conclusions: These data will be discussed in light of known anatomic connections and their role in reward processes. To conclude, specific predictions will be made regarding vulnerability to psychopathology.
Disclosure: M. Ernst, Nothing to Disclose.

55.3 Adolescent Maturation of Cortico-accumbens Circuits and Risk for Addictive Behavior

Patricio O'Donnell*

University of Maryland School of Medicine, Baltimore, Maryland

Background: The modulation of prefrontal cortical projections to the ventral striatum is modulated by dopamine. This modulation is critica for goal-directed and reward-based behaviors. Several lines of evidence suggest dopamine control of information processing matures during adolescence, a critical period in which risky and addictive behaviors may emerge. We explored several aspects of adolescent maturation of prefrontal and ventral striatal information processing using electrophysiological tools, as well as whether adolescent rats exhbit higher risk for addictive behaviors.
Methods: Whole cell recordings were conducted in the medilal prefrontal cortex and nucleus accumbens of juvenile, adolescent and young adult rats. Early adolescent, late adolescent and adult rats were trained to self-administer cocaine or sucrose, and following extinction the rats were tested for cue-induced reinstatiement of cocaine-seeking. Some rats were also tested for electrophysiological changes in nucleus accumbens physiological properties.
Results: D1-NMDA interactions in PFC pyramidal neurons became more efficient during adolescence, allowing the emergence of persistent activity in brain slices. Fast-spiking interneurons were also effectively activated by both D1 and D2 agonists in adult tissue, but not in preadolescent tissue. In the nucleus accumbens, the modulation of medium spiny neurons by D1 and D2 agoninst was also modified during adolescence, yielding an adult striatum with D1 receptors enhancing synaptic responses and D2 receptors attenuating them. The late adolescent period (P45-55) was associated with higher levels of cocaine-self administration and adult rats exposed at that age showed enhanced reinstatement.
Conclusions: The data indicate that the dopamine modulation of prefrontal cortical fundtion and its impact on nucleus accumbens physiology become refined during adolescence, with the emergence of D1-dependent increases and D2-dependent attenuation of infromation throughput. In addition, the late adolescent period showed increased vulnerability for addictive behaviors.
Disclosure: P. O'Donnell, Nothing to Disclose.

55.4 Developmental Impairment of Local Prefrontal GABAergic Circuits by Altered Glutamatergic Transmission during Adolescence

Kuei Y. Tseng*

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Background: Developmental disruption of cortical inhibitory circuits is thought to contribute to the adolescent onset of cognitive deficits observed in many major psychiatric disorders such as schizophrenia and drug abuse. However, the neuronal basis and pathophysiological consequences underlying such changes remains unclear. 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-glutamate interaction and the activity of parvalbumin (PV)-positive fast-spiking (FS) GABAergic interneurons, which exert inhibition over pyramidal output cells and enable synchronous firing in the PFC. Given the crucial role of N-methyl D-aspartate (NMDA) receptors in the regulation of GABAergic interneurons function in the PFC, we examine how repeated administration of the NMDA antagonist MK-801 during the periadolescent transition period (postnatal day -PD- 35 to 40) impacts the normal development of local prefrontal GABAergic network function.
Methods: Using histochemical, biochemical, electrophysiological and behavioral measures, we determined how repeated administration of the NMDA antagonist MK-801 during the periadolescent transition period (postnatal day -PD- 35 to 40; 0.1 to 0.3 mg/kg) impacts the development of local PFC GABAergic microcircuitry and function. Local field potential recordings of medial PFC (infralimbic and prelimbic regions) synaptic response to ventral hippocampal stimulation were compared between saline- and MK-801-treated animals. All electrophysiological measures were collected within 3 to 5 weeks from the last saline or MK-801 injection, and changes in hippocampal train stimulation-induced synaptic facilitation and depression were examined across treatment groups.
Results: We first determined how PV interneuron activity in the PFC changes during postnatal development. Histochemical and biochemical data indicate that PV interneuron function in the normal PFC increases during the periadolescent transition to adulthood, an effect associated with an augmentation of glutamatergic drive onto these interneurons. We next assessed the effect of NMDA receptor blockade during adolescence on these measures. We found that periadolescent (PD 35-40) exposure to the NMDA receptor antagonist MK-801 arrests the characteristic developmental facilitation of PV immunoreactivity in the adult PFC. In contrast, no apparent changes in PV immunoreactivity were observed in rats treated during adulthood (PD 75-80). Electrophysiological analyses of cortical network activity indicate that adolescent MK-801-induced developmental dysregulation of interneuron circuits is sufficient to elicit a sustained disinhibited PFC state. Such PFC disinhibition is associated with a marked and selective attenuation of high frequency-dependent inhibitory control exerted by ventral hippocampal afferents onto the PFC. MK-801 exposure during adulthood did not disrupt the normal pattern of high frequency-induced synaptic depression in the PFC. We next asked the question whether long-term synaptic plasticity within the ventral hippocampal-PFC pathway is also impaired following a history of MK-801 exposure during adolescence. In saline controls, ventral hippocampal HFS elicits a modest ($\sim$20%) but reliable LTD in the PFC. Interestingly, when the same HFS protocol was tested in animals that received MK-801 during adolescence, a shift from LTD to LTP was observed. Again, such a shift was only observed after adolescent MK-801, suggesting that the altered plasticity observed in the adolescent-treated group is also age dependent. All the above electrophysiological observations point to a common age dependent mechanism underlying the onset of prefrontal disinhibition concurrent to the reduced inhibitory control within the hippocampal-PFC pathway following adolescent MK-801. To test this hypothesis, we assessed the impact of local prefrontal microinjection of the GABA-A antagonist picrotoxin on PFC processing of ventral hippocampal drive in adult naïve animals. We found that local picrotoxin administration is sufficient to elicit a PFC state resembling to that induced by adolescent MK-801. These results indicate that the distinctive frequency-dependent synaptic inhibition observed in the adult PFC in response to ventral hippocampal stimulation is mediated by local prefrontal GABAergic transmission. We next examined the behavioral consequences of the decreased prefrontal GABAergic transmission using the discrete paired delayed alternation paradigm to measure deficits in prefrontal-dependent working memory and acquired learning. Adolescent-treated MK-801 rats displayed marked deficits in acquired learning capabilities, relative to controls when tested in adulthood.

Conclusions: Together, these results indicate that glutamatergic activation of PV-positive GABAergic interneurons by NMDA receptors during adolescence is crucial for the development of a functional inhibitory tone in the adult PFC. Such impairments during the periadolescent transition could trigger a sustained disinhibited frontal cortical state and contribute to the onset of prefrontal deficits observed in schizophrenia and related psychiatric disorders.
Disclosure: K. Tseng, Nothing to Disclose.

Panel
56. The Orexins: Bench to Bedside and Beyond

56.1 The Hypocretin/Orexin System: Neuropeptides Involved in Sleep/Wake and Multiple Other Functions

Thomas S. Kilduff*

SRI International, Menlo Park, California

Background: Since our original description of the hypocretins in 1998 and the subsequent description of the orexins and their receptors, an extensive amount of information has accumulated on the neurobiology and function of this system. As a consequence of the link with both animal models of narcolepsy as well as the human disorder itself, the hypocretin/orexin system has become widely recognized as a wakefulness-promoting system. These observations have led to targeting the Hcrt receptors with antagonists for the treatment of insomnia. My presentation will address the necessity of activity of the Hcrt neurons for maintenance of wakefulness and whether the sleep-inducing effects of Hcrt antagonists are mediated through Hcrt receptor 1, receptor 2 or both receptors. I will also present some surprising results obtained from antagonism of Hcrt receptors in narcoleptic mouse models.
Methods: Optogenetics, Pharmacology, EEG/EMG and video recordings, Genetically-manipulated mice.
Results: 1. Maintenance of wakefulness during a period of high sleep pressure is dependent upon Hcrt neuron activity whereas wakefulness is independent of the Hcrt system during periods of low sleep pressure. 2. Sleep induction by Hcrt receptor antagonism appears to be mediated through both Hcrt receptor 1 and 2.3. Hcrt receptor antagonism exacerbates cataplexy in narcoleptic mice and, at high doses, can occasionally induce cataplexy-like events in some wildtype mice.
Conclusions: Hcrt neuron activity seems to be necessary for maintenance of wakefulness under some, but not all, physiological conditions. Blockade of both Hcrt receptors is an effective means of sleep induction.
Disclosure: T. Kilduff, **Part 1:** Since I am not a member of ACNP, I have no idea what "companies doing business with or proposing to do business with ACNP over past 2 years." **Part 4:** CHDIEMD Serono Research InstituteF. Hoffman-La Roche, Ltd.Sunovion.

56.2 Role of Hypocretin/Orexin Receptors in Arousal Control

Luis de Lecea*

Stanford University, Stanford, California

Background: The hypocretin/orexin system, described 15 years ago, consists of two neuropeptides derived from the same precursor, and two receptors (Hcrtr1 and Hcrtr2) that bind the ligands with differential affinity. The Hcrt system has a crucial role in arousal stability, as dysfunction of Hypocretin signaling leads to narcolepsy. Studies with knockout mice deficient for single Hcrt receptors have also revealed differential functions for the two Hcrt receptors. Here we will discuss recent experiments using optogenetics demonstrating multiple functions for Hcrt/orexin system in arousal and hyperarousal. Newly developed small molecules that act as antagonists for Hcrt/orexin receptors may be used as therapeutic tools for a variety of neuropsychiatric disorders.
Methods: We have used optogenetic methods to manipulate the activity of Hcrt/orexin neurons and of norepinephrine neurons in the locus coeruleus as described (Adamantidis et al, 2007, Carter et al, 2010).
Results: Here, we use combinatorial optogenetic tools to test the hypothesis that the LC regulates Hcrt-mediated promotion of wakefulness. We found that photo inhibiting LC neurons during Hcrt stimulation blocked sleep-to-wake transitions. In contrast, when LC neurons were optically stimulated to increase membrane excitability, concomitant photo stimulation of Hcrt neurons significantly increased the probability of sleep-to-wake transitions compared with Hcrt stimulation alone, suggesting that norepinephrine neurons in the LC are major effectors of Hcrt neurons in controlling sleep-to-wake transitions. We have also shown that chronic activation of Hcrt neurons may lead to hyperarousal associated with stress and addiction. The two Hcrt/orexin receptors may have significantly different roles in controlling the transitions from sleep to wakefulness and different levels of arousal.
Conclusions: Our results show that the gain of LC activity bidirectionally regulates Hcrt-mediated sleep-to-wake transitions. We also show that long-term Hcrt/orexin activity may signal different levels of arousal in normal and pathological conditions. Pharmacological interventions that inhibit Hcrt/orexin receptors may improve the symptoms of primary insomnia and several neuropsychiatric disorders.
Disclosure: L. de Lecea, Nothing to Disclose.

56.3 Efficacy and Safety of Suvorexant, an Orexin Receptor Antagonist, in Patients with Primary Insomnia: Results from Three Phase 3 Trials

W. Joseph Herring*

Merck Research Laboratories, North Wales, Pennsylvania

Background: Night-time administration of orexin receptor antagonists is hypothesized to dampen orexin-mediated wakefulness, facilitating sleep. Suvorexant, an investigational orexin receptor antagonist, was effective and well-tolerated in an initial 4-week proof-of-concept study in patients with Primary Insomnia. Here we report results from two 3-month confirmatory efficacy trials (Trial-1 and Trial-2), and one 12-month long term safety trial (Trial-3).
Methods: Three randomized, double-blind, placebo-controlled, parallel-group, trials were conducted in non-elderly (18-64 years) and elderly ($\geqslant$65 years) patients with primary insomnia. A dose of 40 mg for non-elderly patients and 30 mg for elderly patients was evaluated in all three trials; 20 mg for non-elderly patients and 15 mg for elderly patients was also evaluated in Trials-1&2. The elderly dose adjustments were made to match non-elderly exposures. In Trials-1&2, efficacy was assessed at Week 1, Month 1, and Month 3 by patient-reported outcomes (PRO) of subjective total-sleep-time (sTST), time-to-sleep-onset (sTSO), and wake-time-after-sleep-onset (sWASO), and at Night 1, Month 1, and Month 3 by polysomnographic (PSG) endpoints of Latency-to-onset-of-Persistent-Sleep (LPS) and Wakefulness-After-persistent-Sleep-Onset (WASO). In Trial-3, efficacy was assessed over the 12-month treatment and in a 2-month relapse prevention phase by PROs of sTST, sTSO, and sWASO.
Results: The number of patients randomized was 1021 in Trial-1, 1019 in Trial-2, and 781 in Trial-3. In Trial-1 and Trial-2, suvorexant 40/30 mg was significantly superior to placebo on all PRO and PSG endpoints at Night 1/Week 1, Month 1 and Month 3, except that in Trial-2 the effect on LPS at 3 months was not significant, likely due to high placebo response. In both trials, the magnitude of improvement seen for some endpoints was dose-related. In Trial-3, suvorexant was superior to placebo over the first 4-weeks, and also superior to placebo at each month over 12-months. In the relapse phase of Trial-3, insomnia symptoms returned after suvorexant discontinuation. Suvorexant was generally well-tolerated in all three trials, without evidence of clinically important rebound or withdrawal upon discontinuation.

Conclusions: Suvorexant improved sleep onset and maintenance over a 3-month treatment period in two pivotal Phase 3 trials and over the course of a year in a long term study, with a good safety profile and without evidence of clinically important rebound or withdrawal effects following discontinuation.
Disclosure: W. Herring, **Part 1:** Presenter is an employee of Merck, **Part 2:** Merck, **Part 3:** Merck.

56.4 Orexin Agonists and Antagonists Effects beyond Insomnia

Thomas Roth*

Sleep Center Henry Ford Hospital, Detroit, Michigan

Background: Orexin/hypocretin peptides are produced by a cluster of neurons in the hypothalamus. Orexin neurons are responsive to stress, autonomic activity, hunger, satiety, reward system sleep wake homeostasis and circadian timing. These facts suggest that interventions in the orexin system could have important therapeutic effects across a variety of neurospcyhiatric disorders.
Methods: Orexin receptor antagonists have been studied in humans for effects on sleep and in a number of preclinical models of neuropsychiatric disorders. We will review in this talk the available evidence suggesting those disorders in which the clinical utility of orexin receptor agonists and antagonists may be greatest.
Results: The small cluster of orexinergic cells have extensive projections to many brain regions especially thoe associated with arousal and motivation including the histaminergic neurons of the tubueromammilary nucleus noradrenergic neurons of the locus coeruleus, seretonergic neurons of the raphe nucleus and dopaminergic neurons of the ventral tagmental area. Also, thy are responsive to a variety of inputs including the amygdala, nucleus acumbus as well as the dorsomedial nucleus of hypothalamus. Effects on sleep wake are well documented, with antagonist producing sleep, while agonists producing sustained wakefulness and reversal of sleep deprivation effects. Orexin neurons have also been shown to be activated by rewards such as food and drugs. Administration of orexin stimulates food intake. Conversely Orexin antagonists block cocaine self administration. This later effect is thought to be localized at the VTA dopamine neurons. Interestingly, Narcolepsy patients who routinely are treated with stimulants rarely abuse them. The enervation of the PVT has led to research as to the effect of antagonists on anxiety disorders. In understanding the effects of orexin it is important to determine whether the effects are mediated through orexin A or B or both. Also it is important understand whether these clinical effects are direct drug effects, indirect effects of orexin on sleep wake, or the direct effects that are modulated by sleep wake state. Finally, results from clinical studies on effects on co-morbid disorders and neuropsychiatric effects in those patients studied for primary insomnia are can provide evidence about other clinical effects of intervening in the orexin system.
Conclusions: The orexin system is only now beginning to be explored for its clinical importance beyond effects on sleep. Initial data suggest that it may offer important new opportunities to develop novel interventions in areas of great unmet need.
Disclosure: T. Roth, Nothing to Disclose.

Panel
57. Microdomain-specific Proteome Abnormalities in Severe Mental Illness

57.1 Playing in Traffic: Protein Trafficking and Membrane Domains in Polarized Cells

Bettina Winckler*

University of Virginia, Charlottesville, Virginia

Background: Polarized epithelial cells possess differentiated plasma membrane domains that possess very different complements of membrane proteins. The polarized distributions of these proteins are required in order for these cells to mediate vectorial transport of information, in the case of neurons, or solutes and fluid, in the case of epithelia. Polarized cells must utilize molecular signals to encode these polarized distributions and must possess cellular sorting machinery that act upon these signals and interpret the information that they embody.
Methods: We have utilized a novel protein tagging strategy that allows us observe the trafficking of newly synthesized cohorts of membrane proteins and to identify the protein partners with which they associate at various times following their biogenesis.
Results: We find that membrane proteins pursue multiple routes to the plasma membrane that involve transit through distinct intracellular compartments. In addition, a protein's profile of interaction partners changes over the course of the protein's life span.
Conclusions: We have developed tools that permit aspects of protein trafficking in polarized cells to be observed with a high degree of spatial, temporal and biochemical resolution.
Disclosure: B. Winckler, Nothing to Disclose.

57.2 Identification and Characterization of a Putative Subcellular Microdomain: Evidence for Disruption of the Coupling of Glutamate Transporters and Glycolytic Enzymes with Mitochondria in Schizophrenia

Robert McCullumsmith*

The University of Alabama at Birmingham, Birmingham, Alabama

Background: Excitatory amino acid transporter 2 (EAAT2) belongs to a family of sodium-dependent glutamate transporters that maintain low synaptic concentration of glutamate by removing glutamate from the synaptic cleft into astroglia and neurons. Efficient reuptake of glutamate by EAAT2 relies on sodium and potassium gradients generated principally by Na + /K + ATPase and energy intermediates (ATP) that drive glial glutamate reuptake. Hexokinase 1(HK1), an initial enzyme of glycolysis, binds to mitochondrial outer membrane where it couples cytosolic glycolysis to mitochondrial oxidative phosphorylation, producing ATP utilized by the EAAT2/ Na + /K + ATPase complex to facilitate glutamate reuptake. In this study, we hypothesized that EAAT2 is functionally coupled with Na + /K + ATPase and HK1 in a large multiprotein complex and that breakdown of this complex may lead to abnormal glutamate transmission, contributing the pathophysiology of schizophrenia.
Methods: We used double-label immunoflurescence, immunoisolation, mass spectroscopy, subcellular fractionation, and Western blot analysis to interrogate human postmortem samples from the frontal cortex of subjects with schizophrenia and a control group.
Results: EAAT2 protein was found to colocalize with hexokinase 1 (HK1) and Na + /K + ATPase; Analyses of the EAAT2 interactome and an extrasynaptic membrane fraction containing EAAT2 using mass spectroscopy suggest there is a coupling of these and other specific glycolytic enzymes with EAAT2 in this extrasynaptic membrane fraction. We also found an increased ratio of hexokinase 1 in the extrasynaptic/mitochondrial fraction in schizophrenia, suggesting detachment of HK1 from the mitochondrial outer membrane.
Conclusions: We propose that the functional coupling of glutamate transporters with glycolytic enzmyes and mitochondria defines a putative subcellular microdomain present in astrocytic processes adjacent to excitatory synapses. Our data suggest that the integrity of this microdomain may be disrupted in schizophrenia, which could lead to alterations in the buffering and reuptake of glutamate from the synapse, leading to glutamate spillover and activation of extrasynaptic glutamate receptors.
Disclosure: R. McCullumsmith, Nothing to Disclose.

57.3 Evidence from Proteomic Analysis of Schizophrenia Implicates the Cellular Process of Clathrin Mediated Endocytosis in Schizophrenia

David Cotter*

Royal College of Surgeons, Dublin, Ireland

Background: Clathrin mediated endocytosis (CME) is the best characterized mechanism governing cellular membrane- and protein trafficking. Recent evidence implicates CME and related cellular trafficking mechanisms in the pathophysiology of psychotic disorders such as schizophrenia and bipolar disorder. The evidence includes proteomic and genomic findings implicating proteins and genes of the clathrin interactome. Additionally, several important candidate genes for schizophrenia, such as dysbindin, are involved in processes closely linked to CME and membrane trafficking. Also, key aspects of psychosis neuropathology such as synaptic dysfunction, white matter changes, and aberrant neurodevelopment are all influenced by clathrin dependent processes, and other cellular trafficking mechanisms previously linked to psychoses interact with the clathrin interactome in important ways. Furthermore, many antipsychotic drugs have been shown to affect clathrin-interacting proteins. CME influences N-methyl D-Aspartate (NMDA) receptor trafficking and may represent as cellular process underlying the proposed NMDA receptor hypofunction in schizophrenia. We tested the hypothesis that CME associated protein expression is altered in the postsynaptic density in schizophrenia.
Methods: We have used two dimensional gel elctrophoresis and mass spectrometry based proteomic methods to assess subproteomes and fractions of schizophrenia and control candidate brain regions. Brain tissue enrichments were obtained using laser capture microscopy and membrane, myelin and postsynaptic density fractionatiom methods and differential expression between schizophrenia, bipolar disorder and control tissue was characterised. Tissue was donated by the Stanley Medical Reseach Institute and consisted of the well matched Stanley Array series.
Results: Our findings identify core members of the clathrin interactome as differentially expressed in schizophtrenia and bipolar disoder in unenriched tissues. We specifically assessed the postsynaptic density fraction in order test the hypothesis that altered expression of CME proteins is present within this fraction. We demonstrated altered expression of the core CME proteins such as DNM1 and AP2 and of NMDA associated proteins including MAPK3, CYFIP2, SHANK3, SYNPO and EAAT2.
Conclusions: The findings provide convergent evidence which both implicates the NMDA receptor function in the postsynaptic density in schizophrenia and which identifies CME as a cellular process which may underlie this dysfunction in schizophrenia.
Disclosure: D. Cotter, **Part 1:** honorarium research presentation x1 Lilly.

57.4 PSD Protein Partitioning is Altered in the DLPFC of Schizophrenia

Chang-Gyu Hahn*

University of Pennsylvania, Philadelphia, Pennsylvania

Background: Several lines of evidence point to the postsynaptic density (PSD) as a site of convergence for genetic risk factors in schizophrenia. The goal of this project is to investigate prefrontal cortex PSD protein composition in schizophrenic subjects which could provide clues as to the nature of this convergence.
Methods: We developed a proteomics approach for the highly accurate and precise quantification of over 250 selected synaptic proteins. This assay was first used to further validate biochemical fractionation of human postmortem brain tissue in whole tissue homogenates, PSD, vesicular, parasynaptic and synaptosomal preparations prepared from the brain tissue of three normal human subjects and three mice. Next, whole tissue homogenates and PSD enrichments were prepared from the postmortem dorsolateral prefrontal cortex (DLPFC) of 15 matched pairs of schizophrenia and control subjects and analyzed by the proteomics approach.
Results: Validation experiments revealed the enrichment profiles of sub-synaptic preparations from postmortem human and fresh mouse tissue to be highly similar. Proteomic analysis of whole tissue homogenates revealed no difference in protein expression with the exception of two protein families: CAMKIIs and proteolysis. However, enrichment profiles for PSD preparations were significantly different between schizophrenic and control subjects.
Conclusions: Decreased spine density has been repeatedly observed in layers III and IV of the DLPFC in schizophrenic subjects, suggesting a concordant decrease in synaptic protein expression. Our data suggest that altered partitioning of proteins into the PSD microdomain may be an important molecular correlate of postsynaptic dysfunction in the DLPFC of schizophrenia. It will be important to address the relationships between altered partitioning of the PSD proteins and decreased dendritic spine density.
Disclosure: C. Hahn, Nothing to Disclose.