Neuropsychopharmacology (2013) 38, S1–S107

www.neuropsychopharmacology.org

Panel and Study Groups
Monday, December 09, 2013

1. Mini-Panel
Neuronal Immaturity in Schizophrenia

1.1 GABA Signaling in Postmortem Human Brain and Schizophrenia: A Question of Immaturity?

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Background: GABA signaling abnormalities have been reported frequently in postmortem schizophrenia studies. Although the expression in GAD67 expression is probably one of the most replicated findings to date, there have been relatively few studies of this finding with respect to genetic variation or brain development. Similarly, the switch from GABA as an excitatory to inhibitory neurotransmitter in human brain mediated by two genes, NKCC1 and KCC2, has only recently been explored. We have had the opportunity to study both genetic variation and expression in genes in this pathway (GAD1, GAD2, SLC12A2 (NKCC1) and SLC12A5 (KCC2) across human brain development in hippocampus and dorsolateral prefrontal cortex (DLPFC) in a large cohort of normals and patients with schizophrenia, bipolar illness and major depression.
Methods: We have studied over 238 normal human brains in both DLPFC and hippocampus ranging in age from week 14 in the fetus to 80 years of age, using Illumina microchip arrays, qRT-PCR and RNA Seq. In addition there are 30–76 patients with schizophrenia (depending on the brain region and assay) and 63 patients with affective disorders. Each brain has been genotyped with 650K to 1 million SNP chips from Illumina and screened for neuropathology, toxicology and high quality RNA.
Results: First and foremost, there are a number of previously unknown transcripts for each of these genes. Moreover, in every instance there are transcripts that are preferentially expressed in fetal human brain. Second, normal brain development is associated with switching from transcripts that are preferentially expressed in fetal human brain to transcripts that are preferentially expressed postnatally. For example, this is clearly the case with GAD 25 and GAD67, two transcripts expressed by GAD1. Similarly, the switch from GABA being excitatory to inhibitory is mediated by relative ratios that favor NKCC1 prenatally to ones that favor KCC2 postnatally. Moreover, both switches are associated with rs3749034, a SNP in the GAD1 promoter associated with increased risk for schizophrenia ($p < .05$ for GAD25 and GAD67 and $p < .004$)). A second SNP in NKCC1, rs3087889, is also associated with increased risk for schizophrenia and a novel transcripts for NKCC1 ($p < .03$). Moreover, it appears as if there are differences in expression in a number of transcripts for each of these genes especially NKCC1 and KCC2 that confer neuroanatomical and diagnostic specificity with regard to both schizophrenia and affective disorders ($p < .05$).
Conclusions: Human brain development in both PFC and hippocampus is characterized by numerous alternative transcripts whose expression switch dramatically both pre- and postnatally. A number of these changes are associated with genetic variation some of which are associated with increased risk for schizophrenia. Lastly, there is neuroanatomical and diagnostic specificity for both schizophrenia and affective disorders arguing against those that think these are one illness.
Disclosure: J. Kleinman, Nothing to Disclose.

1.2 Immature Dentate Gyrus as a Candidate Endophenotype of Neuropsychiatric Disorders

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Background: Adequate maturation of neurons is crucial for normal cognitive functions and emotional behaviors, with disruptions of this process likely to result in mental health perturbation. Previously, we reported that mice heterozygous for a null mutation of alpha-CaMKII, a key molecule in synaptic plasticity, have profoundly dysregulated behaviors, including hyper-locomotor activity and a severe working memory deficit, which are endophenotypes of schizophrenia and other psychiatric disorders. We found that almost all the neurons in the dentate gyrus (DG) of these mutant mice fail to mature at molecular, morphological and electrophysiological levels. We named this phenotype 'immature dentate gyrus (iDG)'.
Methods: To identify the additional strains of mutant mice that potentially possess the iDG phenotype, we screened approximately 120 additional lines for behavioral phenotypes similar to the ones seen in alpha-CaMKII heterozygous KO mice. We then analyzed gene expression patterns in the dentate gyrus of the mice showing analogous behavioral phenotypes, as well as more detailed analysis using immunohistochemical, electrophysiological and bioinformatics techniques. We also examined the dentate gyrus of non-mutant mouse models displaying analogous behavioral profiles (including chronic fluoxetine and single-dose pilocarpine administration) for iDG, as well as post-mortem brains from schizophrenia and bipolar disorder patients.
Results: We identified several other strains of mutant mice that have a phenotype strikingly similar to iDG, including forebrain-specific calcineurin knockout mice, mice lacking the transcription factor Schnurri-2 (Shn-2), and mice with a point mutation in the SNAP-25 gene. We also found that chronic fluoxetine treatment or a single pilocarpine administration induced 'dematuration,' resulting in an iDG-like phenotype in wild-type mice. Most of the strains of the mice with iDG phenotype have decreased parvalbumin, GAD67 and oligodendrocyte markers. Moreover, gene- and protein expression patterns in the brains of these mice were similar to those found in the post-mortem brains of

the patients of psychiatric disorders, including schizophrenia and bipolar disorder. The brains of iDG mice show mild chronic inflammation, distinct from typical acute inflammation, as revealed by bioinformatics analyses. Anti-inflammatory drugs reversed the cellular/molecular iDG phenotype as well as some behavioral abnormalities in a subset of mutant mice. Surprisingly, the iDG phenotype of Shn-2 knockout mice was not observed at two weeks after birth but emerged after weaning, which may correspond to the human developmental period during which schizophrenia appears in early adulthood. Interestingly, iDG-like phenomena was seen in the post-mortem brains of the patients with schizophrenia and bipolar disorder.
Conclusions: The dentate gyrus is considered to play important role in the regulation of emotion, learning and memory, and the functional disturbance of this region could potentially underlie some symptoms in neuropsychiatric disorders. Thus, iDG represents a promising endophenotype of neuropsychiatric disorders such as schizophrenia, bipolar disorder and epilepsy. We discuss the potential implication of these findings in elucidating the pathophysiology of those neuropsychiatric disorders.
Disclosure: T. Miyakawa, **Part 1:** Advisor/Consultant for Astellas Pharma Inc.

1.3 Immature Neurons in Schizophrenia? Support from Investigations on Proteoglycan Expression

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Background: Converging lines of evidence indicate that chondroitin sulfate proteoglycans (CSPGs) regulate neuronal differentiation, maturation and migration during development. Their cellular pattern of expression and biochemical characteristics have been shown to change during development according to cell differentiation stages. During late postnatal development, CSPGs organize into specialized extracellular matrix structures, ie perineuronal nets (PNNs), surrounding distinct populations of neurons. PNN formation represents a key stage of neuronal maturation and culminates with the closure of critical periods of development. We have shown that PNNs are markedly reduced in the medial temporal lobe of subjects with schizophrenia (SZ). PNN decreases suggest that the neuronal population predominantly associated with them, ie GABAergic neurons expressing parvalbumin, may not have reached full maturity in this disorder. To test the hypothesis that CSPG abnormalities may be associated with a disruption of neuronal maturation, we investigated CSPG expression in the olfactory epithelium (OE) of subjects with SZ. The OE is a continuously regenerating neural tissue where stem cells differentiate and mature into olfactory receptor neurons (ORNs) throughout adult life. Notably, the OE shows neuron lineage abnormalities in subjects with SZ. Among factors potentially contributing to these OE abnormalities, CSPGs represent compelling candidates.
Methods: CSPG expression analysis in OE from non-psychiatric control and SZ subjects in postmortem tissue and OE cell lines was performed using combinations of single and multiple antigen immunocytochemistry, ELISA, and computer-assisted microscopy. Cell lines derived from OE biopsies were tested for CSPG expression in differentiating, phenotypically identified, cells *in vitro*.
Results: 'Cytoplasmic' CSPG (c-CSPG) labeling was detected in sustentacular cells and some olfactory receptor neurons (*c*-CSPG + ORNs), while 'pericellular' CSPG (p-CSPG) labeling was found in basal cells and some ORNs (*p*-CSPG + ORNs). Dual labeling for CSPG and markers for mature and immature ORNs suggests that *c*-CSPG + ORNs correspond to mature ORNs, and *p*-CSPG + ORNs to immature ORNs. Numerical densities of *c*-CSPG + ORNs were significantly decreased in SZ (p *p*-CSPG + ORNs (110.71% increase) and CSPG + basal cells (53.71% decrease), did not reach statistical significance, the ratio of *p*-CSPG + ORNs/ CSPG + basal cells was significantly increased ($p = 0.03$) in SZ. *In vitro* studies have shown thus far a pattern of p-CSPG to c-CSPG expression during differentiation that corresponds to CSPG sulfation patterns and core protein transitions.
Conclusions: Our results suggest that ORN maturation involves a transition of CSPG distribution from the cell surface to the cytoplasm. This pattern is altered in subjects with SZ. CSPG abnormalities were found to affect prevalently putative mature ORNs and the ratio of immature ORN and basal cells expressing CSPGs. Together with previous results (Arnold *et al*., 2001) showing no change of mature ORNs in the OE of subjects with SZ, the present findings suggest that decreased densities of mature CSPG-positive ORNs reflect reduced CSPG expression in these cells. This reduction may be due to reduced synthesis and failure of these cells to fully transition to a mature pattern of CSPG expression. Together with findings in medial temporal lobe, these results are consistent with the hypothesis that maturation of distinct neuronal populations may be disturbed in SZ and point to CSPG abnormalities as a contributing factor.
Disclosure: S. Berretta, Nothing to Disclose.

Mini-Panel

2. Social Processes Initiative in Neurobiology of the Schizophrenia(s)

2.1 Altered Structural and Functional Network Topology in Deficit Schizophrenia

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Background: Intact social cognitive processes are critical for successful social functioning and are considered to be unique and powerful determinants of functional outcome above and beyond neurocognition. Network analysis of structural and functional brain imaging phenotypes has been useful in the identification of brain abnormalities in psychiatric disorders; however, schizophrenia is heterogeneous and results have been inconsistent. We thus examined networks of brain structure and resting state fMRI activity in a subgroup of patients with schizophrenia characterized by strong negative symptom burden and poor social functioning classified as having the 'deficit syndrome.'

Methods: Brain-wide inter-regional correlations in cortical thickness were examined in schizophrenia patients ranked in the top (deficit patients; $N=14$) and bottom (nondeficit patients; $N=14$) quartile of deficit scores and compared to matched healthy volunteers ($n=14$ per group). Networks were derived by thresholding correlation matrices ($p<0.05$ corrected) and node centrality measures were used to identify brain regions that play the most important role in network organization. A subgroup ($N=27$) of patients also completed resting state fMRI exams. We calculated spatial inter-correlations ('expression scores') among independent component templates (Biswal *et al*, 2010) and corresponding individual maps were obtained following dual regression analysis.
Results: Investigation of the structural neuroimaging data revealed a larger number of strong positive correlations in the network among deficit patients compared to healthy volunteers and nondeficit patients. This pattern yielded an overall network that had an increased density of connections. Investigation of brain-wide correlations revealed frontoparietal and frontotemporal correlations that were stronger in the deficit patients compared to the healthy volunteers and correlations with the cingulate gyrus that were stronger in the deficit group compared to the nondeficit group. Analysis of resting state fMRI measures indicated that among patients 'deficit score' correlated significantly ($P<0.05$) with expression scores. Specifically, more severe 'deficit scores' were associated with lower expression scores within cortical midline structures including the posterior cingulate, which is responsible for self-integration and a parieto-fontal network encompassing the parietal lobes, inferior frontal gyrus and posterior temporal lobe that partially overlapped regions found to be abnormal in the structural imaging analysis.
Conclusions: The increase in network density and decrease in resting state fMRI expression scores among patients classified as having the deficit syndrome may reflect decreased specificity and potentially decreased differentiation of brain regions. Many regions of increased centrality and lower resting state fMRI expression in the deficit network are part of the frontoparietal 'mirror neuron' system, which is important for emotion experience sharing. The posterior cingulate cortex is part of the cortical midline circuit that comprises the default mode network important for mental state attribution and correlations with this region distinguish deficit from nondeficit patients. Social cognitive processes that are impaired in the deficit form of schizophrenia and these alterations may serve as neurobiological correlates of this disease subtype.
Disclosure: P. Szeszko, Nothing to Disclose.

2.2 Network Topology in Deficit Schizophrenia, Nondeficit Schizophrenia, and Bipolar Sisorder: From Circuits to Functional Outcome

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Background: Recent data suggests substantial shared etiology for the major psychoses (schizophrenia and bipolar disorder). However, a subset of patients with schizophrenia (with the 'deficit' form of illness) may represent one end of a continuum of neurobiological and social impairment among patients with major psychoses. We sought to compare patients characterized by strong negative symptom burden and poor social function, who have been classified as 'deficit syndrome', to nondeficit patients with minimal negative symptom burden and bipolar disorder patients, using a brain network connectivity approach, and relate abnormal brain circuitry to impairment in social function.
Methods: Following high resolution structural magnetic resonance imaging, and diffusion tensor imaging, brain-wide inter-regional correlations in cortical thickness were examined in schizophrenia subjects ranked in the top ($n=18$ deficit subjects) and bottom ($n=18$ nondeficit subjects) quartile of deficit scores using the same approach as in the accompanying abstract by Szeszko *et al*. Then, $n=32$ deficit, $n=32$ nondeficit subjects, and $n=32$ healthy controls were combined from the Hillside and Toronto samples. In addition, $n=32$ bipolar subjects were compared to $n=32$ controls using the same network topology methods to investigate cortical thickness networks across the major psychoses. A subset of schizophrenia patients ($n=22$) completed the quality of life scale (QLS). Correlations with altered brain network structure in relation to social and functional outcome measures were examined.
Results: Deficit schizophrenia subjects demonstrated a larger number of strong positive correlations among cortical regions compared to individuals with nondeficit schizophrenia, bipolar disorder, or healthy controls subjects resulting in a network with increased density of connections. The network in the deficit subjects contained an increased number of highly central nodes such as the supramarginal, superior temporal and inferior frontal gyri (ie, fronto-parietal) that were less prominent in the nondeficit and control networks. In addition, highly central nodes were also found in cortical midline regions, specifically cingulate and parahippocampal gyrus. No network differences were detected between bipolar and matched healthy control groups. The different network structure identified was significant at Bonferroni corrected p value of 0.05. Mean diffusivity of the right arcuate fasciculus, the white matter tract connecting the fronto-parietal circuit was inversely correlated with the QLS interpersonal subscale ($r=-0.49$, $p=0.02$).
Conclusions: Deficit syndrome subjects demonstrated an increased network density compared to nondeficit subjects, bipolar subjects, and healthy controls, who all demonstrated similar network density. Increased network density in deficit subjects was particularly prominent in fronto-parietal and cortical midline circuitry. Fronto-parietal circuitry in particular has been related to social impairment in autism spectrum disorders. Our findings clearly demonstrate differences in cortical regions comprising fronto-parietal circuitry in deficit patients, who have severe social impairment compared to other patients with major psychoses, who are less socially impaired. We directly related impairment in this circuit to impairment in social function using the QLS. These interregional fronto-parietal and cortical midline alterations may serve as neurobiological correlates of a subgroup of especially socially impaired (deficit) people with schizophrenia. The fronto-parietal circuit should be considered as a useful biomarker and

treatment target of impaired social function in people with schizophrenia spectrum disorders.
Disclosure: A. Voineskos, Nothing to Disclose.

2.3 The Neural Circuitry of Social Impairments in Schizophrenia Spectrum Disorders

Robert W. Buchanan*

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Background: People with schizophrenia spectrum disorders (SSDs) are characterized by marked impairments in social function. These impairments severely impact quality of life, and predict relapse, poor illness course, and unemployment. Over the past decade, considerable progress has been made in delineating the social cognitive processes that underlie these impairments. However, much less is known about the neural circuitry that supports these processes. The NIMH RDoC initiative provides a framework for evaluating the neural basis of social cognition. In the context of the RDoC Systems for Social Processes Domain: the Perception and Understanding of Others, the right fronto-parietal network has been hypothesized to subserve 'lower-level' social cognitive processes necessary for understanding the actions and basic emotions of others, and the cortical midline circuit, has been hypothesized to subserve 'higher-level' processes necessary for understanding the perspective of others (theory of mind). In order to evaluate the hypothesized involvement of the fronto-parietal network in social function impairments, we have conducted diffusion tensor imaging (DTI) studies in three independent samples to examine this circuit in people with the deficit form of schizophrenia, a form of schizophrenia characterized by impaired social function.
Methods: Study 1: Twenty participants with DSM-IV schizophrenia or schizoaffective disorder (deficit: $n = 10$ and nondeficit: $n = 10$) and 11 healthy subjects participated in this study. Study 2: Thirty-six participants with DSM-IV schizophrenia (deficit: $n = 18$ and nondeficit: $n = 18$) and 18 healthy subjects participated in this study. Study 3: Fifty-one participants with DSM-IV schizophrenia (deficit: $n = 14$ and nondeficit: $n = 37$) participated in this study. DTI was used to assess the integrity of the superior longitudinal fasciculus (SLF), the major white matter tract connecting the frontal and parietal lobes.
Results: Study 1: There was a significant FA group difference for the right hemisphere SLF ($F = 3.6$ $df = 2,27$; $p = 0.04$). The deficit group had lower FA than the healthy control group ($p < 0.05$) lower FA compared to the nondeficit group within the right and left SLF, genu of the corpus callosum, splenium of the corpus callosum, posterior cingulate and right and left inferior longitudinal fasciculus.
Conclusions: The finding of lower FA in the right SLF/arcuate fasciculus in three independent samples is consistent with the hypothesis that disruptions in white matter integrity hypothesized to subserve 'lower-level' social cognitive processes play a role in the pathophysiology of the deficit form of schizophrenia.
Disclosure: R. Buchanan, **Part 1:** Advisory Boards: Abbott; Amgen; Janssen Pharmaceutical, Inc.; NuPathe; Pfizer; Roche; Takeda, Consultant: Abbott; Amgen; Bristol-Myers Squibb; EnVivo; Omeros; Pfizer, DSMB: Otsuka; Pfizer.

Panel

3. Autism Spectrum Disorders: From Rare Chromosomal Abnormalities to Common Molecular Targets

3.1 Comprehensive Phenotyping of Mouse Autism Models

Ted Abel*

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Background: Despite the increasing prevalence of autism, developing novel therapeutic approaches to treat this neurodevelopmental disorder has remained a challenge. Behavioral deficits observed in autism and autism spectrum disorders (ASD) span at least four distinguishable symptom clusters: physiological and neurological health; arousal, anxiety, and regulatory systems; social interaction and communication; behavioral inflexibility and cognition. Recent identification of copy number variants associated with autism provides the opportunity to model aspects of this disorder in genetically modified mouse models. A challenge, however, is to develop appropriate, objective behavioral measures that are easily adapted to various labs so that results can be compared across laboratory environments and different mouse models.
Methods: We have developed a novel, automated, high-resolution video analysis software system that enables us to score large amounts of behavioral data, including activity, sleep/wake states, response to novelty, and social interactions. Using these approaches, we have examined the impact of the hemizygous deletion mutants $16p11.2^{del/+}$ on a B6/129 F1 background. We use recently developed algorithms for the high-throughput analysis of video of home cage behaviors and during behavioral tasks to define objectively and quantitatively the impact of these mutations on a battery of behaviors that measure physiological and neurological health; arousal, anxiety, and regulatory systems; social interaction and communication as well as behavioral inflexibility and cognition in three age groups (before, during, and after puberty).
Results: Phenotypic data from the characterization of many lines of genetically modified mice is limited by the use of low-resolution video or infrared beam-break detection systems and by the need to perform labor-intensive manual scoring of the data. We have developed automated approaches to analyze high-resolution video data to characterize a variety of behaviors in mice. We have applied this to genetically modified mice, to models of traumatic brain injury and to models of neurodevelopmental disorders such as autism. We will describe our phenotyping approach (termed 'autotyping') and present our findings on the behavioral impact of the hemizygous deletion in $16p11.2^{del/+}$ mutant mice. Recurrent copy number variation has been linked to a number of neurodevelopmental and

neuropsychiatric disorders. There is particular interest surrounding the 16p11.2 region because copy number variations have been associated with various disorders. Duplication of this region is associated with autism and schizophrenia, whereas deletion of this region is associated with autism. Mouse models were created carrying deletions of the 16p11.2 region (which corresponds to a region on mouse chromosome 7) have been created (Horev *et al*., 2011, PNAS). In our initial studies of 16p11.2$^{del/+}$ mutant mice, we have observed cognitive deficits consistent with altered extinction and behavior flexibility, consistent with some of the cognitive impairments observed in individuals with autism. Using a systems-level approach with more advanced statistical methods, known as principle component analysis, linear discriminate analysis and support vector machines we are identifying combinations of behavioral features that are altered in this genomic deletion model and defining the relationship between the altered behaviors.
Conclusions: Our video-based analysis of mouse behavior enables us to examine complex behavioral phenotypes and define how specific components of these behaviors are altered in mouse models of neurological, psychiatric and neurodevelopmental disorders. We have used this approach to define the impact of hemizygous deletion of genes in the 16p11.2 region of the human genome in mouse models, identifying alterations in behavioral inflexibility and cognition.
Disclosure: T. Abel, **Part 2:** Editor, Neurobiology of Learning and Memory, Elsevier.

3.2 Role of Copy Number Variants in Autism Spectrum Disorders

Santhosh Girirajan*

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Background: Recent studies have suggested a significant role for copy number variants in autism spectrum disorders (ASD) and intellectual disability.
Methods: We analyzed CNV data from individuals with a broad range of developmental disabilities including autism spectrum disorders, intellectual disability, and dyslexia.
Results: Several themes have emerged from these studies comparing the size, distribution, and frequency of CNVs in affected children to those in unaffected controls. First, syndromic disorders including those associated with syndromic ASDs (such as del22q11.2, del22q13, dup7q11.23, and dup17p11.2) associated with congenital malformation syndromes could be distinguished from those with non-syndromic ASD on the basis of the total number of large CNVs and whether the variants are inherited or *de novo*. Second, comparison of CNV data from individuals with dyslexia, ASD, intellectual disability and congenital malformation suggested a strong correlation of total CNV load with the neurological severity of the disorder; individuals having ASD and intellectual disability show increased base pairs of copy number change compared to those having ASD without intellectual disability. Third, comparison of genetic and clinical data from individuals with ASD and severe intellectual disability suggest etiological specificities. For example, a significant association of 1q21.1 duplications with ASD compared to intellectual disability. We also found that as the size of deletions increased, nonverbal IQ significantly decreased, with no impact on the severity of autism features; and as the size of duplications increased, autism severity significantly increased without any impact on nonverbal IQ. Further, a significant increase in base pairs of duplications, but not deletions, was associated with ASD.
Conclusions: These data suggest that a gradation of gene dosage alterations sensitizes individuals to different diseases and that disruption of multiple genes interacting in molecular pathways contribute to the genetic and phenotypic heterogeneity.
Disclosure: S. Girirajan, Nothing to Disclose.

3.3 Tbx1 and Sept5 Contribute to Behavioral and Neuronal Phenotypes in Mouse Models of 22q11.2-Associated ASD

Noboru Hiroi*

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Background: Individuals with 22q11.2 copy number variation (CNV) exhibit high rates of autism spectrum disorder (ASD) and other developmental neuropsychiatric disorders. However, as 22q11.2 CNV minimally includes ~30 genes, specific 22q11.2 genes whose gene-dose alteration causes these disorders cannot be ascertained in humans. We used mouse models and identified a 200-kb region, including *Gnb1l*, *Tbx1*, *Gp1Bb*, and *Sept5*, whose gene-dose alteration causes behavioral phenotypes related to ASD.
Methods: To further evaluate the roles of specific genes within this region, we tested congenic *Tbx1* heterozygous (HT) mice, congenic *Sept5* homozygous (KO) mice, *Sept5* KO mice on two genetic backgrounds, their wild-type (WT) littermates, and C57BL/6J mice that virally over-expressed Sept5 in limbic regions in a battery of behavioral assays. Moreover, we used postnatal neural progenitor cells (pNPCs) derived from the hippocampal dentate gyrus to evaluate the impact of *Tbx1* knockdown by siRNA on proliferation of pNPCs *in vitro*.
Results: Congenic *Tbx1* HT mice exhibited lower levels of social interaction, altered sequences of vocal calls, lower levels of working memory-based behavioral alternation and higher levels of repetitiveness, compared to WT mice. *Sept5* homozygosity selectively reduced levels of social interaction, but this effect was modified by genetic background; conversely, over-expression of Sept5 in the hippocampus or the amygdala elevated levels of social interaction. Tbx1 siRNA reduced the rate of proliferation of pNPCs.
Conclusions: Our data suggest that (1) *Tbx1* and *Sept5* in limbic structures contribute to distinct behavioral phenotypes related to ADS and other developmental neuropsychiatric disorders, (2) phenotypic expression of *Sept5* deficiency is modified by genetic background, and (3) *Tbx1* deficiency reduces the rate of proliferation of pNPCs in the hippocampus.
Disclosure: N. Hiroi, Nothing to Disclose.

3.4 The Translation of Translational Control in Autism Spectrum Disorders

Eric Klann*

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Background: A requirement for *de novo* protein synthesis is one of the hallmarks of long-lasting synaptic plasticity and long-term memory. An increasing number of studies, including several from our laboratory, have identified signaling cascades, including the mTORC1 signaling pathway, that couple neurotransmitter and neurotrophin receptors to the translation regulatory machinery during the formation of long-lasting synaptic plasticity and the consolidation of long-term memory. Interestingly, mutations in negative upstream regulators and downstream effectors of mTORC1, including fragile X mental retardation protein (FMRP) and the eukaryotic initiation factor 4E (eIF4E) are associated with several types of developmental disability and autism spectrum disorder (ASD).
Methods: FXS model mice and eIF4E transgenic (eIF4E Tg) mice were examined for exaggerated protein synthesis and altered translational control. First, we identified the altered translational control mechanisms in the brains of FXS and eIF4E Tg mice. Then we utilized genetic and pharmacological approaches to target the translational control molecules of interest, including eukaryotic initiation factor 4F (eIF4F) and p70 S6 kinase 1 (S6K1).
Results: We found that genetic reduction of S6K1 in FXS model mice corrected exaggerated protein synthesis, abnormal synaptic plasticity, and multiple aberrant behaviors. We currently are determining whether treating FXS model mice with an inhibitor of S6K1 can reverse the aforementioned phenotypes. In addition, we have found that compounds that target eIF4F can reverse exaggerated protein synthesis and synaptic dysfunction in eIF4E Tg mice. Moreover, the eIF4F inhibitors reverse ASD-associated behaviors displayed by eIF4E Tg mice, including repetitive behaviors, behavioral inflexibility, and abnormal social behavior. We currently are determining whether the compounds that target eIF4F have similar effects on aberrant behaviors displayed by FXS model mice. In addition, we are determining the localization of the molecular and cellular alterations in the striatum that might explain the repetitive behaviors and behavioral inflexibiltiy of the eIF4E Tg mice.
Conclusions: Our studies strongly suggest that exaggerated protein synthesis in mice triggers synaptic dysfunction and aberrant behaviors that are associated with ASD. These studies have revealed important links between abnormal translational control and synaptic dysfunction, as well as behaviors associated with ASD. Finally, these studies have provided insight into the molecular basis of certain types of developmental disability and ASD, and have identified a novel class of targets for the development of therapeutics for the treatment of individuals with ASD.
Disclosure: E. Klann, **Part 1:** I am a consultant for Takeda Pharmaceuticals. My wife is employed by Takda Pharmaceuticals., **Part 2:** Consultant for Takeda Pharmaceuticals., **Part 3:** Consultant for Takeda Pharmaceuticals during sabbatical of 2012–13 academic year.

Panel

4. Can Biology Inform Treatment Prediction and Selection in Depression?

4.1 Brain Serotonin 1A Receptor Binding as a Predictor of Treatment Outcome in Major Depressive Disorder

Ramin V. Parsey*

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Background: Psychiatrists currently lack tools that predict antidepressant response to specific treatments for major depressive disorder (MDD). The serotonin 1A ($5\text{-}HT_{1A}$) receptor has been implicated in the pathophysiology of MDD in both animal models and human studies. We previously reported higher $5\text{-}HT_{1A}$ receptor binding in two cohorts of subjects with MDD during a major depressive episode (MDE) using positron emission tomography (PET) imaging with [^{11}C]WAY-100635. This is a state phenomenon, not trait, as we have demonstrated that the effect persists in subjects who are remitted and off of medication. We have also shown that $5\text{-}HT_{1A}$ binding is also associated with treatment outcome after non-standardized antidepressant treatment. Most recently, we examined whether pre-treatment $5\text{-}HT_{1A}$ binding is associated with treatment outcome following standardized escitalopram treatment in MDD. We also compared $5\text{-}HT_{1A}$ binding between all MDD subjects in this cohort and a sample of healthy controls.
Methods: 24 MDD subjects in a current MDE underwent PET scanning with [^{11}C]WAY-100635, acquiring a metabolite-corrected arterial input function and free-fraction measurement to estimate $5\text{-}HT_{1A}$ binding ($BP_F = B_{max}/K_D$, where B_{max} = available receptors and K_D = dissociation constant). Binding estimates were weighted according to their measurement precision, using standard errors estimated by a bootstrapping algorithm incorporating errors associated with fitting the metabolite curve, input function, and time activity curve (TAC) for each region of interest (ROI). MDD subjects then received eight weeks of treatment with escitalopram, initiated at 10 mg, and increased to 20 mg at four weeks for non-responders (those with <10.
Results: 46% of subjects achieved remission following eight weeks of escitalopram. Remitters to escitalopram had 33% higher baseline $5\text{-}HT_{1A}$ binding in the raphe nuclei than non-remitters ($p = 0.047$, $p = 0.033$ with covariates). Across 12 cortical and subcortical regions, $5\text{-}HT_{1A}$ binding did not differ between remitters and non-remitters ($p = 0.86$). $5\text{-}HT_{1A}$ binding was higher in MDD than historical controls across all regions ($p = 0.0003$), a triplication of our finding. Remitters did not differ from non-remitters in several relevant clinical measures.
Conclusions: Elevated $5\text{-}HT_{1A}$ binding in raphe nuclei is associated with subsequent remission with the SSRI escitalopram; this is consistent with data from a separate cohort receivingnaturalistic antidepressant treatment. We confirmed our previous findings of higher $5\text{-}HT_{1A}$ binding

in current MDD compared to controls. Differences with previous findings using different PET outcome measures are discussed.
Disclosure: R. Parsey, Nothing to Disclose.

4.2 Inflammatory Biomarkers Predict Differential Outcome of Depression Treatment with Escitalorpam and Nortriptyline in the GENDEP Project

Rudolf Uher*

Dalhousie University, Halifax, Nova Scotia

Background: The Genome-based Therapeutic Drugs for Depression (GENDEP) project aims to identify predictors of differential response to the serotonin-reuptake inhibiting antidepressant escitalopram and the norepinephrine-reuptake inhibiting antidepressant nortriptyline. We have previously reported the predictive value of symptom dimension of interest and activity, genetic and transcriptomic data, including variation in immunity-related genes (IL11, IL1-beta, TNF-alpha). Here we explore the value of an easily accessible blood biomarker of inflammation: the C-reactive protein.
Methods: We measured the levels of C-reactive protein (CRP) with high-sensitivity nephelometry in peripheral blood samples from 230 treatment-seeking individuals with major depressive disorder who were randomly allocated to treatment with protocol-guided escitalopram ($n=110$) or nortriptyline ($n=120$) for 12 weeks. Blood samples were obtained at baseline, before any study treatment was administered. Severity of depression was measured weekly with the Montgomery-Asberg Depression Rating Scale.
Results: Higher levels of high-sensitivity CRP predicted worse response to escitalopram but not nortriptyline, resulting in a highly significant CRP-by-drug interaction ($b=-1.76$, 95%CI -2.63 to -0.89, $p=0.0001$). Blood levels of CRP in interaction with drug explained 4% in the variation of whether remission was achieved over the 12 weeks of treatment: an effect that is statistically, but not clinically significant. In combination with the symptom dimension of loss of interest and decreased activity, CRP levels explained 6.4% of variation in remission. This combined prediction reaches the simulation-based criterion for clinical significance.
Conclusions: Inflammatory markers in peripheral blood in combination with specific symptom dimensions may clinically meaningfully predict the outcomes of treatment with antidepressants. Individuals with high levels of information may benefit from the noradrenergic nortriptyline more than from serotonin reuptake inhibitor.
Disclosure: R. Uher, **Part 1:** Dr Uher co-chairs a steering board of a research project initiated and funded by Bristol Myers Squibb and collaborates with Pfizer, Glaxo-Smith Kline and Roche as part of the European Union Innovative Medicine Initiative funded NEWMEDS project. Dr Uher has received no personal income from any pharmaceutical or biotech industry and holds no equity in companies active in medicine, pharmaceuticals or biotechnology.

4.3 Initial Results of the NIMH-funded EMBARC Study

Madhukar Trivedi*

UT Southwestern Medical Center, Dallas, Texas

Background: Due to the variable symptom presentation and biological heterogeneity of depression, it is unlikely that a single clinical or biological marker can guide treatment selection. Two types of biosignatures are needed to achieve improved outcomes: (1) biosignatures to maximize the selection of optimal treatment for individual patients at beginning of treatment (moderators) and (2) biosignatures to identify indicators of eventual outcomes early in treatment (mediators). Establishing Moderators and Biosignatures of Antidepressant Response for Clinical Care (EMBARC) aims to address both of these points and this approach has great potential to personalize treatment and to begin to characterize the biology of treatment response. The goal of this multi-site study (conducted across 4 sites) is to link a range of imaging, electrophysiological, blood based markers as well as behavioral/cognitive tasks to treatment response. EMBARC is designed to recruit 400 subjects in a two-step placebo-controlled RCT.Understanding the stability of the identified measures in the form of test-retest reliability and comparability across multiple sites of these measures is critical since detection of meditational effects is dependent on the ability to measure differences related to changes early in treatment.
Methods: As a first step in conducting EMBARC we have completed a reliability study evaluating the performance of the full range of biomarkers in 40 healthy control subjects. This study was designed to provide reliability information (ie, does a given biomarker or test produce the same result each time it is given if there is no change in the subject) and comparability information (ie, does a given assessment or test produce the same result at each site if given to the same subject) about the measures proposed for the main EMBARC study. Measures include: a comprehensive clinical phenotype; cortical thickness—assessed through structural magnetic resonance imaging MRI); Diffusion tensor imaging (DTI) to assess cortical white matter tract integrity; Functional magnetic resonance imaging (fMRI) to test connectivity and brain activation patterns in response to both emotional conflict and reward-dependent learning tasks; pulsed arterial spin labeling (ASL) to measure regional cerebral blood flow; Electroencephalography (EEG) to assess cortical and subcortical brain activation patterns, using advanced EEG processing techniques, collected at both resting state and via loudness dependency of auditory evoked potentials (LDAEP); Behavioral neuropsychological tasks to include reaction time, motor processing speed, cognitive control, working memory, and reward conditioned learning.; DNA, mRNA, and plasma protein samples, before, during and after treatment.
Results: Data have been collected from 40 healthy control subjects tested at each of the 4 EMBARC-HC study sites (up to 10/site). These subjects have no current or past history of psychiatric disorders and have no first degree relative with a current diagnosis or history of mood disorders. In addition we have also conducted analyses to assess comparability across the 4 sites with 3 subjects from each site tested at

each of the other participating sites for a total of 12 travelling subjects.
Conclusions: Results of the reliability assessments indicate that the use of rigorous quality control procedures across sites allows for larger multi-site trials to use complex procedures to assess the biological and psychological underpinnings of treatment response.
Disclosure: M. Trivedi, **Part 1:** Madhukar H. Trivedi is or has been an advisor/consultant to, Alkermes, AstraZeneca, Bristol-Myers Squibb Company, Cephalon, Inc., Cerecor, Concert Pharmaceuticals, Inc., Eli Lilly & Company, Evotec, GlaxoSmithKline, Janssen Global Services, LLC, Janssen Pharmaceutica Products, LP, Johnson & Johnson PRD, Lundbeck, MedAvante, Medtronic, Merck, Naurex, Neuronetics, Otsuka Pharmaceuticals, Pamlab, Pfizer Inc., Rexahn Pharmaceuticals, Ridge Diagnostics, Roche Products Ltd., Sepracor, SHIRE Development, Sierra, Sunovion, Takeda, Targacept, Transcept, and Wyeth-Ayerst Laboratories., **Part 4:** Dr. Trivedi has received research support from: Agency for Healthcare Research and Quality (AHRQ), National Alliance for Research in Schizophrenia and Depression, National Institute of Mental Health, National Institute on Drug Abuse.

4.4 Large-scale Pre-treatment Prediction of Remission with Antidepressants for Individual Patients Based on Cognitive and Emotional Test Performance, as well as Its Neuroimaging Correlates

Amit Etkin*

Stanford University, Stanford, California

Background: Antidepressant medications are one of the most frequently prescribed classes of medications, yet only about one-third of patients achieve remission, and there is no predictive test available in routine practice for guiding choice of which medication is likely to optimize remission for each patient.
Methods: We studied 1,008 adult outpatients with non-psychotic major depressive disorder from the multi-site biomarker-coupled International Study to Predict Optimized Treatment in Depression (iSPOT-D). Medication-free patients were assessed at pre-treatment baseline using a battery of computerized behavioral tests of core cognitive and emotional capacities implicated in depression, and randomized to receive sertraline, escitalopram or venlafaxine extended-release for eight weeks. A pattern classification approach was used to predict symptom remission by patient report on the Quick Inventory of Depressive Symptomatology (QIDS-SR16) and by clinician assessment with the Hamilton Rating Scale for Depression (HRSD17), as well as real-world functional capacity using the Social and Occupational Functioning Assessment Scale (SOFAS). All treatment prediction classifiers controlled for baseline depression severity, age, education, gender and site, and results were verified through cross-validation. Given that the rate of remission in current practice is no more than chance level, we set a criterion of 60% as a clinically meaningful improvement (10%) on existing outcomes. Functional MRI data was acquired on 10% of the total sample and activation was analyzed in working memory and response inhibition (Go-NoGo) tasks in order to determine the neural correlates of treatment prediction.
Results: Prediction using behavioral data was robust when we quantified patient heterogeneity by pre-treatment cognitive and emotional performance, and not for the entire patient sample. For the one-third of patients who showed poor performance pre-treatment (0.5–1.0 standard deviations below the healthy norm), their performance was a robust predictor of treatment outcomes. Symptom remission was predicted with 75% accuracy, and functional capacity status with 73% accuracy. These predictive classifiers significantly differentiated remission rate outcomes between study medications, especially sertraline versus escitalopram or venlafaxine. For the neuroimaging data, dorsolateral prefrontal activation during response inhibition predicted symptom remission while insula activation during working memory predicted functional capacity remission.
Conclusions: Clinically-meaningful pre-treatment prediction of remission was achieved using the behavioral data, primarily for those patients with depression who are also characterized by objective impairments in cognitive and emotional performance. The neuroimaging data provided additional insight into the neural correlates of treatment prediction. These findings can be used to guide antidepressant choice, according to individual patient characteristics and reflect a fundamental advance in the personalization of antidepressant treatment.
Disclosure: A. Etkin, Nothing to Disclose.

Panel

5. Circuitry Underlying Obsessive-Compulsive Disorder: Lessons from Deep Brain Stimulation and Ablative Surgery

5.1 Ablative Limbic System Surgery for the Treatment of OCD

Emad Eskandar*

Harvard Medical School, Boston, Massachusetts

Background: Cingulotomy is a treatment option for patients with chronic severe and medically intractable obsessive-complusive disorder OCD.
Methods: This is a retropstective review of the largest series to date of patients with OCD treated with cingulotomy.
Results: Long-term data demonstrates that ablative limbic system surgery can offer and a significant and durable benefit in patients with severe and medically intractable obsessive-compulsive disorder. At the most recent follow-up (mean 63.8 months), response rates following limbice system surgery (cingulotomy or llimbic leukotomy) were 47% and 22% for full and partial responses, respectively.
Conclusions: Ablative limbic system surgery is a safe and effective option for the treatment of chronic severe and medically intractable obsessive-complusive disorder.
Disclosure: E. Eskandar, Nothing to Disclose.

5.2 DBS of Ventral Striatum in Rodents Modulates Fear Extinction via Prefrontal and Orbitofrontal Projections

Gregory J. Quirk*

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

Background: Many OCD patients exhibit compulsive behaviors that they believe protect them (or others) from imminent danger. These compulsions can be viewed as persistent avoidance responses to perceived threats, suggesting deficient fear extinction. Deep brain stimulation (DBS) of the ventral capsule-ventral striatum (VC/VS) reduces compulsive behaviors in OCD, but little is known about the mechanism. DBS of the ventral striatum (VS) in rats during extinction training can either strengthen or weaken extinction memory, depending on the specific site within the VS. Ventral-VS DBS weakens extinction, whereas dorsal-VS DBS strengthens extinction and induces plasticity in extinction circuits (Rodriguez *et al*., 2012). The marked differences between VS sites suggest that these regions contain distinct sets of myelinated fibers from cortex that are modulated by DBS.
Methods: To test this hypothesis, we injected retrograde tracer WGA into the dorsal-VS or ventral-VS, and measured neuronal labeling in cortical areas using stereological techniques. Acceptable injection sites were analyzed for 4 rats (2 per site). Labeling was assessed in prelimbic (PL), infralimbic (IL), medial orbital (MO), ventrolateral orbital (VLO), and agranular insular (AI) cortices.
Results: There was a robust projection from PL through both VS areas, but thee was no difference between sites (dorsal-VS: 46%; ventral-VS: 44%, $p = 0.64$). MO and VLO projected more through the dorsal-VS site than the ventral-VS site (MO: 22 *vs* 10%; VLO 23 *vs* 3%, $p < 0.05$). In contrast, IL and AI projected significantly more through the ventral-VS site than the dorsal-VS site (IL: 20 *vs* 2%; AI: 19 *vs* 3%, $p < 0.05$). Thus, MO and VLO are likely to be involved in extinction facilitation by DBS, whereas IL and AI are likely to be involved in extinction impairment.
Conclusions: Physiological studies have suggested that DBS of VS can reduce activity in OFC neurons, by driving recurrent inhibition though antidromic activation of corticostriatal axons (McCracken and Grace, 2007). Our anatomical findings are consistent with this idea, because IL inhibition impairs extinction (Sierra-Mercado *et al*., 2010), and IL axons were more abundant in the ventral-VS site. In contrast, fibers from MO and VLO were more abundant in the dorsal-VS site, suggesting that inhibition of these areas would strengthen extinction. We are currently testing this hypothesis. Inhibition of orbitofrontal areas with DBS is consistent with the orbitofrontal hyperactivity seen in OCD (Ursu and Carter, 2009).
Disclosure: G. Quirk, Nothing to Disclose.

5.3 Deep Brain Stimulation for Intractable OCD: Population and Outcomes

Benjamin Greenberg*

Alpert Medical School of Brown University, Providence, Rhode Island

Background: We present data from our ongoing NIMH-supported multicenter controlled trial of DBS for OCD, including: (1) a study of the OCD subgroup meeting DBS criteria, (2) Open phase clinical outcomes to date, and (3) fear conditioning and extinction in OCD patients receiving and not receiving DBS, testing the hypothesis that abnormal fear extinction contributes to OCD and may represent a therapeutic target for DBS.
Methods: (1) Population. We estimated the proportion of DBS candidates in a treatment-seeking OCD sample via stepwise application of key entry criteria to baseline data from the NIMH-funded Brown OCD Longitudinal Study ($n = 325$ adults).

2) DBS. After rigorous multidisciplinary assessments, 15 (of 30 planned) patients had model 3387 VC/VS leads implanted bilaterally, attached to Activa DBS stimulators (Medtronic, Inc). The target has become better defined after clinical experience and considering 3D pathway models derived from nonhuman primates. Patients underwent preoperative targeting and research MRIs, intra- and postoperative macrostimulation, and masked active or sham DBS for 3 months, followed by open DBS.

3) Fear Extinction. At presurgical baseline and 3 and 6 months post-implantation, DBS patients underwent a fear conditioning and extinction paradigm. Two additional OCD groups ($N = 21$ and 36, respectively, plus healthy controls) also underwent fear conditioning and extinction.
Results: (1) Population. Only 0.6% of 325 treatment-seeking patients met DBS criteria, usually because behavioral and medication therapies had not been exhausted (Garnaat *et al*, in press).

2) DBS. Open phase YBOCS OCD severity 6 months after implantation (post 3–6 months of active DBS) showed full response (>35% severity reduction) in 6/15 (40%), partial response (25–35% reduction) in 2/15 (13%) and nonresponse (<35% reduction) in 7/15. At 12 months (which 13 patients have reached) full, partial, and nonresponse rates were 5/13 (38.5%), 3/13 (23%) and 5/13 (38.5%), respectively. The adverse effect profile was as expected: reversible hypomania, infection, and device failure/loss of efficacy. Data from the masked phase will be analyzed on the full sample.

3) Fear Extinction. 21 nonsurgical OCD patients showed a deficit in extinction recall (ER), plus lack of recruitment of ventromedial PFC on fMRI during ER testing (Milad *et al*., in press). We also found an ER deficit in a lifetime OCD group ($N = 36$), whether or not they were above the DSM-IV OCD diagnostic threshold at test (in preparation). In contrast, preliminary analysis of fear extinction paradigm data before and after active DBS for 3–6 months ($N = 4$) suggests fear expression was reduced throughout the paradigm.
Conclusions: DBS may be of benefit in the small proportion OCD cases appropriate for invasive treatments. Our open phase trial data suggest outcomes comparable to those after prior studies of DBS or lesions to the same circuitry (in half or more of patients). We found and replicated defective fear extinction retention in 'nonsurgical' OCD cases. However, in the small sample tested to date we found that DBS might reduce fear expression during extinction as well as extinction retention, something also seen in rodents undergoing DBS-like stimulation in a fear extinction paradigm.
Disclosure: B. Greenberg, **Part 1:** Meeting Travel Expense, Medtronic Inc, **Part 4:** Research Grant Support, Hoffman-LaRoche, Inc.

5.4 The Circuitry of Deep Brain Stimulation and Cingulatomy: Monkey Tracing *vs* Human Tracking

Suzanne Haber*

University of Rochester, Rochester, New York

Background: The 2 cortical regions most associated with obsessive compulsive disorder (OCD) are the orbital prefrontal cortex (OFC) and the dorsal anterior cingulate cortex (dACC). Deep brain stimulation, that targets OFC fibers through the internal capsule (IC) and cingulatomy that targets the cingulate cortex and underlying cingulum bundle, are two neurosurgical therapeutic approaches for treatment resistant OCD. Each of these approaches target different connectivities, involving a different combination of brain regions. Yet both procedures are effective. This raises several questions: Are these 2 cortical regions directly connected; do they have common connections to other cortical areas; and what specific connectivities are involved at each site? Based on monkey tracing experiments we first determined the specific connections between the OFC and dACC, connections with other cortical areas, and the connections that pass through each target. We then tested how accurately we could demonstrate those pathways in the neurosurgically treated-patients.
Methods: Tracing methods were used to define the projection pathways between cortical areas and from them through the internal capsule and cingulum bundle in monkeys. We used dMRI merged with structural MRIs and CT scans from DBS or cingulatomy patients. We placed ROI seeds specific to each patient's electrode contacts or lesion to determine the connections of those targets.
Results: There are direct connections between the dACC and medial OFC, but not lateral OFC. The cingulum bundle carries cingulate to cingulate fibers; cingulate fibers to other cortical areas; other cortical fibers to cingulate cortex, other cortical fibers to non-cingulate cortex, and subcortical fibers to both cingulate and non-cingulate cortex. We previously showed the organization of OFC and dACC fibers through the IC. Using tractography in patients with DBS electrodes or cingulatomies, we found that several of these pathways could be accurately demonstrated in patients. Cingulatomies target a region of the bundle that carries not only cingulate fibers, but also several connections that do not originate or terminate within the cingulate cortex. In contrast, the internal capsule target for DBS captures a combination of vmPFC, OFC, dACC, and vlPFC fibers ascending and descending fibers, but not direct cortico-cortical axons.
Conclusions: Based on the monkey studies we could predict which connections from each seed were likely to be accurate and thus involved in these treatments. We demonstrate that these 2 sites have in common thalamic and brainstem cingulate connections, and ascending amygdala fibers. Of particular interest, cingulatomies capture OFC fibers passing to and terminating in the dACC. The IC site captures both OFC and dACC ascending and descending projections. However, long cortico-cortical connections that are interrupted by the lesion, are not directly involved at the DBS sites.
Disclosure: S. Haber, **Part 1:** Dr. Haber has received consultation fees from Medtronic, Inc and Pfizer, Inc.

Panel

6. Kicking Over the Traces—Noncatecholic Biogenic Amines and Their Receptors

6.1 Evidence from Molecular Modeling, Site-directed Mutagenesis and Behavioral Testing Indicate Trace Amine-associated Receptor 1 is a Methamphetamine Receptor

David K. Grandy*

Oregon Health & Science University, Portland, Oregon

Background: The number of METH abusers continues to grow worldwide and because there is no approved treatment communities are increasingly burdened with demands on healthcare, child welfare and criminal justice services as the drug takes its toll on the emotional and psychological wellbeing of users and their families. Past attempts to explain the physiological and behavioral actions of METH primarily focused on the biogenic amine transporters, in particular the dopamine (DAT) and vesicular monoamine (VMAT) transporters. However, experiments in our laboratory showed METH is also a potent full agonist of TAAR1 *in vitro*. This finding revealed a previously unrecognized gap in our knowledge about the pharmacodynamics of METH and led to our conceiving of the two-hit hypothesis of METH action: The behavioral effects of METH are manifestations of the drug's interaction with DAT *and* TAAR1.
Methods: To test our hypothesis we built molecular models of mouse, rat and guman TAAR1 using Schroedinger, Inc software, used these models in dynamic simulations of ligand-receptor interactions, functionally evaluated (cAMP and chloride conductance-CFTR) forms of mouse and rat TAAR1 mutated based on our models, synthesized in-house TAAR1-selective antagonists, agonists and partial agonists and behaviorally evaluated the ability of these compounds to modify the locomotor effects of methamphetamine on wild type and taar1-deificient mice.
Results: (1) Molecular modeling predicts TAAR1 has three ligand binding domains and therefore allosteric modulation of the receptor is feasible. (2) The aspartate at 102 is essential for agonist binding. (3) The side groups of amino acids in transmembrane domains 6 and 7 determine the receptor's stereoselectivity for the stereoisomers of amphetamine and methamphetamine. (4) The results from behavioral pharmacology studies in wild type and TAAR1-deficient mice using TAAR1-selective compounds conclusively demonstrate the stimulatory effect of methamphetamine is $\sim$60 dependent on TAAR1-mediated signaling and $\sim$40% dependent on the drug's interference with dopamine transporter function thus supportng our 2-hit hypothesis of methamphetmine action.
Conclusions: Trace Amine-Associated Receptor 1 is a methamphetamine receptor and as such should be further investigated for its potential as an important new target in any effort to develop an anti-craving medication.Analogous with other drugs of abuse, specifically cannabis and opiates, the existence of a G protein-coupled methamphetmine receptor implies the drug is interfering with endogenous neurotransmitters/modulators that have pleiotropic effects ranging from gene expression to behavioir.
Disclosure: D. Grandy, Nothing to Disclose.

6.2 Selective TAAR1 Ligands and Transgenic Animal Models Reveal a Role of TAAR1 in Cognitive, Neurologic and Psychiatric Disorders

Raul R. Gainetdinov*

Italian Institute of Technology, Genova, Italy

Background: Structurally related to classical monoaminergic neurotransmitters, trace amines (such as beta-phenylethylamine, tyramine, octopamine and tryptamine) are found at low concentrations in the mammalian brain. Discovery of a group of G protein-coupled receptors, named trace amine associated receptors (TAARs) with some of them being activated by trace amines provided an opportunity to explore their functions. The trace amine associated receptor 1 (TAAR1), which is in part associated with the monoaminergic neuronal circuitry, is the best characterized of the class, though still little is known about its regulation and function. TAAR1 can be activated not only by trace amines but also by a variety of monoaminergic compounds including amphetamines and monoamine metabolites.
Methods: By applying various experimental paradigms aimed to model dopaminergic dysregulation in mice lacking TAAR1 and newly developed selective TAAR1 ligands we investigated the role of TAAR1 in modulating dopamine-related functions such as movement control. Furthermore, we investigated the biochemical mechanism of interaction between TAAR1 and D2 dopamine receptors and the role this interaction plays in D2R-related signaling and behaviors. Finally, we applied *in vivo* microdialysis and fast scan cyclic voltammetry (FSCV) to investigate neurochemical mechanisms involved.
Results: In TAAR1 knockout (KO) mice and by using selective TAAR1 ligands, we observed that TAAR1 generally exerts an inhibitory influence on the locomotion, so TAAR1 agonists inhibit dopamine-dependent locomotor activity, while effects of dopaminergic stimulation is enhanced in TAAR1 knockout mice. In biochemical studies, we observed close functional interaction between TAAR1 and D2 dopamine receptors (D2R). Using a bioluminescence resonance energy transfer biosensor for cAMP in cellular assay, we demonstrated that the D2R antagonists haloperidol, raclopride, and amisulpride were able to enhance selectively a TAAR1-mediated increase of cAMP. Moreover, TAAR1 and D2R were able to form heterodimers when coexpressed in HEK cells, and this direct interaction was disrupted in the presence of haloperidol. In addition, in mice lacking TAAR1, haloperidol-induced striatal c-Fos expression and catalepsy were significantly reduced. A significant modulation of dopamine dynamics following TAAR1 activation was also observed in neurochemical studies.
Conclusions: These data indicate that TAAR1 can affect dopamine neurotransmission via several mechanisms and that this modulatory influence can have important functional consequences *in vivo*. Furthermore, these investigations suggest that TAAR1 may represent a novel target for the pharmacology of dopamine-related disorders such as schizophrenia, ADHD and Parkinson's disease. Other potential therapeutic applications of selective TAAR1 agonists and antagonists will be discussed.
Disclosure: R. Gainetdinov, **Part 4:** I have research grants from F. Hoffmann La-Roche (Basel, Switzerland) on the topic of this presentation.

6.3 The Activation of Intracellular Signaling Systems by Amphetamines: A Potential Role for Trace Amine Receptors

Susan G. Amara*

NIMH, Bethesda, Maryland

Background: Amphetamine has been used therapeutically to treat attention deficit disorders, narcolepsy, and as an appetite suppressant, but like other psychostimulant drugs it also has substantial potential for abuse. D-amphetamine and a variety of other amphetamine-like compounds are well-established substrates for both plasma membrane and vesicular biogenic amine transporters and are also agonists for trace amine-associated receptors (TAARs). Recent work has begun to provide additional insight into how the actions amphetamines on each of these different targets leads to the activation of cellular signaling pathways and regulates neurotransmitter signaling.
Methods: Using real-time monitoring of transporter trafficking in transfected cell lines and in dopamine neurons in the brain we have observed that once amphetamine-like drugs enter dopamine neurons they activate multiple intracellular signaling pathways to trigger changes in the cellular trafficking of the dopamine transporter and other neuronal membrane proteins.
Results: Within the cell amphetamines activate the small GTPases, Rho and Rac1 and trigger endocytosis of the dopamine transporter (DAT) by a RhoA- and dynamin-dependent pathway. Amphetamine also appears to increase intracellular cAMP, most likely through activation of an intracellular trace-amine associated receptor, TAAR1. We have observed that the same amphetamine-activated RhoA-dependent mechanism also activates the internalization of EAAT3, a neuronal glutamate transporter present on DA neurons.
Conclusions: These findings provide a new context in which to consider the actions of amphetamine on dopaminergic and glutamatergic signaling and suggest novel drug targets for modulating the actions of amphetamines.
Disclosure: S. Amara, Nothing to Disclose.

6.4 Trace Amine Associated Receptor 1 Modulation of the Rewarding and Immunological Effects of Drugs of Abuse Supports its Relevance as a Therapeutic Target

Gregory M. Miller*

Harvard Medical School, Southborough, Massachusetts

Background: Trace amine associated receptor 1 (TAAR1) is activated by a spectrum of biogenic amines, thyronamines and amphetamine-like psychostimulant drugs. Recent studies demonstrate that TAAR1 activation alters dopamine and serotonin neuronal firing rates as well as modulates dopamine transporter, norepinephrine transporter and serotonin transporter kinetic and internalization functions, suggesting that it is a potential therapeutic target for the treatment of psychiatric and addictive disorders. Such drugs would function through a distinctively different mechanism than present pharmaceuticals.
Methods: Methamphetamine (METH), morphine or ethanol were administered to wild type (WT) and TAAR1 knockout (KO) mice (75% C57J/BL6 $\times$ 25% 129S1/Sv mixed back-

ground), and assessments were made to determine the role of TAAR1 in modulating the behavioral and reinforcing effects of the different drugs. TAAR1 expression was assessed in rhesus monkey immortalized B lymphoblastoid cell lines as well as in peripheral blood mononuclear cells (PBMC) *in vitro* and the effects of METH in the presence and absence of the specific TAAR1 antagonist EPPTB on TAAR1 signaling was assessed.
Results: TAAR1 KO mice were more sensitive to the behavioral and reinforcing effects of METH and ethanol, but not morphine. TAAR1 KO mice acquired METH-induced conditioned place preference (CPP) earlier than WT mice and retained CPP longer during extinction training. TAAR1 KO mice also showed significantly greater preference for and consumption of ethanol in a two bottle choice paradigm while also showing significantly greater sedative-like effects of acute ethanol manifested as loss of righting observed at a lower dose and for longer time, with similar blood ethanol levels and rates of ethanol clearance. We also observed progressively lower cumulative locomotor activity over 60 minutes in response to increasing doses of ethanol (1.0–2.5 g/kg, i.p.) in TAAR1 KO mice, with no significant difference observed in response to an acute saline challenge between TAAR1 KO and WT mice. TAAR1 was robustly expressed in the immortalized B cells and was inducible upon immune activation in PBMC. TAAR1 stimulation by METH resulted in upregulation/phosphorylation of PKA and PKC, which was blocked by pretreatment with the TAAR1 antagonist EPPTB.
Conclusions: Paralleling its ability to alter brain monoaminergic tone, TAAR1 is a modulator of the behavioral and reinforcing effects of abused drugs, including METH which directly binds to the receptor as well as ethanol which indirectly alters levels of endogenous TAAR1 agonists (eg, dopamine). TAAR1 expression in immune cells is functionally linked to the PKA and PKC signaling pathways, and METH induces activation of these pathways in immune cells via TAAR1. Accordingly, drugs that target TAAR1 are likely to have a significant therapeutic potential for modifying behavior, reward salience and immunological function in the context of drug addiction and other psychiatric disorders.
Disclosure: G. Miller, Nothing to Disclose.

Panel

7. Structural and Functional Brain Changes in Young People at Risk for Severe Mental Illness

7.1 Clinical Stages and Developmental Trajectories of Bipolar Disorder: Family-based Analysis

Martin Alda*

Dalhousie University, Halifax, Nova Scotia

Background: The clinical picture of bipolar disorder (BD) is increasingly recognized as progressing in clinical stages each with specific considerations for diagnosis and treatment selection. Matching the clinical stages with neurobiological (eg brain imaging) studies is important for differentiating between neurobiological vulnerability factors on the one hand and burden of the illness on the other.
Methods: In prospective family studies we investigated the relationships between family history, age at onset, clinical characteristics and psychopathology trajectories in the youngest generation. We analyzed data from over 380 families from the Maritime Bipolar Registry and validated the findings in a similar sample from Sardinia followed by professor Maria del Zompo in Sardinia, Italy.
Results: The early course of BD is characterized by non-specific symptoms such as anxiety, later followed by episodes of depression and only in the late teens/early 20 s by hypomanias and manic episodes. At that point the psychiatric diagnoses become more stable. The family data indicate a partial overlap between mood and psychotic disorders. Rather than following the current diagnostic categories, the familial patterns are suggestive of genetic influences in relation to the clinical course (episodic versus chronic) and specific syndromes (the risk of suicide). The risk of co-morbid anxiety and suicide behaviour is elevated especially in individuals with early onset; the age at onset appears to correlate in families. At later age, the clinical picture of BD starts diverging again as a result of additional factors that include quality of mood stabilization, co-morbid physical conditions and/or neurodegenerative processes.
Conclusions: Our data support the existence of subtypes of severe mental illness that do not necessarily follow the current diagnostic classification, but are familial and likely correlate with response to specific treatments. To understand the pathophysiology of severe mental illness, studies of subjects in early disease stages need to be complemented by investigations of individuals at genetic risk, but who are clinically unaffected.
Disclosure: M. Alda, Nothing to Disclose.

7.2 Neuroanatomical Changes in Bipolar Disorders—Causes Versus Consequences of the Illness

Tomas Hajek*

Dalhousie University, Halifax, Nova Scotia

Background: The onset of BD typically coincides with the structural maturation of the brain during the transition period between childhood and adulthood. The neuroanatomical changes predisposing for BD need to be differentiated from the typical brain maturation and from changes secondary to the presence of the illness. Identifying the brain structural changes predisposing for BD could aid in early diagnosis. Isolating the neuronal sequellae of BD could yield biological outcome measures for prevention and treatment.
Methods: To separate neuroanatomical changes associated with BD into those increasing the risk of the illness and those resulting from it, we acquired MRI scans and prospective clinical data from participants at different stages of BD, including: (1) adolescent/early adult unaffected subjects at genetic risk for BD ($N=50$); (2) adolescent/early adult BD subjects close to the typical age of onset ($N=36$); (3) participants selected for substantial illness burden (minimum 10 years of illness and 5 episodes) and either no lifetime history of Li treatment (non-Li group, $N=19$) or at least 2 years of Li treatment (Li group, $N=37$). We also recruited 99 healthy controls matched to the above mentioned cohorts by age and sex.
Results: Relative to controls, both the adolescent/early adult unaffected at risk subjects and BD participants close to the

typical age of onset showed increased right inferior frontal gyrus (rIFG) volume, as well as comparable hippocampal volume and prefrontal N-Acetyl Aspartate (NAA) levels. Among the unaffected HR subjects, enlarged rIFG volume was associated with an increased risk of conversion to psychiatric disorders within 4 years following the MRI scanning ($b = 1.51$, SE $b = 0.89$, hazard ratio $= 4.5$). In contrast, Li naïve patients with substantial illness burden had smaller hippocampal volumes and prefrontal NAA levels than controls, who showed comparable hippocampal volumes and prefrontal NAA levels to the Li treated subjects with substantial illness burden ($F_{2;102} = 4.97$, $p = 0.009$; $F_{2,61} = 7.23$, $p = 0.002$, respectively). Duration of illness was negatively associated with NAA levels only in the Li naïve ($r = -0.60$, $p = 0.019$), but not in the Li treated group ($r = 0.07$, $p = 0.74$). Even Li-treated patients with episodes of BD while on Li had hippocampal volumes comparable to healthy controls and significantly larger than the non-Li patients ($t = 2.62$, $DF = 43$, $p = 0.006$).
Conclusions: Certain neuroanatomical changes, such as increased rIFG volume, are unique to unaffected subjects at risk for BD or those early in the course of illness. These changes could help identify who among the offspring of bipolar parents is at a particularly high risk of developing psychiatric disorders. Other brain structural or neurochemical alterations, such as decreased hippocampal volume or reduced prefrontal N-acetyl aspartate are found predominantly in subjects with established illness, but not among unaffected relatives of bipolar parents or participants early in the course of BD. Thus, these abnormalities may reflect the neuroprogressive nature of BD, which may possibly be prevented by treatment. Indeed, Li treated subjects did not show these changes despite a substantial illness burden.
Disclosure: T. Hajek, Nothing to Disclose.

7.3 Population Neuroscience and Psychiatric Genetics: A Two-way Street

Tomas Paus*

University of Toronto, Toronto, Ontario, Canada

Background: How can brain imaging carried out in community-based samples contribute to the goals of psychiatric genetics?
Methods: In my presentation, I will provide an overview of our experience with multi-modal datasets acquired in two cohorts: the Saguenay Youth Study (SYS) in Canada and IMAGEN, a multi-site cohort based in France, the United Kingdom, Ireland, and Germany.
Results: In particular, I will focus on one of the challenges of this work, namely the trait-like stability of functional brain phenotypes, and will outline novel approaches for estimating within-subject predictability of the brain response and its heritability.
Conclusions: We believe that this imaging-based approach, applied in community-based samples ascertained in an unbiased manner, provides a window to potential pathophysiological pathways relevant to common brain disorders, such as depression, substance use, and dementia (Paus 2013). Paus T. Population Neuroscience. Springer-Verlag Berlin Heidelberg 2013, ISBN-10: 3642364497.
Disclosure: T. Paus, Nothing to Disclose.

7.4 Vulnerability or Resilience? Brain Developmental Studies in Non-Psychotic Siblings of Childhood Onset Schizophrenia Patients

Nitin Gogtay*

NIMH, Bethesda, Maryland

Background: Converging evidence suggests that schizophrenia is a progressive neurodevelopmental disorder, with childhood onset schizophrenia (COS) cases resulting in more profound brain abnormalities. Siblings of COS patients, which also tend to be younger in age, provide an invaluable resource for differentiating between trait andstate markers, thus highlighting possible endophenotypes for ongoing research.
Methods: In an ongoing prospective study of COS, we mapped longitudinal cortical GM thickness change in non psychotic full COS siblings ($n = 95$, 181 scans; age 5 through 28 years) of patients with COS, contrasting them with age-, sex-, and scan interval-matched healthy controls ($n = 138$, 244 scans). The false-discovery rate procedure was used to control for type I errors due to multiple comparisons.
Results: Younger, nonpsychotic COS siblings showed significant GM deficits in the left prefrontal and bilateral temporal cortices and smaller deficits in the right prefrontal and inferior parietal cortices compared with the controls ($p < 0.05$ for slopes of trajectories). The cortical deficits in siblings disappeared by age 18 years and the process of deficit reduction correlated with overall functioning (GAS scores) at the last scan.
Conclusions: Prefrontal and temporal GM loss in COS appears to be an age specific and subregionally specific endophenotype. Amelioration of regional GM deficits in healthy siblings was associated with higher global functioning (GAS scores), suggesting a relationship between brain plasticity and functional outcome; suggesting the role for resilience factors in non psychotic siblings. Ongoing functional imaging and magnetoencephalography data on the sibling cohort also show overlapping shared abnormalties between COS probands and non psychotic siblings.
Disclosure: N. Gogtay, Nothing to Disclose.

Panel

8. The Role of Inflammation in the Pathophysiology of Mood, Aggressive and Medical Disorders: A Deadly Combination

8.1 Inflammation and Depression: Sleep Disturbance Moderates Induction of Depressed Mood by an Inflammatory Challenge

Michael R. Irwin*

UCLA, Los Angeles, California

Background: Proinflammatory cytokines are thought to contribute, in part, to the onset of depressive symptoms, and possibly to the onset and incidence of major depressive disorder. Meta-analytic results have found that depressive disorders are associated with increases in circulating levels of C-reactive protein (CRP) and interleukin (IL-6), as well as other cellular markers of inflammation. Additionally, elevated levels of systemic inflammation (eg, CRP) are prospectively associated with depression. Recent data also implicate sleep disturbance as an independent prospective risk factor depression. We have found that persistent sleep

disturbance prospectively predicts the recurrence of depression in at risk populations such as older adults. However, the mechanisms that link sleep disturbance and depression are not known. Given that even modest amounts of sleep loss activate cellular and genomic markers of inflammation, due in part to activation of cellular inflammatory signaling pathways (eg, NF-kB) especially in women and those with a history of depression, an activation of inflammatory signaling is hypothesized to be one pathway that links sleep disturbance and depression. Here, we examined the effect of proinflammatory cytokine activation on the neural correlates of socially painful experience and associated depressed mood, and the contribution of sleep disturbance in moderating the induction of depressed mood.
Methods: Participants were randomly assigned to receive either placebo or low-dose endotoxin, which induces increases in proinflammatorycytokine levels in a safe and physiologically relevant manner. Cytokine levels were repeatedly assessed through hourly blood draws; self-reported and observer-rated depressed mood were assessed regularly as well. Two hours after drug administration, neural activity was recorded as participants completed a task in which they anticipated monetary rewards or underwent a task of social exclusion.
Results: Endotoxin led to significant increases (from baseline) in IL-6 and TNF-a levels as well as feelings of depressed mood. Among females, but not males, exposed to endotoxin, increases in IL-6 were associated with increases in social pain-related neural activity (dorsal anterior cingulate cortex, anterior insula) that mediated the relationship between IL-6 increases and depressed mood increases. Additional analyses focused on neural activity associated with anhedonia, another key symptom of depression. These results demonstrated that subjects exposed to endotoxin, compared with placebo, showed greater increases in self-reported and observer-rated depressed mood over time, as well as significant reductions in ventral striatum activity to monetary reward cues. Moreover, the relationship between exposure to inflammatory challenge and increases in observer-rated depressed mood was mediated by between group differences in ventral striatum activity to anticipated rewards. Finally, reports of sleep disturbance prior to the administration of endotoxin was associated with greater increases in depressed mood.
Conclusions: These data are among the first experimental data to show that increases of proinflammatory cytokine activity are associated with increases in feelings of depressed mood, as well as related neural responding. Social pain-related neural activity, as welll as reward-related neural processes mediate the relationship between inflammation and and depressed mood. The presence of sleep disturbance exaggerates increases of depressed mood in response to an inflammatory challenge. Together, these findings raise the possibility that activation of cellular and genomic markers of inflammation, which we have found in association with sleep disturbance, may have recursive, reciprocal effects on depressed mood, and contribute to the risk of depression or its recurrence in those with persistent sleep disturbance.
Disclosure: M. Irwin, Nothing to Disclose.

8.2 Plasma Markers of Inflammation are Elevated in Subjects with Intermittent Explosive Disorder and Correlate Directly with Aggression in Human Subjects

Emil F. Coccaro*

University of Chicago, Chicago, Illinois

Background: Both animal and human studies suggest that behavioral traits related to hostility, anger, and aggressive tendencies are associated with elevations in inflammatory markers. For example, defensive rage in cats is associated with higher levels of IL-6, and mice deficient in cytokine receptors fail to exhibit aggressive and defensive behavior even when threatened11. Human studies suggest similar patterns in which elevations of C-Reactive Protein (CRP) and IL-6 are directly associated with hostility, anger, and aggressive tendency. Despite this, no studies of aggression and inflammatory markers have been reported in psychiatric subjects or in subjects with recurrent, problematic impulsive aggressive behavior.
Methods: Plasma levels of C-Reactive Protein (CRP) and Interleukin-6 (IL-6) were examined in physically healthy subjects with Intermittent Explosive Disorder (IED: $n=69$), non-aggressive subjects with Axis I/II disorders (PC: $n=62$), and non-aggressive subjects without history of Axis I or II disorder (NC: $n=67$).
Results: MANOVA revealed a significant difference among the groups with both inflammatory markers (Wilks $\lambda=0.43$, $F[6,374]=32.66$, $p<0.001$; CRP: $F[2,189]=21.67$, $p<0.001$; Log IL-6: $F[2,189]=70.85$, $p<0.001$). In each case, IED subjects displayed higher inflammatory marker levels than either HC or PC subjects. In addition, both inflammatory markers were directly correlated with composite measures of aggression (CRP: $r=0.39$, $p<0.001$; Log IL-6: $r=0.37$, $p<0.001$) and impulsivity (CRP: $r=0.31$, $p<0.001$; Log IL-6: $r=0.26$, $p=0.001$). Plasma CRP and Log IL-6 were also significantly correlated ($r=0.47$, $p<0.001$). While Composite Aggression and Composite Impulsivity scores were highly correlated ($r=0.66$, $p<0.001$), multiple regression analysis ($F[2,163]=16.25$, $p<0.001$, $R=0.41$, $R2=0.16$) revealed a unique contribution for Composite Aggression but not for Composite Impulsivity for both plasma CRP and Log IL-6 levels. Within Composite Aggression, history of actual aggressive behavior, as opposed to aggressive disposition as a personality trait, accounted for these findings with CRP/IL-6. These findings remained significant even after variables related to body mass index, state depression, psychosocial stress, and other relevant variables, were accounted for.
Conclusions: These data suggest a direct relationship between plasma inflammatory processes and aggression in human subjects. This finding adds to the complex picture of the central neuromodulatory role of aggression in human subjects.
Disclosure: E. Coccaro, **Part 1:** Scientific Avisory Board for Azivan Pharmaceuticals.

8.3 Stress, Trauma, and Inflammation in Non-Psychiatric Subjects

Janice Kiecolt-Glaser*

Ohio State University College of Medicine, Columbus, Ohio

Background: Recent studies from our lab have addressed a number of related questions including (1) whether childhood adversities have lasting, detectable consequences for inflammation and cell aging late in life; (2) whether depressive symptoms influence the magnitude of stress-induced inflammatory responses; and (3) how stress-reducing interventions such as yoga impact inflammation and mood.
Methods: Diverse community samples for these studies have included (1)132 healthy older adults (58 dementia family caregivers and 74 noncaregivers); (2) 138 healthy overweight middle-aged adults; and (3) 200 post-treatment (>2 months and $\leqslant 3$ years) stages 0-IIIA breast cancer survivors (ages 27–76) who were assigned to a 12 week, twice-weekly yoga intervention group ($N=100$) or a wait-list control group ($N=100$) in this 3-month controlled trial with a 3-month follow-up.
Results: Among the older adult sample whose average age was 70, childhood adversities were associated with both heightened IL-6 and shorter telomeres even after controlling for caregiving status; the telomere length difference between individuals reporting no adversities and those reporting multiple adversities could translate into a 7- to 15-year difference in life span. In healthy overweight middle-aged adults, those with higher levels of depressive symptoms produced more IL-6 in response to a standard laboratory stressor, as well as significantly higher levels of IL-6 both 45 minutes and 2 hours after the stressor. Breast cancer survivors who were randomized to the yoga intervention had significantly lower inflammation compared to the wait-list controls; furthermore, more frequent yoga practice produced larger changes in both inflammation and mood.
Conclusions: Adverse childhood events are related to continued vulnerability among older adults, enhancing the impact of chronic stressors like dementia family caregiving on inflammation and telomere length. Inflammation triggers T-cell proliferation, one known cause of telomere shortening, and thus heightened stress- and depression-related inflammation may influence mortality through this pathway, among others. These data are important because even modest levels of depressive symptoms can heighten inflammatory responses to stressors. Interventions that diminish stress or depression may also diminish inflammation. Chronic inflammation has been suggested as one key biological mechanism that may fuel declines in physical function leading to frailty, disability, and, ultimately, death.
Disclosure: J. Kiecolt-Glaser, Nothing to Disclose.

8.4 Transcriptional Signatures Related to Glucose and Lipid Metabolism Predict Treatment Response to the Tumor Necrosis Factor Antagonist Infliximab in Patients with Treatment-resistant Depression

Jennifer C. Felger*

Emory University School of Medicine, Atlanta, Georgia

Background: The tumor necrosis factor (TNF) antagonist infliximab was recently found to reduce depressive symptoms in patients with increased baseline inflammation as reflected by a plasma C-reactive protein concentration >5 mg/L. The current study was designed to further explore genetic predictors and targets of response to infliximab.
Methods: Differential gene expression was examined in peripheral blood mononuclear cells from infliximab responders ($n=13$) versus non-responders ($n=14$) compared to placebo at baseline and 6hr and 24hr and 2 weeks after the first infliximab infusion. Treatment response was defined as 50% reduction in depressive symptoms at any point during the 12-week trial.
Results: One-hundred-forty-eight gene transcripts were significantly associated (1.2 fold, adjusted $p\leqslant 0.01$) with response to infliximab and were distinct from placebo responders. Although there were no differences in infliximab responders and non-responders in body mass index or the presence of manifest metabolic disorders, transcripts predictive of infliximab response were associated with gluconeogenesis and cholesterol transport, and were enriched in a network regulated by hepatocyte nuclear factor (HNF)4-alpha, a transcription factor involved in gluconeogenesis and cholesterol and lipid homeostasis. Of the 148 transcripts differentially expressed at baseline, 48% were significantly regulated over time in infliximab responders, including genes related to gluconeogenesis and the HNF4-alpha network, indicating that these predictive genes were responsive to inflximab. Responders also demonstrated inhibition of genes related to apoptosis through TNF signaling at 6hr and 24hr after infusion. Transcripts down-regulated in responders 2 weeks after infliximab were related to innate immune signaling and nuclear factor-kappa B.
Conclusions: Baseline transcriptional signatures reflective of alterations in glucose and lipid metabolism and potential incipient processes related to the metabolic syndrome predicted antidepressant response to infliximab, and infliximab response involved regulation of metabolic genes and inhibition of genes related to innate immune activation.
Disclosure: J. Felger, Nothing to Disclose.

Study Group

9. Medical and Non-medical Use of Stimulant Drugs for Cognitive Enhancement

James Swanson*, William Pelham, Trevor W. Robbins, Barbara J. Sahakian, James T. McCracken, Susanna N. Visser, Ruben Baler, Kathleen Ries. Merikangas, Raul Gonzalez, James G. Waxmonsky, Tim Wigal, Marc Lerner, Wilson M. Compton

Florida International University, Irvine, California

The stimulant drugs (methylphenidate and amphetamine) affect behavior and cognition. The primary medical use is to treat individuals with Attention Deficit Hyperactivity Disorder (ADHD), but non-medical use intended for cognitive enhancement in non-ADHD individuals occurs in educational, workplace, athletic and other settings (Smith and Farah, 2010). This is a very controversial topic often addressed in newspapers (Schwarz, New York Times, 2013) and magazines (Stix, Scientific American, 2010) for the general public. A discussion of this controversial topic is proposed for a Study Group. James

Swanson will review the trends of overall use of stimulant drugs tracked by prescription records, national supplies, and the Monitoring the Future survey, all of which have information across several decades (Swanson *et al*, 2012). Kathleen Merikangas will present data from the National Comorbidity Survey, which indicates that most adolescents with ADHD are not recognized or treated with stimulants (Merikangas *et al*, 2013). The National Survey of Children's Health has tracked the diagnosis of ADHD and its treatment with stimulant drugs for the past decade (Visser *et al*, 2010), and the most recent findings will be presented by Susanna Visser. William Pelham will summarize the dose-related effects of stimulant drugs on children with ADHD on cognition as well as on symptom-severity in the context of concurrent psychosocial treatment (Strand, Hawk, Bubnik, Shiels, Pelham, and Waxmonsky, 2013). James Waxmonsky will review hypotheses of placebo response and tolerance to the cognitive and behavioral effects of clinical doses of stimulant drugs (Waxmonsky, Waschbusch, Glatt, and Faraone, 2011). Ruben Baler will review the complex mechanisms underlying the effects of stimulant drugs on the brain (Swanson, Baler and Volker, 2011). Marc Lerner will discuss the long-term safety of medical use of stimulant drugs in children (Lerner and Wigal, 2008) and the rules and monitoring of medical use in major league and minor league baseball players. Barbara Sahakian will discuss the neuroethics of the non-medical use of cognitive enhancing drugs that has generated considerable interest and debate and was the topic of a recent book (Illes and Sahakian, 2011). Tim Wigal will discuss differences in medical and non-medical use of stimulant drugs (Swanson, Wigal, and Volow, 2011), especially on college campuses that depend on non-academic factors, such as membership in fraternities, sororities, and athletic teams. The adverse effects of non-medical use of amphetamine may differ than in medical use, and James McCracken will review similarities and differences (Berman, Kuczenski, McCracken and London, 2009). Raul Gonzalez will discuss the cognitive effects of marijuana (Gonzalez *et al*, 2012) and address concurrent use of stimulant drugs. Trevor Robbins will review new compounds that are similar and dissimilar to the stimulant drugs and preclinical studies of some of these (Chamberlain and Robbins, 2013).

Disclosure: J. Swanson, **Part 1:** I have been a consultant with Noven Pharmaceuticals and BLK Pharma, and I recieved indirect support from pharmaceutucal companies from a professional organzation, the European Network of Hyperkinetic Disorders (EUNETHYDIS), to make presentations at annual meetings.; W. Pelham, **Part 1:** Gave a talk at a conference in Japan that was sponsored by Janssen Pharmaceuticals; T. Robbins, **Part 1:** Consultancy: Cambridge Cognition, Lilly, Merck, GlaxoSmithKline, Lundbeck, Teva, Shire Pharmaceuticals, ChemPartners, Royalties: Cambridge Cognition (CANTAB), Editorial Honoraria: Springer Verlag (Psychopharmacology), **Part 2:** Cambridge Cognition, **Part 3:** Cambridge Cognition, **Part 4:** Lilly, Lundbeck, GlaxoSmithKline; B. Sahakian, **Part 1:** Cambridge Cognition Limited, Lundbeck, Janssen/ J&J, Roche, **Part 2:** Cambridge Cognition Limited, **Part 4:** Janssen/J&J, Foresight, BIS, Government Office for Science; J. McCracken, **Part 1:** Research Contracts: Roche, Seaside Therapeutics, Otsuka, Consultant Income: Roche, BioMarin, PharmaNet, Speaker Honoraria: Tourette Syndrome Association, **Part 2:** Research Contracts: Roche, Seaside Therapeutics, **Part 3:** None, **Part 4:** None; S. Visser, Nothing to Disclose; R. Baler, Nothing to Disclose; R. Gonzalez, Nothing to Disclose; J. Waxmonsky, **Part 1:** Research Contract Noven Pharmaceuticals, Research Contract Janssen, Research Contract Shire Inc. , Advisory Board Noven Pharmaceuticals (**Part 4:** Research Contract Noven Pharmaceuticals, Research Contract Janssen, Research Contract Shire Inc. ; T. Wigal, **Part 1:** Otsuka and McNeil, **Part 4:** Eli Lilly, Noven, Shire and Rhodes Pharamceuticals; M. Lerner, Nothing to Disclose; W. Compton, Nothing to Disclose.

Study Group

10. Mental Illness, Violence and the Gun Control Debate: Evidence, Policy, Privacy and Stigma—on Behalf of the ACNP Ethics Committee

David Pickar*, Jerrold Rosenbaum, Emil F. Coccaro, Kenneth Leon. Davis, Paul S. Appelbaum, Brian Frosh, J. Dee Higley

Johns Hopkins, Chevy Chase, Maryland

The tragic killings carried out by individuals with apparent serious mental illness has led to a national dialogue regarding the links between mental illness, violence and gun control. The ACNP Ethics Committee determined it would be timely to explore the clinical knowledge and scientific evidence base regarding violence and mental illness. This study panel will address the following questions: (1) Are patients being unfairly exploited as straw men in a debate to preserve 2nd amendment freedoms or do safety concerns justify singling out those who have suffered mental illness (who represent 6% of the population with severe disorders at any time and up to 45% with any disorder lifetime)? (2) Do concerns including stigmatization and privacy violation outweigh attempts to identify individuals at risk for gun violence? (3) Is there unwarranted generalizing beyond current scientific data and understanding? In this study group data associating mental illness to risk for violence will be briefly summarized, including neuroscientific perspectives (David Pickar, Emil Coccaro, Dee Higley). Maryland State Senator Brian Frosh, Chair Judicial Proceedings Committee, will summarize his legislative efforts and experience relating to gun control and the mentally ill. Paul Appelbaum, Ken Davis and Jerrold Rosenbaum will provide practical and ethical perspective from academic, hospital and institutional medicine. The ethical considerations drawn from privacy violation, civil liberty restriction and profiling will be central in the discussion. The study group is designed for discussion/ debate among the College membership in attendance with speakers briefly highlighting key elements to their topic. The core features of the discussion will then be passed back to the Ethics Committee for their consideration. Panel hopes to hear views of ACNP members on this issue of critical importance to psychiatry, psychology and the behavioral sciences.

Disclosure: D. Pickar, Nothing to Disclose; J. Rosenbaum, Nothing to Disclose; E. Coccaro, **Part 1:** Scientific Avisory

Board for Azivan Pharmaceuticals.; K. Davis, **Part 1:** My wife, Bonnie M. 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:** My wife, Bonnie M. 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:** My wife, Bonnie M. 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.; P. Appelbaum, **Part 1:** Equity interest in COVR, Inc. (violence prediction software)., **Part 2:** Professor, Columbia University, Research Scientist, NY State Psychiatric Institute, Private practice of general and forensic psychiatry; B. Frosh, Nothing to Disclose; J. Higley, Nothing to Disclose.

Study Group

11. New Models of Open Innovation to Rejuvenate the Biopharmaceutical Ecosystem, A Proposal by the ACNP Liaison Committee

Dean F. Wong*, Robert Innis, Lawrence M. Sung, Lisa Gold, Steven Paul, Phillip Phan, Steven Grant, Husseini Manji

Johns Hopkins University School of Medicine, Baltimore, Maryland

Section 1: Primary Purpose of the Study Group This study group will facilitate a unique gathering of academic, government and industry leaders to address the changing product development ecosystem with special emphasis on sharing, intellecutal property (IP) and the importance of translational research for CNS drug development. A major theme that will link the participants and engage the audience is the concept of *open innovation,* first articulated by Henry Chesbrough at Berkeley. The panel will consider how this concept, used successfully in some industries, could be applied to rejuvenate CNS Drug Development. Highlights from recent approaches for IP sharing will be discussed, with the goal of explicating the barriers, benefits and path to an open innovation environment in CNS Translational Research and Drug Development.

Section 2: Experimental Design or Methods Used:We will begin with a brief outline of challenges to CNS translational research sharing and the current climate of drug discovery. This will be followed by discussions from a business and a law academician. The first is an expert on the management of innovation and the use of open innovation in rapid development. The latter will deal directly with how IP sharing can be fostered within a legal framework. The other topics will consist of short examples of how sharing has been facilitated through public/private partnerships including examples from the NIH Extramural or Intramural Neuroscience programs.

Section 3—Summary of Results: Speakers will consist of Husseini Manji (J and J) who will define the ecosystem, describe where new medicines have traditionally come from in the past, and the roles of key members in the evolving ecosystem. Steve Paul, Cornell Medical College, will discuss trends in R and D productivity and then highlight some of the successful and recently initiated public:private partnerships that seek to catalyze innovation in the form of new drugs. Bob Innis, of NIMH-IRP, will discuss the opportunities for leveraging translational research. Phil Phan, of Johns Hopkins Carey Business School, will discuss how open innovation, combined with real options decision models, can accelerate innovation. Frank Pasquale, of the University of Maryland Carey School Of Law, will discuss the factors, with examples such as alltrials.net, that lead to the successful sharing of early stage research in user driven innovator communities. Dean Wong, of Johns Hopkins Medicine, will discuss the challenges for biomarker sharing in an academic setting, while Steve Grant, of the NIH, will describe the efforts by Extramural NIH staff to facilitate data sharing across multiple domains (eg, genetics, brain imaging, phenotype harmonization).

Section 4—Conclusion Statement: Open innovation by leveraging a state-of-the-art biomarker exchange and other sharing processes across the biopharmaceutical ecosystem might help to manage the costs of drug discovery, provide a stimulus for start-up companies trying to exploit scientific research at NIH and academia, and link efforts across academia, government and industry around the focused mission of new CNS therapeutics. Success will fortify Neuroscience Translational research and catalyze CNS Drug Development.

Disclosure: D. Wong, **Part 1:** Consultant for Amgen and Concert Pharmaceuticals, **Part 2:** Johns Hopkins University, School of Medicine, **Part 4:** Avid, Biotie, GE, Intracellular, J + J, Lilly, Lundbeck, Merck, NIH, Otsuka, Roche, Sanofi-Aventis, Synosia, ; L. Sung, Nothing to Disclose; L. Gold, **Part 1:** Full-time employee of Merck and Co, Inc, **Part 2:** Full-time employee of Merck and Co, Inc, **Part 3:** Full-time employee of Merck and Co, Inc; P. Phan, Nothing to Disclose; S. Grant, Nothing to Disclose; H. Manji, **Part 1:** I am a full time employee of Johnson & Johnson, **Part 2:** I am a full time employee of Johnson & Johnson, **Part 3:** I am a full time employee of Johnson & Johnson

Study Group

12. The Assessment Of Suicidal Ideation, Behavior & Risk: At Baseline; As a Measure of Clinical Outcome, and/or as a Treatment Emergent SAE

Eric Youngstrom, Roger E. Meyer*, Ahmad Hameed, John Greist, Phillip Chappell, J. John Mann, David V. Sheehan, Cheryl McCullumsmith, Larry Alphs, Richard C. Shelton, Paula J. Clayton, Kelly Posner

Penn State Hershey Medical Center, Washington District of Columbia

Purpose: FDA requires all participants in clinical trials of CNS-active drugs to be evaluated for treatment emergent suicidal ideation & behavior using a scale that maps to the C-CASA algorithm. The original Guidance Document was modified in 2012 by increasing the granularity of data on ideation & behavior as defined in the C-SSRS. To date, there have been no published comparisons between assessment instruments, & only limited published data comparing self & rater versions of the same scales.

The 'elephant in the room' remains the question of treatment emergent suicidal ideation/behavior & risk as an SAE; along with growing interest in the effects of pharmacotherapy on suicidal ideation/behavior as a primary or secondary outcome. This study group brings together different perspectives to obtain a midcourse evaluation that may advance public health, & encourage ongoing industry interest in the development of new psychotropic drugs.

Approach: Following a 5-minute agenda overview, five 10 minute presentations set the stage for an active discussion:

1) The eC-SSRS: Highlights from clinical trials **J Greist, MD**
2) The S-STS: Highlights from clinical trials **D Sheehan, MD, MBA**

   Each of the first two speakers will address the following:
   1. of trials & research participants
   2. Screening, treatment emergent SAE, signal of Rx efficacy?
   3. Comparison of rater versus self-report versions
   4. Evidence of validity &/or utility of 'severity'
3) Comparison of the C-SSRS & S-STS: Psychometric properties **E Youngstrom, PhD**
4) Comparison of the C-SSRS & S-STS: Operational aspects **A Hameed, MD**
5) Ideation/behavior as primary &/or secondary efficacy endpoints **C McCullumsmith, MD PhD & R Shelton, MD**

The study group will also include invited scientists from industry (P Chappell MD & LAlphs, MD), FDA, and academia (J Mann, MD & P Clayton, MD) to address the following questions:

1. Are the current versions of the above assessment instruments adequate to address pretrial screening, treatment emergent suicidal ideation/behavior, special populations, & treatment efficacy as a primary or secondary endpoint?

2. What data would be useful beyond these instruments? (Risk &/or protective factors; genetic information; other)?

3. Are the pre—marketing clinical trial data adequate to identify treatment emergent suicidal ideation/behavior of a new drug or drug class? If not, what else is necessary?

4. Has the FDA Guidance Document had any effect on industry interest in new psychotropic drugs or drug classes?

5. What is the best strategy to address questions around the 2012 FDA Guidance Document; &, of industry interest in drugs that might reduce the risk of suicide?

Method: With Council approval, the session will be recorded and summarized leading to a document that will be offered to industry & academic members of ACNP through the website.

Conclusion: The summary will include a conclusion statement.

Disclosure: E. Youngstrom, **Part 1:** Eric Youngstrom has consulted with Lundbeck and received past travel support from Bristol-Myers Squibb. He has consulted with Penn State about analyses for a grant funded by Pfizer, and received grant funding from NIMH. ; R. Meyer, Nothing to Disclose; A. Hameed, Nothing to Disclose; J. Greist, **Part 1:** I do not believe questions 2 and 4 are applicable because I do not know whether any/all of the companies with which I do business are 'doing business with or proposing to do business with ACNP' and find that matter confusing. , **Part 2:** eResearch Technology, Healthcare Technology Systems, Possibly Pfizer, though I expect something less than $10,000., If I own any stock in any pharmaceutical or device company it would be in retirement accounts such as the State of Wisconsin Retirement Plan over which I have no control. I have never purchased or held any pharmaceutical stocks in my personal investment account, nor has my spouse., **Part 3:** Please see 2 above., **Part 4:** AstraZeneca, eResearch Technology, Forest, Lilly, Novo Nordisk, Otsuka, Pfizer, Takeda, Transcept, UCB, ; P. Chappell, **Part 1:**; J. Mann, **Part 2:** Royalties from Research Foundation for Mental Health for C-SSRS, **Part 4:** Unrelated past grants from GSK and Novartis; D. Sheehan, **Part 1:** Advisory Board membership to Roche, Sagene Pharma, Otsuka, Forest, Novadel, Labopharm, Neuronetics, International Society for CNS Drug Development (ISCDD). Consultant to Sagene Pharma, Janssen (JNJ), MAPI, Prime Education, Neuronetics, ProPhase, Novadel, xCenda, Targacept, PharmaNeuroBoost. Payments for lectures from Pfizer, Eli Lilly, Glaxo, LaboPharm Angelini, Merck, PharmaNeuroBoost, Quintiles, Hikma, United BioSource, Janssen (JNJ), IncResearch, Otsuka. Payment for manuscript preparation from Quadrant HealthCom Inc. Royalties from eResearch Technology, Pfizer and Simon and Schuster. Stock in Medical Outcomes Systems. Travel Expenses to two ISCDD meetings paid by ISCDD., **Part 2:** Sagene Pharma, Labopharm, Neuronetics, MAPI, Pfizer, Eli Lilly, Glaxo.; C. McCullumsmith, **Part 1:** Jansenn Pharmaceuticals : Suicide Advisory Board; L. Alphs, **Part 1:** Janssen Scientific Affairs Employment and Stock, **Part 2:** 2011–2013 Employed buy Janssen Scientific Affairs a division of Johnson & Johnson; I also received stock from them., **Part 3:** Janssen Scientific Affaris, **Part 4:** None; R. Shelton, **Part 1:** Bristol-Myers Squibb, Cyberonics, Inc., Elan, Corp., Eli Lilly and Company, Euthymics Bioscience, Forest Pharmaceuticals, Janssen Pharmaceutica, Medtronic, Inc., Naurex, Inc., Novartis Pharmaceuticals, Otsuka Pharmaceuticals, Pamlab, Inc., Pfizer, Inc., Repligen, Corp., Ridge Diagnostics, St. Jude Medical, Inc., Takeda Pharmaceuticals, **Part 2:** Pamlab, Inc., **Part 4:** Bristol-Myers Squibb, Elan, Corp., Eli Lilly and Company, Euthymics Bioscience, Forest Pharmaceuticals, Janssen Pharmaceutica, Naurex, Inc., Novartis Pharmaceuticals, Otsuka Pharmaceuticals, Pamlab, Inc., Repligen, Corp., Ridge Diagnostics, St. Jude Medical, Inc., Takeda Pharmaceuticals; P. Clayton, Nothing to Disclose; K. Posner, **Part 1:** Dr. Posner is the director of the Center for Suicide Risk Assessment. The Center, as part of an effort to help execute the FDA suicidality classification mandates, has received support from the following pharmaceutical companies: Abbott, Aerial Biopharma, Albany Molecular Research, Alder Biopharma, Alfresa, Alkermes, Amgen, Astellas Pharm, Astra Zeneca, Biogen, Biomarin Pharmaceutical, Biovail Technologies, Boehringer Ingelheim, Bracket, Bristol Myers Squibb, Cato Research, Celerion, Cephalon, Cetero Research, Chiesi Pharmaceuticals, Covance, CRI Worldwide, Daiichi Sankyo Company, Depomed, Douglas Pharmaceuticals/VersaPharm, EISAI, Elan, EnVivo, Epiomed, Forest, Gilead, GlaxoSmithKline, Grunenthal, GW Pharma Limited, Human Genome Sciences, i3 International, i3 Research, i3 Pharmaceutical Services, ICON Development Solutions, Impax Laboratories, INC Research, Ingenix, IntelGenx Corp, IntraCellular Therapies, Ironwood, IRIS, Isis, Ivax, Janssen, Jazz, Johnson & Johnson, Lilly USA,

Lotus, Lundbeck, MedAvante, MedImmune, Merck, Mochida, Neurocrine Biosciences, Neuronex, Neurosearch, NextWave Pharma, Novartis, Noven, NovoNordisk, Omeros, Orexigen Therapeutics, Orion, Otsuka, Pamlab, Parexel, Pfizer, PGx Health, Pharmaceutical Research Associates, Pharmanet i3, Pierrel Research, PPD, Prana Biotechnology, ProPhase, Psyadon, QED Pharmaceuticals, Quintiles, Receptos, Reckitt Benckiser, Rho, Rhythm, Roche, Sanofi-Aventis, Schering-Plough, Schwarz Biosciences, SCOPE International, Sepracor, Shionogi, Shire, Siena Biotech, SK Life Science, Sunovion, Supernus Pharmaceuticals, Synosia Therapeutics, Takeda Global Research & Development Center, Takeda Pharmaceuticals, TauRx Therapeutics, Theravance, UCB Biosciences, UCB Korea, UCB Pharma, United BioSource Corp, Upsher-Smith Laboratories, Vaccinex, Valeant Pharmaceuticals, Vernalis, Vivus, WorldWide Clinical Trials, Wyeth Ayerst, Wyeth Pharmaceuticals, Wyeth Research, Xenoport and Zalicus. Dr. Posner receives royalty payments from the e-CSSRS, which are distributed to her by her employer, the Research Foundation for Mental Hygiene.

Study Group

13. The Challenges of Designing and Interpreting Clinical Trials with Depot Antipsychotics

W. Wolfgang Fleischhacker*, Raymond Sanchez, Srihari Gopal, Maxine X. Patel, Stephan Heres, Keith H. Nuechterlein, Hiroyuki Uchida

Medical University Innsbruck, Innsbruck, Austria

This proposal aims to provide a forum to discuss some of the controversies around clinical trials with depots and the challenges of translating research into every day clinical practice. (1) Study design: This is where regulatory requirements and 'gold standard' RCTs are difficult to reconcile with every day clinical practice, especially in light of the fact that RCTs with frequent visits and the ensuing care and attention which patients get in such studies are likely to diminish differences between oral and depot antipsychotics. If improved compliance is truly the main advantage of depot over oral antipsychotics, clinical trials, in which indirect compliance enhancing factors, such as the ones inherent in rigorous RCTs (see above) can be controlled for are desirable. Pragmatic randomized trials, could be such an option (discussed by Wolfgang Fleischhacker). (2) Once pertinent studies have been designed the next challenge becomes to run such studies. The enthusiasm with which clinicians will engage in such trials will depend on their general attitude towards depots which, as we know, differs considerably across continents, countries, regions and hospitals. The logistical issues (regulatory requirements, ethics committees, and recruitment challenges) will be reviewed by Ray Sanchez for the US and Sri Gopal for Europe. (3) Maxine Patel will then discuss differences in attitudes towards depot antipsychotics from the perspectives of patients and psychiatrists. In addition, Stephan Heres will review differences in depot utilization across the world and provide potential explanations for them based on the attitudes of prescribers. (4) Keith Nuechterlein will then discuss whether the traditional focus of depot antipsychotic studies on relapse prevention can be usefully broadened to include cognition and work and social functioning based on recent relevant studies. (5) Lastly, Hiroyuki Uchida will provide an overview on neuroimaging data and their potential clinical relevance. As recent imaging studies suggest that depot antipsychotics may differ from their oral counterparts with respect to receptor pharmacology and brain functioning, Hiroyuki Uchida will present pertinent data from PET and SPECT studies. These will also be discussed in light of its potential clinical relevance.

Disclosure: W. Fleischhacker, **Part 1:** Amgen, Lundbeck, Roche, Bristol-Myers Squibb, Otsuka, Janssen, MedAvante, Merck, Vanda, Endo, Takeda, Pfizer, Reckitt-Benckiser, **Part 3:** Janssen, Otsuka, Reckitt-Benckiser; R. Sanchez, **Part 1:** Employee of Otsuka Pharmaceutical Development and Commercialization (Vice President, Global Clinical Development CNS), **Part 2:** Employee of Otsuka Pharmaceutical Development and Commercialization; S. Gopal, **Part 2:** Shareholder Johnson & Johnson (JNJ), Shareholder Merck (MRK); M. Patel, **Part 1:** Consultancy: Janssen; Endo; Amgen; Lundbeck, Principal or Chief Investigator for clinical studies: Amgen; Lundbeck, **Part 4:** Principal or Chief Investigator for clinical studies: Amgen; Lundbeck, ; S. Heres , **Part 1:** I have received honoraria from Janssen-Cilag, Eli Lilly, Sanofi-Aventis and Johnson & Johnson. , I have accepted travel or hospitality payment from Janssen-Cilag, Sanofi-Aventis, Johnson & Johnson, Pfizer, Bristol-Myers-Squibb, AstraZeneca, Lundbeck, Novartis and Eli Lilly. , I have participated in clinical trials sponsored or supported by Eli Lilly, Janssen Cilag, Johnson & Johnson, Bristol-Myers-Squibb, AstraZeneca, Lundbeck, Novartis, Servier, Pierre Fabre, Pfizer, Organon, Roche and Merck. , I have participated in advisory activities and boards for Janssen, Johnson & Johnson, Eli Lilly, Lundbeck and Roche. ; K. Nuechterlein, **Part 1:** Investigator-Initiated Research Grant from Janssen Scientific Affairs, LLC, Research grant from Brain Plasticity, Inc., Consultant and research grant, Genentech, Consultant, Otsuka, **Part 4:** Investigator-Initiated Research Grant from Janssen Scientific Affairs, LLC, Research grant from Brain Plasticity, Inc., Research grant from Genentech.

Tuesday, December 10, 2013

Mini-Panel

14. Adolescent Brain Development and Affective Disorders: The Role of Reward and Threat Circuitry

14.1 Adolescent VTA Neurons Exhibit Latent Neuronal Correlate of Reward Opportunity

Bita Moghaddam*

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: Dopamine neurons in the ventral tegmental area (VTA) are implicated in adolescent behavioral and psychiatric vulnerabilities, but little is known about how adolescent VTA neurons process motivated behavior.

Methods: We recorded from VTA neurons of adolescent and adult rats during learning and maintenance of a cue-driven, reward-motivated instrumental task, and during extinction from this task.

Results: During learning and maintenance of the task, VTA neurons of adolescents and adults similarly responded to cue, instrumental response, and reward. During extinction,

however, despite similar behavioral responses indicating that both age groups had learned the absence of reward availability, the adolescent VTA neurons continued to respond to the cue that had been previously associated with reward. These findings demonstrate the presence of a latent neuronal correlate of reward opportunity in the VTA of adolescents.
Conclusions: Maintaining representation of events that had once predicted reward availability may impact reward seeking behavior and facilitate reinstatement of previously learned contingencies. The latent representation may facilitate renewal and reinstatement of previously associated experience. This would present a mechanism for increased motivation of vulnerability of adolescents to reward-related problems, such as affective disorders and addiction.
Disclosure: B. Moghaddam, Nothing to Disclose.

14.2 Adolescents' Neural Response to Personally Relevant Social Reward is Associated with Severity of Mania and Depression

Erika E. Forbes*

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: Disrupted function in neural reward circuitry has been implicated in affective disorders. To understand the emergence of such disorders, it is critical to investigate reward circuitry during adolescence, the developmental period in which affective disorders emerge and reward circuitry continues to develop. Social reward could be particularly salient to adolescents' affective symptoms because of changes in their social context that involve enhanced value of peer-related rewards. Ecologically valid tasks that capture adolescents' responses to social rewards could be more effective than standard tasks at assessing individual differences in reward function that are relevant to the development of affective problems.
Methods: Participants were 30 adolescents (83% female; mean age 16.35 years; 64% European American, 26% African American, 10% other race) who underwent functional magnetic resonance imaging (fMRI) in a 3T Siemens Trio scanner using a novel paradigm that incorporated personally relevant reward stimuli. Stimuli were 20-second video clips selected from a laboratory-based discussion of intense positive experiences between the participant and his/her closest same-sex friend. Interaction data were coded and selected to represent friends' displays of positive and neutral affect. Control stimuli were video clips of an unfamiliar same-sex peer displaying positive and neutral affect. Participants completed self-report scales of depression (CES-D; Radloff, 1977) and mania (Eckblad & Chapman, 1986). Imaging data were preprocessed and analyzed in SPM8, and AlphaSim was used to control for Type I error by computing minimum cluster sizes required for a corrected $p<0.05$ threshold in each region of interest.
Results: Whole-brain analyses indicated that while viewing a friend's positive affect relative to an unfamiliar peer's positive affect, adolescents exhibited response in a set of regions implicated in reward function and social processing, including the ventral striatum, medial PFC, orbitofrontal cortex, and precuneus. Regression analyses indicated that manic symptoms were associated with greater response in the insula and precuneus, while depressive symptoms were associated with less response in the striatum, dorsal anterior cingulate, and dorsolateral prefrontal cortex.
Conclusions: This novel and innovative fMRI social reward paradigm engaged reward and social processing circuitry and was sensitive to individual differences in affective symptoms. Consistent with previous findings, depression was related to disrupted response to reward in the mPFC, which could reflect altered regulation of reward function and poor flexibility in responding to affective stimuli. Mania was associated with increased response in regions implicated in social cognition, which suggests an enhanced engagement with social rewards.
Disclosure: E. Forbes, Nothing to Disclose.

14.3 Neural Mechanisms of Frustration in Chronically Irritable Youth

Ellen Leibenluft, M.D.*

NIMH, Bethesda, Maryland

Background: Irritability is one of the most common presenting complaints in child psychiatry, yet its mediating neural mechanisms have received little research attention. Irritability can be defined as a decreased threshold for, and maladaptive responses to, frustration, where frustration is the emotion experienced when goal attainment is blocked. Methods for studying the neural mechanisms of frustration typically involve paradigms in which subjects are unable to attain expected rewards and/or receive unexpected punishments. The relevance of reward circuitry to mechanisms mediating frustration is further supported by longitudinal and familial associations between irritability and depression. Such associations have been demonstrated across development, from preschoolers through adolescence and adulthood.The fMRI paradigm used here is a modification of work in press (Deveney *et al*, Am J Psychiatry. 2013 Jun 4. doi: 10.1176/appi.ajp.2013.12070917). Deveney *et al* used a paradigm in which subjects completed a Posner attentional task with and without rewards. During scanning, youth were frustrated by rigged feedback and lost money that they have won on previous trials. Compared to healthy subjects, chronically irritable youth (ie, severe mood dysregulation, or SMD) showed increased subjective responses to frustration, as well as attentional deficits and decreased amygdala-striatal-parietal activation on frustrating, compared to non-frustrating, trials.
Methods: Data will be presented from thirty youth ($N=15$ healthy subjects and 15 SMD) scanned on a modified version of the paradigm in Deveney *et al*. Subjects are scanned during a task which includes trials of the Posner attentional task, first in a condition where they receive rewards on all correct trials, and then in a rigged frustration condition where they are told that they are responding too slowly and lose the previously rewarded money. Jitter is present between trials and between the response and feedback portions of each trial. Data from the frustration condition will be analyzed with a diagnosis (control, MSD) $\times$ trial component (response, positive feedback, negative feedback) ANOVA. Data from both the reward

and frustration conditions will be analyzed with a diagnosis (control, MSD) × trial component (response, feedback) × condition (reward, frustration) ANOVA. Analyses will include ROIs based on prior work and a whole brain analysis. An exploratory analysis including 10 ADHD subjects will examine neural activation across groups as a function of irritability, treated as a dimensional variable measured with the Affective Reactivity Index.
Results: These are pending. Based on prior work, we anticipate that, compared to healthy subjects, SMD will report increased frustration during the frustration condition, and show increased reaction time only on invalid trials during the frustration condition. Further, we anticipate amygdala-striatal-parietal deactivation during frustration trials in SMD *vs* healthy subjects, and an association between irritability and amygdala-striatal-parietal deactivation across diagnoses.
Conclusions: Chronic irritability is associated with increased subjective response to a frustrating task, and decreased attentional control and amygdala-striatal-parietal deactivation during frustration. Frustrating paradigms can be challenging (indeed frustrating!) to design and implement successfully in youth, but represent a promising approach to studying the neural mechanisms mediating irritability in youth.

Unique Data. The paradigm and data presented here have not been published.
Disclosure: E, Leibenluft, Nothing to Disclose.

Mini-Panel

15. After the Trauma: Developmental Trajectories from Childhood to Adult Psychiatric Disorders

15.1 Sex-specific Effects of Childhood Emotional Abuse on Affective Processing in Bipolar Disorder Patients

Katherine E. Burdick*

Mount Sinai School of Medicine, New York, New York

Background: Childhood trauma is associated with affective processing biases in different psychiatric disorders but its effect in bipolar disorder (BPD) has not yet been investigated. Although BPD prevalence is almost equal in males and females, sex is believed to modulate clinical course, severity of illness and is likely to have an influence on affective processing. The aim of this study is to investigate the effect of sex and childhood trauma on affective processing in a sample of BPD patients.
Methods: Presence of childhood trauma was assessed with the Childhood Trauma Questionnaire (CTQ) in fifty-six BPD patients. Affective processing was measured with the Iowa Gambling Task (IGT), evaluating emotional decision making, and with the Affective Go/No-go (AGNG), measuring inhibitory response to negative/positive/neutral conditions. Analysis of Variance (ANOVA) was used to evaluate the effect of sex and childhood trauma on IGT performance and Repeated-Measures ANOVAs were used to compare groups on accuracy and bias measures across conditions on the AGNG.
Results: Significant *sex x Emotional Abuse (EA) interactions* emerged: in the context of prior abuse, females evidenced a more conservative cognitive style than males by selecting fewer cards from the disadvantageous decks [$F(1,49) = 14.218$], *psex x EA interaction* was revealed that was specific to the negative valence stimuli on response bias measures. Abused females scored significantly higher (mean = 8.38, SD = 6.39) than abused males (mean = 0.69, SD = 1.19)[$F(1,46) = 6.348$; $p = 0.015$] and there were no sex-specific effects in the absence of prior abuse.
Conclusions: Emotional abuse differently affects males and females suffering from BPD when engaged in affective processing tasks. Further investigations are needed to elucidate potential pathophysiological mechanisms underlying this interaction.
Disclosure: K. Burdick, Nothing to Disclose.

15.2 The Long-term Consequences of Childhood Maltreatment: Effects on Brain Structure and Subclinical Psychopathology in Healthy Adults

Pamela DeRosse*

Zucker Hillside Hospital, Glen Oaks, New York

Background: Several lines of evidence suggest that maltreated children may be subject to aberrant brain development as a result of the maltreatment. To date, however, few studies have examined whether the changes observed in pediatric samples are still evident in the brains of healthy adults who report a history of childhood maltreatment. Moreover, despite considerable evidence that childhood maltreatment is a risk factor for psychotic disorders, even less is known about how structural differences related to childhood maltreatment contributes to the expression of psychotic symptoms.
Methods: High-resolution T1 magnetic resonance images were acquired for 124 healthy adults characterized for a history of childhood maltreatment. Volumetric profiles of regions previously implicated in studies of children with PTSD were assessed for their relation to a history of childhood maltreatment. Regions showing significant association were then assessed for their relationship to subclinical psychotic-like experiences.
Results: Healthy adults who had a history of childhood emotional neglect were found to have significantly greater lateral ventricle volumes than those without such a history ($F(123) = 5.09$; $p = 0.026$). A second-order correlation coefficient, controlling for total intracranial volume and sex, revealed a significant positive correlation between lateral ventricle volume and subclinical psychotic-like experiences ($r12,3 = -0.20$; $p = 0.03$) but additional analyses revealed that the relation between lateral ventricle volume and subclinical psychotic symptoms was fully mediated by the severity of childhood emotional neglect.
Conclusions: These data suggest that some of the brain structural differences observed in children who have experienced maltreatment persist into adulthood and may have a significant impact on the expression of subclinical psychopathology.
Disclosure: P. DeRosse, Nothing to Disclose.

15.3 The Neurobiology of PTSD Symptoms in Maltreated Children and Adolescents

Michael D. De Bellis*

Duke University Medical Center, Chapel Hill, North Carolina

Background: MRI studies of maltreated children with PTSD suggest adverse brain development. We investigated the neuroanatomy of pediatric maltreatment-related PTSD controlling for maltreatment.
Methods: We recruited 69 maltreated and 59 community non-maltreated children and assessed them with the KSADS-PL for PTSD. Subjects underwent a 3T anatomical MRI brain scan. Exclusionary criteria included IQ118, substance use disorder; (and DSM-IV Axis I disorder in control group only). MRI data were analyzed using automated methods to distinguish grey, white, and CSF volumes. Cerebrum was measured using parcellation procedures to examine specific brain regions. We also measured amygdala and hippocampal volumes.Diffusion Tensor Brain Imaging of the Corpus Callosum were also examined.
Results: Maltreated children with PTSD (mean age 10.1 years, $n = 37$, 19 females) did not differ from maltreated children without PTSD (mean age 9.6 years, $n = 32$, 17 females) and controls (mean age 10.8 years, 33 females) in age, socioeconomic status, or sex. Maltreatment groups did not differ in IQ. After controlling for covariates, total cerebral grey matter volumes were smaller in the PTSD group compared to controls ($F_{2,112} = 5.1$, $p_{2,123} = 4.03$, $p = 0.02$; $F_{2,112} = 7.5$, $pp < .001$). Posterior Grey matter findings persisted when controlling for IQ. These regions include brain areas involved in the developing default or resting state network. TBSS analyses controllng for age and gender indicate that voxels in genu displayed greater FA in Maltreated Adolescents with respect to Controls. Higher Number of DSM-IV PTSD symptoms are Associated with Increased Corpus Callosum FA Values in Genu and Decreased FA in Splenium. Left amgdala volumes were larger in maltreated youth compared with controls and maltreated youth with PTSD. Right hippocampal volumes were larger in maltreated youth compared with maltreated youth with PTSD. More PTSD symptoms predcited smaller amygdala, hippocampal and cerebellar volumes in maltreated youth with subthreshold or threshold PTSD.
Conclusions: Maltreated Children and Adolescents may show Stress-Induced decreased process in leading to Grey Matter (posterior) loss and and suboptimal Myelination Indexes that may limit Neuroplasticity, Cognitive Flexibility, Impairs default network or resting state and result in Increased PTSD symptoms, Co-morbid psychopathology, and Cognitive Compromise in these children and Adolescents. Results Highlight the Importance of Further Imaging Studies in Maltreated Children, particularly regarding the Developing Resting State Network and its Clinical Implications.
Disclosure: M. De Bellis, Nothing to Disclose.

Mini-Panel

16. Biochemical and Behavioral Pharmacology of Synthetic Cathinone Derivatives Found in Psychoactive Bath Salts Products

16.1 Abuse-related and Abuse-limiting Effects of Synthetic Cathinone 'Bath Salt' Derivatives on Intracranial Self-stimulation in Rats

Matthew L. Banks*

Virginia Commonwealth University, Richmond, Virginia

Background: Abuse of synthetic cathinones known as 'bath salts' has increased dramatically in the United States since their debut in 2010. Intracranial self-stimulation (ICSS) may be a useful behavioral procedure for discriminating both DA-mediated abuse-related and 5-HT-mediated abuse-limiting drug effects in a single procedure. Based on the *in vitro* selectivity to promote release or block reuptake of DA/NE versus 5-HT, we predicted that methcathinone and MDPV would display the greatest efficacy to produce an abuse-related facilitation of ICSS, whereas methylone and mephedrone would produce mixed effects that would include both DA-mediated facilitation of low ICSS rates and 5-HT-mediated depression of higher ICSS rates.
Methods: Male Sprague-Dawley rats with surgically-implanted electrodes targeting the medial forebrain bundle responded for multiple frequencies of brain stimulation and were tested in two phases. First, dose-effect curves for methcathinone (0.1–1.0 mg/kg), MDPV (0.32–3.2 mg/kg), methylone (1.0–10 mg/kg) and mephedrone (1.0–10 mg/kg) were determined. Second, time courses were determined for effects produced by the highest dose of each compound.
Results: Methcathinone produced dose- and time-dependent facilitation of ICSS. MDPV, methylone and mephedrone produced dose- and time-dependent increases in low ICSS rates maintained by low brain stimulation frequencies, but also produced abuse-limiting depression of high ICSS rates maintained by high brain stimulation frequencies. Efficacies to facilitate ICSS were methcathinone $\geqslant$ MDPV $\geqslant$ methylone $>$ mephedrone. Methcathinone was the most potent compound, and MDPV was the longest acting.
Conclusions: All cathinone derivatives facilitated ICSS at some doses and pretreatment times, which is consistent with the abuse liability of these compounds. However, efficacies of compounds to facilitate ICSS varied, with methcathinone displaying the highest efficacy and mephedrone the lowest efficacy to facilitate ICSS. These results provide a foundation upon which to assess abuse-related effects of second-generation cathinone derivatives and build structure activity relationships regarding ring substitutions that may influence expression of DA-mediated abuse-related effects and 5-HT mediated abuse-limiting effects. Studies with second generation compounds are in progress.
Disclosure: M. Banks, **Part 1:** Collaborator on a grant from Perdue Pharmaceuticals related to opioid pharmacology in nonhuman primates. , **Part 4:** Collaborator on a grant from Perdue Pharmaceuticals related to opioid pharmacology in nonhuman primates.

16.2 Effects of Newly-emerging Synthetic Cathinone Derivatives on Monoamine Transporter Function in Rats

Michael H. Baumann*

NIDA, NIH, Baltimore, Maryland

Background: The abuse of synthetic stimulants known as 'bath salts' is a growing public health concern. Common constituents of bath salts, including 3,4-methylenedioxypyrovalerone (MDPV) and 4-methylmethcathinone (mephedrone), have been rendered illegal in the US as a means to prohibit sale and use. Unfortunately, new cathinone analogs are now being marketed as legal alternatives to MDPV and mephedrone. The purpose of the present study was to examine the interaction of these newly-emerging cathinones with transporters for dopamine (i.e., DAT) and 5-HT (i.e., SERT).
Methods: Specific drugs were selected for evaluation based on forensic data from the Drug Enforcement Administration. Derivatives of MDPV included á-pyrrolidinovalerophenone (á-PVP) while derivatives of mephedrone included 4-methylethcathinone (4-MEC). *In vitro* assays were carried out in rat brain synaptosomes to assess drug-induced effects on transporter-mediated uptake and release. *In vivo* microdialysis was carried out in n. accumbens of conscious rats to assess drug-induced changes in extracellular dopamine and serotonin.
Results: MDPV and á-PVP displayed low nM potency as DAT blockers, but had no effects on release. Mephedrone was a non-selective transporter substrate with potent releasing ability at DAT ($EC_{50} = 38$ nM) and SERT ($EC_{50} = 98$ nM). 4-MEC had unusual properties, blocking uptake at DAT ($IC_{50} = 546$ nM) while evoking release at SERT ($EC_{50} = 123$ nM). 4-Methylpyrrolidinopentiophenone (4-MePPP) was devoid of releasing activity but blocked uptake at DAT ($IC_{50} = 248$ nM). Microdialysis studies showed that MDPV (0.1–0.3 mg/kg, i.v.) selectively increases extracellular dopamine, whereas mephedrone (0.3–1 mg/kg, i.v.) elevates dopamine and 5-HT. *In vivo* studies with other cathinones are in progress.
Conclusions: Each of the cathinone derivatives examined displays a unique profile of *in vitro* transporter activity. Pyrrolidinophenones like MDPV and á-PVP are potent DAT blockers. Increasing the *N*-alkyl chain length of mephedrone creates compounds with reduced releasing activity, converting them to transporter blockers. The *in vivo* bioactivity of newer cathinones remains to be determined, but *in vitro* effects of the drugs at DAT point to significant propensity for abuse.
Disclosure: M. Baumann, Nothing to Disclose.

16.3 Intravenous Self-administration of 3,4-M<ethylenedioxypyrovalerone (MDPV) and 4-Methylmethcathinone (4-MMC, Mephedrone) in Rats

Michael A. Taffe*

The Scripps Research Institute, La Jolla, California

Background: The recent emergence of cathinone derivative stimulant drugs in recreational use markets recommends controlled laboratory studies to better delineate the risks posed for personal and public health. Although limited, the early evidence shows that some of the more common cathinones, such as 3,4-methylenedioxypyrovalerone (MDPV) and 4-methylmethcathinone (4-MMC; mephedrone), exhibit properties *in vivo* that would not be inferred from structure alone. The purpose of the present work was to determine the extent to which these two compounds support intravenous self-administration in rats.
Methods: Male Wistar and Sprague-Dawley rats were used to assess the intravenous self-administration of MDPV (0.025–0.05 mg/kg/infusion training dose) and 4-MMC (0.5–1.0 mg/kg/infusion training dose) in comparison with d-methamphetamine (MA) and 3,4-methylenedioxymethamphetamine (MDMA), respectively. The 4-MMC and MDMA self-administration was contrasted under 20°C and 30°C ambient temperature conditions.
Results: MDPV was readily self-administered by rats, exhibiting dose dependency under both FR and PR dose-substitution procedures with MDPV(0.01–0.50 mg/kg/inf) exhibiting greater potency and efficacy than MA (0.1–0.25 mg/kg/inf). 4-MMC generated more consistent self-administration across individuals than did MDMA and engendered greater rates of responding across a wide range of ambient temperatures.
Conclusions: The self-administration of MDPV is highly consistent with the emerging pharmacological profile which demonstrates rapid brain entry and activity dominated by dopamine transporter blockade. This compound poses significant threat for compulsive use. 4-MMC self-administration is much more consistent (across individuals) and robust than would be predicted by the reported MDMA-like neuropharmacological effects.
Disclosure: M. Taffe, Nothing to Disclose.

Mini-Panel

17. Emerging Role of the Primary Cilium in Neuropsychiatric Disorders

17.1 Abnormal Response to Stress in Heterozygous AHI1 Knockout Mice: A Consequence of Primary Ciliary Dysfunction?

Bernard Lerer*

Hadassah—Hebrew University Medical Center, Jerusalem

Background: The Abelson helper integration site 1 (AHI1) gene plays a pivotal role in brain development. AHI1 encodes Jouberin (Jbn) which is highly expressed in mammals throughout the developing brain and has been localized to the primary cilium, a highly conserved organelle central to the regulation of cellular signaling pathways. Studies by our group and others have demonstrated association of AHI1 with schizophrenia and autism. To elucidate the mechanism whereby alteration in AHI1 expression may be implicated in the pathogenesis of psychiatric disorders, we studied Ahi1 heterozygous knockout ($Ahi1^{+/-}$) mice on an extensive series of behavioral tests and resting-state functional MRI.
Methods: Heterozygous *Ahi1* knockout mice ($Ahi1^{+/-}$) were generated from chimerae purchased from MMRC (California, USA). $Ahi1^{+/+}$ littermates were used as controls. We

compared performance of male *Ahi1*$^{+/-}$and *Ahi1*$^{+/+}$ mice on behavioral tests that model cognitive, negative and positive aspects of schizophrenia as well as tests evaluating anxiety levels such as elevated plus maze, light dark box and open field. Serum cortisol and core body temperature were measured following behavioral and pharmacological experiments. We examined neural circuits through the use of functional connectivity imaging during resting state (rsfMRI).
Results: Although their performance was not different from wild type mice on tests that model schizophrenia-related endophenotypes, *Ahi1*$^{+/-}$ mice displayed an anxiolytic-like phenotype across converging modalities. Using behavioral paradigms that involve exposure to environmental and social stress, significantly decreased anxiety was evident in the open field, elevated plus maze and dark light box, as well as during social interaction in pairs. Assessment of core temperature and corticosterone secretion revealed a blunted response of the autonomic nervous system and the hypothalamic-pituitary-adrenal axis in *Ahi1*$^{+/-}$ mice exposed to environmental and visceral stress. However, response to centrally-acting anxiogenic compounds was intact. Using resting-state functional MRI, connectivity of the amygdala with other brain regions involved in processing of anxiogenic stimuli and inhibitory avoidance learning, such as the lateral entorhinal cortex, ventral hippocampus and ventral tegmental area, was found to be significantly reduced in the mutant mice.
Conclusions: Taken together, our data link *Ahi1* underexpression with a defect in the process of threat detection; such emotional and cognitive defects have been described in schizophrenia and autism. Alternatively, the results could be interpreted as representing an anxiety-related endophenotype, possibly granting the *Ahi1*$^{+/-}$ mouse relative resilience to various types of stress that is related to abnormal connectivity of the amygdala with brain regions involved in processing anxiogenic stimuli and inhibitory avoidance learning. The extent to which these functional abnormalities are related to primary ciliary dysfunction in *Ahi1*$^{+/-}$ mice, is the focus of our current research efforts.
Disclosure: B. Lerer, Nothing to Disclose.

17.2 Functional Significance of Primary Cilia to GPCR Signaling and Relationship to Neuropsychiatric Disease

Mark Von Zastrow*

University of California, San Francisco, California

Background: Primary cilia are evolutionary ancient structures that, in humans, have well established functions in sensory signaling and in development. It is now clear that a number of diseases involve disruption of ciliary structure or function, and in which dysfunction of primary cilia is likely fundamental to etiology. Many of these 'ciliopathies' include neurological and behavioral signs or symptoms. This suggests the possibility that ciliary dysfunction may be of etiologic significance to major neuropsychiatric disorders or their treatment. Several observations made in the lab support this general hypothesis, and have motivated efforts to understand the functional significance of primary cilia to neural function. My presentation will briefly review these observations and then will discuss recent work investigating the cellular basis of dopamine receptor localization to cilia and the functional significance of ciliary receptor localization to G protein-mediated signaling.
Methods: We have used RNA interference to achieve targeted knockdown of the expression of genes implicated in major neuropsychiatric diseases. We have used fixed and live cell imaging methods to examine receptor localization and dynaicms in cultured neurons relative to cilia. We have developed cilia-targeted signaling biosensors to investigate location of receptor signaling events and thedownstream mediators cyclic AMP relative to cilia.
Results: We find that a significant fraction of genes associated with major neuropsychiatric disrupt cilia when knocked down in cell culture, and that a number of the protein products of these genes localize in or near cilia. We have shown that D1 and D2 dopamine receptors present in cilia represent a diffusionally isolated population that is capable of G protein-linked stimulation of adenylyl cyclase. We have observed that cAMP produced by activation of ciliary D1 receptors is not constrained to cilia and can access the peripheral cytoplasm on a similar time scale as the acute signaling response. We have found a discrete signaling function of ciliary receptor localization to cilia, as well as of exclusion of receptors from cilia, which suggests a form of combinatiorial control of integrated cellular GPCR signaling.
Conclusions: Primary cilia are likely to represent a major site of signal integration in neurons, mediate signaling specificity at the level of closely related GPCRs, and have intriguing disease implications for neurology and psychiatry.
Disclosure: M. Von Zastrow, Nothing to Disclose.

17.3 The Role of AHI1 in Regulating Primary Cilia Signaling

Russell J. Ferland*

Albany Medical College, Albany, New York

Background: While neurons have elaborate dendrites and spines, they also have a newly re-appreciated organelle called the primary cilium (PC). However, little is known regarding the function of PC, particularly as it relates to its role on neurons. Enriched receptor and protein expression and an isolated subcellular compartment make PC a unique organelle for receiving and transducing extracellular signals. The recent identification of a subset of GPCRs present at PC implicates the importance of PC in GPCR signaling. However, it is unclear whether GPCRs at PC and at the cell body have the same ability for efficiently initiating signaling and activating downstream events. In addition, another function for PC on neurons and glia may be in monitoring local osmolarity shifts, particularly given that brain cells are constantly undergoing fluctuations in osmolytes. Since regulation of osmolarity in the brain is critical for normal function, PC may be a focused center for osmolarity sensing that when dysregulated could lead to a variety of pathophysiological brain conditions. In this talk, I will present evidence that AHI1 is required for the proper localization of one of the known neuronal PC-enriched GPCRs, MchR1 to PC, that much of the MchR1 signaling is occurring on PC (and not the cell body), and that MchR1 undergoes an orchestrated trafficking pattern upon ligand

application that does not occur in Ahi1 knockout neurons. Lastly, I will show evidence that PC may be serving as a critical osmolarity sensor for neurons that depends on the presence of Ahi1.
Methods: Mice with targeted deletions of *Ahi1* and wildtype littermate control mice were used. Most experiments utilized neuronal cultures from embryonic day 18.5 brains from wildtype and *Ahi1* knockout animals, in which the following were examined: (1) MchR1 trafficking (via immunolabeling), (2) MchR1 signaling (through calcium imaging and cAMP assays), and (3) quantification of PC number and morphology on neurons. To examine osmolarity sensing of neuronal PC, neuronal cultures from embryonic day 19 rat embryos were used. Changes in neuronal osmolarity were accomplished by increasing the extracellular concentration of osmolytes in the media followed principally by immunolabeling and Western blot experiments (using antibodies to Ahi1 and ciliary proteins). In addition, removal of PC from neurons using chloral hydrate was used to assess the effects of PC on the ability of neurons to sense changes in extracellular osmolarity.
Results: Targeted deletion of *Ahi1* results in a dramatic reduction of MchR1 localization at neuronal PC. Given normal expression and surface targeting of MchR1, the decreased ciliary MchR1 distribution in *Ahi1* knockout neurons is likely a result of defective ciliary membrane protein trafficking. Dynamic redistribution of MchR1 at PC upon ligand stimulation implicates a critical role for PC in MchR1 signaling. Strikingly, *Ahi1* knockout neurons with normal MchR1 plasma membrane expression, but lacking MchR1 expression on PC, have reduced signaling upon ligand stimulation as indicated by an inhibition of forskolin-induced cAMP production. For osmolarity, we identified a novel and unknown hyperosmotic-induced structure that was localized with Ahi1. Both the actin polymerization inhibitor, latrunculin, and the loss of Hap1, one of the main interactors with Ahi1, resulted in an inability to observe the Ahi1-positive structure under hyperosmotic conditions. Moreover, loss of neuronal PC using chloral hydrate eliminated the formation of these hyperosmotic-induced Ahi1-positive structures.
Conclusions: This study suggests a critical role for PC as a requisite site for GPCR localization and ligand reception in order to activate downstream signaling. In addition, our work begins to shed light on how the neuronal PC may help neurons sense and signal osmolarity fluctuations both in the normal and diseased brain.
Disclosure: R. Ferland, Nothing to Disclose.

Panel

18. An Update from the Clinic on mGluR2/3 Approaches for Treating Schizophrenia—Understanding Human Circuit Engagement through to Recent Clinical Trials

18.1 AZD8529—An mGluR2 Positive Allosteric Modulator for the Treatment of Schizophrenia

Alan Cross*

AstraZeneca, Cambridge, Massachusetts

Background: Considerable evidence indicates that hypofunction of prefrontal cortex glutamate signalling and decreased NMDA receptor activation may play an important role in schizophrenia. These receptors are located on GABAergic interneurons, suggesting that hypofunction results in a lack of downstream inhibition and disruption of cortical circuit activity. Activation of the mGluR2 receptor is proposed as a novel therapeutic strategy for schizophrenia, based on the hypothesis that this mechanism would counteract the glutamatergic disinhibition produced by NMDA receptor hypofunction.
Methods: *In-vitro* pharmacological studies used recombinant human mGluR receptors and rat hippocampal slice electrophysiological assays. Preclinical *in vivo* studies examined the effects of AZD8529 on baseline and MK801 induced disruption of firing of mPFC neurons in behaving rats.
Results: AZD8529 acts as a selective positive allosteric modulator of mGluR2, it potentiated the effects of glutamate at the recombinant human receptor with an EC_{50} of 193 nM and an E_{max} of 93% with little effect on other mGluR subtypes. In a rat hippocampal slice assay, AZD8529 enhanced DCG-IV-induced reduction in synaptic transmission at hippocampal Schaffer collateral synapses by 30%, with an EC_{50} of 87 nM. Administration of AZD8529 (10 umol/kg, s.c.) caused a modest ($<10\%$), reduction in baseline firing of mPFC neurons in behaving rats. MK-801 (0.2 mg/kg, s.c.) led to an increase in firing rates of recorded neurons, accompanied by a profound disturbance in organization of cortical firing as measured by a large increase in the variability of firing rates. Administration of AZD8529 significantly reversed the MK-801-induced increase in firing rate variance reflecting a normalization of cortical function.

AZD8529 was well tolerated in human volunteers, with CNS drug exposure confirmed through csf analysis and subseuqently examined in a proof of principle study in symptomatic patients with schizophrenia (Litman et al 2013). Following 7 days washout period, patients received AZD8529 40mg ($n=58$), risperidone 4mg ($n=31$), or placebo ($n=55$) for 28 days, clinical efficacy was assessed using the Positive and Negative Syndrome Scale (PANSS). Whilst risperidone reduced PANSS total score change from baseline compared with placebo (delta $=-9.5$, $p<0.001$), AZD8529 was without effect (delta =1.3, $p=0.491$).
Conclusions: The current data are not consistent with positive modulation of mGluR2 receptors as a mechanism for monotherapy to treat acute schizophrenia. Whether different treatment regimens and particularly adjunct treatment would provide benefit remains to be determined.
Disclosure: A. Cross, Nothing to Disclose.

18.2 Discovery and Early Clinical Development of Novel mGlu2 Receptor PAMs

Hilde Lavreysen*

Janssen Pharmaceutica NV, Beerse, Belgium

Background: Increasing evidence is emerging that several neurological and psychiatric diseases are characterized by disturbed glutamatergic signalling. The mGlu2 receptor is a current focus of research for the treatment of schizophrenia, mood and anxiety disorders. Modulation of the mGlu2

receptor can occur via allosteric or orthosteric ligands. Janssen Research & Development, in collaboration with Addex Therapeutics, have an advanced mGlu2 Positive Allosteric Modulator (PAM) program, and currently have mGlu2 PAM molecules in clinical development.
Methods: Various *in vitro* and *in vivo* preclinical tools were used in the course of the program, including *ex vivo* and *in vivo* mGlu2 receptor occupancy with novel radioligands, sleep-wake EEG recording in rodents, conditioned avoidance responding and 2-deoxyglucose utilization, a lactate-induced rat challenge model and a dominant-submissive rat model. An exploratory treatment study of subtypes of patients with schizophrenia was conducted to generate hypotheses for future proof-of-concept studies.
Results: The talk will cover the discovery and early clinical development of novel mGlu2 receptor PAMs. We will discuss our efforts that have led to the identification of mGlu2 receptor-specific radioligands to measure target engagement and at the same time focus on the challenge of using occupancy studies for this target, because of discrepancies between *in vitro* and *in vivo* labeling of mGlu2 receptors. Though mGlu2 receptor-mediated effects on sleep-wake EEG recording in rodents were found to be a highly reliable, functional read-out of central activity, circadian aspects were found to be of importance for translational clinical testing. The pharmacological properties of different PAMs in models predictive of antipsychotic-like activity will be shown, as well as the effect in animal models evaluating the potential of mGlu2 receptor PAMs in other indications such as anxiety or depression. Hence, we report that mGlu2 PAMs may not be effective in 'classical' models of anxiety or depression but show activity in subchronic models like a lactate-induced rat challenge model and dominant-submissive rat model. The difficulties in determining which *in vivo* read outs are most indicative for allosteric modulation of changes in synaptic glutamate levels, and similarities and differences compared to orthosteric agonists will be discussed.
Conclusions: Based on the observations in various preclinical models, JNJ-40411813 was selected for further clinical development. The translation from preclinical observations to human studies, along with the first data of JNJ-40411813 in a patient trial in schizophrenia will be discussed. Safety of the compound was found to be good, and a signal in patients with residual negative symptoms of schizophrenia was identified.
Disclosure: A. Cross, Nothing to Disclose

18.3 Efficacy of an mGluR2 Agonist (LY354740) and an mGluR2 Positive Allosteric Modulator (AZD8529) in Attenuating Ketamine Effects in Humans

John H. Krystal*

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Background: Experimental medicine studies in healthy human subjects may accelerate the drug development process by providing insights that support the hypothesized mechanism of action of novel therapeutics. In studies that date to the late 1980's, it was hypothesized that the ability of novel schizophrenia therapeutics to attenuate the schizophrenia-like symptoms produced by the NMDA glutamate receptor antagonist, ketamine, might provide this type of experimental medicine assay. Subsequently, as similarities between the effects of ketamine on neural systems and network dysregulation in schizophrenia became more clear, it was hypothesized that the ability of drugs developed for schizophrenia to attenuate ketamine effects on circuit activity related to cognition or symptoms might be reflective of aspects of its therapeutic potential for schizophrenia. The purpose of this presentation is to present our experience using ketamine to study the effects of mGluR agonist/PAMs, as a case study of insights that might emerge from this experimental medicine strategy.
Methods: Two studies are reviewed. We will briefly present a previously published study which evaluated the dose-related (placebo, 100 mg, 400 mg) effects of the mGluR2 agonist, LY354740 on the cognitive and behavioral response to ketamine (0.26 mg/kg bolus followed by 0.65 mg/kg administered over 100 minutes).We will also present the preliminary analyses of an unpublished within-subjects study in healthy subjects of the dose-related (placebo, 50 mg, 180 mg) of the mGluR2 PAM, AZD8529, on the effects of ketamine (bolus of 0.23 mg/kg, followed by 0.58 mg/kg/hr). Each test day was preceded by the oral administraiton of the mGluR2 PAM/placebo 12 hours prior to testing. Test days involved the blinded intravenous infusion of saline and then ketamine in a fixed order while in the Siemens Tim Trio 3T magnet. Imaging took place while subjects received saline/ketamine infusions and during rest (visual fixation) and while they performed a spatial working memory task. A previous study from our group (Driesen *et al*. Biol Psychiatry 2008) showed that schizophrenia patients showed impaired PFC activation while performing this task.
Results: In the initial study, LY354740 produced a significant dose-related attenuation of ketamine-related impairments in working memory. There was also a trend to reduce positive symptoms produced by ketamine.In the second study, AZD8529 did not attenuate the ketamine-related impariment in spatial working memory-related PFC activation or reduce psychotic symptoms produced by ketamine. However, ketamine produced asynchrony in the activation of DLPFC and BA 10 that was associated with the severity of ketamine-induced symptoms. Preliminary analyses suggest that AZD8529 normalized the synchrony between these regions and eliminated the statistical association between synchrony and symptoms.
Conclusions: These data suggest that mGluR2 agonoists or PAMs might be able to reduce some behavioral and circuit effects of ketamine in healthy human subjects. The effects of the mGluR2 modulators are dose dependent and may additionally depend on the ketamine dose and the specific outcome evaluated.
Disclosure: J. Krystal, **Part 1:** Abbott, Amgen, AstraZeneca, BMS, Eisei, Estellas, Forest, Johnson and Johnson, Lilly, Lohocla, Mnemosyne, Naurex, Novartis, Pfizer, Shire, Sunovion, Takedam, Teva, **Part 4:** AstraZeneca, Pfizer

18.4 The Development of Pomaglumetad Methionil as an Innovative Glutamate-based Pharmacotherapy for Schizophrenia

Bruce J. Kinon*

Eli Lilly and Company, Indianapolis, IN

Background: Although dopamine has traditionally received attention as the key neurotransmitter mediating the symptoms of schizophrenia, accumulating evidence implicates a dysregulation of the glutamatergic system as a prominent contributant to the pathophysiology of the disease. Understanding the role of glutamate in schizophrenia may provide a new direction for the development of innovative and hopefully more effective drug therapies for schizophrenia. Pomaglumetad methionil (LY2140023 monohydrate) is a potent and highly selective agonist for the metabotropic glutamate mGluR2 and mGluR3 receptors.We present results of a clinical development program assessing the efficacy of pomaglumetad methionil in reducing psychopathology either as a monotherapy in patients with an acute psychotic exacerbation or as an add-on therapy in patients with prominent negative symptoms. Negative findings from these studies have dampened hope that a glutamate-based therapy for schizophrenia will be forthcoming in the near future, although a new strategic reconceptualization may be evolving.

Methods: The efficacy and safety results of 5 acute monotherapy trials, 3 long-term safety trials, and a subacute add-on trial will be summarized. Post-hoc data analyses at either an individual study level or integrated across trials will be presented to determine whether disease-state (duration of illness; previous antipsychotic drug treatment) or disease-trait (non-Hispanic white patients with schizophrenia carrying T-alleles for the single-nucleotide polymorphism rs7330461 in the serotonin 2A receptor (HTR2A) compared with A/A homozygotes) may influence response to pomaglumetad.

Results: In acute monotherapy trials, the primary outcome PANSS-Total did not demonstrate efficacy for pomaglumetad at doses of 10 mg to 80 mg BID as compared to placebo. Add-on of pomaglumetad (10 mg-40 mg BID) to previous atypical antipsychotic drugs for the treatment of prominent negative symptoms in partially remitted patients did not demonstrate efficacy versus add-on placebo as assessed by the NSA-16. Pomaglumetad was generally well-tolerated across all trials and demonstrated a low association for weight gain and DAD2-mediated adverse events. The incidence of seizures was found to be comparable to currently available antipsychotic drugs. Post-hoc analyses suggested that a subgroup of patients may demonstrate response to pomaglumetad.

Conclusions: Pomaglumetad methionil treatment did not demonstrate efficacy in the *a priori* specified populations studied. The successful development of potential glutamate-based pharmacotherapy for schizophrenia may require an examination of areas of symptom response and brain activity little influenced by present antipsychotic drugs but impacted by modulation of glutamate activity. Further understanding of the unique role of glutamate as a therapeutic target in schizophrenia is needed.

Disclosure: B. Kinon, **Part 1:** Employee of Eli Lilly and Company, Shareholder of Eli Lilly and Company, **Part 2:** Employee of Eli Lilly and Company, Shareholder of Eli Lilly and Company, **Part 3:** Employee of Eli Lilly and Company.

Panel

19. Anxiety and the Striatum, an Unusual Suspect

19.1 Cortico-amygdala Pathways form Hierarchical Networks that Predict Output to the Striatum

Julie L. Fudge*

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Background: Older models of brain organization have emphasized a point-to-point approach, with nodal connections between specific cortical and subcortical regions. Newer models examining whole-brain function show that multiple cortical regions are 'co-activated' with subcortical structures. The tendency of a particular set of afferent inputs to be activated varies by individual, correlating with strength of the putative connection (Saygin, SM 2012). The amygdala is a complex structure with multiple roles in emotional responding, and its activation to specific stimuli varies enormously across individuals.

Methods: To understand the organization of multiple afferent and efferent connections through the amygdala, we used a bidirectional tracer approach in nonhuman primates. We explored the anatomy of connections from both the orbital and medial prefrontal cortex (OMPFC) and insula, two key inputs based on previous work in nonhuman primate. We then analyzed the impact of corticoamygdala organization on the topography of amygdalostriatal outputs within and across animals.

Results: Cortical subregions in both the OMPFC and insula combined to send a layered set of hierarchical connections across different amygdala subregions. Surprisingly, combinations of OMPFC and insula inputs to specific amygdala regions also predicted the organization of outputs in the next limb of the circuit to the striatum. Rather than a point-to-point schema, cortical inputs to the amygdala (1) are organized according to cytoarchitectural features *across* lobes, and (2) are integrated to form an increasingly complex set of inputs from the ventral to dorsal amygdala. In sum, a primitive circuit emanates from the most undifferentiated regions of both insula and OMPFC, influencing all amygdala outputs. Layered upon this in slightly more dorsal regions are projections from slightly more differentiated insula and OMFPC subregions. Finally, relatively differentiated regions of the OMPFC and insula add projections to the most dorsal amygdala, reflecting high levels of information processing in this region. Amygdala-striatal outputs follow suit, with ventral amygdala regions that receive only 'primitive' inputs targeting a restricted region of the dorsomedial shell, while more dorsal amygdala regions that receive all three inputs ('primitive', 'intermediate', and 'developed') project broadly from the ventral striatum to more dorsolateral regions of the striatum

Conclusions: This organization elucidated shows a complex cortical modulation of amygdala function based on cortical architecture, with implications for (1) functional anatomy of the amygdala, and (2) the breadth and complexity of amygdalostriatal outputs.
Disclosure: J. Fudge, Nothing to Disclose.

19.2 Endocannabinoids in the Dopaminergic Control of Punishment and its Avoidance

Joseph Cheer*

University of Maryland School of Medicine, Baltimore, Maryland

Background: Endocannabinoids (eCBs) may provide animals with an adaptive advantage by promoting behaviors that maximize reward while minimizing anxiety. Ventral straital function, which is thought to generate a teaching signal involved in the selection of advantageous behavioral repertoires, is under control of eCBs and; therefore, may contribute to the ability of eCBs to not only strengthen responses leading to the procurement of reward but also those leading to the reduction of harm. We previously demonstrated that disrupting eCB signaling uniformly decreases dopaminergic encoding of reward-predictive cues and reward directed behavior.
Methods: Here, we investigate whether eCBs also modulate dopaminergic encoding of cues predicting either the avoidance of punishment or aversive outcomes. We first used fast-scan cyclic voltammetry to investigate whether disrupting eCB signaling alters dopaminergic encoding of cues predicting the avoidance of punishment during behavior maintained in a signaled shock avoidance procedure. In this task, a stimulus light was presented as a warning signal for 2-s prior to the delivery of recurring foot shocks. During this 2 s warning period, a response lever was extended into the testing chamber which, if pressed, produced a 20 s safety period signaled by a tone. Animals could initiate an avoidance response by pressing the lever within the 2 s warning period, entirely preventing shock. Alternately, once shocks commenced, animals could initiate an escape response by pressing the lever during this punishment period, terminating shock.
Results: Disrupting eCB signaling by treating animals with rimonabant (0.3–1 mg/kg IV) dose-dependently decreased concentrations of dopamine release in the nucleus accumbens that were time-locked to the warning signal while simultaneously weakening shock avoidance behavior, effectively shifting the behavioral outcome from avoidance to escape. We next assessed the effects of disrupting eCB signaling on dopaminergic encoding of cues predicting aversive outcomes using a fear-conditioning model. As previously reported, rimonabant treated rats were resistant to the extinction of fear memories induced by a fear-associated cue when presentations occurred 24hr after conditioning. Impaired fear memory extinction was accompanied by diminished dopaminergic encoding of the fear-associated cue in the nucleus accumbens. In vehicle treated rats we observed a decrease in accumbal dopamine release events during presentation of the fear-associated cue, an effect that was attenuated by rimonabant pretreatment (0.3 mg/kg IV).
Conclusions: Together these data suggest that eCBs might modify distinct behavioral responses related to aversive stimuli by modulating conditioned mesolimbic dopamine release events. This may have implications for conditions encompassing a high anxiety component such as compulsive drug seeking or post-traumatic stress disorder.
Disclosure: J. Cheer, Nothing to Disclose.

19.3 Neural Response in Striatum Varies by Reward Magnitude, Decision Making, and Anxiety Diagnosis in Adolescents

Amanda E. Guyer*

University of California,Davis, California

Background: Previous work suggests dysfunction of reward-related neural substrates in adolescent anxiety and risk for anxiety based on exaggerated striatal activation to as a function of reward magnitude. Past work in diagnosed adolescent anxiety has not yet examined the role of response contingency in reward anticipation. The present work hypothesized that heightened response in striatal regions of interest during a reward task that manipulates both reward magnitude and outcomes dependent upon participants' choice of action would differentiate anxiety and healthy adolescents.

Methods: Thirty-eight adolescents with ($n = 20$, 9 males, 12.0 +/-2.3 yo) and without ($n = 18$, 8 males, 13.3 +/- 2.3 yo) an anxiety disorder were compared on a reward task during fMRI. The task was designed to examine striatal responses to cue/reward anticipation as a function of making a choice *vs* not making a choice to gain a reward, and high *vs* low reward magnitude.
Results: A significant 3-way interaction of Group × Contingency × Reward Magnitude in striatal ROIs was found. A single cluster ($F(1,36) = 16.13$, $p < .0001$) encompassing the right caudate and putamen emerged as well as clusters in the left caudate ($F(1,36)=17.53$, $p < .0002$) and left putamen ($F(1,36)=16.56$, $p < .0003$). In the non-contingent condition, healthy adolescents showed the expected magnitude-related increase in striatal activation from low to high incentive (right caudate/putamen: $p < .000$; left putamen: $p < 0.002$; left caudate: $p < 0.000$), whereas anxious adolescents showed higher activation to low vs. high incentives (right caudate/putamen: $p < 0.05$; left putamen: $p < 0.05$). In the contingent condition, healthy adolescents showed no effects of magnitude on the right striatal cluster. However, in anxious adolescents greater activation to high than low incentive ($p=0.027$) magnitude was found.
Conclusions: These findings reflect unique striatal perturbations in the processing of incentive magnitude that differed in a choice and no-choice situation as a function of anxiety diagnosis. Cognitive processes associated with decision-making play a role in the striatal hypersensitivity to the size of incentive cues encountered by anxious adolescents.
Disclosure: A. Guyer, Nothing to Disclose.

19.4 The Impact of Induced Anxiety on Ventral Striatal Response to Aversive and Appetitive Prediction Error Signals

Oliver J. Robinson*

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Background: Anxiety can facilitate aversive conditioning. This can lead to debilitating emotional responses in post-traumatic stress disorder, but the mechanism is unknown. Computational neuroscience has argued that the detection of a mismatch between expected and observed outcomes within the ventral striatum (i.e., 'prediction errors'), is a critical precursor to the formation of new learned associations. Here we combine a translational model of anxiety with a cognitive neuroimaging paradigm to test whether anxiety can alter prediction error processing.
Methods: 24 subjects underwent threat of unpredictable foot shock whilst completing a simple task designed to elicit matched appetitive (happy face) and aversive (fear face) prediction errors. Neural substrates of prediction errors were established by recording striatal blood-oxygen-level-dependent (BOLD) signal using a functional magnetic resonance imaging scanner. Trait disposition to anxiety disorders was determined using the BIS/BAS questionnaire.
Results: Subjects were significantly more anxious ($F_{1,23} = 255$, $p < 0.001$) during the threat of shock condition which provoked a anxiety*valence interaction in the right ventral striatum [peak voxel $xyz = 28, 10, 10$; P(family-wise error $[\mathrm{FWE}]_{\mathrm{voxel-level}}) = 0.022/P(\mathrm{FWE}_{\mathrm{cluster-level}}) = 0.038$], which was driven by significantly increased aversive PE signal under threat relative to safe ($F_{1,23} = 5.8$, $p = 0.02$) but no comparable change for appetitive PE signal ($F_{1,23} = 1.0$, $p = 0.33$). Threat induced aversive prediction error signal was negatively correlated with both fun-seeking and reward responsiveness subscales of the BIS/BAS questionnaire ($P < 0.03$).
Conclusions: Anxiety significantly increases ventral striatum aversive (but not appetitive) prediction error signal. This may potentially explain trauma reminder in anxiety disorders as well as adaptive recall of aversive events under normative anxious responding. Resilience to psychiatric disorders (as measured by personality scales) may be associated with reduced recruitment of this mechanism.
Disclosure: O. Robinson, Nothing to Disclose.

Panel

20. At the Crossroads of Physics, Physiology, and Psychiatry: Rational Design of Noninvasive Neuromodulation Therapies

20.1 Enhancement of Working Memory in Sleep Deprived Young Adults and in Elderly Adults using rTMS Informed by Covariance-modeled fMRI

Bruce Luber*

Duke University, Durham, North Carolina

Background: The use of covariance-modeled fMRI-guided TMS represents a new and sophisticated means of exploring cortical function. In neuroimaging studies of human cognitive abilities, brain activation patterns that include distributed regions that are strongly interactive in response to experimental task demands are of particular interest. Covariance modeling of fMRI data can capture these patterns of task-related functional activity and provide regional targets for TMS, which can then be further individualized by choosing the peak activity in the target region on an individual basis. The specific form of covariance modeling we use is called the Ordinal Trends model (OrT), which uses a guided PCA approach which expects a range of individual variability in brain imaging data, and makes use of it in a within-subject model that identifies networks that exhibit sustained activity across graduated changes in task parameters (such as increasing memory load). Such an approach produces cortical regions likely to be highly sensitive to TMS modulation, an approach we demonstrate in a series of experiments in which working memory is enhanced using TMS.
Methods: In a first set of experiments, fMRI data from 18 healthy young adults recorded while they performed a delayed-match-to-sample (DMS) letter working memory task before and after two days of total sleep deprivation (SD) were analyzed using OrT. A second group of 15 healthy young adults participated in a TMS session after two days of SD. TMS was applied at three cortical locations determined by the covariance modeling of the fMRI data in the first group. Another 27 healthy adults participated in a third study in which rTMS was applied over four sessions during the two day SD period. In a second set of experiments, the fMRI data of 44 healthy elderly adults and 60 young adults performing the DMS task were analyzed using OrT. Three rTMS target regions based on this analysis were used on 20 healthy elderly adults and 20 young adults over three TMS sessions. Individual peak activity in each target region were chosen for TMS targets.
Results: In the first set of experiments, a cortical network was found that was both significantly activated by the DMS task and whose activation decreased across SD. rTMS applied to cortical sites within that network partially remediated the effects of SD on the DMS task, and the degree of fMRI network deactivation due to SD was significantly coupled with improvement caused by the rTMS. rTMS applied to an occipital node of the network over multiple sessions during SD resulted in complete remediation of the effects of SD in the DMS task in sleep deprived subjects 18 hours after the last TMS session. In the second set of experiments, fMRI networks were found whose activation levels were significantly correlated with individual reaction time (RT) in both elderly and young groups. Three nodes of these networks (in left premotor and occipital cortex and SMA) were used as targets. Significant target × TMS (active *vs* sham) effects were found for both RT and accuracy of DMS performance, with TMS to occipital cortex significantly facilitating RT, and TMS to premotor cortex disrupting performance, more strongly so in the elderly group.
Conclusions: Covariance modeling of fMRI data using OrT uncovered cortical networks related to working memory performance in the context of SD and of aging, and the use of TMS targeting these networks enhanced performance. The use of covariance-modeled brain imaging and TMS are

strongly complementary, with the former providing a sophisticated method of choosing TMS targets using both group and individual information, and use of the latter helping to causally validate interpretation of network function.
Disclosure: B. Luber, Nothing to Disclose.

20.2 Mechanisms of Targeting Cortical State Dynamics with Neuromodulation

Flavio Frohlich*

University of North Carolina Chapel Hill, North Carolina

Background: Non-invasive brain stimulation represents a promising treatment modality for a broad spectrum of psychiatric disorders. Transcranial Alternating Current Stimulation (tACS) targets cortical oscillations that play a fundamental role in cognition and exhibit deficits in patients with psychiatric illness. Elucidating how tACS interacts with cortical network dynamics is crucial for the rational design of individualized brain stimulation for therapeutic purposes. Here, we present our interdisciplinary research that combines computer simulations and *in vivo* electrophysiology in ferrets.
Methods: For the computer simulations, two large-scale cortical model networks were connected by long-range projections that exhibited realistic propagation delays (up to 50 msec). tACS was simulated by application of an equivalent weak somatic current injection. Weak electric fields were applied to deeply anesthetized ferrets (1% isoflurane). Multiunit spiking activity was recorded with laminar probes that spanned all cortical layers. Spectral analysis of multiunit firing was used to evaluate frequency-specific enhancement of network activity. Statistical significance was determined with the Wilcoxon rank sum test.
Results: In the computer simulations ($N = 100$), we found that tACS enhances ongoing cortical oscillations preferentially at the endogenous oscillation frequency (0.65 versus 0.24 normalized enhancement, $p < 0.05$). In further agreement with presence of resonance dynamics, we found that (1) increasing the stimulation amplitude broadened the range of frequencies at which the network was entrained and that (2) entrainment also occurred at the first harmonic frequency. The interconnected cortical networks exhibited spontaneous metastable behavior that was characterized by spontaneous transitions between three qualitatively distinct network states (0.135 ± 0.0134 Hz transition frequency, mean ± s.e.m.). Not only did simulated tACS preferentially switch the networks to the fully synchronized state (transition probability: 86.89% with stimulation versus 43.22% without stimulation) but also did these enhancing effects outlast the stimulation (0.995 correlation in oscillation power enhancement between during and after stimulation). Therefore, our model predicts that tACS modulates cortical network dynamics by switching multistable networks between different cortical states.

Our ferret experiments (N=2, 450 trials/condition) allowed the assessment of tACS on complex, convoluted cortex with invasive electrophysiology. In agreement with our modeling data, we found that weak sine-wave electric fields enhanced network oscillations in a frequency specific way (enhancement ratios: 1.20, 1.14, 1.11, 1.15, 1.17, for stimulation frequencies 0.5 to 2.5 Hz, all $p < 0.00001$,). Superficial network sites showed the strongest enhancement of the ongoing oscillation. The enhancement of the ongoing oscillations was mediated by preferential multiunit firing at the depolarizing phase of the applied electric field.
Conclusions: We used an interdisciplinary approach to demonstrate that resonance dynamics mediate the mechanistic underpinnings of how tACS modulates cortical oscillations. The resulting understanding of the interaction dynamics between applied stimulation and endogenous brain activity provides an important basis for the subsequent rational design of non-invasive brain stimulation that targets individual, patient-specific deficits in cortical network dynamics.
Disclosure: F. Frohlich, Nothing to Disclose.

20.3 Optimizing Stimulus Pulse Characteristics for Transcranial Magnetic Stimulation and Electroconvulsive Therapy via Device Development, Computational Modeling, and Biophysically-motivated Dosing Paradigms

Angel V. Peterchev*

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Background: Electroconvulsive therapy (ECT) is highly effective for the treatment of depression but is associated with cognitive side effects. Repetitive transcranial magnetic stimulation (rTMS) has no significant side effects but is less effective than ECT. ECT is delivered with high, fixed current amplitude that results in excessively strong direct stimulation of most of the brain, potentially contributing to side effects. rTMS is delivered with bidirectional sinusoidal pulses that may be suboptimal for neuromodulation. Two studies illustrate how device development, computational modeling, and biophysically-motivated dosing paradigms could inform improvements of ECT and rTMS technique.
Methods: Study 1: The change of motor cortex excitability resulting from 1 Hz rTMS with 4 different pulse shapes was characterized in 12 healthy human subjects. The pulse shapes included a conventional bidirectional, cosine pulse and 3 rectangular pulses generated with an in-house-built controllable pulse parameter TMS (cTMS) device: bidirectional or unidirectional in either posterior–anterior (PA) or anterior–posterior (AP) induced current direction. Excitability changes were quantified with TMS motor evoked potentials (MEPs). Study 2: Motor threshold (MT) and seizure threshold (ST) were titrated in anesthetized *macaca mulatta* by stepping the current amplitude with bilateral (BL), right unilateral (RUL), bifrontal (BF), and frontomedial (FM) ECT electrode configurations. Seven subjects received BL and RUL stimulation and 4 of them received BF and FM stimulation. The electric field induced by the four electrode configurations was simulated in MRI-based computational head models of the 4 subjects who received all types of ECT. The electric field distribution and the individual MT and ST were used to estimate the directly stimulated brain volume for seizure induction.

Results: Study 1: The 4 rTMS pulse shapes caused differing degrees of change in cortical excitability ($p<0.001$). Two rTMS pulse shapes generated significant ($p<0.05$) reduction in excitability (MEP amplitude reduction by 16% and 9% for unidirectional PA pulse and bidirectional rectangular pulse, respectively). The unidirectional AP pulse produced a nonsignificant MEP reduction by 7%. The conventional bidirectional cosine pulse produced a negligible MEP increase of 1.6%. Study 2: The mean percentage stimulated brain volume at individualized current amplitude ranged from 63% for BL ECT to 25% for RUL ECT. ST and MT were highly correlated for BL, RUL, and FM ECT ($R^2 \geqslant 0.80$, $p \leqslant 0.02$), and had a correlation trend for BF ECT ($R^2 = 0.51$, $p = 0.29$). The coefficient of variation of ST among the subjects was as high as 0.42. The simulated ratio of electrode current to right motor cortex electric field magnitude correlated strongly with the measured ST for RUL ECT ($R^2 = 0.98$, $p = 0.008$).
Conclusions: Study 1 showed that the effectiveness of rTMS can be enhanced substantially by using unidirectional, rectangular pulses with a specific current direction generated by a novel cTMS device. This could inform the study of unidirectional pulses to enhance therapeutic rTMS effectiveness. Study 2 demonstrated that generalized seizures can be induced with current amplitudes that produce more focal stimulation than conventional currents. Focality can be enhanced further via the electrode configuration. More focal stimulation could be explored as a means of lowering side effects. ECT stimulus amplitude should be individualized, since there is a wide spread of ST values across subjects. Both MT and the individual electric field model are strong predictors of ST and should be evaluated as safer alternatives to empirical ST titration for dose individualization.
Disclosure: A. Peterchev, **Part 1:** Dr. Peterchev is inventor on patents and patent applications on TMS technology assigned to Columbia University and Duke University, including technology licensed to Rogue Research; was Principal Investigator on a research grant to Duke from Rogue Research and equipment donations to Columbia and Duke by Magstim, MagVenture, and ANS/St. Jude Medical; has received patent royalties from Rogue Research through Columbia for TMS technology; and has received travel support from Rogue Research through Duke., **Part 3:** Dr. Peterchev has received patent royalties from Rogue Research through Columbia University for TMS technology that he invented., **Part 4:** Dr. Peterchev was Principal Investigator on a research grant to Duke from Rogue Research and of equipment donations to Columbia and Duke by Magstim and MagVenture.

20.4 Targeting of Transcranial Direct Current Stimulation: Insights from Cellular and Computational Models

Marom Bikson*

The City College of New York, New York, New York

Background: Transcranial direct current stimulation (tDCS) is investigated for the treatment of a broad range of neuropsychiatric disorders. Yet, with such broad application the question of mechanistic specificity remains to be addressed. Here we present data on two complimentary methods to achieve specificity, namely targeting of current flow through electrode montage design (including High-Definition tDCS) and by integration with cognitive therapy.
Methods: High-resolution computational models of tDCS current flow were developed from individual MRI. Our modeling work-flow preserves resolution of 1 mm. Current flow through targeted brain regions using *ad-hoc* and optimized tDCS electrode montages are compared. For cellular studies, acute cortical brain slices from rat where exposed to uniform electric fields. Modulation of pathway-specific synaptic activity and oscillation was quantified using field an intracellular recording.
Results: First, specificity of tDCS can be achieved through electrode montage optimization. tDCS dose governs which brain regions are activated and which are spared. Moreover, tDCS dose may be optimized on a subject specific basis. Use if High-Definition tDCS, a technology that replaces conventional tDCS sponge electrodes with an array of smaller electrodes, provides categorical increases in targeting. Second, tDCS can be functionally specific by preferentially modulation co-activated networks. Specificity can arise from network oscillations or synapse specific modulation.
Conclusions: Two distinct and complimentary mechanisms are shown in silico and *in vitro* to allow a relatively 'generic' modulation by direct current to produce specific outcomes. Current flow can be rationally steered to targeted brain regions while selective co-active of neuronal networks allows functional selectivity. These results provide a substrate for further clinical investigations using optimized tDCS montages and matched cognitive therapy activating networks of interest.
Disclosure: M. Bikson, **Part 1:** Marom Bikson has equity in Soterix Medical Inc., **Part 4:** Marom Bikson recieved grant support from Soterix Medical Inc.

Panel
21. Augmentation of Antidepressant Response by Autoreceptor-mediated Mechanisms: Clinical Experience and Mechanisms of Action

21.1 Autoreceptor-mediated Regulation of Neurotransmission: Pharmacological Targets and Potential for Improved Treatment of Major Psychiatric Disorders

Salomon Z. Langer*

Euthymia Ltd., Tel Aviv, Israel

Background: Before the discovery of presynaptic autoreceptors, the traditional view was that neurons communicate only in an anterograde direction: from the nerve terminal to the postsynaptic receptor. The characterization of the physiological role of presynaptic autoreceptors represented a new concept in neurotransmission, as information now was shown to be transferred also from the synaptic cleft to the presynaptic neuron, subserving an autoregulatory function in neurochemical transmission. Work during the last four decades showed that autoreceptors, initially discovered on NE neurons were also present for other neurotransmitters like dopamine, serotonin, acethylcholine, glutamate, and GABA. Autoreceptors were shown to correspond to different receptor subtypes than the corresponding postsynaptic

receptors, generating novel insights into the mechanism of action of drugs used in the treatment of neuropsychiatric diseases.
Methods: The calcium-dependent, electrically-evoked release of NE, dopamine and serotonin was studied in different areas of the rat brain, both *in vitro* and *in vivo* experimental conditions. The effects of agonists and antagonists of release-modulating autoreceptors were determined in the presence and in the absence of drugs which selectively inhibit the neuronal uptake of NE, or of serotonin. Statistical evaluation was made by Student t-test.
Results: Selective antagonists of autoreceptors enhance transmitter release during nerve stimulation, while the agonists reduce the stimulation-evoked release of the transmitter. Partial agonists, like aripiprazole, have a mixed effect on dopamine release which is concentration-dependent. When neuronal uptake of NE, dopamine or 5-HT is inhibited by drugs, autoreceptor antagonists were shown to possess increased efficacy in enhancing the release of NE, dopamine or 5-HT when compared to their effects when neuronal uptake was operational. In addition to the autoreceptor-mediated control of neurotransmission, presynaptic release-modulating heteroreceptors are present on nerve terminals from noradrenergic, serotonergic and dopaminergic neurons. Release-modulatig heteroreceptors respond to mediators other than the neuron's own transmitter. The presynaptic autoreceptors on noradrenergic neurons correspond to the alpha-2 adrenoceptors. Alpha-2 adrenoceptor antagonists like idazoxan and mirtazapine were shown to enhance release of both NE and dopamine in the medial prefrontal cortex. Several atypical antipsychotics possess potent alpha-2 adrenoceptor blocking properties. In the dopaminergic system, pharmacological differences exist between the D-2S presynaptic autoreceptor and the D-2 L postsynaptic receptor as demonstrated with the irreversible antagonist of postsynaptic dopamine receptors: (-)-N-(2-chloroethyl)norapomorphine.The presynaptic autoreceptor on serotonergic nerve terminals correspond to the 5-HT 1D subtype.
Conclusions: Antagonism of central alpha-2 adrenoceptors by drugs like mirtazapine, and several atypical antipsychotics enhance noradrenergic neurotransmission by blocking the negative feedback mechanism mediated by presynaptic alpha-2 adrenoceptors. These drugs also increase the release of dopamine in the medial prefrontal cortex, activating postsynaptic D-1 receptors. Presynaptic autoreceptors for NE, and dopamine are more sensitive to the effects of agonists and antagonists when compared to the corresponding postsynaptic receptor.
Disclosure: S. Langer, Nothing to Disclose.

21.2 Emerging Role of Atypical Antipsychotics as Add-on Therapy in Major Depression

Siegfried Kasper*

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Background: It is apparent that there has been a wide-spread and increasing use of atypical antipsychotics (AAPs) as well as low-potency typical neuroleptics (AP) as add-on for the treatment of depression in clinical practice both in the US and the EU, even at times when controlled studies had not been conducted and health regulatory authorities had not provided treatment guidelines.
Methods: We set out to investigate the use of AAPs in a European sample of depressed inpatients and the potential changes in their drug prescription over the time period 2000 to 2007. On two reference days in the years 2000 (32 psychiatric institutions, $N = 1.078$) and 2007 (54 psychiatric institutions, $N = 1.826$), the following data were recorded for all depressed inpatients (ICD-10: F32.00, F32.01, F32.1, F32.10, F32.11, F32.2, F33.0, F33.00, F33.01, F33.1, F33.10, F33.11 and F33.2) monitored as part of the AMSP (Arzneimittelsicherheit in der Psychiatrie),a drug surveillance program used in participating hospitals in Germany, Switzerland and Austria monitoring age, sex, ICD-10 diagnosis and all medication applied on the reference days. Depressed inpatients with psychotic symptoms were excluded.
Results: We found a significant increase in the number of AAP-treated inpatients from 37.9% in 2000 to 45.8% in 2007 (χ^2: 17.257, p^2: 93.37, p^2: 13.179, p^2: 2.047, $p = 0.15$).
Conclusions: Our study reveals a significant increase in the usage of AAPs in the general treatment of depression within the EU. Moreover, this effect was not only due to augmentation strategies for severely depressed inpatients and the dosage was usually lower than that in mania or schizophrenia. This study is insofar of particular importance since it demonstrates that, although health authorities only recently granted authorization for the use of atypical antipsychotics as add-on treatment in depression, psychiatrists were innovative in both discovering and using this treatment principle long before controlled studies had been conducted.
Disclosure: S. Kasper, Nothing to Disclose.

21.3 Low Doses of Atypical Antipsychotic Drugs Added to Selective Serotonin Inhibitors Produce a Ketamine-like Facilitation of Prefrontal Glutamatergic Neurotransmission

Torgny H. Svensson*

Karolinska Institutet, Stockholm, Sweden

Background: Clinically about 50% of MDD patients respond inadequately to SSRIs and adjunct treatment with low doses of atypical antipsychotic drugs (APDs) may potently augment the antidepressant effect with a fast onset of action, although the mechanisms involved are poorly understood. Preclinical data propose that enhanced catecholamine output and facilitated NMDA-receptor mediated transmission in the medial prefrontal cortex (mPFC) may partly explain this effect. Recent data suggest however that the rapid and potent antidepressant effects of ketamine and scopolamine are critically dependent on AMPA receptor-mediated transmission in the mPFC.
Methods: We used *in vitro* intracellular single electrode voltage clamp recordings to study the effects of low nanomolar concentrations of olanzapine or asenapine, alone or in combination with fluoxetine and escitalopram, respectively, on both NMDA and AMPA induced currents in

pyramidal neurons in the mPFC in rats. Moreover, the effect of a single antidepressant dose of ketamine was analysed on these glutamatergic receptors, 24 h after its systemic administration. Statistical evaluation was made by Students t-test and one-way ANOVA followed by Newman-Keuls test.
Results: Our results show that add-on low dose APD to an SSRI, eg low nanomolar concentrations of olanzapine to fluoxetine or asenapine to escitalopram, may facilitate both AMPA- and NMDA induced responses in pyramidal cells of the mPFC, an effect not attainable by each drug alone, which could be blocked by a selective D1 receptor antagonist. Moreover, analogous effects on both AMPA-and NMDA responses in the mPFC were produced by a systemic antidepressant dose of ketamine 24 h after its administration.
Conclusions: Since essentially analogous effects on AMPA- and NMDA responses were produced by a systemic antidepressant dose of ketamine, the effects on cortical glutamatergic transmission, in particular AMPA induced responses in the mPFC, may potentially explain the clinically well established rapid antidepressant augmentation obtained by adding low doses of APDs to SSRIs in treatment-resistant MDD.
Disclosure: T. Svensson, **Part 1:** Consultant/advisory board: AstraZeneca, Janssen, Lundbeck, Otsuka, Merck Sharp and Dome., **Part 4:** The Swedish Research Council, The Karolinska Institutet, Stockholm (Sweden), The Brain Foundation (Sweden), AstraZeneca, Organon, Schering-Plough, Merck Sharp and Dome, Lundbeck, Otsuka, Astellas.

21.4 Rapid Augmentation of Antidepressant Effect in Treatment-resistant MDD by Add-on Low Dose Aripiprazole

Daniel E. Casey*

Oregon Health and Science University, Lake Oswego, Oregon

Background: In managing major depressive disorder (MDD) with antidepressant treatment (ADT), two important issues regarding augmentation strategies require further research. First, are they effective in minimal or non-responders to adequate ADT regimens? Second, is there evidence to support an early and sustained response (ESusR) to ongoing ADT? We addressed these issues with post-hoc analyses of aripiprazole (ARI) *vs* placebo (PBO) augmentation studies.
Methods: In managing major depressive disorder (MDD) with antidepressant treatment (ADT), two important issues regarding augmentation strategies require further research. First, are they effective in minimal or non-responders to adequate ADT regimens? Second, is there evidence to support an early and sustained response (ESusR) to ongoing ADT? We addressed these issues with post-hoc analyses of aripiprazole (ARI) *vs* placebo (PBO) augmentation studies.
Results: In Study 1, Minimal Improvers (238 ARI; 214 PBO), had a significantly improvement in adjunctive ARI *vs* PBO as early as Week 1 ($p < 0.05$) and in Non-Improvers (126 ARI; 136 PBO) at Week 2 ($p < 0.05$). Improvement was sustained to endpoint in both ARI groups. A significantly greater proportion of patients in both groups responded to ARI compared with PBO at endpoint ($p < 0.01$). In Study 2 the rates of ESusR by MADRS in the adjunctive ARI and adjunctive PBO groups were 11.1% (43/386) and 5.0% (19/384), respectively. The mean weekly ending dose of ARI in those who achieved ESusR was 8.5 mg/day vs. 11.8 mg with adjunctive PBO.
Conclusions: Patients with no or minimal response to standard ADT can benefit from augmentation with low dose ARI and ESusR, using rigorous criteria, was demonstrated with adjunctive ARI at a rate more than double compared with adjunctive PBO to ADT.
Disclosure: D. Casey, **Part 1:** Consultant/advisory board: Bristol-Myers Squibb, Genentech and Merck; Speaker's bureau: Bristol-Myers Squibb, Merck and Sunovion.

Panel

22. Biotypes of Psychosis

22.1 Identification of Distinct Psychosis Biotypes with Multivariate Taxometric Analyses of Neuro-pathologically Relevant Biomarkers

Brett A. Clementz*

University of Georgia, Athens, Georgia

Background: Clinically psychosis diagnoses are characterized by overlap across, and heterogeneity within, categories on symptoms, genetics, neuropathophysiology, and treatment response. Validation of biomarkers and development of biosignatures for psychotic illnesses may be compromised because the gold standard is clinical diagnoses, which often trump biological observations. A solution is to develop neuroscience-based classifications of psychoses. Psychosis subgroups defined by biomarker, not phenomenological, characteristics may yield molecular, systems, and pharmacological signatures that more accurately predict etiology, pathophysiology, and treatment response than do phenomenological diagnoses.
Methods: The B-SNIP1 biomarker panel was used to generate variables that distinguished any psychotic disorder from healthy persons. For this analysis stage, measures at the neurocognitive level of analysis were selected (more classical endophenotypes), and individual dependent variables were screened for yielding the largest effect sizes for differentiating between psychosis (any of schizophrenia, schizoaffective disorder, bipolar disoder with psychosis) and healthy groups. Multiple dependent variables survived this analysis from neuropsychological (BACS), visual attentional and inhibition (prosaccade latency, antisaccade performance, stop signal task), and neural auditory processing (paired stimuli and oddball tasks) paradigms. Data reduction procedures reduced these multiple measures to a set of 10 core characteristics. We then statistically estimated the number of subgroups that efficiently summarized and optimized clustering of the core characteristics among psychosis cases independent of clinical diagnosis (3 subgroups). We then used k-means clustering followed by canonical discriminant analyses among psychosis cases to optimally differetiate the 3 psychosis subgroups (what we here call Biotypes). Comparisons were made between separations of psychosis

biotypes versus clinical diagnostic groups (schizophrenia, schizopaffective disorder, bipolar disorder with psychosis) on the 10 core characteristics.
Results: The core characteristics could be summarized as capturing (i) efficiency of neurocognitive functioning and (ii) sensorimotor regulation. Biotypes were separated in cartesian space by 3–3.5 standard deviation units. Clinical diagnoses were distributed across the three biotypes, so group separations on the core characteristics were all considerably larger for biotypes than for clinical diagnoses. Biotype-1 had severe neurocognitive dysfunction (poor BACS, poor inhibition, low ERP amplitudes, poor auditory suppression, sluggish neuromotor responding); 60% of this group had schizophrenia but 35% had manic psychosis. Biotype-2 had high intrinsic neural activity, moderate neurocognitive dysfunction, and relatively normal auditory ERP amplitudes (but poor signal-to-noise ratios given high intrinsic activities); 45% of this group had schizophrenia and 45% had manic psychosis. Biotype-3 had the most normal biomarker profile but still had moderatly poor antisaccade performance (1.2 SD units worse than normal) combined with faster than normal saccadic reaction times; 60% of this group had a manic psychosis but 30% had schizophrenia.
Conclusions: This B-SNIP1 result demonstrates that meaningful groups of psychosis cases can be generated with homogeneous phenotypic characteristics independent of DSM diagnoses. Considering hereogeneity within the psychosis illness class as a targer rather than a source of unwelcome variance may be useful for understanding unique and shared etio-pathophysiologies within this diagnostic domain.
Disclosure: B. Clementz, Nothing to Disclose.

22.2 Multivariate Fusion Methods Identify Gene Components Associated with Heritable Resting State fMRI Abnomalities in BSNIP Probands and Relatives

Godfrey D. Pearlson*

Yale University School of Medicine, Hartford, Connecticut

Background: Resting state (RS) fMRI patterns are known to be heritable, and abnormal in both schizophrenia (SZ) and bipolar psychotic (PBP) probands and their unaffected relatives. The genetic underpinnings of these abnormalities are unknown, but likely determined by multiple genes acting in concert and not detectable individually by univariate methods such as GWAS due to the weak contribution from each alone. Appropriate multivariate approaches have been used previously in disorders where molecular biology, neuropathology and risk genes are relatively well known (eg Alzheimer's) but not previously in psychoses.
Methods: 1. First we examined 5 min of 3T, RS fMRI data from 300 PBP, 296 SZ, 324 healthy controls (HC) plus 206 unaffected first-degree PBP relatives and 179 SZ relatives from all BSNIP sites to derive intrinsic resting state connections. 2. We then genotyped 240 PBP, 220 SZ and 200 HC using an Illumina Quad 10^6 SNP chip and performed data-driven, multivariate fusion (parallel independent component analysis) to identify clusters of interacting genes related to brain networks of interest. Gene clusters were fed into annotation software (DAVID, IPA) to detect associated molecular biologic processes.3. Analyses were repeated using Biotypes rather than DSM diagnoses, on probands and healthy controls.
Results: 1. After whole-brain correction for multiple comparisons, 7 of 20 total brain networks showed significant (FDR Cor. p^2- one comprising the posterior/inferior default mode network, the other a frontal executive network.2. Parallel ICA of these 2 functional brain circuits, revealed significantly associated SNP networks containing multiple genes associated previously with SZ and BP (plus autism and retardation) eg $Ca2^+$ activated K^+ channel, NPAS3, plus novel genes. Annotation software identified multiple relevant pathways including focal cell adhesion, $Ca2^+$ mediated signaling, axonogenesis, neuronal proliferation and synaptic transmission.3. Corresponding gene/fMRI network correlations for biotypes were greater (p values ranging from $1x10^{-6}$ to $3x10^{-5}$) and more numerous (5 vs 2), than with conventional diagnoses. Additional relevant molecular biological pathways were revealed, including those for neuronal differentiation, neuron projection development and CAM pathways.
Conclusions: Resting state fMRI data in PBP and SZ probands were abnormal; many of these functional circuit abnormalities were similar in both illnesses, revealing a lack of biological specificity with conventional diagnoses. A subset of abnormal connectivity patterns were transmitted to unaffected relatives. This subset was associated with SNPs not significant individually at the GWAS level, but collectively highly significant and contributing together to relevant biological processes. Considering Biotypes rather than DSM diagnoses increased significance levels and revealed additional relevant molecular biological pathways. We will discuss therapeutic implications of these genetic data in terms of possible novel uses of existing drugs based on these biologic processes.
Disclosure: G. Pearlson, **Part 1:** Consultant BMS 2012

22.3 Phenotypic Characterization of the Schizophrenia-Bipolar Disorder Continuum

Matcheri Keshavan*

Harvard Medical School, Boston, Massachusetts

Background: The multi-site Bipolar-Schizophrenia Network on Intermediate Phenotypes (B-SNIP1) was designed to extensively phenotype a large series of psychosis probands, their first-degree biological relatives, and healthy control subjects. Phenotypes included a comprehensive cognitive assessment, evoked electrophysiological responses, oculomotor pursuit, regional brain volume, resting EEG and resting fMRI characteristics.
Methods: We examined the phenotypic differences across categories (Brain structure and function, resting state fMRI data, resting state EEG data, cognition, evoked potential and oculomotor data) as they relate to DSM categories and dimensionally to schizobipolar scale (SBS), a quantitative measure of the schizophrenia- bipolar spectrum dimension,

We also examined these phenotypes between probands, their first degree relatives and healthy controls.
Results: We observed an extensive overlap in phenotypic characteristics (such as abnormalities in gray matter volumes, cognitive performance, saccadic eye movements, and errors on antisaccade tests) across DSM psychosis categories, generally with schizophrenia patients showing the most prominent alterations, followed by schizoaffective and psychotic bipolar disorder. Effect sizes were moderate to large between probands and controls (eg Cognition composite scores, Cohen's d 1- 1.8; antiscaccade errors 1.3-2.5; P300 amplitudes 0.3−0.7; cortical thichmess 0.17-0.8). Similar, but smaller alterations were were also seen in first degree relatives. Several of the phenotypes were abnormal (albeit to a smaller degree) in first degree relatives, and showed moderate to large heritability across several biomarkers (.eg 3–0.8 for cortical thickness).Few robust differences were seen between DSM categories either in probands or in relatives.
Conclusions: These observations suggest that neurobiological dimensions may cut across categories of psychosis spectrum disorders as currently defined using symptom presentations. And may potentially serve as endophenotypes. These data point to the value of developing approaches to a neuroscience- based classification that is agnostic to DSM typology.
Disclosure: M. Keshavan, Nothing to Disclose.

22.4 Validating Psychosis Biotypes

Carol A. Tamminga*

UT Southwestern Medical Center, Dallas, Texas

Background: Phenomenologically-derived categories of psychoses have indistinct biological boundaries. Genetic, electrophysiological, cognitive and oculomotor measures along with structural and functional imaging assessments have all failed individually to provide replicable signatures for diagnoses of psychotic disorders. Meanwhile, basic neuroscience and biomarkers for human neural function have burgeoned. The RDoCs system lays out a system to approach disease classification from normal systems neuroscience. The tools and stage of research are ripe for biologically-based classification. BSNIP1 used a dense battery of phenotypic markers to generate distinct biologically-based clusters within psychosis ('Biotypes'). After creating the novel phenotypic structures, we applied additional measures of brain structure and function, psychosocial performance and family characteristics for validation. An immediate goal is to generate a system that will be useful in genetic and pharmacological research to increase the precision of target-driven research.
Methods: The BSNIP1 network recruited and phenotyped 933 probands and over 1000 of their relatives along with 459 healthy controls. The human volunteers were extensively and similarly phenotyped. Individual phenotype data from all sites were quantified and analyzed within a single expert laboratory. Basic well-accepted psychosis phenotypes were selected as the biotype-defining biomarkers. Additional phenotypes and clinical characteristics were identified as external validators of the biotypes, including brain volume by VBM, psychosocial characteristics (Birchwood SFS) and family phenotypic characteristics.
Results: Structural brain characteristics of the three biotypes using VBM analyses show distinct biotype anatomies. Biotype3 shows only a minimal level of neocortical grey matter volume reduction, mainly apparent in the cingulate cortex and amygdala. Biotype2 shows moderated grey matter reduction localized mainly to limbic cortex, prominent throughout cingulate and hippocampus. By contrast, Biotype1 showed extensive grey matter volume reduction apparent throughout all of cerebral cortex. Relatives of Biotype had no structural difference from normal; Biotype2 relatives showed subtle volume reductions primarily in limbic-hippocampal regions, whereas Biotype1 relatives had volume reductions, albeit subtle, throughout neocortex. Focusing on proband outcome, psychosocial function was the most normal in Biotype3, moderately disturbed in Biotype2 and worst in Biotype1 [131.3(25.0); 123.0(24.0); 116.8(23.4), respectively], paralleling the structural brain changes and cognitive impairment. Relatives of Biotypes1 & 2 show 14.5% and 13.6% spectrum personality disorder, while those of Biotype 3 have only 3.3%. Demographic and clinical characteristics of biotypes will be reviewed.
Conclusions: The goal of generating categories of brain diseases which are based on biological characteristic is widely shared in order to further the goal of genetics and molecular target discovery in psychotic illness. The BSNIP Biotypes are rational products of a dense biomarker characterization within a large psychosis cohort. The validating constructs shown here make the biotypes themselves stronger constructs, and signal a further need for validation with genetic and pharmacological outcomes.
Disclosure: C. Tamminga, **Part 1:** Astellas; Eli Lilly; Intra-Cellular Therapies; Kaye Scholer LLP; Lundbeck; PureTech Ventures; NIMH; Am J Psychiatry; , **Part 2:** Am J Psychiatry; KayeScholer LLP; IntraCellular Therapies, **Part 4:** Sunovion

Panel

23. Neuroactive Steroids and Oxysterols as Endogenous Modulators of GABA and Glutamate Receptors: Basic Mechanisms and Therapeutic Implications

23.1 GABAergic Neurosteroids as Novel Targets for Therapeutic Drug Development in Psychiatry

Charles Zorumski*

Washington University School of Medicine, St. Louis, Missouri

Background: Considerable evidence implicates neural circuit dysfunction in the pathophysiology of psychiatric illnesses, including mood, anxiety and stress-related disorders. This work highlights a key role for imbalances in excitatory and inhibitory transmission within neural systems underlying emotion, motivation and cognition. Endogenous 5-alpha-reduced neurosteroids synthesized in the brain from cholesterol, and related synthetic neuroactive steroids are potent and effective modulators of GABA-A receptors that underlie fast phasic synaptic inhibition as

well as more persistent tonic inhibition. Importantly, chronic stress often associated with neuropsychiatric illnesses appears to down regulate endogenous GABAergic neurosteroids contributing to neural circuit dysfunction. This suggests a mechanistic role for neurosteroids in stress-related disorders and renders these steroids potentially important targets for therapeutic drug development in a range of illnesses.

Methods: Recent studies indicate that excitatory glutamatergic neurons rather than inhibitory interneurons or glia are predominant sites of neurosteroid production under basal conditions in the brain, suggesting that locally-generated neurosteroids are positioned to influence information flow in brain circuits in a paracrine or autocrine fashion. Other studies highlight complex interactions of neurosteroids with specific sites on GABA-A receptors located within receptor membrane spanning regions, and the role of the neuronal plasma membrane in the access of neurosteroids to their sites of action. Our studies use electrophysiological, molecular and cellular imaging methods in combination with medicinal chemistry to study effects of neurosteroids on GABA-A receptors in the hippocampus.

Results: Because of their high lipophilicity, neuroactive steroids readily accumulate in plasma membrane and intracellular pools that influence their effectiveness and time course of action at GABA-A receptors. These observations have important implications for drug design and support the notion that lipid solubility is a significant consideration in functional receptor modulation. These studies challenge traditional views about the mechanisms by which neurosteroids modulate ion channel function. In the case of highly lipophilic steroids, high aqueous potency does not readily translate into high affinity at sites of action within lipid environments where neurosteroids accumulate at concentrations that are up to several orders of magnitude greater than in aqueous environments. Additionally, neurosteroid effects on GABA-A receptors may involve unique interactions with receptor surfaces within lipid domains rather than standard lock-and-key interactions with specific amino acid residues.

Conclusions: This presentation highlights recent developments in understanding molecular and physiological mechanisms contributing to the effects of endogenous neurosteroids and synthetic neuroactive steroids on GABA-A receptors, with emphasis on how these mechanisms influence the development of novel agents to treat stress-related neuropsychiatric illnesses.

Disclosure: C. Zorumski, **Part 1:** I serve on the Scientific Advisory Board of Sage Therapeutics., **Part 2:**.

23.2 Natural and Synthetic Neuroactive Steroids and Oxysterols as Potent NMDA Receptor Allosteric Modulators: Therapeutic Considerations

Steven Paul*

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Background: N-methyl-D-aspartate receptors (NMDARs) areheterotetromeric ligand-gated ion channels implicated in various forms ofsynaptic plasticity that underlie learning and memory. NMDARs have also beenimplicated in the pathophysiology of various neuropsychiatric disorders includingschizophrenia, depression, Alzheimer's disease and epilepsy. Drugs that augmentNMDAR activity may be effective antipsychotics; especially in improving negativeand cognitive symptoms. NMDARs are regulated by various natural substances,including the co-agonists glutamate, glycine and D-serine. Neuroactive steroidsdirectly and rapidly modulate ligand-gated ion channels such as NMDAR andGABA-AR to enhance excitatory or inhibitory neurotransmission. Pregnenolonesulfate (PS) a purported neurosteroid synthesized in brain has also been shownto be an effective positive allosteric modulator (PAM) of NMDARs and improvesmemory in various animal models. However, PS is a relatively weak NMDAR PAM andits presence in brain is controversial. These findings prompted a highthroughput chemical screen by Madau and colleagues to identify othersteroid-like NMDAR PAMs which revealed that several oxysterol derivatives(oxygenated cholesterol metabolites) were potent and selective NMDAR PAMs.-Given the structural similarity between these compounds and natural oxysterolswe screened a series of natural oxysterols and discovered that the brain-specificoxysterol 24(S)-hydroxycholesterol (24(S)-HC) (the major brain cholesterolmetabolite) is a potent NMDAR PAM.

Methods: We used whole cell patch clamp electrophysiologyof primary mouse hippocampal neurons to screen a series of natural oxysterolsto detect any that might modulate NMDARs. We also used hippocampal slices tomeasure LTP a form of synaptic plasticity underlying learning and memory inorder to test the effects of both natural and synthetic oxysterols on synapticplasticity. Finally, we employed medicinal chemistry to discover syntheticdrug-like oxysterol derivatives and tested them in both electrophysiologicaland behavioral assays.

Results: We have shown that the major brain-derivedoxysterol 24(S)-hydroxycholesterol (24(S)-HC) is a very potent and selectivepositive allosteric modulator of NMDARs. At submicromolar concentrations24(S)-HC potentiates NMDAR-mediated EPSCs in mouse hippocampal neurons butfails to affect AMPAR or GABA-AR-mediated responses. In hippocampal slices,24(S)-HC enhances the ability of subthreshold stimuli to induce LTP, a form ofsynaptic plasticity underlying learning and memory. Finally, we have shown thattwo synthetic drug-like derivatives of 24(S)-HC which potently enhanceNMDAR-mediated EPSCs and LTP, reverse memory impairment induced by a NMDARantagonist in mice. Thus, 24(S)-HC may function as an endogenous modulator ofNMDARs acting at a novel oxysterol modulatory site that represents a target fortherapeutic drug development.

Conclusions: Neuroactive oxysterols represent a novel classof NMDAR modulators. The major cholesterol metabolite in brain 24(S)-HC mayfunction as an endogenous NMDAR modulator subserving roles in NMDAR-mediatedsynaptic plasticity and thus may play a role in the pathophysiology of variousneuropsychiatric disorders including schizophrenia and Alzheimer's disease.Importantly, the privileged oxysterol site associated with NMDAR appears to bea good target for drug discovery.

Disclosure: S. Paul, **Part 1:** Alnylam Pharmaceuticals (Board of Directors), Constellation Pharmaceuticals (Board of Directors), Eli Lilly (Stockholder), Karuna Pharmaceuticals

(Board of Directors), Sage Therapeutics (Founder and shareholder), Sigma Aldrich Company (Board of Directors), Tal Medical Pharmaceuticals (Scientific Advisory Board and Board of Directors), Third Rock Ventures (Venture Partner). , **Part 2:** Alnylam Pharmaceuticals (Board of Directors), Constellation Pharmaceuticals (Board of Directors), Eli Lilly (Stockholder), Karuna Pharmaceuticals (Board of Directors), Sage Therapeutics (Founder and shareholder), Sigma Aldrich Company (Board of Directors), Tal Medical Pharmaceuticals (Scientific Advisory Board and Board of Directors), Third Rock Ventures (Venture Partner). , **Part 3:** Alnylam Pharmaceuticals (Board of Directors), Eli Lilly (Stockholder), Sage Therapeutics (Founder and shareholder), Sigma Aldrich Company (Board of Directors), Third Rock Ventures (Venture Partner). , **Part 4:** Alzheimer's Drug Discovery Foundation, AstraZeneca Pharmaceuticals, Johnson & Johnson Pharmaceuticals.

23.3 Neuroactive Steroids Ganaxolone and Allopregnanolone in the Treatment of Epilepsy, Traumatic Brain Injury, and Neurobehavioral Disorders

Michael A. Rogawski*

University of California, Davis, Sacramento, California

Background: I will discuss recent studies on the development of the neuroactive steroids for the treatment of epilepsy, traumatic brain injury (TBI), and other neurological and psychiatric conditions.
Methods: Studies in animal epilepsy and seizure models. Pharmacokinetic studies in animal models. Controlled (randomized and blinded) and open label clinical trials in various patient populations.
Results: I begin with a discussion of the synthetic neuroactive steroid ganaxolone, which has been administered to over 900 patients. I will report the results of a 147 subject double-blind, placebo-controlled clinical trial of orally administered ganaxolone in the treatment of partial onset seizures in adults. This study met a predefined statistical criterion for efficacy. Ganaxolone was found to be safe and well tolerated. There was no increase in the frequency of discontinuations or treatment emergent adverse events in the ganaxolone treatment group compared to the placebo group. Results of the 1-year open label extension study, which demonstrated increasing efficacy over time, will also be presented. I will also provide an update on ongoing studies with ganaxolone in the treatment of anxiety in fragile X and posttraumatic stress disorder (PTSD). Recently, we have begun clinical studies of the natural neurosteroid allopregnanolone. We have developed an intravenous formulation, which is being studied for the treatment of moderate and severe traumatic brain injury. We have developed extensive evidence in animal models supporting the utility of allopregnanolone in the treatment of status epilepticus, when administered intravenously and also intramuscularly. We are embarking on a program to develop allopregnanolone for use in acute status epilepticus and also for the treatment of in hospital nonconvulsive status epilepticus.
Conclusions: Neuroactive steroids have potential utility in the treatment of epilepsy, TBI, and several neurobehavioral conditions including fragile X and PTSD.

Disclosure: M. Rogawski, **Part 1:** Marinus Pharmaceuticals, Sage Therapeutics, Eisai, UCB, Upsher-Smith, **Part 2:** University of California, Davis, Sage Therapeutics, **Part 4:** Gilead Sciences, Eisai, UCB.

23.4 Neurosteroids as Novel Therapeutics and Biomarker Candidates in Schizophrenia and PTSD

Christine E. Marx*

Duke University Medical Center, Durham, North Carolina

Background: Compelling data suggest that neurosteroids hold promise as novel therapeutics and biomarker candidates in schizophrenia and PTSD. They are enriched in brain and demonstrate pleiotropic actions highly relevant to CNS conditions, including neuroprotective effects, learning and memory enhancement, anti-inflammatory actions, neurotrophic effects, and neurogenesis-promoting actions in rodent models. In addition, neurosteroids are altered and appear dysregulated in patients with schizophrenia and PTSD. Restoring equilibrium in neurosteroid pathways by targeting these molecules directly may thus represent a logical pharmacological intervention strategy. Pregnenolone sulfate positively modulates NMDA receptors and could potentially ameliorate NMDA receptor hypofunction in schizophrenia. Allopregnanolone enhances $GABA_A$ receptor responses, demonstrates anxiolytic effects, and modulates the HPA axis, thus exhibiting actions relevant to the neurobiology and therapeutics of PTSD. We are therefore conducting proof-of-concept randomized, controlled, trials (RCTs) utilizing neurosteroid interventions in schizophrenia and PTSD, and investigating neurosteroids as biomarker candidates in Veterans who served in the U.S. Military since September 11, 2001.
Methods: RCT with pregnenolone in schizophrenia: 120 participants randomized to 8 weeks of adjunctive pregnenolone or placebo (fixed escalating dosing). Endpoints were changes in UPSA-B composite scores (functional outcome), MCCB composite scores (cognitive symptoms), and SANS total scores (negative symptoms). Multi-site RCT with ganaxolone in PTSD (an allopregnanolone derivative): Participants randomized to 6 weeks of ganaxolone or placebo in a fixed escalating dosing design, followed by an extension phase of 6 weeks in which all patients receive ganaxolone. Target $n = 80$ participants reaching primary endpoint of change in CAPS scores at 6 weeks. Biomarker investigations in PTSD: Neurosteroid analyses conducted in serum samples from 662 male Veterans and 403 female Veterans who served in the U.S. Military since September 11, 2001.
Results: Participants with schizophrenia randomized to pregnenolone ($n = 56$) demonstrated significantly greater improvements in overall functioning compared to patients randomized to placebo ($n = 55$); change in UPSA-B composite scores, $p = 0.03$ (modified intent-to-treat analysis; 111 of 120 participants received at least one dose of study drug; 95% confidence interval: 0.40–6.10). Pregnenolone was also superior to placebo in improving the communication skills subscale of the UPSA-B ($p < 0.001$; 95% confidence interval 0.45-1.13). No significant changes were demonstrated in MCCB composite

scores or SANS total scores (but pre-randomization SANS scores were very low, mean=11).
Conclusions: Neurosteroids exhibit promise as therapeutic interventions and biomarker candidates. Pregnenolone improved overall functioning in patients with schizophrenia. Ganaxolone in PTSD results are pending (RCT 79% completed). Male Veterans with PTSD demonstrated significantly decreased DHEAS levels, and this neurosteroid was positively correlated with resilience.
Disclosure: C. Marx, **Part 1:** Applicant or co-applicant, pending patents on the use of neurosteroids and derivatives in CNS disorders and for lowering cholesterol (no patents issued, no licensing in place). Unpaid scientific advisor, Sage Therapeutics.

Panel

24. Nutrition, Neurodevelopment, and Risk for Schizophrenia and Autism: From Epidemiology to Epigenetics

24.1 Effects of Periconceptional Folate on Language Delay and Autism Spectrum Disorders: The Norwegian Mother and Child Cohort Study

Camilla Stoltenberg*

Norwegian Institute of Public Health, Oslo, Norway

Background: Prenatal folic acid supplementation reduces the risk of spina bifida and other neural tube defects. Folic acid is also believed to play a critical role in brain development, supplying one-carbon moieties for such vital transmethylation reactions as DNA synthesis, gene expression regulation, and neurotransmitter metabolism. Using a large, prospective observational database, we have examined the relationship between prenatal nutritional supplements and childhood-onset neurodevelopmental disorders. Specifically, we were interested in whether periconceptional folic acid supplements influenced the subsequent risk of severe language delay and autism spectrum disorders.
Methods: The Norwegian Mother and Child Cohort Study (MoBa) recruited 108,841 pregnant women between 1999 and 2008 for self-report questionnaires, biological samples, and prospective followup. Maternal questionnaires administered around week 18 of gestation obtained detailed information about mothers' supplement intake before conception and early in pregnancy. Of note, no foods were fortified with folic acid in Norway during the enrollment period; thus, synthetic supplements and ordinary dietary folate were the only sources of folate intake. Among offspring of this cohort, cases of autism spectrum disorder were identified though questionnaire screening at ages 36 months, 5 years, and 7 years; by professional and parental referrals; and by linkages to the Norwegian Patient Registry. A total of 38,954 children born before 2008 were also assessed for severe language delay at age 3 using a language grammar rating scale.
Results: Compared to children whose mothers took no folic acid supplements from 4 weeks before to 8 weeks after conception, children whose mothers did take periconceptional folic acid supplements exhibited approximately half the rate of severe language delay (OR 0.55, 95% CI, 0.35–0.86), and comparable reductions in autism risk (OR 0.61, 95% CI, 0.41–0.90). The use of other supplements was not associated with reduced risk for either severe language delay or autism spectrum disorders.
Conclusions: The use of periconceptional folic acid supplements resulted in significant reductions in both severe language delay and autism spectrum disorders in the MoBa cohort. While the findings do not establish causal relationships between maternal folate intake and risk of neurodevelopmental disorders, they support additional investigations of genetic and other biological factors that could mediate the effects of folate on brain development.
Disclosure: C. Stoltenberg, Nothing to Disclose.

24.2 Longitudinal Effects of In Utero Folate Exposure on Cortical Thickness: Implications for Neurodevelopmental Disorders

Joshua L. Roffman*

Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts

Background: Reduced blood folate levels, and low-functioning genetic variants within the folate metabolic pathway, have both been associated with schizophrenia risk. Several clinical trials involving patients with chronic schizophrenia suggest a modest benefit from folate supplementation. Greater potential benefit, including the possibility of prevention, may be realized through earlier intervention with folate. Mandatory folate fortification of grain products was implemented by the US government by 1998, which doubled mean blood folate levels among women of childbearing age. Here, we determined whether this intervention was associated with altered cortical development, as detected by cortical thickness changes in children who gestated over the course of the folate fortification roll-out period.
Methods: Using a large repository of clinical and MRI data (the Partners Research Patient Data Registry) we identified 386 children who gestated during and after the roll-out of grain folate fortification and who underwent clinical brain MRI scans at 9 to 13 years of age at Massachusetts General Hospital. After reviewing radiology reports and MRI images, we included 44 children with high resolution T1 scans that were read as clinically normal, and for whom no motion or other artifact was detected. Using FreeSurfer, scans were pre-processed and cortical thickness was determined across the entire cortical mantle.
Results: Among children who gestated during the folate fortification roll-out period in New England (born from 9/1/96 to 6/30/97), cortical thickness within the left inferior frontal gyrus correlated significantly with date of birth ($r = 0.72$, p
Conclusions: Population-wide folate fortification of grain products was associated with increased thickness within the inferior frontal gyrus among the first children who were exposed to this intervention in utero. Previous studies involving healthy siblings of children with childhood-onset schizophrenia demonstrated reduced cortical thickness in same region, suggesting that it may be a marker of genetic liability for early onset psychosis. The current findings, although limited by the use of a clinically heterogenous

cohort, suggest that population-wide folate fortification efforts influence an MRI phenotype that is relevant to schizophrenia susceptibility. They are also consistent with epidemiologic studies that demonstrate a protective effect of increased maternal folate intake on subsequent risk for neurodevelopmental disorders.
Disclosure: J. Roffman, **Part 4:** Pamlab.

24.3 Periconceptional Folic Acid and Neurodevelopmental Disorders: Historical Context and Current Research

Ezra Susser*

Columbia University, New York, New York

Background: Several large-scale epidemiologic studies have identified reduced periconceptionalfolic acid intake as a risk factor for neurodevelopmental disorders, includingautism and schizophrenia. This talk willpresent an overview of findings that led up to the current research onpericonceptional folic acid and neurodevelopmental disorders. The subsequent talks will then focus oncurrent specific findings in depth.
Methods: A series of studies based on the Dutch Hunger Winter of 1944–1945 and the Chinese Famine of 1959–1961 examined the relationship between famine and subsequent risk for schizophrenia among individuals who gestated under these conditions. These results are presented in the context of translational work that points to potential genetic and epigenetic mechanisms, as well as ongoing prospective studies.
Results: The offspring of mothers exposed to periconceptional famine- approximately one month before to 8 weeks after the start of pregnancy- had an increased risk of schizophrenia. This concordance was found despite substantial differences in the context and nature of these two famines and the results are in many ways complementary, as each setting had advantages and disadvantages for examining this question. In the Dutch Famine, the increase in schizophrenia was largely coincident with an increase in neural tube defects, which are known to be related to folate deficiency, and to be largely preventable by maternal intake of folic acid supplements from about one month before to 4 weeks after conception. Folate plays a key role in the 'one carbon pathway', in processes that may be relevant to early origins of neurodevelopmental disorders including schizophrenia, for example, in DNA synthesis and repair, and in the epigenetic process of methylation. Genetic variants (eg MTHFR677TT) that influence folate metabolism have been associated with schizophrenia as well as with other neurodevelopmental disorders such as autism.
Conclusions: Thus far, it has not been possible to examine directly whether periconceptional folic acid supplements have a role in schizophrenia prevention. This is primarily because it is unethical to initiate randomized clinical trials of these supplements in high income countries, where they are recommended for all women of reproductive age, and because such trials would need a very long term follow-up. Investigators have therefore turned to: (i) large observational prospective pregnancy cohorts that collect data on both periconceptional supplements and neurodevelopmental outcomes. These are currently able to examine the relation of periconceptional folic acid supplements to neurodevelopmental disorders in children, and will later be able to examine their relation to schizophrenia, ii) research designs that can help to refute or confirm results from these prospective studies using specialized designs such as sibling studies and 'Mendelian randomization' iii) randomized controlled trials of periconceptional folic acid in low-income countries done within clearly recognized ethical guidelines, and iv) studies of potential mechanisms in both human and animal work, for example, epigenetic pathways.
Disclosure: E. Susser, Nothing to Disclose.

24.4 The Placental and Neuronal Methylomes at the Interface of Genetic and Environmental Risk and Protective Factors in Autism

Janine LaSalle*

University of California Davis School of Medicine, Davis, California

Background: Autism is an increasingly common disorder of complex etiology, affected by multiple genetic and environmental influences. Epigenetic mechanisms such as DNA methylation represent a critical link at the interface between genetic and environmental factors. Recent next-generation sequencing efforts have now given us access to the whole human DNA methylome at base resolution, revealing striking differences in the epigenomic landscapes. In brief, these studies have confirmed that the human genome is highly methylated ($>70\%$ methylation) except for CpG island promoters that are strongly depleted for DNA methylation. However, large-scale partially methylated domains (PMDs, 70% average methylation). Since environmental exposures can impact the human methylome by reducing the supply of methyl donors and preconception levels of methyl-donor nutrients such as folate are protective for autism, neural tube defects, and schizophrenia, we are investigating global levels of DNA methylation by whole genome approaches.
Methods: Using bisulfite conversion followed by high-throughput sequencing (MethylC-seq), we developed a novel hidden Markov model (HMM) to computationally map the genomic locations of PMDs in human IMR90 fibroblasts, SH-SY5Y neuronal cells, and human placenta. To examine a protective effect of prenatal vitamin use on DNA methylation in a prospective epidemiological study, mothers in the MARBLES (Markers of Autism Risk in Babies: Learning Early Signs) study who have at least one child with ASD, and who became pregnant with another child were included. LINE-1 methylation was measured in DNA extracted from maternal whole blood samples collected at first and second trimester and the child's cord blood using bisulfite conversion and pyrosequencing (averaged across 4 CpG sites).
Results: We found that genomic regions marked by cell line specific PMDs contain genes that are expressed in a tissue-specific manner, with PMDs being a mark of repressed transcription. Genes contained within neuronal highly methylated domains (N-HMDs) were significantly enriched for calcium signaling, synaptic transmission and neuron differentiation functions. Autism candidate genes were

significantly enriched within N-HMD regions and include genes such as *CHRNA7, GABRB3, CNTNAP2, EN2, NLGN3, HTR2A, RELN, GRIK3, KCNN3*, and *NRXN1*. Mothers of children who met criteria for ASD on the ADOS were significantly less likely to have taken a prenatal vitamin before or during the first month of pregnancy. Taking a prenatal vitamin during this time was associated with significantly higher LINE-1 DNA methylation in the mother's second trimester blood. Prenatal vitamin use was also protective for ASD in the prospective MARBLES study, similar to the protective effect previously seen in the case-control CHARGE study.
Conclusions: Our results suggest that large-scale methylation domain maps could be relevant to interpreting and directing future investigations into genetic and epigenetic etiologies of autism. Preliminary findings from the MARBLES study of autism risk suggest that taking prenatal vitamins before and during the first month of pregnancy could reduce risk for autism symptoms and impact maternal methyl donors that saturate the human methylome in early development.
Disclosure: J. LaSalle, Nothing to Disclose.

Panel

25. Pathophysiology and Treatment of Obesity and Glucose Dysregulation in Schizophrenia

25.1 Dopamine, Clean Up Your 'AKT!' Restoring Insulin Signaling in Brain

Aurelio Galli*

Vanderbilt University, Nashville, Tennessee

Background: Schizophrenia is a disorder caused by multiple genetic and environmental variables. Current conceptions about schizophrenia suggest that despite the disease's heterogeneous causes, a crucial common pathway involves dysfunction of dopamine (DA) networks. One schizophrenia candidate gene encodes the protein kinase Akt, which is regulated *via* phosphorylation in response to hormones (eg insulin), growth factors, and neurotransmitter receptors. The data we have generated illuminate a molecular link between the recognized association of Akt signaling with brain disorders and feeding dysfunctions, including obesity and type 2 diabetes (T2DM).
Methods: Recently, we have developed a new model using germ-line altered mice that we believe lends novel insight into pathogenesis. We used conditional gene targeting in mice to dramatically impair neuronal phosphorylation of Akt at Serine 473 which controls Akt signaling specifically in monoaminergic neurons.
Results: Our molecular model linking brain dysfunction to diabetes relies on the observation that insulin, a key glucoregulatory hormone in the periphery, has a pivotal homeostatic role in the brain. We and others have shown that neuronal insulin action, *via* signaling pathways involving Akt, regulates key elements in monoamine homeostasis, including the trafficking and function of the DA transporter as well as norepinephrine transporter. We have shown that impaired Akt function, due to either insulin deficiency (type 1, T1DM) or neuronal insulin resistance (T2DM), results in impaired central DA homeostasis and contributes to hyperphagia. We propose that this DA dysfunction also contributes directly to the monoaminergic impairment associated with mental health disorders that are highly co-morbid with diabetes.
Conclusions: We show that dysregulated brain monoamine homeostasis leads to disordered feeding.
Disclosure: A. Galli, Nothing to Disclose.

25.2 Dysglycemic Signals in Antipsychotic-treated Children and Adolescents with Schizophrenia-spectrum Disorders: Trajectories, and Moderating and Mediating Factors

Christoph U. Correll

The Zucker Hillside Hospital, Glen Oaks, New York

Background: Antipsychotics are being used increasingly in children and adolescents for the treatment of psychotic and non-psychotic disorders. Antipsychotic-related weight gain has attracted considerable attention, especially in the vulnerable pediatric population. However, life-shortening effects are related to perturbations in lipid and, especially, in glucose metabolism. The focus of this presentation is on the acute and chronic effects of antipsychotics on glucose metabolism, including insulin resistance, prediabetes and diabetes in youth.
Methods: First, data from the ongoing Second-generation Antipsychotic Treatment Indications, Effectiveness and Tolerability in Youth (SATIETY) study were collected as part of a prospective inception cohort study of 272 antipsychotic-naïve youth. At baseline, week one, 4, 8, 12 and three-monthly thereafter, body weight, waist circumference, and fasting assessments of blood glucose and lipid parameters were obtained. Primary outcome for these analyses was insulin resistance, measured as the homeostatic model assessment (HOMA). Data were compared in youth with schizophrenia-spectrum disorders (29.8%) and those with mood spectrum disorders (26.7%) or aggressive spectrum disorders (43.5%). Moderators and mediators of insulin resistance were analyzed. Second, in a Danish nationwide, retrospective longitudinal register linkage case control study, all 36,141 psychiatrically ill youth children and adolescents exposed to antipsychotics over a ten-year period were compared retrospectively with non-antipsychotic users with at least one psychiatric diagnosis on the time to onset of diabetes. Schizophrenia-spectrum diagnosis was assessed as a potential moderator of diabetes risk.
Results: In the prospectively assessed antipsychotic youth (mean age: 13.9 years) body weight increased after a mean of 10.8 weeks by 19.0 (95% CI:16.4, 21.5) lbs $=15.2$ (13.2, 17.2)% with olanzapine ($N=45$), 13.5 (10.9, 16.0) lbs $=10.4$ (8.5, 12.3)% with quetiapine ($N=36$), 11.9 (10.7, 13.1) bs $=10.4$ (9.4, 11.3)% with risperidone ($N=135$), and 9.9 (8.2, 11.5) lbs $=8.1$ (7.0, 9.5)% with aripiprazole ($N=41$). Weight gain $>7\%$ occurred in 84.4% ($n=38$) of patients on olanzapine, 64.4% ($n=87$) on

risperidone, 58.4% ($n = 24$) on aripiprazole, and 55.6% ($n = 20$) on quetiapine. Increasing insulin resistance, measured as HOMA, was not significantly associated with sex, age, race, diagnostic group or leptin:fat mass ratio at baseline. HOMA change was significantly associated with various measures of body weight increase, being the most associated with fat mass change ($p = 0.0001$). Individually, only olanzapine was associated with significant HOMA increase at 3 months, with glucose increase being mediated by olanzapine dose > 10 mg/day ($p < 0.001$).
Conclusions: Antipsychotics are associated with a relevant risk for insulin resistance and diabetes development. Not all antipsychotics carry the same risk and both the indication for antipsychotic use and the specific antipsychotic prescribed require careful consideration. Moreover, proactive monitoring, risk factor identification, and the development of novel treatments for the amelioration and, ideally, prevention of glucose metabolism perturbations in antipsychotic treated youth and adults are urgently needed.
Disclosure: C. Correll, **Part 1:** Actelion, Alexza; Bristol-Myers Squibb, Cephalon, Eli Lilly, Genentech, Gerson Lehrman Group, IntraCellular Therapies, Lundbeck, Merck, Janssen/J&J, Otsuka, Pfizer, ProPhase, Roche, Sunovion, Takeda, Teva, and Vanda. He has received grant support from BMS, Janssen/J&J, and Otsuka, **Part 2:** Bristol-Myers Squibb, Cephalon, Merck, Otsuka, Pfizer, ProPhase, **Part 4:** BMS, Janssen/J&J, and Otsuka

25.3 Effect of Metformin on Weight in Patients with Schizophrenia with Impaired Fasting Glucose

Scott Stroup*

Columbia University, New York, New York

Background: Obesity is epidemic in schizophrenia and a contributor to excess morbidity and premature mortality. Evidence-based treatment approaches are needed to reduce risks of cardiovascular disease and premature death in individuals diagnosed with schizophrenia who are treated with antipsychotic medications.
Methods: In a randomized double blind trial we compared metformin to placebo to reduce weight in people with schizophrenia who were overweight (BMI > 27). 146 patients with schizophrenia or schizoaffective disorder who were taking stable doses of any one or combination of two antipsychotic medications were enrolled and followed for 16 weeks. 43 patients had impaired fasting glucose defined as FBG 100–125 mg/dL. Metformin was titrated to 2000 mg/day.
Results: Overweight patients with schizophrenia with impaired fasting glucose had a unique profile of changes in glucose and lipid metabolism. The subgroup of patients with impaired fasting glucose at baseline had significantly larger reductions in weight (3 kg, $p = 0.0005$), non-HDL cholesterol (14 mg/dL, $p = 0.021$), and total cholesterol (14 mg/dL, $p = 0.028$) compared to placebo while the subgroups with normal fasting glucose at baseline had only trend-level advantages over placebo on these measures.
Conclusions: This subgroup analysis suggests that metformin, when used as an adjunctive treatment for individuals with schizophrenia who take antipsychotics and are overweight, has larger benefits among patients with impaired fasting glucose than for those with normal fasting glucose. Measures of glucose metabolism may be helpful when deciding whether to use metformin to reduce metabolic risk factors for cardiovascular disease.
Disclosure: S. Stroup, Nothing to Disclose.

25.4 No Effect of Adjunctive, Repeated Dose Intranasal Insulin Treatment on Body Metabolism in Patients with Schizophrenia

Xiaoduo Fan*

University of Massachusetts Medical SchoolWorcester, Massachusetts

Background: One important action of insulin in the brain is on food intake and weight control. This study examined the effect of adjunctive intranasal insulin therapy on body metabolism in patients with schizophrenia.
Methods: Each subject had a DSM-IV diagnosis of schizophrenia or schizoaffective disorder and had been on stable dose of antipsychotic agent for at least one month. In an 8-week randomized, double-blind, placebocontrolled study, subjects received either intranasal insulin (40 IU 4 times per day) or placebo. The whole body dual-energy X-ray absorptiometry (DXA) was used to assess body composition. Lipid particles were assessed using nuclear magnetic resonance (NMR) spectroscopy. All assessments were conducted at baseline, and repeated at week 8.
Results: A total number of 39 subjects completed the study (18 in the insulin group, 21 in the placebo group). There were no significant differences between the two groups in week 8 changes for body weight, body mass index, waist circumference, as well as various measures of lipid particles (p's > 0.100). The DXA assessment showed no significant differences between the two groups in week 8 changes for fat mass, lean mass or total mass (p's > 0.100).
Conclusions: In the present study, adjunctive therapy of intranasal insulin did not seem to improve body metabolism in patients with schizophrenia. The implications for future studies were discussed.
Disclosure: X. Fan, **Part 1:** Eli Lilly—advisory board, **Part 4:** Eli Lilly—investigator initiated clinical trial grant

Panel

26. Peripheral Immune and Endocrine Pathways as Markers of PTSD Risk and Symptom Development: Evidence from Prospective Studies

26.1 Blood-based Gene-expression Predictors of PTSD Risk and Resilience among Deployed Marines: A Pilot Study

Stephen J. Glatt*

SUNY Upstate Medical University, Syracuse, New York

Background: Susceptibility to PTSD is determined by both genes and environment. Similarly, gene-expression levels in peripheral blood are influenced by both genes and environment, and expression levels of many genes show good correspondence between peripheral blood

and brain. Therefore, our objectives were to test the following hypotheses: (1) pre-trauma expression levels of a gene subset (particularly immune-system genes) in peripheral blood would differ between trauma-exposed Marines who later developed PTSD and those who did not; (2) a predictive biomarker panel of the eventual emergence of PTSD among high-risk individuals could be developed based on gene expression in readily assessable peripheral blood cells; and (3) a predictive panel based on expression of individual exons would surpass the accuracy of a model based on expression of full-length gene transcripts.
Methods: Gene-expression levels were assayed in peripheral blood samples from 50 U.S. Marines (25 eventual PTSD cases and 25 non-PTSD comparison subjects) prior to their deployment overseas to war-zones in Iraq or Afghanistan.
Results: The panel of biomarkers dysregulated in peripheral blood cells of eventual PTSD cases prior to deployment was significantly enriched for immune genes, achieved 70% prediction accuracy in an independent sample based on the expression of 23 full-length transcripts, and attained 80% accuracy in an independent sample based on the expression of one exon from each of five genes.
Conclusions: If the observed profiles of pre-deployment mRNA-expression in eventual PTSD cases can be further refined and replicated, they could suggest avenues for early intervention and prevention among individuals at high risk for trauma exposure.
Disclosure: S. Glatt, **Part 1:** I serve as a scientific consultant to SynapDx Corp.

26.2 Evidence for Plasma C-Reactive Protein Concentration as Biomarker of PTSD Risk

Dewleen G. Baker*

University of California, San Diego, California

Background: Observational studies largely support an association of post-traumatic stress disorder (PTSD) with increased peripheral inflammation. Given the cross-sectional nature of the evidence, it is not known whether the observed association is due to PTSD-induced changes promoting inflammation (as sometimes postulated) or to inflammation predisposing to PTSD. Based on prospectively and longitudinally collected data from a military cohort, our aim was to determine whether plasma concentration of the inflammatory marker, C-reactive protein (CRP), predicts future PTSD symptoms.
Methods: These data were collected as part of the Marine Resiliency Study (MRS), a prospective field study of $\sim$2,600 war zone-deployed Marines. PTSD symptomatology and various physiological and psychological phenotypes were measured pre-deployment and three and six months following a seven month deployment. Subjects were male infantry Marines imminently deploying to a war-zone, of whom PTSD symptomatology, assessed using the Clinician Administered PTSD Scale (CAPS) was available from 1,861 (72.8%) and 1,609 subjects (63.0%) at three and six months following deployment, respectively. In addition to collection of relevant physiological covariates, eg waist circumference, height, weight, baseline high-sensitive CRP plasma levels were measured on these Marines by using a sandwich enzyme-linked immunosorbent assay (ELISA, ALPCO Diagnostics, Salem, NH) at all three time points.
Results: We determined the effects of baseline plasma CRP concentration on post-deployment CAPS using Zero-inflated negative binomial regression. This approach is designed for distributions which have an excess of zeros in addition to being positively skewed. Adjusting for baseline CAPS, trauma exposure, and other relevant covariates, we found baseline plasma CRP to be a highly significant overall predictor of post-deployment CAPS ($p = 0.002$): each 10-fold increment in CRP concentration was associated with an odds ratio of non-zero outcome (presence *vs* absence of any PTSD symptoms) of 1.51 (95% CI, 1.15–1.97; $p = 0.003$) and a fold-increase in outcome when non-zero (extent of symptoms when present) of 1.062 (95% CI, 0.99–1.14; $p = 0.086$).
Conclusions: A marker of peripheral inflammation, plasma CRP concentration may help predict risk of PTSD symptom emergence.
Disclosure: D. Baker, Nothing to Disclose.

26.3 Exaggerated Threat Sensitivity and Avoidance as Contributors to Elevated Inflammation in Posttraumatic Stress Disorder: Data from the Mind Your Heart Study

Aoife O'Donovan*

University of California, San Francisco, California

Background: Trauma exposure and posttraumatic stress disorder (PTSD) have now been linked with elevated inflammation in a number of studies. However, the relationship between posttraumatic stress symptoms and inflammation remains poorly understood. It is possible that exaggerated threat sensitivity and avoidance could promote inflammation through effects on biological stress responses and health behaviors.
Methods: Our sample included 735 Veterans Affairs outpatients (35% with current full /partial PTSD) who participated in the Mind Your Heart Study (mean age = 58 ± 11; 94% male). The Clinician Administered PTSD Scale was used for diagnosing PTSD and assessing threat sensitivity and avoidance. High sensitivity C-reactive protein (CRP), white blood cell (WBC) count and fibrinogen were used as indices of inflammation. We first ran models adjusting for age, sex, race, kidney function and socioeconomic status, and then added health behavior factors including body mass index, smoking, sleep quality, alcohol use and physical activity.
Results: Patients with current PTSD had elevated CRP ($p = 0.04$) and WBC ($p = 0.03$) but not fibrinogen ($p = 0.12$). Within patients with PTSD, higher threat sensitivity was associated with elevated CRP and WBC ($p < 0.02$), while greater avoidance was associated with elevated fibrinogen ($p = 0.03$). However, after adjusting for health behavior factors, only the threat sensitivity findings remained significant.

Conclusions: Both exaggerated threat sensitivity and avoidance may promote inflammation in the aftermath of trauma exposure. While avoidance may influence inflammation primarily through adverse health behaviors, exaggerated threat sensitivity may promote inflammation through repeated and prolonged activation of the biological stress response.
Disclosure: A. O'Donovan, Nothing to Disclose.

26.4 Longitudinal Plasma Testosterone Trajectory and its Relation to Combat, Temperament and PTSD

Eric Vermetten*

Military Mental Health—Research, Defense, Utrecht, Netherlands

Background: Circulating testosterone levels are suppressed by physical and psychological stress. Plasma levels of testosterone can increase during stressful events and may be elevated in combat-related posttraumatic stress disorder (PTSD). While the knowledge on functioning of the HPA-axis has been well understood in PTSD, the hypothalamic-pituitary-gonadal (HPG) system has received much less attention. We assessed several neurohormones in a longitudinal cohort study in soldiers prior to deployment to a combat zone. In earlier analyses we found high GR number, high GILZ mRNA expression, low FKBP5 mRNA expression, high sensitivity of T-cells for regulation by dexamethasone to predict development of PTSD as well as correlation of GR with amygdala reactivity after combat.
Methods: We recruited 1032 servicemen prior to deployment and collected blood prior to deployment to a combat zone. They also participated in psychological assessment (eg depressive symptoms, temperament and character, fatigue). They were followed up for 2 additional time points post deployment (1 month and 6 months post deployment) in which blood was assessed in combination with the same psychological assessment.
Results: Mean concentration of testosterone in the sample was 18.7 nmol/l (SD 6.2 nmol/l). PTSD symptoms occurred in approx 5 % of the sample, depressive and fatigue symptoms were higher. We analyzed testosterone in plasma at these three time points and correlated these to the developmental trajectory of PTSD symptoms. We found significant correlations between testosteron and 'novelty seeking', and negative correlation between 'coöperativeness and testosteron both before as after deployment. High testosterone correlated with stressful experiences during deployment.
Conclusions: A typical repsonse to anticipating imminent combat showed lowered excretion of testosterone suggesting that men responded to the potential threat of these situations with an inhibition of testosterone secretion. In this dataset comparison of plasma testosterone levels at the three different time points may indicate alterations in testosterone levels after combat as an acute stress response of the HPG axis, in contrast to an adaptation of the HPG-axis under chronic psychological stress. As has been found in earlier studies we also reporeted that novelty seeking correlated with testosteron, a correlation that was stable before as well as after combat.
Disclosure: E. Vermetten, Nothing to Disclose.

Panel

27. Posttraumatic Stress Disorder: From Markers to Mechanisms

27.1 Allele Specific Epigenetic Modifications—A Molecular Mediator of Gene–environment Interactions in Stress Related Psychiatric Disorders?

Torsten Klengel*

Max Planck Institute of Psychiatry, Munich, Germany

Background: Understanding the underlying molecular mechanisms of gene-environment interactions (GxE) in psychiatric disorders will particularly contribute to treatment and prevention. However, the molecular events behind the statistical interaction remained elusive. Here we show a potential common mechanism of how environmental factors, moderated by genetic predisposition, influence long-term epigenetic states that lead to psychiatric disorders.
Methods: In the framework of the Grady Trauma Project, we collect data on early life trauma exposure in an inner city, low socioeconomic and highly traumatized population. We investigate the interaction of simple nucleotide polymorphisms (SNPs) in the glucocorticoid receptor (GR) modulating gene FKBP5 in interaction with early trauma. We further elucidate the molecular mechanisms behind this GxE using pyrosequencing of bisulfite converted DNA to determine DNA methylation changes in and around glucocorticoid response elements (GREs) in FKBP5. The functional impact of common, less common and rare variants in FKBP5 is determined by luciferase reporter assays. We further use chromatin conformation capture (3C) assays to show the impact of the functional variant in FKBP5 on the three-dimensional structure of the gene locus.
Results: *FKBP5* rs1360780 interact with child abuse exposure on the development of current PTSD symptoms in adulthood. Risk allele carriers exhibit a significant demethylation of CpGs around GREs in intron 7 of FKBP5 whereas carriers of the protective genotype retain a stable epigenetic profile. Chromatin Conformation Capture (3C) experiments revealed a directional three-dimensional (3D) structure of the FKBP5 locus with strong interaction of intron 7 and intron 2 with the transcription start site in risk allele carriers but without interaction of intron 2 in carriers of the protective genotype. The physical 3D property of the FKBP5 locus will be further described by chromatin conformation capture seq (4C) experiments. In addition to common variants in FKBP5 interacting with childhood abuse we identified less common and rare variants in and around GREs in the promoter, intron 2, intron 5 and 7 by targeted resequencing of the FKBP5 locus. The identified variants further alter the responsiveness of FKBP5 to glucocorticoids either enhancing or repressing the enhancer properties of the GREs tested in luciferase reporter assays. We present the effect of these variants on risk for PTSD in our large clinical cohort. In a multipotent human hippocampal progenitor cell line we show that GR-activation by dexamethasone leads to a stable DNA demethylation in FKBP5 when the cells are treated in

the proliferation and differentiation phase. In contrast, treatment after differentiation does not result in a demethylation highlighting the presence of vulnerable developmental phases.
Conclusions: This is the first example of an allele-specific, childhood trauma-dependent epigenetic mechanism that lead to a stable epigenetic memory of early trauma moderated by genetic predisposition and an increased risk for PTSD in adulthood. We highlight the effect of common and rare genetic variants on the 3D structure of the DNA and their impact on DNA methylation as well as the enhancer function of GREs.
Disclosure: T. Klengel, Nothing to Disclose.

27.2 Contextual Processing Deficits in PTSD: Translational Studies

Israel Liberzon*

University of Michigan, Ann Arbor, Michigan

Background: Pervasive feelings of impending danger are characteristic of PTSD, and patients report that they do not feel safe even when no objective threats to their safety are present. The brain mechanisms that underlie this sensitivity and enduring sense of threat are not yet understood, and may lie at the core of the pathophysiological mechanisms that perpetuate fear responses and cause or sustain this disorder. We had hypothesized that deficits in processing of contextual information are at the core of these symptoms and performed functional neuroimaging studies in PTSD subjects as well as translational studies in animal model of PTSD, to identify brain regions and molecular mechanisms involved in contextual processing deficits.
Methods: Experiments involving fear conditioning, extinction, renewal and reinstatement had been performed in 3T fMRI scanner in 28 OIF/OEF veterans (PTSD subjects and combat controls), over two consecutive days.Fear conditioning, extinction and renewal had been performed in Single Prolong Stress (validated rodent model of PTSD) exposed animals and control groups. Prefrontal, hippocampal and amygdala tissue were processed to assess glucocorticoids receptor levels as well as glutamate and glutamine concentrations
Results: PTSD subjects unimpaired exhibited acquisition of fear conditioning and extinction learning, however altered BOLD signal in prefrontal and hippocampal regions during contextual processing in fear reinstatement and renewal experiments. Exaggerated fear renewal was also demonstrated in Single Prolonged Stress (SPS) animals. Subsequent studies linked upregulation in glucocorticoid receptors (GR) in hippocampus and prefrontal cortex to exaggerated fear renewal in these animals. In parallel studies SPS animals were also found to have decreased glutamate and glutamine concentrations in prefrontal regions.
Conclusions: The findings from both human neuroimaging studies and animal models suggest that contextual processing abnormalities are present in PTSD patients and may lead to abnormal renewal of learned fear responses. Animal model implicated upregulation of glucocorticoid receptors in both mPFC and hippocampus in contextual processing abnormalities, and suggest that further investigation of glutamatergic signaling in PFC in PTSD is warranted as well as potential link between GR upregulation and glutamatergic abnormalities
Disclosure: I. Liberzon, Nothing to Disclose.

27.3 Identification of Novel Gene Regulatory Networks Associated with PTSD: Evidence from Whole Genome Studies Examining DNA Methylation

Douglas E. Williamson*

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

Background: Post-traumatic stress disorder (PTSD) is a chronic and disabling anxiety disorder that occurs in response to a severe traumatic event. Recent research has identified several genetic loci and alterations in gene regulatory networks associated with PTSD. However, little is known about epigenetic changes, particularly the role of DNA methylation, in the development of PTSD after exposure to a traumatic event.
Methods: Under the auspices of the STRONG STAR PTSD Consortium, we are actively engaged in pre-clinical, clinical, and epidemiologic research to identify specific as well as novel genetic markers of PTSD. Here we will describe aspects of this research focused on the interrogation of post-mortem tissue in PTSD cases/controls and selected brain areas; the posterior cingulate cortex (pCC) and the medial orbital frontal cortex (mOFC). Whole genome DNA methylation patterns of PTSD and control post-mortem tissue were surveyed using methyl-CpG binding domain-based capture sequencing (MBDCap-Seq). MBDCap-seq libraries were sequenced using the Illumina HiSeq2000.
Results: A total of 25 genes were differentially methylated in the pCC, 4 hypermethylated in PTSD cases. Conversely, 355 genes were differentially methylated in the mOFC, 344 hypermethylated in PTSD cases. In the mOFC, the glutamate receptor, ionotropic, AMPA 2 (GRIA2) and the lysine (K)-specific demethylase 6B (KDM6B) were identified as two of the top genes hypermethylated in PTSD cases (fold changes of 3.39 and 4.53 respectively). In a subsequent study, we ran the Illumina Human Methylation450 array in DNA samples from the mOFC in 8 PTSD cases and 8 controls and identified additional genes and CpG sites uniquely methylated in PTSD. Interestingly, we identified several additional genes that were hyper- or hypomethylated in PTSD cases that have previously been linked with dementia including ephrin A5 (EFNA5) a protein that regulates growth cone survival. Additional studies will be described using pre-clinical models to examine the specific effect of stress, shown to result in fear conditioning, on changes in DNA methylation in the pCC and mOFC.
Conclusions: This is one of the first reports using NextGen sequencing and microarray strategies to identify novel genes and DNA methylation patterns associated with PTSD in post-mortem tissue. The link between PTSD and dementia will be described and the implication of the genes

identified will be discussed. The relevance of these findings as they inform our ongoing research identifying novel gene candidates associated with the onset of PTSD and changing in response to successful treatment will be discussed.
Disclosure: D. Williamson, Nothing to Disclose.

27.4 Opioid Receptor-Like 1 (OPRL1) is Involved in Amygdala-dependent Fear in Mice and Humans with PTSD

Kerry J. Ressler*

Emory University School of Medicine, Atalnta, Georgia

Background: The amygdala-dependent molecular mechanisms driving the onset and persistence of Post-traumatic Stress Disorder (PTSD) are poorly understood. Recent clinical studies have suggested that opioid analgesia in the aftermath of trauma may decrease the development of PTSD. Using a hypothesis-neutral gene discovery approach, we identified a gene within the opioid regulation pathway that is involved in amygdala-dependent fear consolidation.
Methods: Animals All experiments were performed on adult (2–3 months old) wildtype strain C57BL/6J from Jackson Labs, male mice.RNA extraction and Microarray hybridization Total RNA was isolated and purified from the tissue with the RNeasy Mini Kit catalog (Qiagen). Illumina Mouse WG-6 v2 Expression BeadChip microarray (Illumina, Inc.) was assayed for 45,281 transcripts.cDNA synthesis and quantitative PCR (q-PCR) Total RNA was reverse transcribed using the RT2 First Strand Kit (Qiagen). The primer used for the q-PCR was the TaqMan Oprl1 Mm00440563_m1 from Applied Biosystems (Carlsbad, CA).SR-8993 administration SR-8993 was dissolved in physiological saline. Systemic intraperitoneally (i.p.) volume of injection was 8ul/g and the dose was 3 mg/kg for behavioral experiments and 10 mg/kg for brain penetration assays. Intramygdalar SR-8993 volume of injection was 0.5 μl with 100 ng dose per side. For stereotaxic surgery, administration of a volume of 0.5 ul/side of SR-8993 was delivered over a period of 60 seconds.Human subjects Detailed trauma interviews using the PTSD symptom scale (PSS) were collected on approximately 2,000 highly traumatized males and females and the Clinician Administered PTSD scale (CAPS) on a smaller subset. The subjects were adult (average age $\sim$40 years old), primarily female (60%), highly traumatized, impoverished, primarily African American, and with very large rates of current and lifetime PTSD. Other phenotype measurements included in the data collection were the Childhood Trauma Questionnaire (CTQ) as our primary child abuse measure, and current substance abuse.GWAS All DNA for genotyping was quantified by gel electrophoresis using Quantity One (BioRad, Hercules, CA) and then normalized to 400 ng. Using the Illumina Human Omni1-Quad BeadChip (Illumina Inc.), SNP genotyping was performed according to instructions by the manufacturer. Data was analyzed with PLINK.Neuroimaging Eight fearful and eight neutral (4 male and 4 female) faces were selected from the stimulus set of Ekman and Friesen. Faces were presented in a random order for 500 ms with a 500 ms presentation of a fixation cross separating each face stimulus. Brain imaging data were acquired on a Siemens 3.0-Tesla Magnetom Trio TIM whole-body MR scanner. Structural images were acquired using a gradient-echo, T1-weighted pulse sequence (TR = 2600 ms, TE = 3.02 ms; 1 mm × 1 mm × 1 mm voxel size).Statistics Statistics were performed with IBM SPSS Statistics 19.0. Detection of outliers was performed, and when necessary, removed from analyses. Repeated measures of ANOVA or Student's ttest (two-tailed) for independent samples were tested with Bonferroni Post-hoc analysis. The results are presented as mean ± or + standard error of the mean (SEM) and statistical significance was set at $P < 0.05$.
Results: Using a mouse model of dysregulated fear, we found altered amygdala expression of the *Oprl1* gene (Opioid receptor-like 1, encoding the amygdala nociceptin/NOP/orphanin FQ receptor))($p < 0.05$). *Oprl1* is found in mice in the central amygdala (CeA) whereas its expression in other amygdala regions is relatively low. Systemic and central amygdala infusion of SR-8993, a novel and highly selective NOP receptor agonist, impaired fear memory consolidation (p*OPRL1* interacts with self-reported childhood trauma to predicts PTSD symptoms ($F(2,1793) = 6.9$, P 0.001)). This SNP also associates with physiological startle measures of fear discrimination , ($F(1,69) = 12.3$, P 0.001), and fMRI analysis of amygdala-insula functional connectivity ($p_{corr} < 0.05$).
Conclusions: Together, these data suggest that *Oprl1* is associated with amygdala function, fear processing,and PTSD symptoms. Further, our data suggest that activation of the OPRL1/NOP receptor may interfere with fear memory consolidation, with implications for prevention of PTSD following trauma.
Disclosure: K. Ressler, Nothing to Disclose.

Panel

28. The Future of Translational Research in Addiction

28.1 Cross-species Behavioral Tests for Investigations of Addictive and Psychiatric Disorders

Mark A. Geyer*

University of California, San Diego, California

Background: Animal models are essentialfor drug discovery and development in psychiatry. Over the past 3 decades, the 'modeling' of complex psychiatric and addiction disorders has shifted from ill-conceived attempts to recapitulate complete diagnostic syndromes to focus instead on specific dimensions of psychopathology. Given that rodents cannot talk (at least to us), animal studies must rely on measuring non-verbal phenomena. These phenomena in turn need to be demonstrable and measurable in humans and linked to parallel measures in the relevant patient population. Presumably, such measures are closer to biological and genetic substrates and more likely to be predictable from animal to human experiments. Fortunately, specific constructs of relevance to psychiatric and addictive disorders can be assessed using cross-species measures in non-verbal tasks.
Methods: Measures of locomotor and exploratory behavior in rodents have been used for decades to assess

arousal and curiosity and define stimulants and depressants. Rodent versions of the Behavioral Pattern Monitor (BPM) have identified empirical profiles that discriminate among a variety of stimulant drugs of abuse in keeping with their disparate mechanisms of action. To extend this paradigm across species, a human BPM (hBPM) was developed to assess exploratory behavior in psychiatric and drug-abusing populations. The hBPM is an office-size room containing furniture, but no chair, and an assortment of interesting objects to engender exploration. The subject is tracked by an overhead camera and movements are monitored by accelerometers for a 15-min session. No specific instructions are provided other than to 'wait in here'. The same subjects are also tested in an Iowa Gambling Task (IGT) to assess risky behavior. Ongoing studies in the hBPM are examining methamphetamine (METH) abusers with and without comorbid HIV infection.
Results: In the hBPM on the locked inpatient Psychiatry Unit, manic BD patients exhibit a specific phenotypic profile that differs from other clinical groups and is characterized by increased distance-covering activity, increased specific exploration, and reduced spatial d. In the hBPM in the outpatient Psychiatry Unit, preliminary results from subjects with HIV infection and comorbid history for METH abuse revealed some increases in motor activity and exploratory interactions with objects. In this comorbid group, object interactions were correlated with increased measures of risky behavior in the IGT.
Conclusions: These findings support the use of the BPM as a reverse-translational paradigm to investigate phenomena relevant to addiction and other psychiatric populations in both humans and rodents. The subtle abnormalities seen in subjects comorbid for HIV and METH abuse are being used to validate related animal models which can support studies of underlying neurobiological mechanisms and, potentially, novel treatments. For example, the hBPM profile exhibited by acutely ill manic patients has been used to assess the validity of genetic and pharmacological models of mania and its treatment. Similarly, the interaction of METH and HIV is being assessed in mutant mice expressing HIV-related proteins, with and without exposure to a METH binge regimen. The further development of cross-species models is critically important, given the recent concerns that preclinical animal models have not been adequately predictive of Phase III clinical trials. The alternative strategy recommended here is to design parallel animal and human paradigms to test putative treatments across species. These human experimental medicine measures must in turn be selected to be predictive of efficacy in larger trials in the relevant patient populations.
Disclosure: M. Geyer, **Part 1:** San Diego Instruments, Omeros Inc., Lundbeck, Dart Neuroscience, Neurocrine, Abbott, Cerca, Takeda, **Part 2:** San Diego Instruments, Omeros Inc.

28.2 Identifying the Molecular Determinants of Inhibitory Control Problems in Addictions

J. David Jentsch*

University of California Los Angeles, California

Background: Over the past 10 years, it has become increasingly clear that the neural substrates required for the effortful suppression of contextually-inappropriate reward-seeking behaviors are impaired in behavioral addictions. Moreover, several influential conceptual models now recognize these impairments as crucial to the loss of control over drug-seeking and -taking that characterizes substance dependence. That said, much remains unclear about the relationship between inhibitory control deficits and addictions. Whether these deficits anticipate addiction or are a consequence of it is an open question, and the underlying biological mechanisms that lead to impaired control are generally underexplored.
Methods: Stimulant drug-naive and -experienced non-human primates have been studied using translational laboratory measures of inhibitory control (eg, reversal learning), temperament and spontaneous motor behaviors, as well as with non-invasive MR- and PET-based neuroimaging. Inbred mouse strains have been used in genome-wide linkage and association analyses to detect genetic influences on inhibitory control, as well as the causal influence of these genetically-influenced behavioral phenotypes on sensitivity to drug reinforcement (measured with operant self-administration procedures).
Results: Our studies in stimulant drug-naive non-human primates have indicated that individual differences in inhibitory control abilities relate strongly to brain dopamine D2-like receptor availability; monkeys with naturally-occurring low levels of brain dopamine D2 receptors exhibit the most difficulty with suppressing inappropriate responses. Once experience with methamphetamine is commenced, brain dopamine D2 receptors decline and structural hypertrophy of the putamen occurs as a consequence of the drug, and this translates into escalating impairments in self control. Studies in inbred mice reveal the same relationship; strains with genetically determined low dopamine D2 receptor levels exhibit poorer inhibitory control and greater sensitivity to the reinforcing effects of cocaine. Variation in candidate genes (syn3, nt5dc3, rgnef) have been linked and associated with these phenotypes.
Conclusions: Problems with inhibitory control and addictions appear to exist in a bidirectional relationship with one another. Genetically influenced low dopamine D2 receptor availability leads to problems with self control and to enhanced susceptibility for drug-reinforced behaviors. Additionally, experience with the pharmacological actions of the drug further suppresses D2 signaling and impairs inhibitory control. These data suggest that intervening to counteract these molecular and behavioral adaptations will be useful in enabling effective suppression of drug-seeking and -taking behaviors.
Disclosure: J. Jentsch, Nothing to Disclose.

28.3 Neurocircuitries for Social Stress and Drug Abuse: Novel Targets for Intervention

Klaus A. Miczek*

Tufts University, Medford, Massachusetts

Background: Extra-hypothalamic CRF, its receptor subtypes and binding protein modulate aminergic activity. Several of the CRF1 receptor populations in dorsal raphe nucleus, ventral tegmental area and locus coeruleus and at limbic and cortical terminals of the aminergic projections are sites for reducing escalated intake of alcohol and other drugs of abuse. The current studies investigate (1) the critical characteristics of social stress that engender escalated drug intake, (2) the CRF receptor subtype-specific interventions

that putatively prevent and reverse the stress-induced escalation of drug seeking and taking.
Methods: Intermittent episodes of social stress were studied in mice or rats that confronted an aggressive resident opponent for 5 min in 24–72 h intervals. *in vivo* Microdialysis of samples from the prefrontal cortex and n. accumbens shell were assayed for biogenic amines, acids and peptides, Microinjections of CRF R1 antagonists were performed via permanent cannulae into the VTA, n. accumbens or medial prefrontal cortex. The experimental design consisted of comparing socially stressed with non-stressed control animals. Within the socially stressed animals the actively coping animals were differentiated from the passively coping ones as defined by response latencies and durations. Alcohol consumption was studied in a binge model of drinking-in-the-dark as well as in a dependence-inducing model of intermittent access to 20% ethanol. Cocaine self-administration was implemented using intravenous catheters and protocols that comprised the acquisition, maintenance, abstinence and reinstatement phases. In addition, animals were studied during a 24-h continuous access binge.
Results: 1. Brief episodes of social defeat stress induce behavioral sensitization in mice and rats. 2.Continuous social defeat stress engendered salient features of depressive-like behavior such as reduced body weight gain, anhedonia-like impaired saccharine preference and exploration, disrupted estrous cyclicity in females; (3) Intravenous cocaine self-administration was escalated in intermittently stressed mice and rats, at low doses during the maintenance phase and during the 24-h binge, whereas continuous subordination stress suppressed cocaine taking. (4) Intermittent access, like social stress, escalated alcohol consumption leading to dependence. (5) Microinjection of CRF R1, but not CRF R2 antagonists into the VTA prior to each defeat episode prevented the subsequent sensitized motor activity, escalated cocaine self-administration during the 24-h binge, and phasic DA release in the n. accumbens shell. (6) CRF R1 antagonists in the VTA selectively prevented escalated alcohol drinking without affecting concurrent water intake.
Conclusions: The selectivity of CRF R1 antagonist action on preventing and reversing escalated alcohol and cocaine taking identifies this receptor subtype as important target for intervention. In addition to the behavioral selectivity, the data point to receptor selectivity and anatomical selectivity for the modulation of dopaminergic activity in projections from the VTA. The precise interaction between CRF R1 and DA cellular activity awaits further characterization. Also, the differences between actively and passively stress-coping individuals appear to be linked to differential neuroadaptations as evidenced by BDNF and DA activity.
Disclosure: K. Miczek, Nothing to Disclose.

28.4 Towards Consilience in Animal and Human Behavioural Models in Addiction

David Stephens*

University of Sussex, Brighton, United Kingdom

Background: Comparison of data from human and animal studies of drug reward are bedevilled by differences in methodological approach. While human studies often exploit the ability of people to report subjective evaluations, animal studies obviously cannot, and rely upon interpretation of behavioural endpoints, and often use measures of drug self-administration. Importantly, in human studies, self-administration measures of reward value do not always correlate with subjective reports of liking, indicating that these measures do not reflect a single (or perhaps even a related) phenomenon. Furthermore, human self-administration studies differ markedly from animal self-administration studies, so that linking animal and human research is problematic. For this reason, we have sought to develop methods that are not only superficially similar in humans and animal studies (face validity) but which are, to at least some extent homologous across the species. Conditioned reinforcement represents a relatively discrete and psychologically well-characterized behavior, with a well established neurobiology in rodents, it would seem to be an excellent candidate for translational work between animals and humans. Indeed, similar approaches have already been applied in human imaging studies, revealing the involvement of the same brain circuitry, so that there appears to be an emerging argument for homology between the rodent and the human phenomenon, and thus for a useful candidate for comparative studies between rodents and humans.
Methods: In the mouse, animals were trained to associate a tone cue with delivery of a food pellet into a receptacle. Following training over 6 sessions, two levers are inserted into the chamber, activation of one lever leading to presentation of the tone cue, while the other has no effect. In our human studies, the CS was an abstract picture presented on a video display, and predicted gain of money. An alternative stimulus did not predict reward. Following ca. 100 trials, individuals were introduced to a test session in which one button press gave rise to the conditioned cue presentation, while an alternative gave rise to the unpaired stimulus. These approaches were used to study pharmacological effects, and genetic associations with conditioned reward.
Results: In both mouse and human studies, subjects worked to obtain a cue that was previously associated with reward (food or money, respectively). In the mouse, the ability of psychostimulants to facilitate such responding for a conditioned reinforcer was markedly influenced by manipulations of GABAergic systems within the accumbens. Preliminary data also indicate psychostimulant effects on performance of humans that resemble the rodent data.
Conclusions: By studying homologous behaviours in rodents and humans, it may be possible to make more reliable predictions regarding human performance from animal studies.
Disclosure: D. Stephens, **Part 1:** Research Contract with GSK, Cambridge, UK, Consultant with Merz & Co, Frankfurt, Germany, **Part 2:** Research Contract with GSK, Cambridge, UK

Panel

29. Treating Addiction: Should We Aim High or Low?

29.1 Rapid LTP in Accumbens is a Common Feature of Relapse to Multiple Classes of Addictive Drug

Peter Kalivas*

Medical University of South Carolina, Charleston, South Carolina

Background: Chronic use of addictive drugs produces enduring cellular changes in the prefrontal and striatal

circuitry that regulate behavioral responding. While these changes are thought to contribute to the enduring vulnerability to relapse that characterizes addiction, different classes of addictive drug produce opposite forms of synaptic plasticity. For example, while heroin leaves the prefrontal to accumbens synapses in a state of long-term depression, cocaine produces long-term potentiation (LTP). Thus, it seems unlikely that these baseline drug-induced changes in synaptic strength mediate the vulnerability to relapse that is shared by all the drugs of abuse. The research to be presented shows that synaptic plasticity associated with the actual relapse event itself is shared between cocaine, nicotine and heroin, posing a causal cellular mechanism and shared molecular targets for treating relapse.

Methods: Rats were trained to self-administer cocaine, nicotine or heroin for 2 weeks, and after 2 weeks of extinction training were presented with a light/tone drug-associated cue that reinstates lever pressing. Rats were sacrificed at different times after initiating reinstatement, and one of 3 measurements was made in the accumbens (1) dentritic spine morphology, (2) AMPA and NMDA currents, or (3) Western blotting for various glutamate associated proteins.

Results: Across nicotine, cocaine and heroin trained animals a number of common adaptations were identified, notably during the first 15–45 min of relapse induced by a drug-associated cue, glutamate synapses in the accumbens underwent LTP as measured by an increase in dendritic spine diameter and the AMPA:NMDA ratio. These changes paralleled and were positively correlated with the intensity of the behavioral response, and depended on activity in the prefrontal cortex. It was noticed that after withdrawal from the self-administration of all 3 drugs, the nucleus accumbens harbored a marked down-regulation in glial glutamate uptake via GLT1. It was hypothesized that the reduced glutamate elimination promotes spillover from glutamatergic synapses during cue-induced drug seeking, and that this mediates both the rapid LTP-like changes and behavioral reinstatement. To test this hypothesis, GLT1 was restored by administering N-acetylcysteine. N-acetylcysteine prevented reinstatement induced by cues after all three drugs, and in the experiments conducted to date has also consistently inhibited the measures of LTP.

Conclusions: These data demonstrate that rats trained to self-administer 3 distinct classes of addictive drug, cocaine, heroin and nicotine, all share in common reduction in glutamate transport via GLT1 in the nucleus accumbens. The reduced elimination of synaptically release glutamate produces a spillover of glutamate into the extrasynaptic space that is associated with rapid, transient LTP-like changes in synaptic physiology and morphology in the accumbens. Importantly, when GLT1 protein and function was restored with N-acetylcysteine, cue-induced reinstatement to all three addictive drugs was prevented, as were the reinstatement associated LTP-like increases in dendritic spine head diameter and the AMPA:NMDA ratio. These data describe a shared mechanism of relapse between different classes of addictive drug, providing a new model for developing treatments for individuals who abuse multiple drugs.

Disclosure: P. Kalivas, Nothing to Disclose.

29.2 Silent Synapse-based Circuitry Reorganization in Cocaine Craving

Yan Dong*

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: Relapse to drug addiction can occur after prolonged abstinence and is often precipitated by exposure to drug-associated cues that provoke drug craving. In rat models of drug relapse and craving, cue-induced drug craving progressively increases after withdrawal from cocaine and other abused drugs, a phenomenon termed 'incubation of drug craving'. Incubation of cocaine craving is partially mediated by delayed time-dependent drug-induced accumulation of GluA2-lacking, calcium permeable AMPAreceptors (CP-AMPARs) in nucleus accumbens (NAc), a brain region critical for cocaine reward and relapse. The molecular triggering events and the specific glutamatergic projection to NAc that are involved in this form of long-lasting cocaine-induced synaptic plasticity are unknown. We and others previously reported that, in the NAc, non-contingent exposure to cocaine causes the formation of silent excitatory synapses, potentially immature synapses that express stable NMDA receptors with AMPA receptors either absent or highly labile. Abundant in the newborn brain, silent synapses are formed during early developmental stages and subsequently 'mature' into fully functional synapses by recruiting AMPA receptors. The functional significance of cocaine-induced formation of silent synapses in the NAc in animal models of drug reward and relapse is unknown, but it has been hypothesized that generation and subsequent unsilencing of silent synapses may be a critical cellular process through which exposure to cocaine re-develops/re-organizes the related brain circuit to produce long-lasting addiction-related behaviors. Here, we studied the role of cocaine-induced formation of silent synapses in the basolateral amygdala (BLA)-to-NAc and prefrontal cortex (PFC)-to-NAc glutamatergic projection in the incubation of cocaine craving.

Methods: We employed *in vitro* and *in vivo* optogenetic approach combined with slice electrophysiology and operant behavioral tests (cocaine self-administration) to examine the role of two critical pathways, BLA-to-NAc and PFC-to-NAc afferents in cue-induced incubation of cocaine craving.

Results: Following cocaine self-administration, silent excitatory synapses are detected in these two projections. Our subsequent results suggest that these cocaine-generated silent synapses mature into fully functional synapses by recruiting calcium-permeable AMPA receptors. Furthermore, optogenetic reversal of the maturation of silent synapses within the BLA-to-NAc projection reverses incubation of cocaine craving, whereas reversal of the maturation of silent synapses within the PFC-to-NAc projection promotes cocaine craving.

Conclusions: Silent synapses play a critical role in synaptic and circuitry development. Our results suggest that the potential silent synapse-based circuitry re-organization play a differential roles in cocaine craving; reorganization of the BLA-to-NAc projection contribute to, whereas reorganization of PFC-to-NAc compromise, incubation of cocaine craving.

Disclosure: Y. Dong, Nothing to Disclose.

29.3 Synaptic Depression via Positive Allosteric Modulation of mGluR1 Suppresses Cue-induced Cocaine Craving

Marina E. Wolf*

Rosalind Franklin University of Medicine and Science, North Chicago, Illinois

Background: Long after achieving abstinence, cocaine addicts remain vulnerable to cue-induced craving leading to relapse. Thus, a pharmacological treatment to reduce cue-induced craving could prolong abstinence. In a rodent model of addiction, rats show a progressive intensification ('incubation') of cue-induced craving after withdrawal from extended access cocaine self-administration. We showed previously that high conductance Ca^{2+}-permeable AMPA receptors (CP-AMPARs) accumulate at synapses in the nucleus accumbens (NAc) during prolonged withdrawal and mediate the expression of 'incubated' craving. Then, using slice recordings, we showed that mGluR1 stimulation produces a postsynaptically expressed form of synaptic depression in the NAc of 'incubated rats' that is mediated by CP-AMPAR removal from synapses. The goal of the present study was to determine if systemic treatment with an mGluR1 positive allosteric modulator (PAM) would remove CP-AMPARs from NAc synapses and thus reduce cue-induced cocaine craving.
Methods: Rats self-administered cocaine or saline (6 h/d for 10 d). To assess craving, cue-induced seeking tests were conducted on withdrawal day (WD) 45 or greater. During the test, nose-poking in the active hole (previously associated with cocaine and cue delivery) now delivers only the cue. Thus, responding provides a measure of cue-induced craving. Some rats were killed at different times after the test, and slices were prepared for patch clamp recordings. Levels of CP-AMPAR transmission were assessed based on the rectification index and sensitivity to naspm. Biotinylation and co-IP studies evaluated mGluR1 surface expression and association with Homer proteins.
Results: First, we showed that intra-NAc infusions of the group I agonist DHPG or an mGluR1 PAM attenuated the expression of incubated cue-induced cocaine seeking. Next, in behavioral studies followed by patch-clamp recordings, we showed that systemic administration of an mGluR1 PAM also reduced seeking, an effect accompanied by removal of CP-AMPARs from NAc synapses. This protective effect persisted for $\sim$1 day. These findings led us to hypothesize that mGluR1 normally exerts inhibitory tone on CP-AMPAR levels in the NAc and that loss of mGluR1 tone during withdrawal enables CP-AMPAR accumulation. Based on the time-course of CP-AMPAR accumulation during withdrawal, we selected 3 time-points for study: WD14 (before any change), WD25 (when CP-AMPARs are beginning to increase) and WD48 (when CP-AMPARs are stably expressed). While saline and cocaine groups did not differ on WD14, the cocaine group exhibited decreased mGluR1 surface expression on WD25 and WD48, suggesting that persistently decreased mGluR1 surface expression contributes to CP-AMPAR accumulation. Homer protein levels and mGluR1-Homer interactions were not altered. Restoring mGluR1 tone during this critical period (WD15-33), by administering repeated mGluR1 PAM injections, blocked CP-AMPAR accumulation and the incubation of cue-induced cocaine seeking, effects which persisted 2–3 days after the last PAM injection.
Conclusions: Acutely stimulating mGluR1, after incubation has occurred, reduces CP-AMPAR transmission and prevents expression of incubated cocaine craving. Furthermore, repeated mGluR1 PAM injections during a critical period of withdrawal oppose the decrease in surface mGluR1 that normally occurs, thereby maintaining mGluR1-mediated inhibitory control over CP-AMPAR accumulation. Thus, mGluR1 PAMs could be used by recovering addicts to control cue-induced craving and prolong abstinence. This has major translational significance as no treatment is presently available that can provide such protection.
Disclosure: M. Wolf, **Part 1:** I have 50,000 shares ($\sim$\$50,000) in a non-publicly traded entity: Grace Laboratories LLC, 1755 Logans Knoll NE, Atlanta GA 30329. I do not receive any income at this time. There is no linkage to my research or to ACNP., I have 50,000 shares ($\sim$\$50,000) in a non-publicly traded entity: CIS BIotech Inc, 2701 North Decatur Rd, Decatur, GA 30033. I do not receive any income at this time. There is no linkage to my research or to ACNP., **Part 2:** I have 50,000 shares ($\sim$\$50,000) in a non-publicly traded entity: Grace Laboratories LLC, 1755 Logans Knoll NE, Atlanta GA 30329. I do not receive any income at this time. There is no linkage to my research or to ACNP., I have 50,000 shares ($\sim$\$50,000) in a non-publicly traded entity: CIS BIotech Inc, 2701 North Decatur Rd, Decatur, GA 30033. I do not receive any income at this time. There is no linkage to my research or to ACNP.

29.4 Withdrawal from Acute Amphetamine Potently Down-regulates VTA Dopamine Neuron Activity: Reversal by Ketamine

Anthony A. Grace*

Dopamine Neurophysiology, Schizophrenia, Translational Research, Pittsburgh, Pennsylvania

Background: Amphetamine administration is known to produce a rewarding, positive affective state in subjects that take the drug, presumably through its ability to elicit dopamine release. However, following 18 hours withdrawal from amphetamine, subjects often report a negative affect, which causes the subject to take additional drug to offset this condition. This is consistent with the opponent process theory, in which the drug-induced positive state leads to a homeostatic, long-lasting down-regulation of the same system upon withdrawal. A similar condition also exists following acute restraint stress, in which the dopamine neuron activation observed 2 hours after restraint is replaced by a substantial down-regulation of dopamine neuron firing 24 hours later; an effect that is mediated via the amygdala. We tested whether ketamine, a novel rapidly-acting antidepressant, could reverse this amphetamine-driven attenuated dopaminergic state.
Methods: Male Sprague-Dawley rats were given 2.0 mg/kg amphetamine, and 18 hours later were anesthetized with choral hydrate and dopamine neurons were recorded by passing an electrode through the ventral tegmentum in a 9-track pattern and counting active dopamine neurons. Dopamine neuron population activity (i.e., number of

neurons firing per electrode track), firing rate and firing pattern were assessed. A subgroup of rats was administered 5.0 mg/kg ketamine prior to recording.
Results: 18 hours post-amphetamine, there was a greater than 50% reduction in dopamine neuron population activity in the ventral tegmental area, with no change in average firing rate or pattern. When the amphetamine-treated rats were injected with ketamine immediately before recording, dopamine neuron activity was restored to baseline levels.
Conclusions: The dopamine system is regulated in opposite manners depending on the state of the organism. Conditions that result in reward or sensitization produce an increase in dopamine system activity. However, following withdrawal of these treatments, there is an extended, time-dependent compensatory down-regulation of dopamine neuron firing. Specifically, a decrease in the number of dopamine neurons firing would limit the amplitude of phasic responses to stimuli. Such a down-regulation would lead to an attenuated response of the dopamine system to rewarding or activating stimuli; this resultant negative affective state could then drive additional drug taking to offset these consequences. Ketamine administration appears to reverse this attenuated state, and therefore may prove beneficial in circumventing the cycle of drug taking/withdrawal/readministration that leads to abuse.
Disclosure: A. Grace, **Part 1:** Johnson & Johnson, Lundbeck, Pfizer, GSK, Puretech Ventures, Merck, Takeda, Dainippon Sumitomo, Otsuka, Lilly, Roche, Asubio, **Part 4:** lilly, lundbeck

Wednesday, December 11, 2013

Mini-Panel

30. Are the Putative Therapeutic Effects of Kappa-opioid Antagonists Explained by Anti-stress Actions?

30.1 Disruption of Kappa-opioid Receptor Actions Reduces Stress Effects on Cognitive Function and Anxiety-like Behavior

Ashlee Van't Veer*

McLean Hospital, Belmont, Massachusetts

Background: Stress disrupts attention in humans and can precipitate psychiatric conditions including anxiety, depression and drug addiction. Corticotropin-releasing factor (CRF) is a key mediator of the stress response and administration of CRF produces stress-like effects in humans and laboratory animals. Indeed, levels of CRF are elevated in individuals with PTSD and depression and hypersecretion of CRF has been hypothesized to be the primary contributing factor in the development of these disorders. Recent work suggests that kappa-opioid receptor (KOR) antagonists can block stress and CRF effects, raising the possibility that at least some stress effects are mediated via KORs. The present studies were designed to further characterize interactions between KOR systems and stress-induced behaviors following CRF or footshock.
Methods: Cognitive function was assessed using the 5-choice serial reaction time task (5CSRTT) in rats food deprived to 85% free-feeding weight. Rats received an intracerebroventricular (ICV) infusion of vehicle and were tested 1 hr later to obtain a performance baseline. On the following day, rats received an injection of vehicle or JDTic, a KOR antagonist with long-lasting effects (>14 d) in rodents. In subsequent test sessions, rats received ICV infusions of CRF (0.25, 0.5 or 1 μg) or aCSF and were tested 1 hr later. Anxiety-like behavior was assessed in mice using the acoustic startle reflex following CRF infusion or footshock. Mice were matched into groups with equivalent baseline startle and given an injection of the KOR antagonist JDTic or vehicle prior to testing. In the test session, mice received either an ICV infusion of CRF (1 μg) or ascending footshock amplitudes each followed by a startle test. Mutant KOR mice were subjected to the same behavioral protocols without administration of JDTic. Data were analyzed by ANOVAs and *post hoc* tests.
Results: CRF produced dose-dependent decreases in correct responses and increases in omission errors in the 5CSRTT and JDTic attenuated these effects. In mice, blockade of KORs by JDTic attenuated CRF and footshock induced increases in startle. In comparison, constitutive KOR knockout (KO) mice had similar levels of potentiated startle compared to controls and JDTic did not have an effect on startle in these KOs. Conditional KOs in which KORs were ablated only in dopamine transporter (DAT) expressing neurons had significantly lower levels of footshock-induced potentiation, but similar levels of CRF-enhanced startle compared to controls.
Conclusions: These data suggest KOR antagonists may prevent stress effects that can degrade performance and contribute to the development of psychiatric illnesses such as anxiety and PTSD. Interestingly, constitutive KOR KO mice show equivalent levels of CRF and footshock-potentiated startle compared to wild type littermates, whereas KOR deletion restricted to DAT expressing neurons was sufficient to reduce footshock effects. Lack of JDTic effects in the constitutive mutants indicates that the actions of KOR antagonists are due to on-target effects. This pattern of results suggests compensatory adaptations in KOR function occurring during development may account for the dissociation between JDTic-treated and constitutive KOR KO mice. Further, KOR function in regions outside dopamine neurons likely plays an important role in regulating CRF effects on startle. Overall, these data provide additional evidence that disruption of KOR function reduces stress-induced behaviors and support the idea that a general ability for disruption of KOR function to reduce the effects of stress underlies the beneficial effects of KOR antagonists in a diverse range of disease models.

Disclosure: A. Van't Veer, Nothing to Disclose.

30.2 Dynorphin-kappa Systems in Compulsive-like Responding with Extended Access to Elicit Drugs

George F. Koob*

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Background: Drug addiction has been defined as a chronically relapsing disorder characterized by (1) compulsion to seek and take the drug, (2) loss of control in limiting intake, and (3) emergence of a negative emotional state (eg, dysphoria, anxiety, irritability), reflecting a motivational

withdrawal syndrome, when access to the drug is prevented (defined here as dependence). The compulsivity associated with drugs of abuse has been hypothesized to derive from several mechanisms, one of which involves increased drug seeking that results from negative reinforcement. Multiple mechanisms can be envisioned to explain the role of kappa systems in the compulsivity associated with addiction, including interactions with the dopamine system (a within-system neuroadaptation where dynorphin drives decreases in dopamine function) and interactions with other brain stress systems (between-system neuroadaptation where dynorphin activates or is activated by corticotropin-releasing factor). Under this framework, the emergence of a negative emotional state with the development of dependence provides the aversive stimulus to drive drug seeking to remove the negative emotional state (negative reinforcement).
Methods: Animal models of excessive drug taking associated with extended access have been developed where animals increase drug seeking with extended access/exposure. Here, animals are allowed access to intravenous self-administration of drugs for either 1 h per day (short access group) or 6–21 h per day (long access groups).
Results: Long, 6 h access to cocaine and methamphetamine, 12 h access to heroin, and 21 h access to nicotine results in an increase in responding over days, whereas short access produces no escalation in intake. Blockade of kappa opioid receptors can block the escalation in intake of cocaine, methamphetamine, heroin, and nicotine. Evidence to date suggests that one site for these actions is the shell of the nucleus accumbens, but other data suggest a role for the central nucleus of the amygdala.
Conclusions: One viable hypothesis to explain these results is that nucleus accumbens dynorphin mediates the dysphoric-like effects of drug withdrawal, and the central nucleus of the amygdala mediates the anxiety-like effects of drug withdrawal. Preliminary evidence also suggests that once the kappa system is engaged, the neuroadaptations persist and may be resistant to kappa antagonists. Thus, the dynorphin-kappa opioid system may be a key component of the transition to dependence, and different brain areas may be responsible for the multiple effects of activation of the dynorphin/kappa opioid system in the compulsivity associated with addiction.
Disclosure: G. Koob, Nothing to Disclose.

30.3 The Dissociable Effects of Kappa-Opioid Receptor Activation on Intolerance to Delay and Response Inhibition

Brendan Walker*

Washington State Univeristy, Pullman, Washington

Background: Alterations in opioid peptides following chronic alcohol exposure contribute to excessive alcohol seeking and consumption. The ê-opioid receptor (KOR) is the primary target for the endogenous opioid peptide dynorphin (DYN) and KORs within nuclei comprising the central extended amygdala, prefrontal cortex (PFC) and orbitofrontal cortex (OFC) participate in the complex integration of information related to different behavioral domains such as decision-making and negative affect. Recent evidence has indicated that the DYN / KOR system in the PFC and OFC of humans and animals is dysregulated in alcohol dependence in a manner that could contribute to maladaptive behavioral regulation. The primary purpose of these studies was to assess the involvement of KORs in the development of impulsive- or withdrawal / KOR agonist stress-induced negative affective-like phenotypes as assessed by measurement of response inhibition, intolerance to delay or 22-kHz ultrasonic vocalizations (USVs) following central KOR agonist exposure or during alcohol dependence-induced acute withdrawal, with both conditions being challenged by the KOR antagonist nor-binaltorphimine (norBNI).
Methods: Separate groups of male Wistar rats were trained in the stop-signal reaction time (SSRT) and delay discounting (DD) paradigms. The impact of the centrally-administered KOR agonist U50,488 on performance in the SSRT and DD paradigms was assessed using a within-subject dosing design and the effects of norBNI on the pro-impulsive effects of U50,488 was determined. Lastly, withdrawal / KOR agonist stress-induced negative affective-like behavior was assessed via measurement of 22-kHz ultrasonic vocalizations during acute withdrawal in alcohol-dependent animals following norBNI infusion or following U50,488 infusion with or without norBNI pre-exposure in alcohol-naïve animals.
Results: The results showed a dissociable effect of KOR agonists on impulsive phenotypes related to response inhibition or intolerance to delay with selective effects in the SSRT that were not confounded by locomotor effects of U50,488 or a lack of motivation to engage in the task. Importantly, the pro-impulsive effects of U50,488 infusion were rescued by pretreatment with norBNI. Acute withdrawal-induced 22-kHz USVs were dose-dependently reduced by norBNI and U50,488-induced increases in 22-kHZ USVs were rescued by norBNI.
Conclusions: When 'mimicking' alcohol dependence and withdrawal via central KOR agonist infusions, for the first time the DYN / KOR system was implicated as a mediator of impulsive responding, selectively related to response inhibition, in a norBNI-reversible manner. These results identify novel canonical targets involving the DYN / KOR system for the treatment of neuropsychiatric disorders involving impulse control, the increased impulsivity observed in substance use disorders and withdrawal stress-induced negative affect.
Disclosure: B. Walker, **Part 4:** Completed a cooperative research agreement on 9/24/2011 with H. Lundbeck A/S, Copenhagen on the kappa-opoid mechanisms of nalmefene.

Mini-Panel

31. Developing Imaging Biomarkers for Treatment Development: Beyond CNTRICS, CNTRaCs and NEWMEDS

31.1 Imaging Biomarkers for Psychiatric Disorders: The NEWMEDS Experience

Andreas Meyer-Lindenberg*

Central Institute of Mental Health, Mannheim, Germany

Background: NEWMEDS (Novel Methods leading to New Medications in Depression and Schizophrenia—is an

European Union funded international consortium of scientists that represents one of the largest research academic-industry collaboration projects to find new methods for the development of drugs for schizophrenia and depression. NEWMEDS brings together scientists from academic institutions with nearly all major biopharmaceutical companies. One focus of he project has been to develop standardised paradigms, acquisition and analysis techniques to apply brain imaging, especially fMRI and PET imaging to drug development.
Methods: We investigated human and rodent fMRI methods usable for drug discovery. In humans (Plichta *et al*. 2012, Neuroimage) we assessed both within-subject and group-level reliability of a combined three-task fMRI battery targeting three systems of wide applicability in clinical and cognitive neuroscience: an emotional (face matching), a motivational (monetary reward anticipation) and a cognitive (n-back working memory) task. FMRI reliability was quantified using the intraclass correlation coefficient (ICC) applied at three different levels ranging from a global to a localized and fine spatial scale: (1) reliability of group-level activation maps over the whole brain and within targeted regions of interest (ROIs); (2) within-subject reliability of ROI-mean amplitudes and (3) within-subject reliability of individual voxels in the target ROIs. In rodents, we developed resting state fMRI methods and assessed effects of the drug, haloperidol (Gass *et al*. ENP 2013). Sprague-Dawley rats received either haloperidol (1 mg/kg, s.c.) or the same volume of saline a week apart. Resting-state functional magnetic resonance imaging data were acquired 20 min after injection. Connectivity analyses were performed using two complementary approaches: correlation analysis between 44 atlas-derived regions of interest, and seed-based connectivity mapping.
Results: In humans, eeliability of group level activation was excellent for all three tasks with ICCs of 0.89–0.98 at the whole brain level and 0.66–0.97 within target ROIs. Within-subject reliability of ROI-mean amplitudes across sessions was fair to good for the reward task (ICCs = 0.56–0.62) and, dependent on the particular ROI, also fair-to-good for the n-back task(ICCs = 0.44–0.57) but lower for the faces task (ICC = -0.02–0.16). In rodents, presence of haloperidol led to reduced correlation between the substantia nigra and several brain regions, notably the cingulate and prefrontal cortices, posterodorsal hippocampus, ventral pallidum, and motor cortex. Haloperidol induced focal changes in functional connectivity were found to be the most strongly associated with ascending dopamine projections. These included reduced connectivity between the midbrain and the medial prefrontal cortex and hippocampus, possibly relating to its therapeutic action, and decreased coupling between substantia nigra and motor areas, which may reflect dyskinetic effects.
Conclusions: In humans, the newly developed task battery was well suited to between-subject designs, including imaging genetics. When specific recommendations are followed, the n-back and reward task are also suited for within-subject designs, including pharmaco-fMRI. In rodents, be showed the feasibility of connectivity functional imaging data data for further characterizing the functional circuits modulated by antipsychotics that could be targeted by innovative drug treatments.

Disclosure: A. Meyer-Lindenberg, **Part 1:** Speaker and Advisory boards: AstraZeneca, J + J, Lundbeck, Servier, Lilly

31.2 New Neuroscience Based Cognitive Paradigms for Biomarker Research in Schizophrenia

Deanna M. Barch*

Washington University, Saint Louis, Missouri

Background: Over the past decade there has been a growing awareness of the disabling effects of impaired cognition in schizophrenia. At the same time, cognitive neuroscience has seen an explosion of technical advances and new knowledge regarding the neural basis of cognition. The Cognitive Neuroscience Research To Improve Cognition in Schizophrenia initiative conducted a series of conferences to develop a consensus on the constructs and paradigms from cognitive neuroscience that are ripe for translation, with a number of research efforts ongoing designed to achieve the goals outlined by CNTRICs. This presentation will overview of the results the Cognitive Neuroscience Task Reliability & Clinical Applications (CNTRACs) Consortium. CNTRaCs developed measures of Goal Maintenance (Dot Probe Expectancy Task; DPX), Episodic Memory (Relational and Item Specific Encoding Task; RISE), Visual Integration (Jittered Orientation Visual Integration Task: JOVI), and Gain Control (Contrast-Contrast Effect Task; CCE). We present information on the development and validation of these new tools, data on sensitivity to the cognitive impairments in schizophrenia, relationships to functional outcome and symptoms, test-retest reliability and comparison to MATRICs measures.
Methods: The CNTRaCs Consortium conducted two large-scale studies across five sites. Study 1 included 138 participants with schizophrenia and 136 healthy demographically matched controls, and compared multiple versions of each of the four tasks to examine optimal detection of group differences, interpretability as a specific construct, length and tolerability, and relationships to functional status and symptoms. Study 2 included 103 participants with schizophrenia and 132 healthy demographically matched controls, and examined test-retest reliability and practice effects across 3 timepoints (baseline, day 7, day 21), relationships to functional status and symptoms, and comparison to the Hopkins Verbal Learning Test and BACS Symbol Coding subtests of the MATRICS battery.
Results: In Study 1, the DPX and the RISE elicited large effect sizes for detecting cognitive deficits in schizophrenia, the JOVI detected moderate effect size deficits, with all three tasks providing data that showed excellent interpretability in terms of the constructs of interest. The CCE revealed a small effect size deficit, and performance appeared to be confounded by time on tasks effects, making it difficult to interpret the results as reflecting gain control deficits. In schizophrenia, performance on the DPX and the RISE were correlated with performance on the UPSA-B as a proxy measure of function, and performance on the DPX was correlated with informant ratings of functional status and negative symptoms. In Study 2, the DPX

and the RISE once again elicited large effect size cognitive deficits, and the JOVI a moderate effect size. The DPX and the JOVI showed some improvement from baseline to day 7, but no further practice effects from day 7 to day 21. The RISE did not show practice effects. The DPX showed good test-rest reliability (ICC = 0.77), the RISE measures showed moderate reliability (ICCs 0.55 to 0.62), and the JOVI measures good to moderate reliability (ICCs 0.56 and 0.73 for threshold and accuracy). The CNTRaCS and MATRICS were both related to proxy measure of function. When controlling for shared variance with the MATRICs, the DPX and RISE continued to identify cognitive impairments in schizophrenia and the DPX continued to correlate with function.
Conclusions: These results provide evidence of the validity, reliability and utility of these new neuroscience based measures of Goal Maintenance (DPX), Relational Encoding and Retrieval (RiSE) and Visual Integration (JOVI).
Disclosure: D. Barch, **Part 1:** Consultant for Pfizer and Amgen, Research Grants from Novartas and Dainippon Sumitomo Pharma Co., Ltd, **Part 4:** Research Grants from Novartas and Dainippon Sumitomo Pharma Co., Ltd

Mini-Panel

32. Human Brain Evolution and Comparative Epigenomics

32.1 Decoding the Molecular Evolution of Cognition

Genevieve Konopka*

UT Southwestern Medical Center, Dallas, Texas

Background: It has been hypothesized that one of the consequences of the highly evolved cognitive capacity of the human brain is the development of increased vulnerability to cognitive disorders. Thus, elucidating the origins of human-uniqueness in terms of our enhanced cognitive abilities has been a long-standing goal of many avenues of neuroscience research. Recent technical breakthroughs in genomics have allowed us to begin to identify genetic and molecular signatures in the central nervous system that distinguish humans from non-human primates.
Methods: Using RNA-seq in a comparative genomics approach, we are elucidating human-specific patterns of gene connectivity in the brain. We are examining gene expression signatures across multiple cortical regions to better ascertain the contribution of specific cortical circuits to cognitive function. We have also begun to combine these data with functional methods ranging from human cell culture to brain imaging to prioritize and more fully understand the most salient pathways for the evolution of human cognition. Finally, we are implementing methods to modulate gene expression in rodents in human-specific patterns followed by behavioral testing.
Results: We have identified novel human-specific patterns of gene expression and regulation in the neocortex. These data suggest that the human brain has undergone rapid modifications of gene expression patterns to support our enhanced cognitive abilities. In addition, we identify an enrichment of cognitive disease related genes that demonstrate unique gene expression changes in the human brain.
Conclusions: These approaches have led to important insights into signaling cascades involved in language, molecular mechanisms driving neuronal plasticity, and gene expression changes underlying cortical arealization. Together, these data combined with other work on comparative genomes and epigenomes will pioneer an innovative approach for understanding the functional consequences of genes, gene evolution, and gene expression on human cognition.
Disclosure: G. Konopka, Nothing to Disclose.

32.2 Divergent Whole Genome Methylation Maps of Human and Chimpanzee Brains Reveal Epigenetic Basis of Human Regulatory Evolution and Disease Susceptibility

Soojin V. Yi*

Georgia Institute of Technology, Atlanta, Georgia

Background: DNA methylation is a pervasive epigenetic modification of DNA that strongly affects chromatin regulation and gene expression. To date, it remains largely unknown how patterns of DNA methylation differ between closely related species, and whether such differences contribute to species-specific phenotypes.
Methods: To investigate these questions, we generated nucleotide-resolution whole-genome methylation maps of the prefrontal cortex of multiple humans and chimpanzees. We then integrated these newly generated data with data on gene expression. By doing so, we have investigated genomic, epigenomic and transcriptomic differences between human and chimpanzee brains.
Results: Levels and patterns of DNA methylation vary across individuals within species, according to the age and the sex of the individuals. We also find extensive species-level divergence in patterns of DNA methylation, and hundreds of genes exhibit significantly reduced levels of promoter methylation in the human brains compared to chimpanzee brains. Furthermore, we investigated the functional consequences of methylation differences in humans and chimpanzees by integrating data on gene expression generated using next-generation sequencing methods, and found a strong relationship between differential methylation and gene expression. Finally, we found that differentially methylated genes are strikingly enriched for loci associated with neurological disorders, psychological disorders, and cancers.
Conclusions: Our results demonstrate that differential DNA methylation may be an important molecular mechanism driving gene expression divergence between human and chimpanzee brains and potentially contribute to the evolution of disease vulnerabilities. Thus, comparative studies of humans and chimpanzees stand to identify key epigenomic modifications underlying the evolution of human-specific traits.
Disclosure: S. Yi, Nothing to Disclose.

32.3 Neuronal Epigenome Mapping in Human and Non-human Primate Prefrontal Cortex

Jogender Singh Tushir*

University of Massachusetts Medical School, Worcester, Massachusetts

Background: Cognitive abilities, higher order functions and disorders unique to humans are thought to result from adaptively driven changes in brain transcriptomes, but little is known about the role of epigenetic signatures affecting transcription start sites (TSS).
Methods: Neuronal nuclei sorting from human, chimpanzee and macaque brain; next generation sequencing of nucleosomes trimethylated at histone H3-lysine 4; next generation sequencing of prefrontal transcriptomes, RNA immunoprecipitation, *in situ* hybridization
Results: We mapped in human, chimpanzee, and macaque prefrontal cortex the genome-wide distribution of histone H3 trimethylated at lysine 4 (H3K4me3), an epigenetic mark sharply regulated at TSS, and identified 471 sequences with human-specific enrichment or depletion. Among these were 33 loci selectively methylated in neuronal but not non-neuronal chromatin from children and adults, including TSS at DPP10 (2q14.1), CNTN4 and CHL1 (3p26.3), and other neuropsychiatric susceptibility genes. Regulatory sequences at DPP10 and additional loci carried a strong footprint of hominid adaptation, including elevated nucleotide substitution rates and regulatory motifs absent in other primates, with evidence for selective pressures during more recent evolution and adaptive fixations in modern populations. Chromosome conformation capture at multiple H3K4me3 peaks in the 2q14.1 region (DPP10 neurodevelopmental susceptibility locus) revealed higher order chromatin structures demonstrating physical contact among peaks spaced 0.5–1 Mb apart. In addition, we describe a novel human specific, cis-bound RNA (Loc389023) that physically associates with PRC2 (Poly-comb repressor complex 2) at a bivalent DPP10 promoter. Loc389023, which was expressed in a small subset of cortical neurons, increases H3K27me3 profiles (a repressive histone mark) in 2q14.1 and may be associated with downregulation of DPP10 at both the protein and RNA levels.
Conclusions: Taken together, our results suggest that coordinated epigenetic regulation via newly derived TSS chromatin could play an important role in the emergence of human-specific gene expression networks in brain that contribute to cognitive functions and neurological disease susceptibility in modern day humans.
Disclosure: J. Singh Tushir, Nothing to Disclose.

Mini-Panel

33. Intergenerational Transmission of Trauma—From Animal Models to Humans

33.1 Behavioral and Neural Mechanisms of the Intergenerational Transmission of Trauma

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University of Michigan, Ann Arbor, Michigan

Background: Anxiety disorders, such as posttraumatic stress disorder and specific phobias may be transmitted to subsequent generations. Despite the clinical evidence, we lack animal models explaining behavioral, neural and molecular mechanisms of this transmission. We have recently demonstrated in a rat model that infants acquire fear responses to a specific odor following an exposure to the mother expressing fear to that odor. We have shown that in our model the intergenerational transmission of fear is mediated by alarm pheromone signaling. Here, we will discuss behavioral and neural mechanisms involved in the intergenerational transmission of fear.
Methods: Rat pups and mothers were exposed to the olfactory cue presentations eliciting maternal fear (mother received prior fear conditioning training to that cue). Maternal behavior during the cue exposure was videotaped, scored and classified into fearful/defensive, rough/abusive, nurturing and neutral behaviors. Neural activity in the pup's brain during the exposure to a frightened mother was assessed using a C14 2-deoxyglucose autoradiography. Pups responses to the cue eliciting maternal cue were tested in the absence of the mother using a Y-maze odor aversion test. In each experiment one experimental group (pups exposed to a frightned mother) was compated to two control gorups (pups exposed to unfrightened mother with or without a history of a learned fear).
Results: Our analysis indicates that maternal fearful/defensive behaviors but not rough/abusive behaviors during the conditioned cue exposure with the pups support intergenerational transmission of a cue odor aversion. The analysis of neural activity in the pup's brain indicates the involvement of two olfactory subsystems: the vomeronasal organ-accessory olfactory bulb ($F_{(2,14)} = 6.090$; $p < 0.02$) and the Grueneberg ganglion-necklace glomeruli pathways ($F_{(2,14)} = 8.438$; $p < 0.004$), as well as the amygdala ($F_{(2,14)} = 7.545$; $p = 0.007$) in the intergenerational transmission of odor aversion.
Conclusions: Our results demonstrate how specific fear responses may be transmitted across generations through parental emotional communication. These findings provide a model explaining how parental adaptive and maladaptive fear responses may be transmitted to the offspring, such as in posttraumatic stress disorder and specific phobias.
Disclosure: J. Debiec, Nothing to Disclose.

33.2 Epigenetic Markers in the GR and FKBP5 Genes in Children of Holocaust Survivors

Rachel Yehuda*

Mount Sinai School of Medicine, New York, New York

Background: Adult children of Holocaust survivors are at increased risk for mood and anxiety disorders such as posttraumatic stress disorder. Prior studies have demonstrated alterations in glucocorticoid receptor (GR) responsiveness in Holocaust offspring that are similar to those observed in PTSD, and occur largely in association with maternal PTSD.
Methods: In the first study, cytosine methylation of the 1F exon promotor of the GR gene and GR gene expression were examined in lymphoytes of offspring of Holocaust survivors and Jewish comparison subjects. Other measures reflecting glucocorticoid responsiveness (lysozyme IC50, dexametha-

sone suppression test), urinary cortisol and cortisol metabolism were also obtained. In the second study, we examined FKBP5 methylation at intron 7 in blood samples of offspring and their parents.
Results: In the first study, GR gene methylation was lower in Holocaust offspring relative to comparison subjects [$t(94) = 2.46$, $p = 0.016$), and was significantly lower in association with maternal PTSD [$F(1,94) = 7.54$, $p = 0.007$]. In the second study, FKBP5 methylation was found to be lower in 1 out of 6 sites in Holocaust offspring compared to comparison subjects [$F(1,30) = 4.91$, $p = 0.035$]. Comparable reductions in FKBP5 methylation were not observed in Holocaust survivor parents compared to demographically-comparable controls [$F(1,37) = 0.01$, $p = 0.916$]. There was a significant correlation between FKBP5 methylation in mothers and offspring ($r = 0.63$, $p = 0.018$, $n = 15$), whereas FKBP5 methylation between fathers and offspring were not associated ($r = 0.19$, $p = 0.645$, $n = 8$).
Conclusions: The findings suggest that GR and FKPB5 methylation changes in Holocaust offspring result from intergenerational transmission along the maternal lines, possibly owing to in utero or early maternal behavioral changes associated with maternal PTSD following Holocaust exposure. GR and FKBP5 methylation correlated with several neuroendocrine indices suggesting that previously observed changes in cortisol and GR levels may have epigenetic origins.
Disclosure: R. Yehuda, Nothing to Disclose.

33.3 Transgenerational Imprints on Structure and Function in the Mammalian Nervous System

Brian Dias*

Emory University, Atlanta, Georgia

Background: Responding to environmental stimuli is crucial to the survival of organisms, and species. When and how environmental information results in experience-dependent alteration of nervous system structure and function is a fundamental question in behavioral neuroscience. Ancestral experience is a rich source of information for future generations. Model systems are important to further our understanding of such intergenerational transmission including that of trauma and its sequelae. To address this, we asked whether learning by an ancestral generation (F0) can be inherited by the F1 and F2 generations, using an olfactory fear conditioning protocol wherein an odor is paired with a mild foot-shock.
Methods: To address the transgenerational inheritance of parental trauma, we asked whether learning by an ancestral generation (F0) can be inherited by the F1 and F2 generations. For this purpose, we subjected F0 mice to an olfactory fear conditioning protocol wherein an odor is paired with a mild foot-shock.
Results: We find that fear conditioning adult mice (F0 generation) to Acetophenone causes subsequently conceived F1 and F2 male offspring to display a behavioral sensitivity to Acetophenone, despite them having no prior exposure to this odor ($p = 0.043$, $t(27) = 2.123$). Acetophenone is detected by the M71 odorant receptor in the Main Olfactory Epithelium and we find that the F1 and F2 generations have larger M71 glomeruli in the olfactory bulbs {Dorsal M71 Glomerular Area in F1 generation: ($p < 0.0001$, $F(2,91) = 15.53$). Medial M71 Glomerular Area in F1 generation: ($p < 0.0001$, $F(2,84) = 31.68$)}. Epigenetic analyses in the olfactory epithelium of both generations indicate that these neuroanatomical changes may result from increased transcription of the M71 gene that detects the F0 conditioned odor. Cross-fostering and *in vitro* fertilization (IVF) studies suggest that the transgenerational effects are inherited.
Conclusions: We conclude that parental olfactory experience before conception can be inherited at the level of structure and function in the nervous system. From a translational perspective, this work allows us to appreciate how parental experience prior to conceiving offspring profoundly influences the nervous systems of future generations—an influence that might contribute to the manifestation of neuropsychiatric disorders such as phobias, anxiety, and Post Traumatic Stress Disorder (PTSD).
Disclosure: B. Dias, Nothing to Disclose.

Panel

34. Alterations of the Glutamate Cycle in Severe Mental Illness

34.1 Abnormalities of Glutamate Transporter Expression in Schizophrenia: Evidence for Increased Glutamate Reuptake and Altered Subcellular Partitioning of EAAT2 Interacting Proteins

Robert E. McCullumsmith*

University of Alabama, Birmingham, Alabama

Background: Glutamate transporters remove glutamate from the synaptic cleft, facilitating excitatory neurotransmission by maintaining extrasynaptic glutamate levels. Captured glutamate may be converted to glutamine and cycled back to the presynaptic terminal, where it is converted back into glutamate. One of the transporters, excitatory amino acid transporter 2 (EAAT2) is expressed at high levels adjacent to synapses. Since the capture efficiency of EAAT2 is estimated to be about 50%, this transporter may act like a sponge, limiting glutamate spillover from perisynaptic regions to the extracellular space. We hypothesize that abnormalities of glutamate synapses extend to the glutamate transporters, and that there are changes in the activity, localization, and cellular distribution of these molecules in schizophrenia.
Methods: We used electron microscopy, immunoisolation, immunofluorescence, mass spectroscopy, Western blot analysis and subcellular fractionation to investigate the cellular and subcellular localization of glutamate transporters in the human frontal cortex. We used [3H]-glutamate to assess glutamate reuptake in synaptosomal membranes isolated from postmortem brain. We used two cohorts of subjects; an older cohort from the Bronx VA brain bank (18 pairs of subjects), and a younger cohort from the Alabama Brain Collection (10 pairs of subjects).
Results: We found alterations in the spatial distribution of EAAT2, suggesting that EAAT2 is not properly localized to perisynaptic regions in schizophrenia. We also found an

increase in the expression of the EAAT2 splice variant EAAT2B in a biochemically purified fraction containing cytosolic proteins and extrasynaptic membranes in subjects with schizophrenia. EAAT2B mRNA expression is selectively increased in neurons, and decreased in astrocytes. We also confirmed that EAAT2 interacts with hexokinase, Na + /K + ATPase, and aconitase in human brain. We found changes in aconitase levels in a mitochondria enriched fraction, and a shift in hexokinase 1 expression from mitochondria to the cytosol. Finally, we found an increase in Na + dependent glutamate reuptake in a mixed synaptosomal preparation containing astrocytiic and presynaptic markers, in samples with an overall decrease in EAAT2 expression.

Conclusions: Our data support the hypothesis that the cellular and subcellular distribution of EAAT2 is abnormal in schizophrenia. As EAAT2 faciltates the glutamate/glutamine cycle, changes in the activity or localization of this molecule would disrupt the cycle, leading to inefficient recovery of glutamate for presynaptic release. Our data also suggest that coupling of EAAT2 to glycolytic enzymes and mitochondria is disrupted. EAAT2 co-transports Na + and K + with their gradients, and the Na + /K + ATPase maintains these gradients in the face of high throughput glutamate transport. Tight coupling to mitochondria provides ATP for this process. Finally, we found an increase in Na + dependent glutamate reuptake; in the context of decreased total EAAT2 expression, these data suggest a compensatory increase in reuptake capacity by other transporter isoforms, including the neuronal transporter EAAT3, and the EAAT2 splice variant found in neurons, EAAT2B. Taken together, our data argue for an increase in cycling glutamate, with altered localization of EAAT2 and a global increase in reuptake capacity.

Disclosure: R. McCullumsmith, Nothing to Disclose.

34.2 Functional Implications of Altered *In Vivo* Glutamate and GABA Systems in Schizophrenia

Laura M. Rowland*

University of Maryland School of Medicine, Baltimore, Maryland

Background: Glutamate abnormalities are implicated in schizophrenia but *in vivo* investigation remains challenging, especially at conventional MR field strengths such as 3T. We used a novel, very short echo time proton magnetic resonance spectroscopy (1H-MRS) sequence to investigate glutamate, glutamine, the major metabolite of synaptic glutamate, and glutamine/glutamate ratio in schizophrenia (SZ) at 3T. The glutamine/glutamate ratio may reflect glutamate-glutamine neurotransmitter cycling. Reproducibility of this technique was first assessed. We also report GABA levels that are not contaminated by macromolecule signal to provide a comprehensive picture of excitatory and inhibitory neurotransmitter function. Since previous reports suggest glutamatergic and GABA measures may vary with SZ illness duration we investigated younger and older groups. The relationships between glutamatergic and GABA measures, psychiatric symptom severity, mismatch negativity (MMN) thought to reflect glutamate NMDAR function, and cognition were examined.

Methods: Fifty subjects participated in this study [SZ: younger (n=13) and older (n=12)] and age matched HC (n=25). MR scanning was conducted on a 3T Siemens Tim Trio. For detection of glutamate and glutamine, spectra were acquired using phase rotation STEAM: TR/TM/TE = 2000/10/6.5-ms, VOI ~ 24cm^3, NEX=128, 2500-Hz spectral width, 2048 complex points, and $\Delta\phi1 = 135^\circ$, $\Delta\phi2 = 22.5^\circ$, $\Delta\phi3 = 112.5^\circ$, and $\Delta\phi ADC = 0^\circ$. For detection of GABA, spectra were acquired from the same voxel using macromolecule suppressed MEGA-PRESS: TR/TE=2000/68 ms, 14 ms editing pulses applied at 1.9 and 1.5 ppm, and 256 averages. Spectra were quantified with LCModel and GANNET. Working memory, processing speed, and ERP MMN were assessed.

Results: Test-retest of glutamate and glutamine was excellent (Mean CVs: Glu = 2.3%, Gln = 9.3%). We observed lower glutamate ($p = 0.027$) and GABA ($p = 0.05$) and a trend for higher glutamine/glutamate ratio ($p = 0.06$) in the older SZ group only. GABA was negatively related to glutamine ($p = 0.002$) and glutamine/glutamate ($p = 0.008$) in both groups suggesting a homeostatic balance between excitatory and inhibitory activity. Glutamate, glutamine, glutamine/glutamate levels were not related to positive or negative symptom severity. However, strong associations were found between glutamine/glutamate ratio and MMN amplitude ($p < 0.001$). Better working memory performance was correlated with higher GABA levels in both groups ($p = 0.047$).

Conclusions: Spectroscopic data acquired at very short echo times enables the reproducible detection of complex multiplets such as glutamate and glutamine, which are very difficult to detect at 3T. Our results suggest MRS measures of excitatory and inhibitory neurotransmitter function, obatined by optimized spectroscopic methods, are associated with specific brain functions linked to the pathophysiology of schizophrenia. We found that illness course may affect glutamatergic and GABA measures since they were altered in older subjects with SZ. Glutamine/glutamate ratio is also altered in the older SZ group. The strong association between the glutamine/glutamate ratio and MMN suggests that glutamine/glutamate may be a good marker of glutamatergic function.

Disclosure: L. Rowland, Nothing to Disclose.

34.3 Glutamatergic Abnormalities in Medicated and Unmedicated Patients with Schizophrenia

Adrienne C. Lahti*

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Background: The glutamate (Glu) hypothesis of schizophrenia proposes that blockade of N-methyl-D-aspartate (NMDA) receptors on ã-aminobutyric acid (GABA) inhibitory neurons results in excess Glu release leading to excitotoxicity (Olney & Farber, 1995). Consistent with this model, recent proton magnetic resonance spectroscopy (^{1}H-MRS) studies have reported higher glutamate + glutamine (Glx) levels in unmedicated or drug-naïve patients with schizophrenia (SZ). While MRS studies have focused on Glx and *N*-acetyl-aspartate (NAA), a metabolite also found abnormal in SZ, separately less attention has been paid to the relationship between them. We compared Glx levels and the correlation of Glx and NAA in three cohorts of patients:

(1) in medicated SZ (Kraguljac *et al*, 2012), (2) in unmedicated SZ, and (3) in unmedicated SZ evaluated longitudinally during treatment.
Methods: Using 1H-MRS we compared: (1) stable, medicated SZ ($n=50$) and matched controls ($n=48$); (2) unmedicated SZ ($n=27$) and matched controls ($n=27$); (3) unmedicated SZ ($n=20$) scanned unmedicated, and after 1 and 6 weeks of treatment. Voxels were placed in the dorsal anterior cingulate cortex (ACC) and hippocampus. Spectra were acquired using the point resolved spectroscopy sequence (PRESS; TR/TE = 2000/80 ms) and analyzed using jMRUI.
Results: (1) In medicated SZ, relative to controls, there were no significant differences in Glx/Cr in either voxel. In the hippocampus, NAA and Glx ratios were significantly correlated in controls ($r=0.40$, $p<0.01$), but not in schizophrenia ($r=-0.06$; $p=0.71$) ($z=2.22$, $p=0.02$). (2) In unmedicated SZ, relative to controls, Glx/Cr was significantly elevated in hippocampus ($p=0.04$). (3) ANOVA revealed no effect of treatment on Glx/Cr or NAA/Cr levels in either region. Post-hoc paired-samples t-tests revealed trends towards decrease in Glx/Cr [paired $t(19)=1.56$, $p=0.14$] and increase in NAA/Cr [paired $t(19)=1.88$, $p=0.08$] in the hippocampus. In the ACC, the correlation between Glx and NAA was not significant when patients were unmedicated ($r(18)=0.28$, $p=0.24$); however, these metabolites were significantly positively correlated after one week ($r(18)=0.69$, $p=0.001$) and 6 weeks ($r(18)=0.55$, $p=0.01$) of treatment. In the hippocampus, correlations between Glx and NAA were non-significant at all 3 time points (all $p>0.05$).
Conclusions: In medicated patients, there were no differences in Glx in either the ACC or hippocampus. However, in unmedicated patients Glx was elevated in the hippocampus. These findings are consistent with (1) reports of higher Glx in unmedicated or drug naïve patients (Kegeles *et al*., 2012; de la Fuente- Sandoval *et al*., 2011 ; (2) report of no difference or decreased Glx in medicated patients (Marsman *et al*., 2011; Rowland *et al*., 2012). While studies of healthy controls have consistently reported a positive correlation between Glx and NAA, we found the correlation to be absent in unmedicated patients in both hippocampus and ACC. In ACC, the correlation appears to strengthen with treatment. In hippocampus, the correlation is absent whether or not patients are medicated. Because NAA and Glx are linked through the tricarboxylic acid and the glutamate-glutamine cycles, the lack of correlation between these metabolites suggest possible underlying pathologies of these cycles. We identified two forms of glutamatergic abnormalities in SZ: (1) elevated Glx in unmedicated SZ and (2) absence of correlation between Glx and NAA in both unmedicated and medicated SZ. These abnormalities might contribute to a progressive negative effect on brain structure and function.
Disclosure: A. Lahti, Nothing to Disclose.

34.4 Psychosis and Cognition Related to Different Brain Glutamate Pools in Schizophrenia

Juan Bustillo*

University of New Mexico, Albuquerque, New Mexico

Background: The NMDA hypofunction model of schizophrenia predicts a paradoxical increase in synaptic glutamate release. *In-vivo* measurement of glutamatergic neurotransmission in humans is challenging, but glutamine, the principal metabolite of synaptic glutamate, can be quantified with proton magnetic resonance spectroscopy (H-MRS). Although a few studies have measured glutamate, glutamine and glutamine/glutamate ratio, it is not clear which of these H-MRS indices of glutamatergic neurotransmission are altered in schizophrenia. Furthermore, the relationships of these measures to the the core psychopathological domains of the illness remain unclear.
Methods: Conventional ^{1}H-MRS spectra were acquired using the standard PRESS with a TE = 40 ms and a TR = 1.5 s at 3T. The voxel was prescribed to include mostly grey matter in the dorsal anterior cingulate. Spectra were quantified using LCModel, with fits $<30\%$ for Gln and $<20\%$ for all other metabolites and partial volume corrected.

Eighty-four patients with DSM-IV-R diagnosis of schizophrenia and 81 psychiatrically-healthy volunteers were matched in age, gender, ethnicity and occupational level of the head of household of family of origin.
Results: Glutamine was increased in the schizophrenia group ($p=0.01$) as well as the glutamine/glutamate ratio ($p=0.007$) but not glutamate ($p=0.9$). Glutamine levels were positively correlated with severity of psychotic symptoms ($p=0.02$) not with negative symptoms or antipsychotic dose. Glutamate levels were negatively correlated with Attention/Vigilance ($p=0.04$) and Working Memory ($p=0.01$) in the schizophrenia group but not in the healthy controls.
Conclusions: Elevated glutamine, is consistent with increased glutamatergic synaptic release in schizophrenia, as predicted by the NMDA hypofunction model. The differential relationship between glutamatergic indices and clinical/cognitive manifestations of the illness, suggest a dissociation of function for the glutamate pool: increased synaptic release (glutamine) with psychosis and increased glutamate metabolism (glutamate) with cognitive impairment. Further understanding the underlying mechanism of glutamatergic dysfunction in schizophrenia, may lead to new pharmacological strategies to improve cognition and treat psychosis.
Disclosure: J. Bustillo, **Part 1:** Speakers Bureau and advisory consulting for Otsuka America Pharmaceutical Inc

Panel

35. Early Stress and Emotion Dysregulation

35.1 Effect of Direct Eye Contact in PTSD Related to Interpersonal Trauma: An fMRI Study of Activation of an Innate Alarm System

Ruth Lanius*

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Background: In healthy individuals, direct eye contact initially leads to an activation of a fast subcortical pathway, which then modulates a cortical route eliciting social cognitive processes. The aim of this study was to gain insight into the neurobiological effects of direct eye-to-eye contact using a virtual reality paradigm in individuals with

posttraumatic stress disorder (PTSD) related to prolonged childhood abuse.
Methods: We examined 16 healthy comparison subjects and 16 patients with a primary diagnosis of PTSD related to childhood abuse using a virtual reality fMRI paradigm involving direct versus averted gaze as developed by Schrammel *et al*. (2009). Subtraction and functional connectivity analyses were conducted.
Results: Irrespective of the displayed emotion, controls exhibited an increased blood oxygenation level-dependent response during direct *vs* averted gaze within the dorsomedial prefrontal cortex, left temporoparietal junction, and right temporal pole. Under the same conditions, individuals with PTSD showed increased activation within the superior colliculus (SC)/periaqueductal gray (PAG) and locus coeruleus. Functional connectivity analyses revealed increased connectivity of the salience network with the left amygdala and right insula during direct vs averted gaze in subjects with PTSD as compared to controls.
Conclusions: Our findings suggest that healthy controls react to exposure to direct gaze with an activation of a cortical route which enhances evaluative 'top-down' processes subserving social interactions. In individuals with PTSD, however, direct gaze leads to a sustained activation of a subcortical route of eye contact processing, an innate alarm system involving the SC and underlying circuits of the PAG. Altered connectivity of the salience network with the amygdala and insula was also observed during direct gaze in PTSD subjects as compared to controls. Implications of these data for under- and overmodulation of emotions states in PTSD will be discussed.
Disclosure: R. Lanius, Nothing to Disclose.

35.2 Examining the Genetic Underpinnings of the Amygdala Habituation Deficit in Borderline Personality Disorder

M. Mercedes Perez-Rodriguez*

Mount Sinai School of Medicine, New York, New York

Background: Borderline personality disorder (BPD) patients have emotion-processing deficits and lack the normal habituation of amygdala activity to repeated negative emotional stimuli seen in healthy volunteers. The Met allele of the rs6265 SNP of the brain derived neurotrophic factor (BDNF) gene has been shown to increase neural reactivity to emotional stimuli in the amygdala and other areas involved in emotion processing and to impair extinction learning in both mice and humans. We have shown that patients with BPD lack amygdala habituation to repeated emotional pictures. In the present study we expand and refine our prior finding by using an imaging genetics framework to investigate the genetic underpinnings of this neural deficit. Specifically, we aimed to test the impact of BDNF rs6265 SNP genotypes on amygdala reactivity to repeated emotional pictures in BPD patients compared to healthy and psychiatric controls.
Methods: Event-related functional magnetic resonance imaging (MRI) was employed in 3 groups: unmedicated BPD ($n = 19$) and schizotypal personality disorder (SPD, $n = 18$) patients and healthy control subjects ($n = 20$) during a task involving viewing unpleasant, neutral, and pleasant pictures each presented twice within their respective trial block/run. The amygdala was hand-traced on each participant's structural MRI scan and co-registered to their MRI scan. Amygdala responses were examined with a mixed-model multivariate analysis of variance including BDNF rs6265 SNP genotype (ValVal vs Met-carriers).
Results: We found a Diagnostic group (BPD, healthy control, SPD) × Genotype (BDNF rs6265 SNP ValVal vs Met-carriers) × Picture type (unpleasant, neutral, pleasant) × Picture repetition (Novel/Repeat) × Time interaction ($F[40,64] = 1.68$, $p = 0.031$, Wilks) indicating that Met-carrying BPD patients (but not SPD patients or HCs who are Met carriers) showed increased and prolonged amygdala reactivity during repeated unpleasant pictures, but not during the novel presentation, representing a failure to habituate.
Conclusions: BDNF pathway genes may mediate the amygdala hyper-reactivity to unpleasant emotional stimuli found in BPD, which is consistent with the clinical feature of high sensitivity to emotional stimuli with unusually strong and long-lasting reactions, and supports a model of emotional undermodulation in BPD, related to hyperarousal and limbic hyperactivation during exposure to unpleasant emotional stimuli. BDNF has been implicated in emotion processing and extinction learning, closely related to habituation. The BPD-specific diagnosis-by-genotype interaction that we report here supports BDNF agonists as a novel therapeutic avenue for BPD, a disorder which lacks FDA-approved medications.
Disclosure: M. Perez-Rodriguez, Nothing to Disclose.

35.3 Influence of Dissociation on Emotional Distraction in Borderline Personality Disorder

Annegret Krause-Utz*

Central Institute of Mental Health, Mannheim, Germany

Background: Emotion dysregulation is a core feature in borderline personality disorder (BPD) and has been associated with amygdala hyperreactivity. Beyond that, individuals with BPD report dissociative experiences. It has been proposed that dissociation is characterized by an over-modulation of affect associated with amygdala underactivity. We investigated the influence of dissociation on emotional distraction in un-medicated interpersonally traumatized patients with BPD.
Methods: During fMRI, BPD patients and healthy participants (HC; matched for age and education) performed a working memory task, while being distracted by negatively arousing and neutral pictures from the International Affective Picture System. In a first study, 22 BPD patients were assigned to two subgroups (with high *vs* low dissociation) based on a median split of their ratings on the Dissociation Stress Scale 4 (as a measure of state dissociation). In study 2, BPD patients were exposed to either a personalized script inducing dissociation ($n = 15$) or a neutral script ($n = 15$) before task performance.
Results: In study 1, BPD patients with high dissociation showed significantly lower activation in the amygdala and insula after emotional distraction compared to BPD patients

with low dissociation. In study 2, similar patterns of limbic brain activation were observed in BPD patients, who had been exposed to the dissociation script.
Conclusions: Findings suggest that dissociative states in (interpersonally traumatized) individuals with BPD are associated with dampened limbic activation during emotional distraction.
Disclosure: A. Krause-Utz, Nothing to Disclose.

35.4 Weakening Fear Memories as a Potential Treatment for Posttraumatic Stress Disorder

Karim Nader*

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Background: In some individuals, exposure to traumatic events can lead to posttraumatic stress disorder (PTSD). This condition is characterized by several symptoms including hypervigilance, avoidance behaviours, intrusive memories and emotional dissociation. It is also linked with medial prefrontal cortex dysfunction and impaired extinction recall. Current PTSD treatments typically only produce partial improvement. Hence, there is a need for preclinical research to develop novel therapeutic approaches. Animal studies have indicated that fear memories can be weakened by pharmacologically blocking reconsolidation (memory restabilization after retrieval) or by extinction learning (learning that a danger signal is safe). Evidence suggests, that among other physiological alterations, there is increased noradrenergic activity in PTSD patients. Consequently, drugs that specifically target this system and are safe for human can be of clinical interest. Here, we investigated the efficacy of an á2-adrenoreceptor agonist, to block memory reconsolidation in an animal model of intrusive and persistent traumatic memories. We also extended our research by examining the dynamics of extinction retention in this model.
Methods: Using an auditory fear conditioning paradigm in rats, we tested the efficacy of clonidine to weaken fear memory retention when administered systemically after fear memory retrieval, evaluating dosage, number of treatments and specificity in reconsolidation blockade. Additionally, using a similar paradigm, conditioned rats were extinguished with repeated non-reinforced presentations of a tone. Extinction retention was tested 1, 4, 8, or 24 hours after extinction learning.
Results: We found that post-retrieval administration of clonidine disrupted fear-related memories in a dose-dependent manner and that two treatments were sufficient to obtain maximal memory impairment. Furthermore, we determined that this effect is long-lasting and specific to reconsolidation processes. In extinction, short retention intervals (1 and 4 h test) resulted in greater fear recovery compared to the longer retention intervals. Expression of extinction may be impeded by endocrine and physiological changes that occur as a result of the aversive experience of extinction training. Although blocking adrenergic transmission has been used to prevent fear in rats and humans, the β-adrenergic antagonist propranolol was unable to prevent fear recovery.
Conclusions: Our results demonstrate that systemic administration of clonidine following retrieval is efficient to persistently disrupt fear memory retention through reconsolidation blockade. This provides preclinical parameters for future therapeutic strategies to be developed using reconsolidation blockade. In addition, results from the extinction study suggest that there is impaired recall of extinction at short retention intervals following extinction learning. We can use this paradigm to test how processing of extinguished cues by the medial prefrontal cortex may be impaired in PTSD. Identifying the mechanisms involved in this behavioural dysregulation may reveal additional pharmacological approaches to facilitate extinction-based therapies.
Disclosure: K. Nader, Nothing to Disclose.

Panel

36. Epigenetic Mechanisms in Neuropsychiatric Disorders

36.1 Global Transcriptome Analysis of Human Cerebrospinal Fluid

Claes Wahlestedt*

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Background: Cerebrospinal fluid (CSF) is an accessible body fluid that coalesces with the brain interstitial fluid and acts as an essentially nuclease-free repository where RNA transcripts shed by brain tissues can reside for extended periods. Therefore, disease related RNA transcripts can conceivably be monitored in CSF.
Methods: RNA deep sequencing techniques, related protocols and data analysis pipelines are emerging technologies. We have optimized sequencing protocols such that we now obtain almost 170 million reads per CSF sample, utilizing pair-end and directional techniques.
Results: Using next generation RNA sequencing we have discovered that a wide variety of RNA transcripts are indeed detectable in CSF and that some are differentially expressed in Alzheimer's disease (AD) patients when compared to controls. A large fraction of the differentially expressed RNAs that we identified are noncoding transcripts, which represent putative biomarker candidates for early detection of AD pathology.
Conclusions: Our method of RNA extraction and directional RNA sequencing from low input RNA samples is suitable for the identification and quantification of CSF extracellular RNA transcripts. We believe that our method will have important implications for future studies that interrogate fluctuations of CSF RNA profiles across a variety of disease conditions.
Disclosure: C. Wahlestedt, Nothing to Disclose.

36.2 Insights into the Roles of the Methyl-DNA Binding Protein MeCP2 in Addictive-like Behaviors

Anne E. West*

Duke University Medical Center, Durham, North Carolina

Background: The methyl-DNA binding protein MeCP2 is emerging as an important regulator of drug reinforcement processes. Psychostimulants induce phosphorylation of MeCP2 at Ser421 (pMeCP2), however the functional

significance of this posttranslational modification for addictive-like behaviors was unknown.
Methods: To determine the requirement for pMeCP2 in neuroadaptive responses to repeated psychostimulant drug exposure we utilized a strain of knockin (KI) mice in which Ser421 of MeCP2 has been replaced by the nonphosphorylable amino acid Ala. We tested locomotor activity induced by acute amphetamine or cocaine, behavioral sensitization to repeated amphetamine, and cocaine self-administration in the KI mice and their WT littermates. We also examined cellular electrophysiology correlates of these behaviors in striatal slice preparations.
Results: We found that MeCP2 Ser421Ala KI mice displayed both a reduced threshold for the induction of locomotor sensitization by investigator-administered amphetamine and enhanced behavioral sensitivity to the reinforcing properties of self-administered cocaine. Behavioral differences were accompanied in the knockin mice by an enhanced psychostimulant-dependent reduction of medium spiny neuron excitability, which is a neural adaptation strongly associated with the rewarding properties of these drugs.
Conclusions: We propose that phosphorylation of MeCP2 at Ser421 functions to limit the circuit plasticities in the nucleus accumbens that underlie addictive-like behaviors.
Disclosure: A. West, Nothing to Disclose.

36.3 MicroRNA 135 is Essential for Chronic Stress Resiliency, Antidepressant Efficacy and Intact Serotonergic Activity

Alon Chen*

Weizmann Institute of Science, Rehovot, Israel

Background: The link between dysregulated serotonergic activity and depression and anxiety disorders is well established, yet the molecular mechanisms underlying these psychopathologies are not fully understood. Here, we explore the role of microRNAs in regulating serotonergic (5HT) neuron activity.
Methods: To this end, we determined the specific microRNA 'fingerprint' of 5HT neurons, studied the regulation of selected microRNA following administration of antidepressants and established genetically modified mouse models, expressing higher or lower levels of miR135.
Results: We identified a strong microRNA-target interaction between microRNA135 (miR135), and both serotonin transporter and serotonin receptor-1a transcripts. Intriguingly, miR135a levels were upregulated following administration of 5HT-linked antidepressants. Genetically modified mouse models, expressing higher or lower levels of miR135 demonstrated major alternations in anxiety and depression–like behaviors, 5HT levels and metabolism and behavioral response to antidepressant treatment. Finally, miR135a levels in blood of depressed human patients were significantly lower, and increased following cognitive behavioral therapy.
Conclusions: The current results suggest a potential role for miR135 as an endogenous antidepressant and provide a new venue for potential treatment and novel insights into the onset, susceptibility and heterogeneity of stress-related psychopathologies.
Disclosure: A. Chen, Nothing to Disclose.

36.4 MicroRNAs and Drug Addiction

Paul Kenny*

The Scripps Research Institute—Scripps Florida, Jupiter, Florida

Background: MicroRNAs are small noncoding RNAs that are emerging as key regulators of almost all apsects of brain function and experience-dependent neuroplasticity. Drug addiction is considered a disorder of neuroplasticity, but little is currently known about the role for microRNAs in addiction. Circulating microRNAs in plasma are stable and may serve as useful biomarkers of various disease states, including neuropsychiatric disorders. Nevertheles, their usefulness as biomarkers for addiction has not been explored. We explored the role for microRNAs in regulating compulsive-like responding for cocaine in rats and mice. In addition, we also explored the potential utility for plasma microRNAs as biomarkers of addiction with diagnostic and prognostic value.
Methods: Compulsive-like repsonding for cocaine was induced in rats by permitting them extended (6 h) daily access to intravenous cocaine self-administration. Control rats were permitted either restricted (1 h) daily access to the drug, or remained cocaine naive throughout. Expression profiling of microRNAs in the striatum of rats demonstrating compulsive-like responding for cocaine was assessed using microarrays. Individual microRNAs whose expression was dysregulated in the brains of these rats was verified by real-time PCR. MicroRNAs were overexpressed in the striatum of rats using lentivurus vectors. MciroRNAs were also overexpressed using a transgenic overexpression approach in mice. Conversely, microRNA funciton was disrupted in striatum using antisense oligonucleotides or by using lentivirus vectors to express 'sponge' inhibitors.
Results: We found that two microRNAs, miR-212 and miR-132, were upregulated in the striatum of rats demonstrating compjlsive-like cocaine responding. Overexpression of miR-212 in striatum profoundly decreased the motivational value of cocaine in rats, but only in those with extended daily access to the drug. Conversely, disruption of miR-212 signaling in striatum increased cocaine intake in rats. We found that miR-212 controls cocaine intake through at least two mechanisms in striatum: first, it increases the activity of the transcription factor CREB; second, it knocks down expression of the transcriptiona repressor MeCP2. In contrast to the effects of miR-212 overexpression, we found that miR-132 overexpression increased the motivational porperties of cocaine. Currently, we investigating the mechanisms by which miR-132 regulates cocaine intake. Finally, we assessed microRNA expression profiles in the blood samples of human treatment-seeking drug addicts at the time of their admission to a treatment facillity in West Palm Beach, and at the end of their treatment 4 weeks later. We identified unique plasma expression patterns of microRNAs in those with substance abuse disorders

compared with control samples collected from the local community.
Conclusions: MicroRNAs in striatum play a key role in regulating sensitivity to the motivational properties of cocaine and in driving the development of compulsive-like cocaine intake. MicroRNAs may also serve as useful biomarkers for addiction in human drug addicts with diagnostic and prognostic value.
Disclosure: P. Kenny, **Part 1:** Consultant to Pfizer, Inc., Co-founder of Eolas Therapeutics.

Panel

37. Glutamate-dopamine Interactions in Nicotine and Cocaine Dependence: Biomarkers and Therapy Opportunities

37.1 Dopamine Activity and Reward Processing in Smokers Before and After Smoking Cessation: Combined [18F]FDOPA/fMRI Studies

Gerhard Gründer*

RWTH Aachen University, Aachen, Germany

Background: Research on nicotine addiction indicates greater ventral striatal activity in smokers compared to non-smokers in response to smoking-associated cues but blunted reactivity to non-drug rewards. Furthermore, the only available small PET study on dopamine synthesis capacity in nicotine-dependent subjects demonstrated an increase in [18F]FDOPA uptake in the striatum in smokers. It is completely unexplored, however, whether reward processing and dopamine metabolism change after smoking cessation. The aim of the present study was to examine dopamine metabolism, neural correlates of reward anticipation and cue reactivity in non-smokers and nicotine-dependent smokers before and three months after smoking cessation in a combined [18F]FDOPA/fMRI paradigm.
Methods: 30 male nicotine dependent subjects and 15 healthy control never-smokers (matched for age, cognition, education) were included. The control group underwent a single [18F]FDOPA-PET scan (124 min data acquisition after bolus injection). One half of the patient-group underwent a first scan in withdrawal (>5 hours without smoking), the remaining subjects were scanned under continued smoking conditions. Subsequently, all patients participated in a smoking cessation program consisting of six psychotherapeutic interventions. 16/30 patients were able to remain abstinent for at least three months. This group participated in a second imaging session. All subjects underwent arterial blood sampling during the scanning procedure for obtaining activity input curves and [18F]FDOPA/[18F]OMFD ratios. The reversible inlet/outlet model was applied in order to obtain the net blood/brain clearance (dopamine synthesis capacity, K), the loss of fluorinated metabolites (dopaminergic turn-over, kloss), and the total distribution volume (storage capacity, VD). In addition, all subjects performed two paradigms on a 1.5 T MR scanner: Monetary and social reward anticipation were investigated using the Monetary and Social Incentive Delay task. The second paradigm examined cue reactivity by presenting blocks of smoking-related, neutral or sexually arousing pictures. fMRI data were analyzed with SPM.
Results: Previous findings of a positive correlation between dopamine synthesis capacity and cognitive performance could be replicated (TMT-B *vs* VD in ventral caudate: $r=-0.631$, $p=0.016$). In continuously smoking patients, dopamine synthesis capacity was strongly and significantly blunted in particular in the right (-16%, $p=0.045$) and left ventral caudate (-16%, $p=0.050$). kloss was reduced on a trend-level. Three months abstinence normalized all presynaptic dopaminergic parameters in every region. Patients in acute withdrawal demonstrated a tendency toward reduced K and kloss. During both monetary and social reward anticipation smokers showed weaker activity of the NAcc compared to non-smokers. However, in response to smoking-associated pictures stronger neural responses were found in the caudate nucleus. No effect of smoking cessation could be detected.
Conclusions: Contrary to previous findings, our PET data indicate that smoking is associated with reduced dopamine synthesis capacity and turnover rather than elevations. The complete normalization after 3 months of abstinence was unexpected and does not claim an inefficient dopamine system to be a strong trait towards nicotine dependence. Our fMRI data implies that striatal activation during anticipation of non-smoking rewards is decreased in smokers while reactivity is increased for smoking-associated pictures. The findings further suggest that neural activation during reward processing is not affected by smoking cessation. Thus, while neurochemical markers of nicotine dependence are normalized, functional activation in cue-related paradigms are not.
Disclosure: G. Gründer, **Part 1:** Dr. Gründer has served as a consultant for Bristol-Myers Squibb (New York, NY), Cheplapharm (Greifswald, Germany), Eli Lilly (Indianapolis, Ind), Forest Laboratories (New York, NY, USA), Lundbeck (Copenhagen, Denmark), Otsuka (Rockville, Md.), Roche (Basel, Switzerland), and Servier (Paris, France). He has served on the speakers' bureau of Bristol-Myers Squibb, Eli Lilly, Gedeon Richter (Budapest, Hungary), Otsuka, Roche, and Servier. He has received grant support from Alkermes, Eli Lilly, and Roche. He is co-founder of Pharma-Image —Molecular Imaging Technologies GmbH, Düsseldorf. , **Part 2:** 2011: Eli Lilly, **Part 3:** 2011: Eli Lilly, **Part 4:** Dr. Gründer has received grant support from Alkermes, Eli Lilly, and Roche. He is co-founder of Pharma-Image—Molecular Imaging Technologies GmbH, Düsseldorf.

37.2 *In Vivo* Imaging of Human mGluR5 and nAch Receptors with PET: Dynamic Duo for Abuse Studies and Drug Occupancy?

Dean F. Wong*

Johns Hopkins University School of Medicine, Baltimore, Maryland

Background: Nicotine dependence (NIC-D) pathophysiology and treatment remain highly elusive. The availability of new potential mGluR5 and nAchR treatments for clinical trials allow exploration in (NIC-D). This presentation will describe current and evolving biomarker opportunities that

will facilitate progress in NIC-D translational research and drug development.
Methods: First-in-human safety and tolerability PET was conducted with the mGluR5 antagonist, [^{18}F]FPEB, which is now gaining widespread global use (Wong, *et al*. JNM 2013). We carried out radiation dosimetry in 6 healthy adults (3 M, 3F) and measured brain binding potentials (BP_{ND}) with 90 min. scans ($n=2$ per each of 5 subjects) using the high resolution research tomograph (HRRT) ($\sim$ 2 mm resolution). Test/retest (TRT) (BP_{ND}) variability and comparison was made with the widely used tracer [^{11}C]ABP 688 ($N=11$). We also carried out occupancy (OCC) using [^{11}C]ABP688 and [^{18}F]FPEB with mGluR5 negative allosteric modulators (NAMs) fenobam and STX107 in baboons and humans, respectively. We also tested the first in human safety and dynamic brain imaging of [^{18}F]AZAN, a selective á4â2 nAchR tracer on the HRRT with ($N=9$) non-smokers and with nicotine exposure ($N=3$) smokers. Also additional subjects were imaged while on varenicline or placebo. TRT ($N=5$) was also estimated.
Results: Radiation dosimetry of FPEB was 62 mrem/mCi, allowing multiple PETs/ yr. FPEB had BP_{ND} from 0.5 (globus palladus) to 3.5 (insula). The TRT BP_{ND} show a measurable advantage for [^{18}F]FPEB ([^{11}C]ABP 688 ($>10\%$). Baboons using [^{11}C] ABP688 show an OCC of 90% after i.v. administration of 1.33 mg/kg of fenobam. Human OCC using [^{18}F] FPEB and STX107 are ongoing. [^{18}F]AZAN has an radiation effective dose of 52 mrem/mCi, allowing multiple PETs / yr. Vol of dist (V_T) ranged $\sim$6 (CC) to $\sim$20 (thalamus) and BP_{ND} $\sim$ 0.5 cingulate to $\sim$ 2.6 (thalamus). The TRT of BP_{ND} was excellent ($<10\%$). Development of AZAN is a dramatic improvement in logistics for imaging nAChR because of the shortened scan time to 90 min. compared to existing human radiotracers.
Conclusions: First in human PET ligands for mGluR5/ nAchR to study NIC-D pathophysiology and OCC by mGluR5 for NAMs show these ligands can measure target engagement. Partial agonists as varenicline and nicotine from smoking can displace AZAN suggesting this optimal nAchR á4â2 PET ligand can be utilized in studies of NIC-D within a 90 min. PET.
Disclosure: D. Wong, **Part 1:** Consultant for Amgen and Concert Pharmaceuticals, **Part 2:** Johns Hopkins University, School of Medicine, **Part 4:** Avid, Biotie, GE, Intracellular, J + J, Lilly, Lundbeck, Merck, Otsuka, Roche, Sanofi Aventis, Synosia

37.3 Reduced mGluR5 Receptor Binding in Smokers and Ex-smokers Determined by [11C]ABP688 Positron Emission Tomography: Clinical and Scientific Relevance

Gregor Hasler*

UPD Waldau, Bern, Switzerland

Background: Nicotine addiction is a major public health problem due to the development of primary glutamatergic dysfunction. We have previously shown that the metabotropic glutamate receptor 5 (mGluR5) is markedly reduced in smokers and ex-smokers. In this follow-up study we will evaluate the significance of these findings. Becauseantagonism of the metabotropic glutamate receptor 5 (mGluR5) reduced nicotine self-administration in rats and mice, we hypothesized that low mGluR5 was a protective factor against relapse.
Methods: We used positron emission tomography (PET) with the radiolabeled mGluR5 antagonist 3-(6-methylpyridin-2-ylethynyl)-cyclohex-2-enone-O-11C-methyl-oxime ([^{11}C]ABP688) (15), which binds with high selectivity to an allosteric site, to measure mGluR5 availability in 30ex-smokers with a mean duration of nicotine abstinence between 4 weeks and 10 years. We also continuously assessed relapse of nicotine consumption in the sample participants.
Results: In our previous studies, we found a marked global reduction (20.6%; $p<0.0001$) in the mGluR5 distribution volume ratio (DVR) in the gray matter in current smokers. The most prominent reductions were found in the bilateral medial orbitofrontal cortex. In ex-smokers, we also found marked global reductions in the average gray matter mGluR5 DVR, which were less prominent than that in current smokers. We will analyze the mGluR5 DVR in ex-smokers regarding duration of abstinence at the time of the scan and duration to relapse after the scan.
Conclusions: Our findings suggest that reduced mGluR5 binding may not reflect a simple consequence of nicotine consumption but instead may represent a trait-like pathogenetic or compensatory change associated with nicotine addiction. The result of this study will elucidate the role played by mGluR5 in the maintenance of nicotine abstinence.
Disclosure: G. Hasler, **Part 1:** Servier (Suisse) SA, Lundbeck (Schweiz) AG, Schweizerische Gesellschaft für Bipolare Störungen, AstraZeneca, Eli Lilly (Suisse) SA, **Part 4:** Novartis Switzerland

37.4 Understanding Glutamate, Acetylcholine and Dopamine Interactions in Nicotine Dependence using Animal Models

Manoranjan S. D'Souza*

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Background: Nicotine is the main ingredient in tobacco that leads to continued tobacco smoking. The reinforcing effects of nicotine are partially mediated by dopaminergic neurons originating in the ventral tegmental area (VTA) and projecting to cortical and limbic nuclei including the nucleus accumbens (NAcc). Nicotine increases the activity of dopaminergic neurons by binding directly to nicotinic cholinergic receptors (nAChRs) located on dopaminergic neurons and indirectly by increasing glutamate transmission by binding to nAChRs located on glutamatergic afferents regulating the activity of mesolimbic dopaminergic neurons. Using a combination of behavioral, neurochemical, pharmacological and anatomical techniques we investigated glutamate, acetylcholine and dopamine interactions in nicotine-seeking behavior.
Methods: First, the effects of blockade of nicotine-induced increases in glutamate transmission on intravenous nicotine self-administration and cue-induced reinstatement of nicotine seeking were evaluated. In addition, *in vivo* microdialysis was used to determine the effects of blockade of glutamate transmission on nicotine-induced increases in dopamine in the NAcc shell. Finally, the effects of direct injections of glutamate receptor antagonists in mesolimbic

brain sites, such as the NAcc and the VTA, on nicotine seeking were investigated.
Results: Systemic, intra-NAcc shell and intra-VTA administration of the metabotropic glutamate receptor 2/3 (mGlu2/3) agonist LY379268 attenuated nicotine self-administration. In addition, systemic LY379268 administration attenuated cue-induced reinstatement of nicotine seeking. Interestingly, *in vivo* microdialysis studies indicated that LY379268 pretreatment blocked nicotine-induced increases in NAcc shell dopamine in nicotine-experienced rats only in the presence of a nicotine-associated context. In addition systemic, intra-NAcc shell and intra-VTA administration of the mGlu5 receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) attenuated nicotine self-administration. Finally, systemic and intra-VTA administration of the N-methyl-D-aspartate (NMDA) receptor antagonist LY235959 attenuated nicotine self-administration. Interestingly, LY235959 administration in the NAcc shell and core increased nicotine self-administration and cue-induced reinstatement of nicotine seeking, respectively.
Conclusions: Overall blockade of glutamatergic neurotransmission either via activation of the predominantly presynaptic mGlu2/3 receptors or blockade of postsynaptic NMDA and mGlu5 receptors, attenuated nicotine seeking. The *in vivo* microdialysis data demonstrate that mGlu2/3 receptors negatively modulate the combined effects of nicotine and nicotine-associated contexts/cues on NAcc dopamine. Moreover, the data suggest that mGlu2/3 and mGlu5 receptors in the NAcc shell and the VTA play an important role in regulating the reinforcing effects of nicotine. The increase in nicotine self-administration and cue-induced nicotine seeking after injections in the NAcc shell and core is intriguing and suggests an inhibitory role of NMDA-mediated glutamatergic transmission in these two regions on nicotine seeking. Finally, the opposite effects of the NMDA receptor antagonist in the NAcc shell compared to the VTA on nicotine self-administration suggests that the NMDA receptors play a differential role in nicotine seeking in these two mesolimbic brain sites. Taken together, the data highlight a complex interaction of nicotinic receptor activation by exogenously administered nicotine with glutamatergic and dopaminergic transmission in mesolimbic brain sites.
Disclosure: M. D'Souza, Nothing to Disclose.

Panel

38. β4α2-Nicotinic Acetylcholine Receptors in Schizophrenia: Implications for Smoking Cessation and Therapeutics

38.1 Examining the α4β2 Nicotinic Partial Agnoist Varenicline on the Tobacco Abstinence Syndrome in Schizophrenia Versus Control Smokers

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Imperial College London, Toronto, Ontario, Canada

Background: People with schizophrenia are more likely to smoke cigarettes than the general population. Varenicline tartrate (Chantix®) is a $\alpha_4\beta_2$ nicotinic partial agonist and effective smoking cessation medication, which has recently been shown to be effective in smokers with schizophrenia. However, varenicline's mechanism of action in schizphrenia versus healthy control smokers has not been characterised. We therefore evaluated the effects of varenicline on tobacco reinforcement and features of the tobacco abstinence syndrome, including craving and cognitive dysfunction, in schizophrenia and control smokers.
Methods: Over the three separate test weeks, schizophrenia ($n = 14$) and control ($n = 14$) smokers were co-treated (using a counterbalanced sequence) with varenicline (0, 0.5 and 1 mg BID) during a 3-day laboratory paradigm incorporating assessments of smoking topography, craving, withdrawal and cognition (sensorimotor gating, attention, processing speed, visuospatial working memory (VSWM), verbal learning and memory and impulsivity) at baseline smoking, overnight abstinence and smoking reinstatement conditions.
Results: Overnight abstinence from smoking increased craving in both control and schizophrenia smokers (p'spp's < 0.05).
Conclusions: Establising varenicline's effects on intermediate markers of addiction and relapse (eg, craving and cognitive impairment) provides important information about its potential mechanism of action as a smoking cessation medication. This study provides the first systematic evaluation of varenicline in a human labortatory study of schizophrenia versus control smokers and suggests that its effects may differ dependent on psychiatric diagnosis. This has important implications for the development of $\alpha_4\beta_2$ nicotinic partial agonists as treatments for tobacco dependence and cognitive enhancement in schizophrenia.
Disclosure: V. Wing, **Part 4:** Pfizer IIR Operating Grant 2012–2014 ($50,000), Pfizer IIR 2010–2013 (medication supply only), Pfizer GRAND Operating Grant 2013–2015 ($20,000)

38.2 Extended Duration Pharmacotherapy with Varenicline Prevents Relapse to Smoking in Adult Smokers with Schizophrenia

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Background: Standard pharmacotherapies for smoking cessation are effective for smokers with schizophrenia, though relapse rates are high after treatment discontinuation. We sought to evaluate the effect of extended duration pharmacotherapy on relapse in smokers with schizophrenia spectrum disorders. We used varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist that binds at the same site on the receptor as nicotine and may decrease the rewarding properties of nicotine.
Methods: Stable, treated adult outpatients with schizophrenia or schizoaffective disorder who smoked $\geqslant 10$ cigarettes/day enrolled in a 12-week trial of varenicline plus weekly group cognitive behavioral therapy (CBT) for smoking cessation. Those who attained $\geqslant$ 14 days of continuous abstinence at week 12 enrolled in a relapse prevention intervention in which participants were randomly assigned to continue pharmacotherapy with varenicline or identical placebo added to a tapering schedule of CBT for 40-weeks.

Participants were then followed for 24 weeks after treatment discontinuation.
Results: Of 183 participants with schizophrenia entering open treatment, 78 (43%) attained abstinence and enrolled in the double-blind, relapse prevention phase. Those assigned to continued pharmacotherapy had relapse rates that were less than half that of those assigned to placebo at end of treatment at week 52, and 3, 12, and 24 weeks after treatment discontinuation in analyses that considered those who terminated the study early to have relapsed. This was a large, significant effect at each time point.There was a 69.1% reduced relapse rate with extended duration pharmacotherapy. Time to 50% relapse (conservative definition) was 4 wks on placebo *vs* 28 wks on varenicline.
Conclusions: Extended duration pharmacotherapy plus CBT significantly improved relapse rates over and above group CBT in smokers with schizophrenia spectrum disorders who attained initial abstinence with open treatment. This suggests that despite evidence for nicotinic receptor abnormalities in this population, extended duration pharmacotherapy may support sustained tobacco abstinence in this population.
Disclosure: A. Evins, **Part 1:** Pfizer: Supplemental research support for the NIDA funded trial: R01 DA021245 Extended Duration Varenicline for Prevention of Smoking in Schizophrenia, Envivo Pharmaceuticals: Supplemental research support for the NIDA funded R01 DA030992 Proof of Concept Trial of an Alpha-7 Nicotinic Agonist for Nicotine Dependence, GSK: Supplemental research support for NIDA funded U01 DA019378 Cooperative Drug Discovery Group for Nicotine Dependence, **Part 4:** Pfizer: Supplemental research support for the NIDA funded trial: R01 DA021245 Extended Duration Varenicline for Prevention of Smoking in Schizophrenia, Envivo Pharmaceuticals: Supplemental research support for the NIDA funded R01 DA030992 Proof of Concept Trial of an Alpha-7 Nicotinic Agonist for Nicotine Dependence, GSK: Supplemental research support for NIDA funded U01 DA019378 Cooperative Drug Discovery Group for Nicotine Dependence

38.3 *In Vivo* Evidence for β2*-nAChR Upregulation in Smokers as Compared to Nonsmokers with Schizophrenia

Irina Esterlis*

Yale University, West Haven, Connecticut

Background: Schizophrenia is associated with very high rates of tobacco smoking. The latter may be related to an attempt to 'self-medicate' symptoms and/or to alterations in function of high affinity nicotinic acetylcholine receptors (β_2^*-nAChRs).
Methods: Smoking and nonsmoking subjects with schizophrenia ($n=31$) and age-, smoking- and sex-matched comparison subjects ($n=31$) participated in one [^{123}I]5-IA-85380 single photon emission computed tomography (SPECT) scan to quantify β_2^*-nAChR availability. Psychiatric, cognitive, nicotine craving and mood assessments were obtained during active smoking as well as smoking abstinence.
Results: There were no differences in smoking characteristics between smokers with and without schizophrenia. Subjects with schizophrenia had lower β_2^*-nAChR availability relative to comparison group, and nonsmokers had lower β_2^*-nAChR availability relative to smokers. Relative to smokers without schizophrenia, smokers with schizophrenia had higher $\hat{a}_2^*$-nAChR availability in limited brain regions. In smokers with schizophrenia, higher $\hat{a}_2^*$-nAChR availability was associated with fewer negative symptoms of schizophrenia and better performance on tests of executive control. Chronic exposure to antipsychotic drugs was not associated with changes in β_2^*-nAChR availability in schizophrenia.
Conclusions: Although subjects with schizophrenia have lower β_2^*-nAChR availability as compared to comparison group, smokers with schizophrenia appear to upregulate in the cortical regions. Lower receptor availability in smokers with schizophrenia in the cortical regions is associated with a higher number of negative symptoms and worse performance on tests of executive function; suggesting smoking subjects with schizophrenia who upregulate to a lesser degree may be at risk for poorer outcomes. These data strongly support medications that target the cholinergic system for treatment of symtpoms of schizophrenia.
Disclosure: I. Esterlis, Nothing to Disclose.

38.4 Nicotinic CHRNA4 Exon 5 Genotype Predicts Clinical Outcome in Schizophrenia and Neuroleptic Drug Treatment-Response

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Charite Berlin, Germany

Background: The self-medication hypothesis of schizophrenia posits that patients smoke to improve cognitive performance and protein expression of nicotinic high-affinity alpha4beta2 receptors is diminished in schizophrenia. Based on experimental work, i.e., voltage clamp investigations of nicotinic channel properties in Xenopus Oocyte, electrophysiological and functional imaging studies in healthy probands, we have recently demonstrated that the common, synonymous CHRNA4 exon 5 variant rs1044396 is 1) functional (dose-dependent change of receptor sensitivity in response to acetylcholine) and 2) is associated with selective attention and (prefrontal) brain function. We now predicted that rs1044396 is associated with clinically relevant quantitative traits of schizophrenia (clinical outcome, drug treatment response).
Methods: 1) We investigated a mixed sample of $N=903$ clinically stable schizophrenia out-patients (Caucasians) using the Positive and Negative Syndrome Scale (PANSS) for clinical outcome prediction. Age at onset of illness was 24.1 (SD 8.7) years, the total number of inpatient admissions was 5.6 (SD 5.7). 2) For association analysis with neuroleptic drug treatment response over a treatment period of four weeks, we used two independent Caucasian samples of acutely ill schizophrenia patients (incl. one sample of $N=70$ first-episode patients) from two randomized, double-blind comparisons of risperidone and haloperidol.

Results: Using linear regression analyses, in clinically stable out-patients rs1044396 was significantly associated with clinical outcome: PANSS TOTAL: $P = 0.0038$, b = 2.913 (95%-CI = 0.942–4.883), PANSS GENERAL: $P = 0.0023$, b = 1.693 (95%-CI = 0.608–2.777), PANSS NEGATIVE: $P = 0.0188$, b = 0.837 (95%-CI = 0.139–1.535), PANSS POSITIVE: ns. rs1044396 was also significantly associated with drug treatment response in acutely ill schizophrernia patients. Repeated-measures ANOVA revealed a genotype-dependent treatment-associated improvement of PANSS TOTAL ($F(2,208) = 4.53$, $P = 0.01$) and the two subscales PANSS POSITIVE ($F(2,208) = 11.26$, $P < 0.0001$) and PANSS NEGATIVE ($F(2,208) = 3.26$, $P = 0.04$). There were no differences regarding PANSS symptom scores at admission between the genotypes ($P > 0.05$). Re-analyzing first-episode patients ($N = 70$) and the remaining $N = 142$ patients separately, significant genotype effects on treatment-associated PANSS score changes were still seen in both cohorts.
Conclusions: The CHRNA4 exon 5 variant rs1044396, which we have previously shown to affect nicotinic alpha4beta receptor sensitivity and prefrontal brain function, predicts clinical outcome and drug treatment response in three independent samples of schizophrenia patients. Our quantitative trait study further supports the notion of a potential clinical relevance of nicotinic neurtransmission via alpha4-beta2 receptors in schizophrenia.
Disclosure: G. Winterer, **Part 1:** PharmaImage—Biomarker Solutions GmbH (CEO), Janssen Pharmaceutica (consulting, services), Lundbeck (consulting, services), Boehringer Ingelheim (consulting), UCB Pharma (consulting, services), Pfizer (speaker bureau, grant), Focus Drug Development (consulting, services), Dritte Patent Portfolio Beteiligungsgesellschaft mbH & Co KG (consulting), Ratiopharm (consulting), **Part 2:** PharmaImage—Biomarker Solutions GmbH (CEO), Janssen Pharmaceutica (consulting, services), Lundbeck (consulting, services), UCB Pharma (consulting, services), Focus Drug Development (consulting, services), **Part 3:** PharmaImage—Biomarker Solutions GmbH (CEO), Janssen Pharmaceutica (consulting, services), Lundbeck (consulting, services), Focus Drug Development (consulting, services), **Part 4:** Pfizer/McNeill

Panel

39. Legal Damages: New Insights into Chronic Marijuana Effects on Human Brain Structure and Function

39.1 Effect of Long-term Cannabis use on Axonal Fiber Connectivity

Andrew Zalesky*

The University of Melbourne, Melbourne, Victoria, Australia

Background: Cannabis use typically begins during adolescence and early adulthood, a period when cannabinoid receptors are still abundant in white matter pathways across the brain. However, few studies to date have explored the impact of regular cannabis use on white matter structure, with no previous studies examining its impact on axonal connectivity. I will describe recent neuroimaging work conducted in Australia, where we aimed to examine axonal fibre pathways across the brain for evidence of microstructural alterations associated with long-term cannabis use and to test whether age of regular cannabis use was associated with severity of any microstructural change.
Methods: Diffusion-weighted magnetic resonance imaging (42 gradient directions) and brain connectivity mapping techniques were performed in 59 cannabis users with longstanding histories of heavy use and 33 matched controls. Whole-brain axonal fiber tracking (tractography) was performed in each study participant to trace out millions of streamlines following the trajectories of axonal fiber bundles, thereby reconstructing the connectome. The network-based statistic was used to identify between group differences in axonal white matter connectivity.
Results: Axonal connectivity was found to be impaired in cannabis users in the right fimbria of the hippocampus (fornix; 84% reduction in streamline count)
Conclusions: Our findings indicate long-term cannabis use is hazardous to the white matter of the developing brain. Delaying the age at which regular use begins may minimize the severity of microstructural impairment. Some of the white matter structures implicated, particularly the corpus callosum and commissural fibres, have been implicated by previous schizophrenia studies, providing preliminary evidence for a common origin in connectome pathology between cannabis abuse and schizophrenia.
Disclosure: A. Zalesky, Nothing to Disclose.

39.2 Impact of Chronic Marijuana Use on Reward and Control Brain Networks

Francesca M. Filbey*

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Background: Addiction models such as the opponent process theory and somatic marker hypothesis have suggested that drug-seeking behavior is due to a 'hypersensitive' reward system that is out of balance with a 'hyporesponsive' control system. These models imply a failure of the frontal control system to keep responses to salient rewards in check. While these systems have been well described, how communication or functional connectivity within and between these systems changes as a result of chronic marijuana use has yet to be determined.
Methods: Here, we describe three studies that examined functional connectivity in these networks as a result of chronic marijuana use. First, we examined functional connectivity within the reward-motivation network using an fMRI cue-elicited craving paradigm in 38 SCID-dependent (DEP) and 33 non-dependent (N-DEP) chronic marijuana users. Second, we looked at the inhibitory control network using an fMRI Stop-Signal task to determine inhibitory control network connectivity in 44 DEP and 30 N-DEP chronic marijuana users. Third, we looked at functional connectivity during resting state fMRI (fcMRI) in the salience (SN) and executive control networks (CEN), in addition to the default mode network (DMN), between chronic marijuana users ($N = 51$) and non-using controls ($N = 53$).

Results: Using psychophysiological interaction (PPI) analysis on BOLD response to marijuana cues (*vs* control cues), DEP had greater connectivity between the nucleus accumbens (NAc; seed region) and orbitofrontal cortex (OFC), anterior cingulate gyrus (ACG), and, insula relative to N-DEP (cluster-corrected $p<.05$, $z=2.3$). Of note, regional activations via general linear modeling did not differ between the groups suggesting that that degree of functional alteration within the reward-motivation network is associated with degree of severity of use. Similar to the results during cue-elicited craving, there was no significant difference in regional activation between DEP and N-DEP in any of the inhibitory control networks. However, the PPI analysis showed that during successful response inhibition, DEP had greater connectivity between right frontal control network (seed region) and substantia nigra/sub-thalamic nucleus (STN) network compared to N-DEP (SVC, FWE-corrected $p<.05$). Multiple regression analyses on the PPI maps showed modulatory effects of age of onset and quantity of marijuana use in N-DEP, but not DEP. Taken together, these findings suggest that functional connectivity between frontal control and substantia nigra/STN networks during response inhibition is sensitive to the effects of degree of severity unlike behavioral task performance and regional activation in inhibitory control networks. Further, modulators of this connectivity, such as onset and quantity of marijuana use, show attenuated effects with progression to dependence. Lastly, the fcMRI findings showed no significant difference among the nodes of DMN ($p=0.94$) and SN ($p=0.82$) connectivity between the marijuana users and the controls. However, the functional synchrony among CEN nodes was significantly lower in marijuana users compared to controls ($p=0.005$). Further, the CEN regions showed significantly negative association with the Impulsive Sensation Seeking Scale (ImpSS) suggesting that greater impulsivity was associated with decreased coupling of CEN regions.
Conclusions: Our findings suggest that chronic marijuana use impacts functional connectivity in reward and control networks. Moreover, alterations in connectivity are modulated by severity of marijuana use such that clinical dependence is associated with greater connectivity in both reward and control networks. To conclude, differences in connectivity may be used as a diagnostic metric that could inform therapeutic outcomes in cannabis use disorders.
Disclosure: F. Filbey, Nothing to Disclose.

39.3 Multimodal MR Imaging in Adolescent MJ Users

Deborah Yurgelun-Todd*

University of Utah, Salt Lake City, Utah

Background: Converging evidence from neurobiological, neuroimaging and behavioral studies in humans suggests that individuals with acute and chronic marijuana (MJ) exposure exhibit alterations in brain integrity and behavioral inhibition. However, there is limited information regarding the extent to which these brain-based effects represent acute or chronic brain changes, are associated with risk factors for marijuana use, or are the result of marijuana exposure. We examined the effects of MJ on the development of frontal lobe systems in MJ-smoking adolescents compared to healthy non-using adolescents by applying a complementary set of magnetic resonance imaging techniques to further characterize MJ-related brain changes.
Methods: Thirty-nine (39) adolescents with marijuana use (MJ aged 18.0 ± 2.2 years), and 38 healthy controls (HC aged 18.0 ± 0.9) completed an MR imaging protocol which included sMRI, rsFMRI, and task induced BOLD fMRI on a 3T MRI system. A subset of participants also completed proton MRS in order to measure GABA concentration in the anterior cingulate. Cortical reconstruction and volumetric segmentation was performed to obtain brain volumes utilizing the Freesurfer image analysis suite. Functional MRI data were analyzed using general linear model and the SPM5 software package in Matlab. GABA-edited MEGA-PRESS proton MRS methods were used to determine GABA concentration. Participants also completed diagnostic interviews and behavioral ratings.
Results: MJ using adolescents showed decreased orbitofrontal volume and decreased cortical thickness in bilateral insula and superior frontal coritces compared with HC. Activation measures during a finger-tapping task indicated that MJ users produced significantly less BOLD activation in the cingulate and cerebullum than HC. Furthermore, we found that age of onset of MJ use was significantly correlated with the structural measures of orbitofrontal volume and prefrontal cortical thickness, while the fMRI activation of the cingulate cortex appeared to be associated with chronic MJ exposure. We also found a 22% reduction in GABA within the cingulate of adolescent MJ users, which showed a moderate association with MJ exposure ($r=-0.43$, $p=0.10$) When MJ users were divided by total lifetime MJ smokes, MJ users with greater than 1250 lifetime smokes had smaller left rostral anterior cingulate cortex (ACC) volumes ($p=0.01$), and smaller right and left ($p=0.01$) posterior cingulate cortex (PCC) volumes. Heavy smokers showed positive correlations between left rostral ACC ($r=0.44$, $p=0.06$) and age of onset and a negative correlation between total lifetime MJ use and left PCC ($r=-0.47$, $p=0.04$).
Conclusions: The finding of a significant association between reduced frontal regional brain volumes and age of onset of MJ use is consistent with the hypothesis that development of brain regions may be related to the initiation of marijuana use or that early initiation may lead to reduced volumes. Frontal brain changes may be most pronounced in adolescents with heavy MJ use patterns. The negative correlations between lifetime MJ use and BOLD signal suggest that MJ use results in functional alterations of cingulo-cerebellar circuits in the developing brain. Reduced cingulate GABA concentration may reflect a neurotoxic effect of MJ use, as a trend toward a negative relationship was evident with lifetime MJ exposure. Overall these imaging results appear to support both neurodevelopmental factors and neurotoxic effects associated with heavy MJ use in adolescents.
Disclosure: D. Yurgelun-Todd, Nothing to Disclose.

39.4 Unmotivated? Signatures of Blunted Dopaminergic Responsiveness in Chronic Marijuana Abuse

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Background: Marijuana is the most frequently abused illicit substance yet it is not understood if its abuse is associated with similar changes in brain dopamine (DA) function as reported for other addictions (ie cocaine, alcohol). Notably, DA function has been linked to willingness to expend effort for rewards, and heavy marijuana users are anecdotally characterized by motivational deficits, where this may result from decreased dynamic dopamine functioning. Specifically, imaging studies of drug abusers have reported decreases in striatal DA D2 receptor (D2R) availability associated with reduced activity in fontal regions and reduced DA increases when challenged with a stimulant drug. Here we evaluate whether marijuana abusers show similar disruptions in dynamic DA responsiveness.
Methods: Forty participants (*20 marijuana abusers and 20 controls*) were scanned with positron emission tomography (PET) and [^{11}C]raclopride to assess D2 receptor availability before and after methylphenidate (0.5 mg/kg iv), which is a stimulant drug that increases DA by blocking DA transporters; and with [^{18}F]fluoro- deoxyglucose to assess brain glucose metabolism (marker of brain function) before and after methylphenidate (MP). In parallel we measured MP's behavioral and cardiovascular effects.
Results: Baseline striatal D2 receptor availability did not differ between controls and marijuana abusers, and MP significantly increased DA (measured as reduction's in [^{11}C]raclopride specific binding) to the same extent in both groups. In contrast, MP effects in regional brain glucose metabolism were significantly attenuated in marijuana abusers when compared to controls. Similarly MP's behavioral and cardiovascular effects were markedly attenuated in the marijuana abusers.
Conclusions: Though marijuana abusers showed normal tonic striatal D2R availability and normal DA increases with MP, their regional brain metabolic and behavioral responses to the DA increases were markedly attenuated. These suggest that marijuana abuse may be associated with a dampened neuronal reactivity to DA stimulation. Future research can relate individual differences in blunted dynamic DA responsiveness to measures of motivation and life function.
Disclosure: N. Volkow, Nothing to Disclose.

Panel

40. Manipulating BDNF-TrkB Signaling in Brain Disorders: Complex Regulation and Cellular & Systems Level Interactions as Novel Substrates for Translational Medicine

40.1 Convergence of BDNF and Glucocorticoid Receptor Signaling

Moses V. Chao*

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Background: The actions of glucocorticoids and neurotrophins, such as BDNF, have been implicated in numerous psychiatric disorders. However, the mechanisms of how glucocorticoids and BDNF influence maladaptive actions are not well understood. We have previously shown that genetic disruption of glucocorticoid signaling in the hypothalamus resulted in disinhibition of the HPA axis, upregulation of hypothalamic levels of BDNF and increased CRH expression. Our present studies show there is a close relationship between BDNF signaling and the actions of the glucocorticoid receptor (GR), a ligand-activated transcription factor through post-transcriptional modifications by phosphorylation.
Methods: Mass spectrometry analysis of the glucocorticoid receptor isolated from cortical neurons treated with BDNF revealed new phosphorylation sites. To test the significance of these events, we have examined the impact of BDNF signaling on glucocorticoid function using gene expression microarray and real time quantitative PCR in primary rat cortical neurons stimulated with the selective GR agonist dexamethasone (Dex) and BDNF, alone or in combination.
Results: We found that BDNF treatment induces the phosphorylation of the glucocorticoid receptor (GR) at serine 155 (S155) and serine 287 (S287). Expression of a non-phosphorylatable alanine double mutant (S155A/S287A) impaired the induction of a subset of BDNF and Dex regulated genes. Moreover, BDNF-induced GR phosphorylation increased GR occupancy and cofactor recruitment at the promoters of selective genes. Therefore, BDNF signaling acts to specify and amplify GR-mediated transcription by a phosphorylation-dependent mechanism.
Conclusions: The interactions between BDNF and glucocorticoids include specific phosphorylation of GR by BDNF. We have identified several new serine phosphorylation sites in GR, which result in an amplification of transcriptional responses by BDNF signaling.
Disclosure: M. Chao, Nothing to Disclose.

40.2 Differential Contribution of Individual BDNF Splice Variants to Brain and Behavioral Functions

Keri Martinowich*

Lieber Institute for Brain Development, Baltimore, Maryland

Background: Brain-derived neurotrophic factor (BDNF) is implicated in diverse brain functions including the survival, maturation and differentiation of numerous neuronal cell types as well as regulation of synaptic plasticity, synaptogenesis and dendritic morphology. BDNF is regulated at multiple levels, which likely contribute to its ability to exert such a wide range of functions. Production of multiple transcripts represents an additional level of regulation: the rodent *Bdnf* gene produces more than 20 different transcripts, which are composed of a 5′ untranslated region (UTR) exon spliced to a common coding exon, which uses either of two alternative 3′ UTRs. Upstream of each 5′UTR are unique promoters, which are not only sensitive to different signaling cascades, but also exhibit divergent transcription kinetics. Previous work has investigated activation of individual promoters, showing that which *Bdnf* transcripts are produced in response to a given stimulus depends on the nature of the stimulus and the cell signaling pathways that are activated. Additional studies

have documented expression patterns of individual *Bdnf* transcripts in different brain regions and across neurodevelopment. Collectively, the transcripts encoding the 5′ exons I, II, IV and VI represent ~95% of the total *Bdnf* mRNA produced in both the rodent and human brain. While it is clear that existence of unique promoters allows for precise temporal and stimulus-specific regulation of BDNF production, the functional consequences of multiple transcripts encoding the same protein is not well understood.
Methods: In order to assess the *in vivo* consequence of selective loss of *Bdnf* variants we recently developed and validated four transgenic mouse lines in which transcription from *BDNF* promoters I, II, IV and VI, (BDNF-e1, BDNF-e2, BDNF-e4 and BDNF-e6, respectively) is selectively disrupted. To engineer these lines, we inserted an enhanced green fluorescence (eGFP)-STOP cassette directly upstream of the splice donor site at the end of the respective 5′UTR. In order to determine whether individual splice variants differentially contribute to functions attributed to BDNF-TrkB signaling, we used a combination of cellular and molecular biology, neurophysiology and animal behavior to assess selected brain and behavioral functions attributed to BDNF-TrkB signaling in these animals.
Results: We elucidated differential phenotypes between knock-out lines on several brain and behavioral functions that have previously been attributed to BDNF-TrkB signaling. In particular, we found significantly differential contribution of individual exons to phenotypes related to energy regulation and obesity, extinction learning and development of the excitatory/inhibitory balance.
Conclusions: Loss of BDNF production from individual splice variants differentially contributes to various brain and behavioral functions that are mediated by BDNF-TrkB signaling.
Disclosure: K. Martinowich, Nothing to Disclose.

40.3 Role of Slitrk5 in Regulating BDNF Dependent Signaling

Francis Lee*

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Background: Recent clinical strategies have focused on using growth factors as potential therapeutic agents for neuropsychiatric disorders. In particular, brain-derived neurotrophic factor (BDNF) has been posited to be a candidate due to its roles in neuronal survival and plasticity. In the CNS, these functional effects are mediated through TrkB receptor tyrosine kinases. It has been less well–established whether BDNF-dependent TrkB signaling in the CNS relies on additional modulatory proteins in order to elicit optimal responses in brain circuits. In the peripheral nervous system (PNS), a well-established co-receptor for Trk receptor modulation is $p75^{NTR}$, a member of the tumor necrosis factor (TNF) receptor family. However, $p75^{NTR}$ receptor expression is restricted in the adult CNS. Identification of additional modulatory components of the TrkB receptor complex in the CNS would provide compelling insights not only into the basic regulatory mechanisms of BDNF-dependent signaling, but also inform efforts to optimize these signaling pathways for therapeutic benefit, for example, by providing novel targets for drug development. Our preliminary studies indicate that a specific Slitrk—Slitrk5, binds to TrkB and sorts TrkB receptors in neurons preferentially to the endocytic recycling pathway. With these new results, we postulate that a nopvel cell surface neuronal protein, Slitrk5, is a modulatory component of the TrkB receptor complex and mediates TrkB signaling and trafficking and subsequent biological responses.
Methods: We performed analyses of BDNF dependent TrkB signaling and trafficking in cultured neurons obtained from Slitrk5 knock-out mice.
Results: We have deteromined that Slitrk5 interacts with with TrkB receptors through the extracellular TrkB leucine rich repeat (LRR) domain and modifies subsequent BDNF-dependent signaling, and identifies a new type of interaction with TrkB via its extracellular domain, which has not been shown previously, and provide a potential long-sought role for its LRR domain. We have also found that this protein-protein interaction is important for subsequent endocytic trafficking and signaling from this TrkB receptor complex.
Conclusions: While extensive information regarding neurotrophin-dependent signaling has been established, less is known of how signaling diversity is generated for the predominant CNS neurotrophin, BDNF. Our preliminary studies provide compelling evidence that a neuron-specific cell surface protein, Slitrk5, is capable of regulating BDNF generated signaling cascades. The ability to regulate BDNF-dependent survival and plasticity related signaling suggests Slitrk5 may be a valid target for drug development for enhancing region specific trophic responses.
Disclosure: F. Lee, Nothing to Disclose.

40.4 Synaptic Repair: Translating BDNF Biology into New Medicines for Psychiatric Diseases

Bai Lu*

GlaxoSmithKline, R&D China, Shanghai, China

Background: Despite significant progress in identifying novel targets for psychiatric diseases, these efforts have not been translated into superior treatments over existing therapies. Increasing evidence suggests that synapse and circuit dysfunctions underlying the pathophysiology of mental illnesses. Studies of BDNF, the best known 'synaptogenic' molecule proven in human, may pave the way for a paradigm shift in treating psychiatric disorders.
Methods: Emerging evidence on BDNF regulation of memory and emotion, the impact of BDNF genotype on psychiatric endophenotypes, and the progress in tools to measure synaptic dysfunction in humans all suggest that time is ripe to target synaptic repair by the BDNF pathway in the clinic.
Results: In this talk, I will highlight evidence for BDNF regulation of synaptic plasticity, its role in cognitive functions such as memory and extinction. In particular, I will talk about 1) Activity-dependent BDNF transcript enhances GABAergic transmission in the prefrontal cortex (PFC), controlling fear memory extinction and behavioral flexibility; 2) proBDNF- $p75^{NTR}$ signaling controls anxiety and stress-coping behaviors, by regulating stress-induced long-term

depression (LTD) in the hippocampus. I will then discuss efforts in translating BDNF biology into medicines, with emphasis on how to measure synaptic changes in human *in vivo*.
Conclusions: Through experimental medicine in humans, we hope that a paradigm-shifting 'synaptic repair' strategy will bring innovative medicines for the treatment of psychiatric diseases.
Disclosure: B. Lu, Nothing to Disclose.

Panel

41. Multidimensional Data Integration and Causality: A Systems Approach for Unraveling the Molecular Architecture of Mental Disorders

41.1 Cis and Trans Data Integration to Find Mechanisms causing Psychiatric Disorders

Edwin van den Oord*

Virginia Commonwealth University, Richmond, Virginia

Background: During the past decade, the data available to study the genetic basis of psychiatric conditions have grown exponentially. Typical examples include gene expression data, meta-analyses of linkage scans, published candidate gene studies, disease-specific biochemical pathways, and genome-wide association studies. Integrating all these existing data has huge potential to increase the likelihood of identifying genes that affect susceptibility to psychiatric conditions and better understand the underlying disease mechanisms.
Methods: Traditional data analysis techniques cannot be used to integrate the results from all these studies as they require that data are obtained from the same subjects. Investigators therefore often resort to looking for simple concordance between results from different studies for the same target ('cis'). Concordance can be compelling when found. However, such an approach does not take full advantage of the data and cannot shed light on the underlying disease mechanisms as it does not consider biological relations between genes ('trans'). We propose a novel data integration strategy that allows data integration in statistically optimal fashion. As our method can perform independent tests of biological relationships across all available datasets, disease mechanisms can be studied in very large samples.
Results: To illustrate our approach we present results of a proof of concept study where we integrated a variety of data sources such as gene expression data, candidate genes from the literature, and linkage data, into a meta-analysis of genomewide association studies of schizophrenia. Through a specifically designed experiment where we replicate 6,544 genetic markers in 6,298 other subjects, we show that our method identifies effects that may otherwise require sample sizes that are 2.5 times larger or replication studies with up to 10 times as many markers. We replicated SNPs in TCF4 ($P = 2.5 \times 10–10$) and NOTCH4 ($P = 3.2 \times 10–7$) that are among the most robust SCZ findings. More novel findings included POM121L2 ($P = 3.5 \times 10–7$), AS3MT ($P = 9.0 \times 10–7$), CNNM2 ($P = 6.0 \times 10–7$), and NT5C2 ($P = 4.1 \times 10–7$). The most significant pathways involved neuronal function (axonal guidance, neuronal systems, L1CAM interaction) and the immune system (antigen processing, cell adhesion molecules relevant to T cells, translocation to immunological synapse).
Conclusions: Data integration provides a powerful approach to enhance the study of the genetic basis of psychiatric conditions.
Disclosure: E. van den Oord, Nothing to Disclose.

41.2 Computational Analysis of Complex Human Disorders

Andrey Rzhetsky*

University of Chicago, Chicago, Illinois

Background: Focusing on autism, bipolar disorder and schizophrenia, my talk will touch a set of computational approahes and questions.
Methods: The questions touched in my talk will include: How understanding of genetics and epidemiology of disease can be advanced through modeling and computational analysis of very large and heterogeneous datasets? What are the bottlenecks in analysis of complex human maladies? How can we model and compute over multiple data types to narrow hypotheses about genetic causes of disease? How collaborations across multiple fields of science can bring translational results to initially purely academic studies?
Results: I will outline how large-scale clinical record data and legacy GWAS and linkage results can be used for comparison of competing genetic models for complex phenotypes, with examples in neuropsychiatric disorders.I will provide examples of specific predictions of putative new genetic associations for a list of phenotypes.
Conclusions: A large set of in silico predictions can be generated from joint analysis of large lgacy datasets.
Disclosure: A. Rzhetsky, Nothing to Disclose.

41.3 Elucidating the Complexity of Psychiatric Disorders via the Integration of High-dimensional, Multiscale Data

Eric E. Schadt*

Icahn School of Medicine at Mount Sinai, New York, New York

Background: The causal chain of events that lead to the development of psychiatric disorders such as schizophrenia remains elusive. Such psychiatric disorders are complex, resulting from the interplay of potentially hundreds (or thousands) of genetic loci and environmental factors. Genetic and environmental perturbations induce changes in the molecular interactions of cellular pathways whose collective effect may become clear through the organized structure of multiscale biological networks.
Methods: We have developed a novel systems approach to study psychiatric disorders such as schizophrenia that models the global molecular, functional, and structural changes in the affected brain that in turn can lead us to the root causes of the disease. To characterize the molecular, cellular, and physiological systems associated with psychiatric disorders, I will discuss the analysis on schizophrenia

and addiction-related phenotypes using novel human data generated across multiple data modalities, including DNA variation, RNA sequencing, functional and structural imaging, and high-content clinical characterizations. By integrating these diverse data collected across multiple cohorts comprised of many hundreds of individuals, I will describe the construction of gene regulatory networks, functional and structural MRI based networks, high-content phenotypic networks, in addition to the integration of these network models. Because DNA variation was systematically assessed across all cohorts, it provides a common set of perturbations that can be leveraged to not only infer causal relationships among different molecular and higher order traits, but that can help link networks at different scales (eg, molecular and imaging) across cohorts.
Results: Through this integrative network-based approach, I will present a rank-ordering of the resulting network structures for relevance to different psychiatric disorders, highlighting both known and novel biological pathways involved in disease pathogenesis and progression. I will further demonstrate that the causal network structures we construct from this big data integration exercise is a useful predictor of response to gene perturbations and presents a novel framework to test models of disease mechanisms underlying psychiatric disorders. I will furtherdemonstrate that our approach can offer novel insights for drug discovery programs aimed at treating psychiatric disorders by screening our disease-associated networks against molecular signatures induced by marketed and novel compounds across a number of cell-bases systems, including those derived from stem cells isolated from patients with psychiatric disorders.
Conclusions: We cannot hope to understand complex human diseases such as schizophrenia by focusing on single dimensions such as DNA, RNA, or imaging data, even if those single dimensions of data are generated on many thousands of individuals. Instead, we must integrate the diversity of data that inform on different disease conditions using advanced mathematical algorithms to construct predictive models of disease as I will demonstrate here for schizophrenia. By enabling experimentalists to leverage and iterate on these models, we can experimentally validate, invalidate or refine them to deliver ever more accurate representations of our understanding of disease.
Disclosure: E. Schadt, **Part 1:** SAB for Pacific Biosciences and SAB for Berg, **Part 2:** SAB for Pacific Biosciences and SAB for Berg.

41.4 The ENIGMA Consortium: Meta-analyzing Neuroimaging and Genetic Data from 125 Institutions

Paul Thompson*

UCLA School of Medicine, Los Angeles, California

Background: We report recent discoveries made by the ENIGMA Consortium (http://enigma.loni.ucla.edu), which meta-analyzes neuroimaging and genetic data worldwide, discovering how disease risk genes affect the brain. The recent flood of discoveries in psychiatric genetics makes it imperative to understand how disease risk genes disrupt or re-wire the brain, and why, and which kinds of abnormalities can be prevented.
Methods: The ENIGMA project unites 125 institutions worldwide with genome-wide scans, brain MRI and DTI ($N = 21{,}151$ participants) to (1) discover how disease risk genes affect the brain, (2) find unknown determinants of brain integrity and factors that promote or disrupt brain function and brain networks.
Results: The ENIGMA Project has now found and replicated genomic regions associated with regional brain structure (Stein Nat Gen 2012; Hibar *et al*., in preparation); we also present new data revealing implicating these same genomic regions in psychosis and mental retardation, and joint work with the Psychiatric Genomics Consortium to follow up genetic discoveries rapidly and in new ways. Genome-wide connectome-wide scans have now discovered genetic variants that affect brain organization, and some are promising targets to treat neurodegeneration (Jahanshad PNAS 2013).
Conclusions: We also report updates from the ENIGMA schizophrenia and bipolar working groups, that have uncovered unsuspected patterns in disease profiles, by integrating neuroimaging and genetics on a worldwide scale.
Disclosure: P. Thompson, Nothing to Disclose.

Panel

42. Neurobiological Regulation of Palatable Food Binging and Seeking

42.1 Cholinergic Control of Food Intake: Mechanisms Highjacked by Nicotine

Marina Picciotto*

Yale University School of Medicine, New Haven, Connecticut

Background: Tobacco smoking in humans and nicotine administration in animals decreases appetite and body weight. Many individuals report that they are reluctant to quit smoking because they are afraid to gain weight, and subsets of smokers, including teenage girls, report that they initiate smoking to control their weight. Nicotine, the primary psychoactive substance in tobacco, stimulates nicotinic acetylcholine receptors (nAChRs) on pro-opiomelanocortin (POMC) expressing neurons in the arcuate nucleus of the hypothalamus (ARC), and this is required for the ability of nicotine to decrease food intake in mice. Acetylcholine is the endogenous neurotransmitter that activated nAChRs, but its effects on food intake are not well understood.
Methods: We identified the nicotinic acetylcholine receptor subunits expressed in ARC using semi-quantitative PCR. We have also used viral-mediated delivery of fluorescent tracers and anatomical techniques to identify the source of cholinergic input to the ARC. Finally, we are using optogenetic methods to identify the functional consequences of regulating acetylcholine signaling in the ARC on food intake.
Results: We have already identified beta 4 subunit containing nAChRs on POMC neurons in the ARC, and that stimulation of beta 4 nAChRs can decrease food intake acutely, and decrease body weight chronically; however, the partner subunits for beta 4 have not yet been identified. RT-

PCR has identified a large number of subunits, including the alpha 3, alpha 4, alpha 6, alpha 7, beta 2 and beta 4 subunits, that are expressed in the ARC. Neither the alpha 2 nor the alpha 5 subunit can be detected in ARC homogenates. *In situ* hybridization studies will help identify the subunits expressed specifically in POMC neurons. Immunocytochemistry for choline acetyltransferase (ChAT, a marker of cholinergic neurons), examination of GFP expression in ChAT-GFP mice and visulalization of channelrhodopsin in ChAT-Cre mice have all shown that there are no cholinergic cell bodies in the ARC in C57BL6/J mice. This is contrary to one published study suggesting that there are ChAT-positive neurons in the rat ARC. Instead, it appears that brain stem cholinergic nuclei project to the ARC. We are currently using transgenic mice expressing channel rhodopsin in cholinergic neurons or viral delivery of channel rhodopsin to subsets of cholinergic neurons in ChAT-Cre mice to determine the role of acetylcholine in the ARC on food intake.

Conclusions: Molecular genetic studies have shown that nAChRs containing the beta 4 subunit are essential for the ability of nicotine to decrease food intake in mice. Further, nAChRs on POMC neurons in the ARC, and stimulation of MC4 receptors in the paraventricular nucleus are essential for these appetite-suppressing effects. Despite these pharmacological effects of nicotine, the role of acetylcholine signaling in the ARC on food intake is not known. These studies show that a large number of nAChR subunits are available to transduce the effects of acetylcholine in ARC, and that local neurons within the ARC are not responsible for cholinergic innervation of these nAChRs. The ability to stimulate cholinergic terminals in ARC selectively using optogenetic techniques will be essential to understanding how acetylcholine regulates this circuit, and will help identify the physiological role for this neurotransmitter in regulation of appetite.

Disclosure: M. Picciotto, Nothing to Disclose.

42.2 Extended Amygdala-hypothalamic Inhibitory Circuits Regulate Feeding

Garret D. Stuber*

University of North Carolina, Chapel Hill, North Carolina

Background: Binge eating is one of the most prevalent types of eating disorders. Therefore, experiments aimed to determine the causal relationship between discrete neural circuit elements and maladaptive feeding behaviors are clearly warranted. The bed nucleus of the stria terminalis (BNST), a component of the extended amygdala, is a key integrator of diverse motivational states through its interactions and connectivity with various synaptic targets in anatomically distinct brain regions. We previously demonstrated that BNST-GABAergic neuron projections targeting the ventral tegmental area modulate reward- and anxiety-related behaviors. However, the function of the BNST is not limited to these discrete motivational processes, as it may also play a vital role in other motivated states such as feeding behavior, via inhibitory projections to the lateral hypothalamus (LH). Gross neuroanatomical manipulations have demonstrated that electrical stimulation of the LH elicits robust feeding responses, while direct infusions of GABA and glutamate agonists suppress and promote feeding respectively. While these studies demonstrated that the LH is an important neuroanatomical substrate for controlling feeding, the precise connectivity and function of genetically distinct LH neurons has not been systematically dissected. In addition, the specific presynaptic inputs to functionally distinct LH neurons and their role in engaging motivated feeding behaviors are completely unknown.

Methods: Here, we used excitatory and inhibitory optogenetic methods to selectively activate and suppress BNST-GABAergic fibers that innervate the LH in order to assess the behavioral sufficiency and necessity of this pathway for regulating feeding in vgat-ires-cre mice. In addition, we performed functional neurocircuit mapping experiments to determine the functional connectivity between BNST GABAergic inputs and genetically defined postsynaptic neurons in the LH.

Results: Photostimulation of BNST-GABAergic terminals within the LH produced robust feeding behavior in satiated mice, while photoinhibition of this projection suppressed feeding in hungry mice. In addition, direct optogenetic activation of postsynaptic GABAergic LH neurons also produced robust feeding, while stimulation of the glutamatergic neurons in the LH suppressed feeding in hungry mice and was associated with aversive-like behavioral phenotypes. Ongoing experiments detailing the precise synaptic connectivity between BNST GABAergic inputs and postsynaptic LH neurons will also be presented.

Conclusions: These data suggest that inhibitory BNST-GABAergic projections likely attenuate the activity of distinct LH neurons to facilitate feeding and may help to explain how disruption of specific circuit interactions can lead to the misrepresentation of hunger and thus promote excessive caloric intake.

Disclosure: G. Stuber, Nothing to Disclose.

42.3 Neural Correlates of Craving, Cognitive Control and Reward Processing in Obesity and Binge-eating Disorder

Marc N. Potenza*

Yale University School of Medicine, New Haven, Connecticut

Background: There currently exists debate regarding the extent to which obesity might be considered within an addiction framework. To best address the obesity epidemic, an improved understanding is needed of the relationships between metabolic measures implicated in obesity (eg, insulin resistance) and constructs linked to addictions (eg, food craving). Further, binge-eating disorder (BED), an eating disorder with greater prevalence than anorexia or bulimia nervosa, has been hypothesized to be particularly closely linked to addictions, yet few brain imaging studies have examined similarities and differences between obese people with and without BED.

Methods: Functional magnetic resonance imaging (fMRI) was used to study lean and obese people in a guided-

imagery task involving three different cues (favorite-food, stress and neutral-relaxing). Laboratory measures of fasting plasma glucose and insulin were obtained to calculate a homeostatic measure of insulin resistance (HOMA-IR) to relate to subjective and fMRI responses. Additional fMRI measures (Stroop Color-Word Interference Task and Monetary Incentive Delay Task (MIDT)) were obtained in obese people with and without BED and in lean people.
Results: Obese but not lean people showed increased activation of striatal, insular and hypothalamic brain regions in response to favorite-food and stress cues. In obese but not lean people, HOMA-IR measures correlated with corticolimbic striatal brain activations during the favorite-food and stress conditions. HOMA-IR measures correlated with favorite-food-cue-induced food craving in obese but not lean people, and thalamic activations mediated this relationship. When examining subjects of similar body mass indices (BMIs), tobacco smokers as compared to non-smokers showed relatively diminished corticolimbic striatal brain activations to favorite food cues. Obese people with BED (as compared to obese people of similar BMIs without BED and lean comparison subjects) showed relatively diminished activation of the ventromedial prefrontal cortex (vmPFC), inferior frontal gyrus (IFG) and insula during Stroop performance, with vmPFC and IFG activation in the BED group correlating inversely with dietary restraint scores. During MIDT performance, obese people with BED as compared to obese people without BED showed relatively diminished activation of the ventral striatum during reward anticipation and vmPFC and insula during reward outcome, similar to findings in alcohol dependence and pathological gambling. Amongst the group with BED, relatively diminished activation of the ventral striatum during reward anticipation and mPFC during reward outcome was associated with poorer treatment outcome.
Conclusions: Findings indicate similarities between BED and addictions and suggest that hypoactivation of corticolimbic striatal regions during cognitive control and reward processing may link to poorer impulse control, representing possible treatment targets in BED. The relationships between metabolic measures, brain activations and food craving in obese but not lean people suggest a particularly strong link between metabolic measures and food-related motivations in obesity, suggesting novel targets for medication development. The tobacco-related findings suggest a mechanism for tobacco's influence on appetite and strategies for managing food consumption during smoking cessation.
Disclosure: M. Potenza, **Part 1:** Consulting to Boehringer-Ingelheim and Lundbeck; financial interests in Somaxon. Grant from Psyadon, **Part 4:** Grant from Psyadon.

42.4 Serotonin Control in the Proclivity for High Impulsive Action and Binge Eating

Kathryn Cunningham*

University of Texas Medical Branch, Galveston, Texas

Background: Binge eating disorder (BED) is the most prevalent eating disorder in the U.S. and is marked by recurrent, brief episodes (eg, two hours) of overeating [esp., highly palatable sugar/fat ('sweet-fat') food] and a loss of control over food intake. Impulsivity, which is defined as a predisposition toward rapid unplanned reactions to stimuli without regard to negative consequences, is a factor in the multifaceted determinants that underlie the etiology of BED and its pathogenesis. Our *central hypothesis* is that imbalanced serotonin (5-HT) signaling through the 5-HT_{2A} receptor (5-HT_{2A}R) and 5-HT_{2C}R predicts inherent impulsivity which enhances the extent of binge eating on sweet-fat food.
Methods: Two assays were established to explore this hypothesis: (1) rats were identified as high (HI) or low impulsive action (LI) phenotypes in the 1-choice serial reaction time (1-CSRT) task in which performance was reinforced with sweet-fat pellets (17% kcal by sucrose/45% kcal fat), and (2) the propensity for binge eating was assessed upon 2-hr access to sweet-fat chow. Pharmacological and genetic manipulations were employed to evaluate the role of 5-HT function in these behaviors.
Results: The upper and lower 25% of rats, respectively, were identified as high impulsive (HI) or low impulsive (LI) phenotypes based on premature responses in the 1-CSRT task. Levels of impulsive action positively correlated with the ratio of the 5-HT_{2A}R to 5-HT_{2C}R protein in the medial prefrontal cortex, *suggesting a potentially-causal role for a 5-HT2AR:5-HT2CR imbalance in this phenotype*. HI rats consumed significantly more kcal during the 2-hr binge *vs* LI rats, *experimentally linking the propensity for impulsive action to the magnitude of binge eating*. The combination of a subthreshold dose of a selective 5-HT_{2A}R antagonist *plus* a selective 5-HT_{2C}R agonist synergistically transformed HI rats to LI rats, *suggesting that a perturbed 5-HT2AR:5-HT2CR balance may contribute to these behaviors.*
Conclusions: These data support the hypothesis that an imbalance in 5-HT_{2A}R:5-HT_{2C}R homeostasis may be an antecedent to high trait impulsivity which then promotes binge eating behavior. This innovative contribution is the first step in a continuum of research that could lead to the targeted development of pharmacological strategies to restore serotonergic homeostasis and minimize deleterious behaviors that promote BED.
Disclosure: K. Cunningham, Nothing to Disclose.

Panel

43. New Directions for Optogenetics: Investigating Plasticity Mechanisms Underlying Psychiatric Disorders

43.1 Brief Repeated Cortico-striatal Stimulation Leads to Persistent OCD-Relevant Behaviors

Susanne E. Ahmari*

Columbia University, New York, New York

Background: Obsessive Compulsive Disorder (OCD) is a chronic, severe mental illness that affects 2–3% of people worldwide, yet the pathophysiology remains unclear. Though multiple lines of evidence indicate that dysregulation within cortico-striato-thalamo-cortical (CSTC) circuits is correlated with OCD, causation cannot be tested in humans.
Methods: We used optogenetic technology in mice to simulate CSTC hyperactivation observed in OCD patients.

Mice were injected with AAV-channelrhodopsin-EYFP in orbitofrontal cortex (OFC), and implanted with fiberoptics in ventromedial striatum (VMS). 473 nm 10 Hz stimulation of OFC-VMS projections yielded light-evoked field responses. 5 minutes of stimulation was performed daily for 5 consecutive days; behavioral measures included grooming and open field. Data was analyzed using repeated-measures ANOVAs and post-hoc tests. *In vivo* electrophysiology was performed to examine electrophysiologic correlates of behavioral responses.
Results: Repeated hyperactivation of OFC-VMS projections over 5 days induced repetitive grooming, a mouse behavior linked to OCD ($p < 0.05$). Increased grooming persisted for 2 weeks after cessation of stimulation ($p < 0.03$), and was reversed by chronic fluoxetine. Development of persistent grooming was correlated with increased evoked activity at OFC-VMS synapses ($p < 0.02$), which was also reversed by chronic fluoxetine. No differences were observed in anxiety or prepulse inhibition.
Conclusions: This is the first evidence that repeated hyperactivation of cortico-striatal projections directly generates OCD-like behaviors. Furthermore, repetitive grooming, once established, persists without further direct circuit hyperactivation, but is resolved using a treatment regimen effective in reducing OCD symptoms. Finally, plasticity at OFC-VMS synapses correlates with the observed behavioral change. This approach may provide a general template for modeling plasticity-based disease states using optogenetics.
Disclosure: S. Ahmari, Nothing to Disclose.

43.2 Cortical Control of Brainstem Neuromodulatory Systems in Motivated Behavior

Melissa R. Warden*

Cornell University, Ithaca, New York

Background: Major depression is common, yet is poorly understood, and current treatments are often inadequate. Evidence suggests an important role for the prefrontal cortex (PFC). Of particular interest are PFC projections to dorsal raphe nucleus (DRN), the major source of serotonin to the forebrain, and lateral habenula (LHb), a structure recently implicated in negative reward processing and a novel target for deep brain stimulation in depression. We describe a combined projection-specific optogenetic and electrophysiological approach to investigating these circuits in behaving rats, and report on both acute and long-lasting changes in depression-related behaviors and circuit properties.
Methods: We employ both high-speed neurophysiological readout and optical control to probe circuit properties of the depressed brain. High-speed readout is carried out with *in vivo* tetrode recordings. High-speed control is implemented by optogenetics, with single-component microbial opsins delivered by viral vectors to targeted circuit elements.
Results: When DRN-projecting mPFC axons were illuminated, rats showed an acute antidepressant-like phenotype in the forced swim test (FST) ($p < 0.01$, Wilcoxon signed-rank). However, inhibition of this projection with eNpHR3.0 instead induced a long-lasting decrease in FST mobility ($p < 0.01$ Mann-Whitney U). Stimulation of the mPFC-LHb projection acutely decreased FST mobility ($p < 0.01$, Wilcoxon signed-rank). Single mPFC neurons encoded depression-related behavioral changes during the FST.
Conclusions: Fast optical and neurophysiological methods were used to probe aspects of circuit function in both acute and long-lasting depression-related behavioral states. We find altered circuit properties in depression-related states, including altered neural activity in the medial PFC (mPFC), and present evidence suggesting causal significance of these neurons in acute and long-lasting depression-related behaviors.
Disclosure: M. Warden, **Part 1:** Stanford University has filed for patent protection on technology invented by Dr. Melissa R. Warden and Dr. Karl Deisseroth.

43.3 Different Patterns of Stimulation in Projections from VTA to PFC Exert Distinct Effects on Behavioral Flexibility

Vikaas S. Sohal*

University of California, San Francisco, California

Background: Detailed pharmacology studies have revealed that level of dopamine in the prefrontal cortex (PFC) is critical for working memory and that activating or blocking specific PFC dopamine receptors can modulate memory retention as well as behavioral flexibility. An important open problem is to connect these pharmacology results with the known firing patterns of midbrain dopamine neurons.
Methods: TH-cre mice expressing either cre-dependent ChR2 or eYFP in the ventral tegmental area and substantia nigra were trained in an association/set-shifting task. Mice were assayed on the extent to which stimulating prefrontal TH-positive fibers affected their ability to maintain a reward association, as well as to perform extradimensional and intradimensional attentional shifts.
Results: Mice were stimulated with one of three stimulation patterns: control (no light), tonic (regular repeated flashes) or phasic (bursts of light flashes). During extradimensional and intradimensional shifts, tonically stimulated mice expressing ChR2 showed profound perseveration, selecting the previous cue in 87% of trials. This perseveration persisted even in the absence of the reward. Phasically stimulated mice showed the opposite behavior; they lost any previous association and were unable to form new associations above chance levels.
Conclusions: Our results suggest that DA fibers from the VTA act as a potential gatekeeper in the PFC, either locking in the mouse's current strategy or releasing it to allow for behavioral flexibility.
Disclosure: V. Sohal, Nothing to Disclose.

43.4 Molecular and Circuit Basis of Impaired Hippocampal-prefrontal Synchrony in a Mouse Model of Schizophrenia Predisposition

Joshua A. Gordon*

Columbia University, New York, New York

Background: The 22q11 microdeletion is a copy number variation that confers a significant risk of developing schizophrenia. *Df(16)A*$^{+/-}$ mice, engineered to lack the

chromosomal locus syntenic to the 22q11 deletion, evince a number of schizophrenia-related phenotypes, including deficits in spatial working memory (SWM). We have previously linked SWM deficits in *Df(16)A*$^{+/-}$ mice to deficits in long-range functional connectivity between the medial prefrontal cortex (mPFC) and the hippocampus (HPC) (Sigurdsson *et al*., *Nature*, 2010); however, the molecular and circuit level mechanisms underlying these deficits are unclear. Mice with a haploinsufficiency of *Dgcr8* (*Dgcr8*$^{+/-}$), a gene within the microdeletion region involved in microRNA biogenesis, also have SWM deficits, suggesting that this line might be a useful tool with which to explore these mechanisms. Here we use these *Dgcr8* haploinsufficient mice to examine the molecular and circuit basis for impaired hippocampal-prefrontal functional connectivity in the 22q11 microdeletion model.
Methods: Neural recordings were obtained from *Dgcr8*$^{+/-}$, *22q11*$^{+/-}$, and wildtype littermates implanted with microelectrodes in the ventral HPC (vHPC), dorsal HPC and mPFC under baseline conditions and during performance of a T-maze delayed non-match-to-sample test of SWM. Single units from the mPFC, as well as local field potentials from all three brain regions, were analyzed for synchrony between and within each region using custom-written MATLAB software. To manipulate vHPC terminals, we used an additional cohort of wildtype animals injected with an adeno-associated virus carrying the optogenetic silencer enhanced Arch3.0 or a control virus carrying mCherry, both under the control of the CaMKII promotor. Virus was injected into the vHPC, and optical fibers were implanted overyling the mPFC, both bilaterally. These mice were trained in the t-maze task, and performance was assayed during interleaved light-on and light-off trials.
Results: First, we measured functional connectivity within the HPC and between the HPC sites and the mPFC. While *22q11*$^{+/-}$ mice had a general phenotype of decreased functional connectivity across all three recording sites, *Dgcr8*$^{+/-}$ mice had a specific deficit in the vHPC-mPFC pathway. Both theta-frequency coherence and phase-locking of mPFC cells to the vHPC theta oscillation were decreased in *Dgcr8*$^{+/-}$ mice. Moreover, vHPC-mPFC coherence at baseline predicted how long each animal took to learn the task. Next, to determine whether disrupting vHPC-mPFC connectivity is sufficient to disrupt SWM, we utilized an optogenetic approach to inhibit synaptic transmission at vHPC axon terminals in the mPFC. Optogenetic silencing of vHPC terminals in the mPFC decreased mPFC multiunit responses to electrical stimulation of the vHPC *in vivo*, proving the efficacy of the manipulation. Finally, in behaving animals, illumination of the vHPC terminals with light impaired SWM performance only in the active virus group.
Conclusions: These results demonstrate the necessity of vHPC-mPFC transmission in normal SWM performance, and suggest a central role for this circuit in the behavioral and physiological impairments associated with the 22q11.2 microdeletion. Furthermore, they point to the *Dgcr8* gene and microRNAs as potential molecular mediators of long-range connectivity deficits seen in this model of schizophrenia predisposition.
Disclosure: J. Gordon, **Part 1:** Speaker at Pfizer Basic Research Division Neuroscience Symposium, honorarium, 2011.

Panel

44. Public-private Repositioning Partnerships: A New Path to Achieve Target Validation and Proof of Concept for Novel CNS Indications

44.1 Drug Repositioning Through Open Innovation—An Industry Perspective

Donald Frail*

AstraZeneca, Waltham, Massachusetts

Background: Most marketed drugs are approved for indications in addition to what they were originally developed, a reflection of the role of a common biology across diseases. We have explored ways to 'crowd source' emerging breakthroughs that may identify additional new uses of potential new drugs that are being developed. In parallel, there is a greater focus on translational research by academic investigators and therefore a need to access the appropriate compounds to evaluate and validate new concepts, ideally in humans. This provides the opportunity for mutually beneficial partnerships developed through Open Innovation.
Methods: In Dec, 2011, AstraZeneca and the Medical Reserach Council of the UK announced a groundbreaking partnership focus on evaluating and validating new concepts using AstraZeneca comopunds that have been in development. This was subsequently followed by a similar partnership with NCATS at the NIH. We now have significant experience to understand the opportunities and challenges involved in these types of partnerships.
Results: Fifteen different collaborations have been established as a result of the AstraZeneca MRC partnership, including preclinical and clinical studies. In additional, the NCATS NIH program has been implemented. Our extensive efforts in drug repositioning, both within our company and with external partners like the MRC investigators, has provided significant experience with the opportunities and the challenges involved. These will be discussed in detail. Examples of specific drug repositioning programs will be provided to illustrate the breadth of biology across diverse biological systems and diseases, including diseases of the nervous system. Specific drug development topics will include the challenges related to patents, including the value of methods of use patents, regulatory challenges and the opportunities for 'data exclusivity', choosing the dose, particularly if central exposure is required, the limitations of safety data packages, and the management of patient safety information.
Conclusions: We are very encouraged by these types of partnerships, with advantages for the acadmic and industry collaborators, and most importantly for the unmet needs of patients.
Disclosure: D. Frail, **Part 1:** I am an employee of AstraZeneca., **Part 2:** I am an employee of AstraZeneca., **Part 3:** I am an employee of AstraZeneca and was an employee of Pfizer.

44.2 NCATS/NIH-Industry Pilot Program on Drug Repositioning

Christine Colvis*

National Center for Advancing Translational Sciences, Bethesda, Maryland

Background: Last year, the National Center for Advancing Translational Sciences (NCATS) launched a pilot program, 'NIH-Industry Pilot Program: Discovering New Therapeutic Uses for Existing Molecules' that tested a new model for PPP collaborations including template agreements to shorten the time it would take to establish collaborations between an academic institution and a pharmaceutical company and move more rapidly into the actual research.
Methods: For the pilot, NCATS established memoranda of understanding with eight pharmaceutical companies, AbbVie, AstraZeneca, Bristol-Myers Squibb Company, Eli Lilly and Company, GlaxoSmithKline, Janssen Pharmaceutical Research & Development, L.L.C, Pfizer, and Sanofi. Collectively, these companies made 58 assets that have undergone significant research and development, including safety testing in humans, available for the pilot.
Results: Within 60 days after publication of the Funding Opportunity Announcement, NCATS received almost 160 pre-application proposing new uses for these well-developed assets. Collaborations established between academic institutions and the company will test ideas for new therapeutic uses, with the ultimate goal of identifying promising new treatments for patients.
Conclusions: This presentation will review some of the lessons learned from the pilot thus far, including the utility of template agreements in speeding the collaborative process.
Disclosure: C. Colvis, Nothing to Disclose.

44.3 Open Innovation and Mobile Health Technology to Improve and Accelerate Clinical Development

Tomasz Sablinski*

Transparency Life Sciences, Short Hills, New Jersey

Background: Open innovation and mobile health technology are anticipated to improve and accelerate the clinical development of promising drugs for unmet medical needs with unprecedented efficiency and productivity. A scalable, replicable model to develop a portfolio of drug candidates, across many therapeutic areas has been developed. Although the model is broadly applicable to all chronic diseases, its relevance to testing new treatments for CNS disorders is particularly high. A key element of the TLS approach is reducing clinical trial costs by at least 50%, by partnering with new technology providers and minimizing costly infrastructure and patient visits that make trials so expensive.
Methods: A web-based platform was developed to enable the optimal use of crowdsourcing, mobile health and transparency. *Crowdsourcing* is used to enable patients, physicians, researchers and other stakeholders to contribute to the design of the company's clinical trials, resulting in protocols that are focused on parameters most relevant to clinical decision-making and patient needs. *Advances in information technology and mobile health* are leveraged to implement trials that are far more cost-effective than current approaches, while reducing burdens on subjects and sponsors and enhancing data quality. *Transparency* throughout the development process is implemented to build credibility with diverse stakeholders and produce better results.
Results: At the time of this abstract preparation (April 8, 2013) the Transparency's website had close to twenty one thousand visits, (67.58% from new visitors, and 32.42% from returning visitors). Over 700 individuals registered as contributors, and either completed at least one Protocol Builder, or at least one Indication Finder, or expressed interest in doing so in the near future. A clinical study protocol, posted on TLS website, was designed by the community of approximately 70 MS patients and around 30 clinical researchers, practicing neurologists, and key opinion leaders in MS. As a result of this curated open source approach the design is highly innovative: 1. Only first and last visit will be traditional site visits; all data in the 12 months between 1st and last visit will be collected from patients remotely with the use of variety of telemonitoring devices, and digital (virtual visits), 2. Primary efficacy endpoint of the study is disability as measured by average change in composite functional score on the Multiple Sclerosis Functional Composite (MFSC); this score is considered an optimal reflection of disease progress; telemonitoring makes it possible to administer easily. 3. The protocol, submitted to the FDA as part of IND was accepted with 'the study may proceed' letter, and no changes were requested by the agency. In December of 2012 TLS has received FDA clearance to proceed with a Phase 2 lisinopril MS trial—the first-ever that relies on crowdsourcing and telemonitoring.
Conclusions: New scientific knowledge accessed through a combination of open innovation and key opinion leaders input will improve clinical study protocol design issues and identify promising new indications for repositioning. TLS' first repositioning candidate is lisinopril as a potential treatment for multiple sclerosis. Preclinical data suggest this widely used anti-hypertensive may have utility in combination with existing MS drugs to increase the time until relapse.
Disclosure: T. Sablinski, **Part 5, Auven Therapeutics, Transpartency Life Sciences**

44.4 The MRC/AstraZeneca Mechanisms of Disease Compound Sharing Initiative

Christopher Watkins*

Medical Research Council (MRC), London

Background: The Medical Research Council (MRC) is the largest public funder of biomedical research in UK, with an annual budget of over Ł750 m. The MRC supports and advances medical research in three main ways: by providing research grants and career awards to scientists in UK universities and hospitals, by funding research centres in partnership with universities, and through its own research facilities. The partnership provided academic researchers with unprecedented access to a high quality collection of clinical and pre-clinical AstraZeneca compounds, which had

been deprioritised. Researchers were able to use these for studies that investigated mechanisms of human disease and the development of potential therapeutic interventions. The MRC has invested Ł7 M in a world leading, award winning open innovation collaboration with AstraZeneca.

Methods: The initiative was launched in December 2011, and funding decisions announced in October 2012. The pool of assets included compounds that had completed Phase 1 where evidence of target engagement, pharmacological activity *in vivo* and appropriate toxicity profile was available, and candidate drug compounds which had completed Phase 2a/2b and had been halted for efficacy/portfolio reasons within AstraZenenca. A two-stage process was used to identify projects that were feasible, did not duplicate existing studies, and did not directly contribute to AstraZeneca's own development programmes. At outline stage, targets of all the compounds were made available to researchers together with sufficient information to allow assessment of the utility of these prior to submission of an outline proposal. At this stage researcher's IP was protected through a CDA between MRC and AstraZeneca. Following the outline stage, AstraZeneca researchers became full co-applicants on the proposal and provided researchers with more information on the compounds once individual CDAs were signed. The rights to IP generated using the compounds varied between projects but were similar to those currently used in academically-led research. AstraZeneca retained rights over the chemical composition of the compounds, and any new research findings will be owned by the academic institution with AstraZeneca having first right to negotiate.

Results: 15 research projects were awarded funding. A broad range of projects studying common diseases like Alzheimer's, cancer and lung disease through to rarer conditions such as motor neurone disease and muscular dystrophies. Eight of the projects involve clinical trials of potential new therapies, and seven focus on earlier work in laboratory and animal models.

Conclusions: This initiative was transformational in stimulating relationships between academia and industry. It served to support MRC's Translational Research Strategy and provided AstraZeneca the opportunity to engage with a larger section of the academic community across a range of disease areas that may have fallen outside of its core focus.

Disclosure: C. Watkins, Nothing to Disclose.

Panel

45. The Ventromedial Prefrontal Cortex in Conditioning and Extinction in Chronically Relapsing Disorders

45.1 Deficits in Ventromedial Prefrontal Cortex Group1 Metabotropic Glutamate Receptors Underpin Cognitive Dysfunction During Protracted Cocaine Withdrawal

Karen K. Szumlinski*

University of California, Santa Barbara, California

Background: Anomalies in prefrontal cortex (PFC) function are posited to underpin difficulties in learning to suppress drug-seeking behavior during abstinence, which may underpin the 'incubation of drug craving' purported to occur in both laboratory animals and humans during protracted drug withdrawal. A history of extensive cocaine self-administration dysregulates PFC extracellular glutamate in laboratory animals, suggesting deficits in glutamate signaling within this region might underpin the intensification of drug-seeking during protracted withdrawal. As Group1 metabotropic glutamate receptors (mGluRs) regulate drug-related learning, we hypothesized that cocaine-induced reductions in Group1 mGluR function within the PFC might impair behavioral suppression during cocaine abstinence to promote drug-seeking.

Methods: We first assayed the short- and long-term consequences of extended access to intravenous cocaine (6 hrs/day; 0.25 mg/infusion for 10 days) upon the expression of Group1 mGluRs within the major ventral and dorsal PFC subdivisions by immunoblotting at 3 versus 30 days withdrawal. We then employed neuropharmacological approaches to examine the effects of mimicking (using selective mGluR1 and/or mGluR5 antagonists) or 'reversing' (using an mGluR1/5 agonist) cocaine-induced changes in Group1 mGluR function within the ventromedial aspect of the PFC (vmPFC) for cocaine-seeking behavior during withdrawal. Follow-up studies examined for cocaine-elicited changes in down-stream regulators of Group1 mGluR function (eg, Homer proteins, PKCs, ERK, PI3K/AKT) and, when appropriate, assayed also their relevance for cocaine-seeking behavior.

Results: Following protracted withdrawal, cocaine-experienced animals exhibited a time-dependent intensification of cue-induced cocaine-seeking behavior and an impaired extinction of this behavior. These behavioral phenomena were associated with a time-dependent reduction in mGluR1/5 expression, as well as an imbalance in Homer2 versus Homer1 expression, during protracted withdrawal and both of these protien changes were inversely related to the activational state of ERK, AKT and PKC epsilon within this region. Pharmacological manipulations of vmPFC mGluR1/5 produced no immediate effects upon cue-induced cocaine-seeking behavior, but produced residual effects on a subsequent test for cocaine-seeking. At 3 days withdrawal, cocaine-experienced rats infused intra-vmPFC with mGluR1/5 inhibitors, either before or after an initial test for cocaine-seeking, persisted in their cocaine-seeking akin to cocaine-experienced rats in protracted withdrawal. Conversely, cocaine-experienced rats infused with an mGluR1/5 agonist before the initial test for cocaine-seeking at 30 days withdrawal exhibited a facilitation of extinction learning. In contrast, virus-mediated reversal of the cocaine-induced imbalance in Homer1 versus 2 expression failed to alter cocaine-seeking behavior in the absence of cocaine, but prevented cocaine-primed reinstatement of cocaine-seeking. Peptide inhibitor-mediated inhibition of PKCepsilon translocation failed to alter cocaine-seeking.

Conclusions: These data indicate that cue-elicited deficits in vmPFC Group1 mGluRs mediate resistance to extinction during protracted withdrawal from a history of extensive cocaine self-administration. Moreover, these data indicate that while cocaine-elicited imbalances in vmPFC expression of different Homer isoforms do not contribute to learning to suppress drug-seeking behavior during cocaine abstinence, they are critical for the reinstatement of that drug-seeking behavior by cocaine. If relevant to humans, these data pose pharmacological stimulation of mGluR1/5 as a potential

approach to facilitate learned suppression of drug-seeking behavior which may aid drug abstinence and protect against relapse.
Disclosure: K. Szumlinski, Nothing to Disclose.

45.2 Factors that Impact the Functional Reactivity of the Fear Extinction Network Across Disorders

Mohammed R. Milad*

Harvard Medical School, Charlestown, Massachusetts

Background: Many studies have now examined fear extinciton in rodents and humans. These studies implicate many cortical and subcortical regions in the acquisition and expression of the extinciton memory. How does the function of these brain regions differ across psychiatric disorders characterized by elevated fear and anxiety remains unlcear. Also unclear is how sex differences and sex hormones may or may not play a role here.
Methods: We used a validated two-day fear conditioning and extinction paradigm across different populations of patients diagnosed with PTSD, OCD, SCZ, and more recently acorss GAD and SAD. We used fMRI, and psychophysiological reactivity to examine the differences and similarities of the functional reactivity of the network across disorders. We also measure sex hormones in some of the suties to examine how those hormones may interact with fear extinction
Results: The results will be presented across the disorders showng the common impairment across disorders, which appears to be mainly in the vmPFC. Differences in the reactivity of the fear extinction network during extinction recall, however, was noted and will be discussed. This is especially noted in the insular cortex, striatum, and amygdala
Conclusions: The conclusion is too premature to be reached at this stage but clearly suggest that our analysis will likely to lead us to some specific neural signature related to fear extinction that may be unique to the different disorders mentioned above.
Disclosure: M. Milad, Nothing to Disclose.

45.3 Retention of Extinction Learning for Monetary Reward in Cocaine Addiction: Role of the Amygdala and VMPFC

Rita Goldstein*

Department of Psychiatry, New York, New York

Background: Addiction is characterized by continued drug-seeking despite reduced pleasure derived from the drug and even in the face of catastrophic consequences, suggesting that addicted individuals may have diminished ability to form and/or maintain new associations for stimuli that previously predicted rewards.
Methods: Here, we used classical appetitive conditioning to examine the psychophysiological and neural correlates of extinction learning and its retention in 37 individuals with cocaine use disorders (CUD) and 16 healthy controls. Subjects learned to associate a cue, the conditioned stimulus (CS), with monetary reward ($4, CS+) or no reward ($0, CS-). Extinction training was conducted following acquisition and involved repeated presentation of the CS without the paired reward. Retention of extinction learning was assessed a day later.
Results: Differential skin conductance responses (SCR) to the CS+ versus CS-, indexing the success of reward acquisition, extinction, or extinction retention, did not differ between the groups for any of the learning phases (F0.22). In a subset of subjects who underwent functional MRI (29 CUD, 9 controls), extinction learning and retention were associated with a progressive reduction in activation of the ventral striatum to the CS+ versus CS- (Day 1 > Day 2; $F = 5.16$, $p = 0.008$). While in controls, a similar pattern was observed for the amygdala, in CUD it was not, and this represented a significant group $\times$ learning phase interaction (CUD > Controls, Day 2 > Day 1, $F = 5.55$, $p = 0.006$). In addition, although the ventromedial prefrontal cortex (VMPFC) did not show any significant group or learning phase effects (F0.62), suppression of activity of this region during extinction learning on Day 1 was related to Day 2 extinction retention (differential SCR CS+ > CS-: Spearman-$r = 0.48$, $p = 0.009$).
Conclusions: Thus, consistent with studies demonstrating that the amygdala and ventral striatum are involved in the expression of learned associations and the VMPFC is critical for the retention of extinction of aversive conditioning (eg, involving electric shock), our results provide preliminary evidence for the involvement of these regions in the retention of extinction of appetitive conditioning involving monetary gain, highlighting common neural correlates of aversive and appetitive learning in humans.
Disclosure: R. Goldstein, Nothing to Disclose.

45.4 Role of Ventral Medial Prefrontal Cortex (mPFC) and Its Projections to Accumbens Shell on Context-induced Reinstatement of Heroin Seeking in Rats

Jennifer M. Bossert*

NIDA/NIH, Baltimore, Maryland

Background: In rats, exposure to heroin-paired contexts after extinction of drug-reinforced responding in a different context reinstates heroin seeking. This reinstatement is attenuated by inhibition of glutamate or dopamine transmission in nucleus accumbens shell, but not core (Bossert *et al.*, 2006, 2007). Here, we examined the role of ventral mPFC, a brain area that projects to accumbens shell, in this reinstatement. We first studied the role of ventral mPFC by using Fos immunohistochemistry, reversible inactivation with muscimol + baclofen, and a novel 'Daun02 inactivation method' (Bossert *et al.*, 2011). We then used an anatomical asymmetrical disconnection procedure to examine the interaction between glutamatergic projections from ventral mPFC to accumbens shell and local dopamine D_1 postsynaptic receptors in this reinstatement. Finally, we combined Fos with the retrograde tracer Fluoro-Gold (FG) to assess activation of this pathway during context-induced reinstatement (Bossert *et al.*, 2012).
Methods: Rats were trained to self-administer heroin for 12 days; drug infusions were paired with a discrete tone-light cue. Lever-pressing was subsequently extinguished in a

non-drug-associated context in the presence of the discrete cue. Rats were then tested in the heroin- or extinction-associated contexts under extinction conditions.
Results: Reversible inactivation of ventral mPFC decreased context-induced reinstatement, an effect that was mimicked by selectively inactivating ventral mPFC neurons using the Daun02 procedure. We also found that context-induced reinstatement was associated with increased Fos expression in ventral mPFC neurons, including those projecting to accumbens shell, and that inactivation of ventral mPFC in one hemisphere combined with dopamine D_1 receptor blockade into contralateral or ipsilateral accumbens shell decreased this reinstatement.
Conclusions: Our findings demonstrate that selectively activated ventral mPFC neurons mediate context-induced reinstatement of heroin seeking, and that activation of glutamatergic projections from ventral mPFC to accumbens shell, previously implicated in inhibition of cocaine relapse, promotes heroin relapse.
Disclosure: J. Bossert, Nothing to Disclose.
Thursday, December 12, 2013

Panel

46. Applying Animal and Human Models of Risk Avoidance and Impulsivity to Understanding Eating Disorders

46.1 A Translational Assessment of Reward-based Learning in Adolescents with Bulimia Nervosa

Rachel Marsh*

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

Background: Bulimia Nervosa (BN) is characterized by recurrent binge-eating episodes that are associated with a loss of control, followed by self-induced vomiting or another compensatory behavior to avoid weight gain. Our previous findings from women and adolescents with BN suggest that self-regulatory processes are impaired due to their failure to engage frontostriatal circuits appropriately that may be due, in part, to reductions of local volumes on the surface of ventrolateral prefrontal cortices (VPFC) in the BN compared to control participants. VPFC disturbances may also affect reward processing within mesocorticolimbic circuits in BN, thereby decreasing the rewarding relief that normally results from eating and further driving urges to binge-eat. We probed the functioning of these circuits in adolescents with BN using an fMRI paradigm analogous to a task used to define the neural basis of spatial learning in rodents (Packard *et al*, 1989; 1991), tailored to a virtual reality (VR) environment.
Methods: We compared BOLD response in 22 adolescents with BN and 20 age-matched controls (Mean age, SD: BN 16.7, 1.6; HC 15.8, 2.1; $p = 0.15$) while they used an MRI-compatible joystick to navigate an 8-arm radial maze surrounded by extra-maze cues (eg, mountains, trees, sunset) with rewards ($) hidden at the end of the maze arms. In the learning condition, all maze arms contained a reward and participants had to learn to use the cues to navigate and find rewards (revisits were scored as errors). In the control condition, the same spatial cues were interchanged randomly among the same locations at the end of each attempt, thereby destroying any possibility spatial learning. Participants were rewarded randomly at the same frequency as in the learning condition, but without regard to their actual performance. The control condition thus shared all salient features with the learning condition (i.e., visual stimuli, motor demands, cognitive effort, and reward/punishment frequencies). We assessed group differences in task performance and fMRI signal associated with searching the maze and reward feedback (reward or no reward), reporting voxels with a posterior probability of $>98.75\%$ (analogous to $p<0.0125$) and a cluster filter $\geqslant 25$.
Results: Both groups demonstrated spatial learning on the task and both activated temporoparietal and occipital areas when searching the maze. Group differences were detected in mescorticolimbic circuits: whereas control adolescents activated hippocampus when searching in the active condition, BN adolescents instead activated bilateral ventral and dorsal striatum, medial PFC, amygdala, and hippocampus during the control condition, when using the cues for navigation (i.e., spatial learning) was disabled. Group differences associated with reward feedback were also detected in medial temporal lobe and striatum, such that BN, but not control, adolescents activated amygdala and hippocampus during reward, and the ventral striatum during no reward, in the control condition.
Conclusions: Adolescents with BN activated mesocorticolimbic circuits when searching the maze and receiving unexpected rewards when learning was attempted but rendered impossible due to the randomization of spatial cues in the control condition. These findings suggest alterations in the dopaminergic mesolimbic system early in the course of BN, perhaps pointing to a target for early intervention that can be tested in parallel animal and human studies with this translational paradigm. Together with findings of deficient frontostriatal functioning in BN, these data suggest that both oversensitivity to reward and deficient self-regulation likely contribute to impulsivity in BN.
Disclosure: R. Marsh, Nothing to Disclose.

46.2 D1- and D2-like Dopamine Receptors, Impulsive Temperament and Corticostriatal Function as Related to Risky Decision-making: Multimodal Imaging in Healthy Research Participants

Edythe D. London*

University of California, Los Angeles, California

Background: Disordered cognitive control can lead to maladaptive decisions, including those that involve risky behaviors; and impulsivity is a feature of several neuropsychiatric disorders that exhibit dopaminergic dysfunction. While studies of clinical populations that display deficits in risk-based decision-making and pharmacological findings from clinical and animal studies have strongly implicated a role of dopamine in risk-based decision-making, direct evidence of the links between different dopamine receptor subtypes, impulsive temperament, and neural function during risk-based decision-making has been lacking.

Methods: Following prior observations of negative correlations between D2-like receptor BPnd and measures of impulsiveness, Study 1 used PET to assess D1-like binding in 20 healthy volunteers who gave self-reports on the Dickman Impulsivity Inventory and Barratt Impulsiveness Scale. In Study 2, 60 healthy volunteers were tested using the Balloon Analogue Risk Task (BART) during fMRI. In the BART, participants can pump a virtual balloon to increase potential monetary reward or cash out to receive accumulated reward; each pump presents greater risk and potential reward (represented by pump number). At a different time, striatal D2-like dopamine receptor binding potential (BPnd) was measured with positron emission tomography (PET) in 13 of the participants.
Results: Study 1: Consistent with findings in animals, D1-like receptor BPnd in the human midbrain showed a robust, negative correlation with behavioral impulsivity, as indexed by the Barratt Impulsiveness Scale ($r = 0.71$, $p = 0.003$). In addition, exploratory voxelwise analysis provided preliminary evidence of positive associations between D1-like BPnd in the orbitofrontal cortex and impulsivity, as measured by the Dickman scales. In Study 2, losses were followed by fewer risky choices than wins; and during risk-taking after a loss, amygdala and hippocampal activation exhibited greater modulation by pump number than after a cash-out event. Striatal D2-like BPnd was positively related to modulation of ventral striatal activation by pump number when participants decided to cash out and negatively to the number of pumps in the subsequent trial, but inversely related to modulation of prefrontal cortical activation by pump number when participants took risk, and to overall earnings.
Conclusions: Study 1 provides the first concrete evidence for an inhibitory function of midbrain D1-like receptors in behavioral impulsiveness in humans, and suggests an opposing function of D1-like receptors in the orbitofrontal cortex. Interventions that influence signaling through dopamine D1 receptors, therefore, may impact neuropsychiatric disorders that feature impulsive behavior. Although there is substantial evidence that frontolimbic and dopaminergic brainregions influence decision-making, how choice-outcome, dopamine neurotransmission and frontostriatal activity are integrated to affect choices is unclear. Study 2 findings provide *in vivo* evidence for a potential mechanism by which dopaminergic neurotransmission may modulate risk-taking behavior through an interactive system of frontal and striatal activity.
Disclosure: E. London, Nothing to Disclose.

46.3 Harm Avoidant Behaviors and Altered Limbic and Executive Neural Function in Anorexia Nervosa

Walter Kaye*

University of California San Diego, La Jolla, California

Background: Individuals with anorexia nervosa (AN) restrict food consumption and become emaciated. They tend to be ahendonic, inhibited, over-concerned with planning and consequences, and overly sensitive to risk and uncertainty. AN have elevated harm avoidance (HA), a construct with elements of anxiety, inhibition, and inflexibility, that is present pre-morbidly in childhood and persist after recovery, and which associated with altered dopamine D2/D3 receptor activity in the dorsal caudate, part of executive frontal-striatal neural circuitry. Altered eating in AN may be a consequence of dysregulated reward processing and/or awareness of homeostatic needs, perhaps related to a bias of the executive neural system to be overly sensitive to risk and thus inhibit incentive motivational drives subserved by the limbic system. We used fMRI to determine if AN have altered limbic and executive neural function in relationship to the incentive motivational aspect of money.
Methods: We used a region of interest analysis based on known functional distinctions. Study 1 compared 10 ill adolescent AN to 12 matched controls to assess neural response to negative and positive monetary feedback (Delgado 2000). Study 2 compared 19 recovered adult AN to 15 matched controls, after fasting and after being fed, to examine the effects of hunger and satiety on neural correlates of time discounting while subjects made a series of choices between monetary reward options that varied by delay to delivery (McClure 2004).
Results: Study 1) Consistent with the literature, controls tended to show a stronger BOLD response to positive compared to negative (eg wins vs losses) feedback in limbic and executive striatal and cingulate regions. While ill adolescent AN showed a normal response to wins and losses in limbic regions, they had a significantly stronger BOLD response to negative feedback compared to positive feedback in executive and motor striatal and cingulate regions, and was also related to measures of perseveration. Study 2) A significant group by satiety interaction was detected in the delayed discounting paradigm. Controls showed greater response when fasted, compared to being fed for the beta response (the value placed on immediate outcomes) in the anterior ventral striatum , and for the delta response (which reflects a more consistent weighting of time periods) in executive frontal , cingulate , and striatal regions. In contrast, recovered AN had the opposite response in these regions: they exhibited a greater BOLD response in the fed condition relative to the fasting condition. For both studies, corrections for multiple comparisons used false discovery rate (FDR) control at $q = 0.1$; all p's $< .05$.
Conclusions: Study 1: ill AN participants exhibited an abnormal pattern, with an exaggerated response to losses compared to wins in posterior executive and sensorimotor striatal regions. Individuals with AN tend to be overly responsive to negative feedback such as criticism or making mistakes. These findings may reflect the neural processes responsible for a bias for AN to be more sensitive to risk than reward, and have difficulty with set-shifting. Most people are uncomfortable when hungry and describe pleasure when eating, whereas those with AN tend to be anxious when eating, and feel better when starving. Study 2: the contrast between controls and AN showed they had opposite responses after being fed or fasting. AN had diminished responses in limbic regions to immediate reward when fasting and increased activation of executive circuits after eating. In summary, these studies further support the possibility that AN individuals have reduced reward and enhanced activity of executive circuitry that may contribute to anxious and inhibited behaviors.
Disclosure: W. Kaye, Nothing to Disclose.

46.4 Striatal Dopamine D2 Receptor Modulation of Risky Decision Making

Barry Setlow*

University of Florida, Gainesville, Florida

Background: Disordered risk taking is a feature of several neuropsychiatric conditions including anorexia nervosa, and in some cases 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 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 increases. However, there is considerable and stable variability among individuals' performance in this task, such that rats can be reliably classified according to their degree of risk taking. This task was used to determine the relationship between risk taking/aversion and cognitive flexibility, as well as the contributions of striatal dopamine signaling to risky decision making.
Methods: All experiments were conducted in male Long-Evans rats. Study 1 examined the relationship between risky decision making and cognitive flexibility in an attentional set-shifting task. Study 2 employed systemic pharmacological modulation of dopamine signaling to determine the dopamine receptor subtypes involved in risky decision making. Study 3 examined relationships between risky decision making and dopamine receptor mRNA expression in the striatum. Study 4 determined the effects of direct activation of striatal dopamine receptors on risky decision making.
Results: In Study 1, a t-test revealed that rats characterized as 'risk-averse' made more perseverative errors in the set-shifting task compared to their 'risk-taking' counterparts (p.72, ps
Conclusions: High levels of risk aversion in the risky decision making task were associated with worse set-shifting performance and greater striatal D2 receptor expression, consistent with the clinical profile of anorexia nervosa. Pharmacological activation of D2-like receptors (either systemically or directly within ventral striatum) increased risk aversion, suggesting that blockade of these receptors could be a useful target for attenuating maladaptive risk aversion.
Disclosure: B. Setlow, Nothing to Disclose.

Panel

47. Behavioral, Endocrine, and Neural Plasticity Changes Reflecting Stress Associated with Mouse and Monkey Models of Heavy Alcohol Drinking

47.1 Behavioral and Endocrine Adaptations in a Monkey Model of Heavy Alcohol Drinking

Kathleen A. Grant*

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Background: Excessive alcohol consumption and stress are highly co-morbid, but mechanisms that underlie this co-morbidity are not well understood. Non-human primates (NHP) can uniquely address the alcohol-stress interactions due to similar endocrine systems to humans and the propensity to drink alcohol excessively. Here we present the effect of chronic ethanol drinking on (a) habituation of ethanol drinking following repeated abstinence; (b) altered endocrine responsiveness to stress challenge; and (c) the ability of stress to modulate ethanol intakes in heavy alcohol drinking compared to non-heavy alcohol drinking monkeys.
Methods: Adult rhesus monkeys ($n = 32$) were housed individually with a panel that contained 2 drinking spouts, stimulus lights, a food receptacle and a response button. Fluid and food intake were accurately measured. Monkeys were trained to comply with veinipuncture for endocrine measures and blood ethanol concentrations (BEC) without anesthesia. A schedule-induced polydipsia (SIP) procedure was used to induce water and then ethanol (4% w/v) drinking with a fixed-time schedule (FT-5 min) of food delivery. The amount of fluid consumed under the FT-5 min was predetermined and increased every 30 sessions as follows: water, 0.5 g/kg ethanol, 1.0 g/kg ethanol, and 1,5 g/kg ethanol. After induction, the monkeys were allowed concurrent access to 4% ethanol (w/v) and water in daily, 22 hr , 'open-access' sessions for at least 12 months. BECs were taken every 5 days at 7 hrs into the 22 hr session. Forced abstinence (28 consecutive days) were then imposed, with at least 4 months of open-access to ethanol between each abstinence.
Results: There are wide spread individual differences in the daily intake of ethanol across the 12 months of 22 hr/day access, with 35% of the population defined as heavy drinkers. In these heavy drinkers, imposing abstinence resulted in signs of dependence. Repeated abstinence did not affect the average, but lowered the variance, of daily ethanol intakes. Examination of daily drinking patterns reveal consistently rapid intakes of high does of ethanol early in the session followed by nearly continuous drinking of smaller doses of ethanol throughout the rest of the session, including in the dark phase. Examination of endocrine status shows a dose-related decline in cortisol over the year of drinking, with a loss of diurnal rhythm and very high rebound during the abstinence periods. During the chronic open-access to ethanol, a relatively mild stressor of being removed from the housing cage led to a significant increase in ACTH, but not cortisol, in heavy compared to non-heavy drinkers.
Conclusions: This monkey model of SIP induction and then 'open-access' to ethanol reveal key individual differences in the risk for future heavy drinking. Heavy drinkers were more likely to: rapidly drink intoxicating amount of ethanol during the SIP induction; show signs of physical dependence; show disruptions in the HPA axis; have an exaggerated HPA response to mild stress; and to develop a stereotypic pattern of ethanol self-administration after forced abstinence. Collectively, the data highlight the value of this monkey model for disentangling mechanisms that underlie the co-morbidity of heavy alcohol consumption and adverse stress response.
Disclosure: K. Grant, Nothing to Disclose.

47.2 Behavioral and Neural Adaptations Linked to Stress Associated with a Mouse Model of Ethanol Dependence and Relapse Drinking

Howard C. Becker*

Medical University of South Carolina, Charleston, South Carolina

Background: The interaction between stress and ethanol drinking behavior is complex and mechanisms underlying this interaction in the context of dependence are not well understood. We have employed a mouse model of ethanol dependence and relapse drinking to examine mechanisms by which stress associated with chronic ethanol exposure and withdrawal experience promote/mediate escalation of voluntary ethanol drinking. Ethanol dependence is known to alter brain reward and stress pathways, and adaptations in corticotropin releasing factor (CRF) within cortical-basal ganglia-limbic-HPA circuitry have been implicated in stress responsiveness and ethanol self-administration. This presentation will first describe behavioral adaptations in the model, and then data on the role of CRF in mediating/contributing to (a) escalation of ethanol drinking in dependent mice; (b) altered behavioral responsiveness to stress challenge; and (c) the ability of stress to modulate ethanol consumption in dependent compared to nondependent mice.
Methods: Adult C57BL/6J mice were trained to drink 15% (v/v) ethanol using a limited access (2 hr/day) 2-bottle choice paradigm. Once stable baseline intake was established, mice received several weekly cycles of chronic intermittent ethanol (CIE) vapor exposure (EtOH group) or air exposure (CTL group) in inhalation chambers (16 hr/day $\times$ 4 days) alternated by 5-day limited access drinking test cycles. Behavioral responsiveness to a 10-min inescapable forced swim test (FST) was assessed in separate groups of EtOH and CTL mice 3 or 7 days after the final inhalation treatment. In a separate study, EtOH and CTL mice were exposed to FST 4 hr prior to drinking test sessions to examine effects of stress on drinking in the CIE model. In another study, the effect of administering the CRF1R antagonist MTIP (5–40 mg/kg, ip.) on drinking was examined in EtOH and CTL groups after escalation of drinking was established.
Results: Repeated cycles of CIE exposure produced significant escalation of voluntary ethanol drinking in EtOH mice relative to their baseline level as well as intake in nondependent CTL mice that remained relatively stable over the all test cycles. Further, EtOH mice exhibited a faster rate of intake during drinking sessions, which produced a 2–3 fold increase in blood ethanol levels and significantly elevated brain ethanol concentrations that persisted beyond the 2-hr drinking session. EtOH mice also exhibited significantly less immobility than CTL mice when the 10-min FST exposure was given at 3 or 7 days into withdrawal. A similar effect was observed following CRF infusion (50–200 ng, icv.) in CTL mice, suggesting that the CIE effect of altering FST responsiveness may be mediated by increased CRF activity. FST treatment given during each drinking test cycle produced significantly greater escalation of drinking in dependent (EtOH) mice with little effect on drinking in nondependent (CTL) mice. Finally, MTIP reduced drinking in a dose-related manner in EtOH mice, with the highest dose effective in both EtOH and CTL groups.
Conclusions: These results indicate that repeated cycles of CIE exposure produce escalation of ethanol drinking, altered behavioral response to a stress (FST) challenge, and FST stress experience uniquely interacts with CIE exposure to further augment ethanol self-administration. Further, CIE-induced alterations in CRF activity appear to underlie these effects. Escalated drinking is responsive to CRF1R antagonist treatment, and CIE exposure compromises behavioral response to FST stress, an effect mimicked by exogenous CRF administration. On-going studies are measuring CRF peptide levels (ELISA) and Crf1R and Crf2R mRNA levels (qPCR) in various key structures within reward and stress circuitry in relation to drinking associated with the CIE $\pm$ FST stress model. Collectively, these results implicate adaptations in CRF activity in mediating/contributing to escalation of drinking and altered stress responsiveness associated with dependence.
Disclosure: H. Becker, **Part 1:** Dr. Becker has conducted contractual work and served as a consultant for Eli Lilly and Company, but those activities have no relationship to work that will be presented in this symposium., **Part 4:** Dr. Becker engaged in a research contract with Eli Lilly and Company.

47.3 Chronic Ethanol Exposure Increases Output from the Sensorimotor Striatum in Mouse and Monkey Models via Changes in Neuronal Excitability and Synaptic Transmission

David Lovinger*

NIAAA, Bethesda, Maryland

Background: Corticostriatal circuits control action learning and performance, and have been implicated in several aspects of drug seeking and addiction. While a great deal of attention has been focused on the 'limbic' circuitry that includes the Nucleus accumbens, recent studies suggest that the associative circuitry including dorsomedial striatum (DMS) in mice and the caudate nucleus in monkeys, and the sensorimotor striatum including the dorsolateral striatum (DLS) in mice and putamen nucleus in monkeys, also have roles in the neural actions of drugs of abuse.
Methods: We have examined effects of chronic ethanol exposure on neuronal excitability and synaptic transmission in the DMS and DLS using brain slice electrophysiological recordings in 3 animal models: 1) Mice given chronic intermittent ethanol (EtOH) vapor exposure for 4 weeks; 2) Mice consuming EtOH under a Drinking in the Dark (DID) schedule for 6 weeks; and 3) Monkeys consuming EtOH on a 22 hr per day schedule for 1–3 years.
Results: The excitability of striatal spiny projection neurons (SPNs) in the DLS and putamen is increased in slices from chronic EtOH-exposed mice and monkeys, as measured by increased action potential firing in response to postsynaptic current injection. The frequency of glutamatergic miniature excitatory postsynaptic currents (mEPSCs) is increased in both caudate and putamen nuclei from monkeys that consumed EtOH, and dendritic spine numbers are increased in putamen, but not caudate, of monkeys that consumed EtOH for 3 years. The increased mEPSC frequency may thus

reflect increased numbers of glutamatergic synapses, at least in putamen. No changes in mEPSC frequency or amplitude were observed in mice given EtOH exposure with either procedure. The frequency of GABAergic miniature inhibitory postsynaptic currents (mIPSCs) was decreased in DLS and putamen SPNs in all of the alcohol exposure models. The mIPSC frequency correlated especially well with indices of alcohol intake in the monkey models. Chronic EtOH-induced decreases in mIPSC frequency were observed in mouse DMS, but increases were observed in monkey caudate nucleus. These findings indicate that a presynaptic decrease in GABAergic transmission in sensorimotor striatum arises relatively early in the course of chronic EtOH exposure and can persist for years. This synaptic alteration does not depend on voluntary consumption as is it observed with exposure by either vapor or consumption.

Conclusions: The overall pattern of findings in our chronic EtOH exposure models indicates that SPNs in striatum are disinhibited and show greater excitability, with a later developing increase in glutamatergic drive. Effects in associative striatum are more mixed. The net effect of these neurophysiological changes would be to facilitate throughput in the sensorimotor circuitry, perhaps facilitating habitual alcohol seeking and intake.

Disclosure: D. Lovinger, Nothing to Disclose.

47.4 Similar Dopamine System Adaptations in Mouse and Monkey Models of Excessive Alcohol Exposure

Sara R. Jones*

Wake Forest University School of Medicine, Winston Salem, North Carolina

Background: Ethanol has complex effects on the dopamine system when given acutely, with both excitatory and inhibitory aspects leading to an overall modest enhancement of dopamine signaling. Repeated exposure to ethanol and withdrawal attenuates both the set-point of dopamine system function and the response to ethanol challenge. We have examined dopamine dynamics in two animal models of chronic ethanol exposure and withdrawal, a mouse model of chronic intermittent ethanol vapor exposure and a monkey model of chronic excessive drinking. Ethanol has been shown to interact with both reward and stress pathways, and ethanol dependence-induced drinking escalation has been linked to increased activity of the kappa opioid receptor system, which is activated by stress. Alterations in nucleus accumbens dopamine parameters such as release, uptake, autoreceptor activity, ethanol-induced elevations in dopamine levels and release activity evoked by tonic vs phasic stimulations will be described. In addition, the sensitivity of kappa opioid receptors to activation by the agonist U50,488 in the nucleus accumbens will also be described.

Methods: Adult C57BL/6J mice received several weekly cycles of chronic intermittent ethanol (CIE) vapor exposure (EtOH group) or air exposure (CTL group) in inhalation chambers (16 hr/day × 4 days). Adult monkeys had free access to an ethanol solution for more than 12 months following an induction procedure. All monkey experiments took place in the laboratory of Dr. Kathleen Grant at Oregon Health Sciences University. For microdialysis experiments, surgeries were conducted 2 days prior to starting the EtOH vapor exposure regimen. Mice were anesthetized with ketamine (20 mg/kg, ip) and xylazine (100 mg/kg, ip)and guide cannulae for probes were implanted above the NAc. Following the final 16 h vapor exposure, microdialysis probes were inserted into the NAc and aCSF was infused at a rate of 1.0 íL/min with at least a two hour period of equilibration prior to the start of the experiment. Samples were collected at 20-min intervals and analyzed by HPLC-EC. For voltammetry experiments, mice or monkeys were sacrificed and brains were sectioned into 400 ím-thick coronal slices which were transferred to a submersion recording chamber perfused at 1 ml/min at 32 °C with oxygenated aCSF. A carbon fiber microelectrode (approximately 150 íM length, 7 íM radius) and a bipolar stimulating electrode were placed into the NAc core and dopamine was evoked by single electrical pulses applied every 5 min. Cumulative dose-response curves were obtained for drugs. To determine kinetic parameters, dopamine signals were modeled using Michaelis–Menten kinetics, as a balance between release and uptake (Wightman *et al.*, 1988). All voltammetry data were collected and modeled using Demon Voltammetry and Analysis software.

Results: After chronic exposure to ethanol, either via vapor inhalation in mice over repeated cycles of exposure and withdrawal, or 12–18 months of free access to ethanol drinking in monkeys, there were several changes in dopamine function that were similar or identical across the two species. First, the maximal rate of dopamine uptake, or Vmax, was greater in ethanol-exposed animals. This would be expected to decrease extracellular levels of dopamine. In addition, presynaptic D2-type presynaptic dopamine autoreceptors, which provide feedback inhibition of dopamine release, were found to be supersensitive after ethanol exposure in both species. This would also be expected to reduce extracellular dopamine levels. The activity of kappa opioid receptors, which inhibit presynaptic dopamine release, was found to be increased in mouse brain, with monkey experiments ongoing. Acute ethanol challenges produced decreases rather than increases in extracellular dopamine in mice, potentially through a supersensitive dynorphin/kappa opioid receptor mechanism. Finally, the levels of electrically evoked dopamine release were found to be lower in the monkey model of excessive drinking, but unchanged in the mouse ethanol exposure model.

Conclusions: These findings indicate that there are many similarities between the neurobiological consequences of chronic, intermittent ethanol exposure across species, specifically mouse and monkey models. All of the adaptations documented in these models are aimed at reducing extracellular dopamine levels, and augmenting the effects of stress on the dopamine system, such that during withdrawal both mice and monkeys would be expected to be experiencing a hypodopaminergic state with supersensitivity to stress through increased sensitivity to kappa opioid receptor activity.

Disclosure: S. Jones, Nothing to Disclose.

Panel

48. Brain on Fire: Inflammation in Neurological and Psychiatric Illness

48.1 Monitoring Neuroinflammation and Demyelination using Magnetic Resonance Imaging in a Preclinical Setting

Danielle L. Graham*

EMD Serono Research & Development Institute, Billerica, Massachusetts

Background: Multiple Sclerosis (MS) is a disease that is characterized by an inflammatory process resulting in demyelination and axonal injury. Current treatments, acting primarily at the level of the peripheral immune system, effectively reduce the number and frequency of relapses in the relapsing-remitting form of MS (RRMS), but do not delay the progression of symptoms and accumulation of neurological disability in secondary-progressive MS (SPMS). There is currently a poor understanding of SPMS and no effective treatment. Novel therapies that cross the blood brain barrier to target neuroinflammation and reverse or halt neurodegeneration would bring tremendous benefit to MS patients. A key hurdle in the development of such therapies is the availability of readouts to monitor CNS activity. In order to address these challenges, we established a unique animal model of inflammation-driven neurodegeneration that mimics several clinical and pathological features of MS. Utilizing this model we specifically evaluated the use of preclinical translational endpoints such as magnetic resonance imaging (MRI) and optical coherence tomography (OCT) to longitudinally monitor disease progression.
Methods: Female rats were immunized with full length myelin oligodendrocyte glycoprotein (MOG) at Day 0 and assessed daily by monitoring neurological score (gait, paralysis, etc) for the remainder of the study. Roughly 40 days post-induction (dpi) animals were euthanized and the level of spinal cord and brain inflammation, demyelination, and axonal damage determined by histology. In a second cohort of animals, disease progression was monitored longitudinally using MRI techniques including T1-weighted, T2-weighted and magnetization transfer (MT). In addition, the thickness of the retinal nerve fiber layer (RNFL) was measured using OCT at select time points during disease progression.
Results: Immunization with MOG protein in rats induced significant neurological symptoms including abnormal gait and limb paralysis ($p<0.05$ as compared to controls) as well as increased inflammation (326% as compared to controls normalized at 100%; $p<0.05$), a reduction in myelin (54% vs 100%; $p<0.05$) and axonal damage (30% vs 100%, $p<0.05$) in the spinal cord. Significant inflammation, demyelination, and axonal damage were also present in the cerebellum, a finding unique to this model. In the cervical spinal cord, MRI T2-weighted (T2-W) and MT images correlated with neurological disability, reflecting the underlying inflammation and demyelination occurring during the disease course. Neuroinflammation within the cerebellum developed dynamically with approximately 50% of animals having lesions by the 1st relapse and more than 80% developing lesions by 39 dpi ($p<0.05$ as compared to control animals). In addition, total lesion load in the cerebellum showed a steady increase during the chronic phase of the disease, likely reflecting increased neurodegeneration. Finally, the extensive neuroinflammation in the optic nerve evident via histology at the end of the study was first detected by MRI in 80% or rats during the first relapse. Similarly, in a separate cohort of DA rats, OCT revealed a decrease in RNFL thickness as the disease progressed.
Conclusions: These data indicate that the clinical disease course in DA rats mimics several aspects of the neurological and pathological symptoms present in MS including significant neuroinflammation and ultimately neurodegeneration. They also demonstrate the potential of using translational readouts such as MRI and OCT in preclinical model systems. Such technologies will enhance efficacy testing and may ultimately improve the probability of success in developing novel CNS therapeutics.
Disclosure: D. Graham, **Part 5:** EMD Serono Research Development Institute.

48.2 Pruning Developing CNS Synapses: An Active Role for Glia and Immune Molecules

Beth Stevens*

Boston Childrens Hospital, Boston, Massachusetts

Background: During development, neural circuitry undergoes a remodeling process in which excess synapses areeliminated or pruned and the remaining synapses are strengthened. While it is clear that this is an activity-dependent process, the precise molecular mechanisms have not been elucidated. Work from our laboratory has demonstrated a surprising new role for glia and molecules traditionally associated with innate immune system function. We found that complement cascade proteins, in elimination and refinement of CNS synapses;however themechanism by which complement mediates synapse removal hasvremained elusive. The primary role of thecomplement cascade in the innate immune system is to opsonize or tag unwantedcells or debris for removal by phagocytic macrophages via specific complementreceptors.Our recent studies support a model in which 'weaker' or less active synapses in the developing brain are similarly tagged by complement and then eliminated by microglia, the primary phagocytic immune cells in CNS and the only resident brain cell to express complement receptor 3 (CR3/cd11b)
Methods: Using high resolution imaging and assessment of mice deficient in complement-related proteins, we provide evidence that microglia activelyparticipate in synaptic pruning by phagocytosing developing synapse. Specifically. we used immuno-electron micropscopy and several super-resolution Imaging techniques (SIM, Array Tomography) to assess microglia-synapse interaction and synaptic engulfment. In addtion, we used immunohisyological approaches to determine the expression and localization of complement (C1q, C3) and other immune-related molecules at developing CNS synapses.
Results: In this study, we demonstrate that microglia are critical mediators of synaptic pruning in the normal,

developing brain. Furthermore, we have identified an underlying cellular-based mechanism of complement-dependent synapse elimination: CR3/C3-dependent engulfment by microglia. Here we show that: 1) Specialized postnatal microglia engulf RGC presynaptic terminals undergoing active synaptic remodeling. 2) Engulfment of inputs is regulated by neuronal activity such that microglia selectively phagocytose 'weaker' RGC inputs. 3) Engulfment of RGC inputs by microglia is dependent upon complement cascade component C3, which is present at high levels during this precise time in the developing dLGN. 4) The receptor for C3, CR3, is specifically expressed by microglia in the P5 dLGN and is also necessary for microglia-mediated engulfment of excess RGC inputs.5) Genetic (CR3 KO or C3 KO) or pharmacological (minocycline) perturbations that disrupt microglia-mediated engulfment of RGC inputs result in deficits in structural remodeling and pruning of synaptic inputs. 6) Defects in synaptic circuitry are sustained into adulthood in CR3 and C3 KO mice. In addition, our preliminary experiments reveal defects in microglia phenotype and interactions with synapses in two mouse models of autism

Conclusions: These results identify a new role for microglia in the healthy, developing brain and identify complement-dependent phagocytosis of synapses by microglia as a novel mechanism by which inappropriate synaptic connections are physically eliminated.Our data offer new insight into mechanisms underlying synaptic pruning in the developing CNS, provide a novel role for microglia in the healthy brain, and provide possible important mechanistic insight into synaptic pathology associated with developmental and degenerative CNS disease.

Disclosure: B. Stevens, Nothing to Disclose.

48.3 Role of the Peripheral Immune System in Stress-induced Depressive Behavior

Georgia E. Hodes*

Mount Sinai School of Medicine, New York, New York

Background: Interleukin-6 (IL-6), a pro-inflammatory cytokine, is elevated in the blood of subjects with depression and may reflect hyperactivity of the peripheral immune system. We utilized repeated social defeat stress (RSDS), an animal model of depression, to examine individual differences in the peripheral immune response to stress. After exposure to RSDS some animals termed susceptible show a spectrum of depression-like behavior, whereas resilient animals behave more akin to controls.

Methods: Blood was sampled from mice throughout the entire social defeat stress and IL-6 levels were measured. In a separate group of animals, peripheral mononuclear cells (PBMCs) were isolated before RSDS and stimulated with lipopolysaccharide (LPS) to examine the pre-stress IL-6 response. To test the functional relevance of the peripheral immune response to stress *in vivo*, we ablated the peripheral immune system of naïve mice using irradiation. We then replaced it by transplanting bone marrow from a susceptible mouse following 10 days of social defeat stress that exhibited a strong IL-6 response to LPS stimulation, or a control mouse with little or no IL-6 response to LPS challenge. We then exposed bone marrow recipient mice to a sub-threshold micro-defeat and tested for depression and anxiety-associated behavior. Additionally we transplanted bone marrow from IL-6 knockout mice into naïve mice and exposed them to 10 days of RSDS to examine whether IL-6 was necessary to induce depression-associated behavior.

Results: A time-course analysis of peripheral IL-6 indicated that susceptible mice exhibit heightened IL-6 levels following their first defeat, which remain elevated 48 hours after the end of RSDS. Pre-stress LPS stimulation of PBMCs taken from animals that went on to show depression-associated behavior following RSDS, exhibited greater release of IL-6. Bone marrow transplants revealed a functional role for the peripheral immune system in the development of susceptibility to social defeat stress. Transplants from susceptible mice induced social avoidance following a sub-threshold microdefeat in naïve recipient animals, while transplantation of bone marrow from an IL-6 knockout mouse promoted resilience to RSDS.

Conclusions: We report that there are individual differences in the IL-6 response to stress. Animals that develop a susceptible phenotype following RSDS exhibit exaggerated release of the pro-inflammatory cytokine IL-6. Furthermore, LPS stimulation of IL-6 can be used to predict which animals will display social avoidance following RSDS. Functionally, replacement of the bone marrow can be used to induce or block a depression-like phenotype depending upon the cytokine profile of the donor. These studies indicate that individual differences in the inflammatory response to stress underlie the development of depression-like behavior in the social defeat model.

Disclosure: G. Hodes, Nothing to Disclose.

48.4 Testing the Cytokine Hypothesis of Depression: Trials and Tribulations

Andrew H. Miller*

Emory University School of Medicine, Atlanta, Georgia

Background: To determine whether inhibition of the inflammatory cytokine tumor necrosis factor (TNF) reduces depressive symptoms in patients with treatment resistant depression (TRD) and whether increased baseline plasma inflammatory biomarkers predict treatment response, we conducted a randomized double-blind, placebo-controlled trial in 60 medically-stable outpatients with TRD.

Methods: Patients were administered 3 infusions of the TNF antagonist infliximab (5 mg/kg)($n = 30$) or placebo ($n = 30$) over 12 weeks. Depression was assessed using the 17-item Hamilton Depression Rating Scale (HAM-D-17).

Results: No difference in change of HAM-D-17 scores between treatment groups across time was found. However, there was a significant interaction between treatment, time and log baseline c-reactive protein (CRP)($p = 0.01$), with change in HAM-D-17 scores (Baseline to Week 12) favoring infliximab-treated patients with a baseline CRP > 5 mg/L. Indeed, patients with a baseline CRP > 5 mg/L exhibited a

treatment response ($\geqslant$50% reduction in HAM-D-17 at any point during treatment) of 62% (8/13) in the infliximab group versus 33% (3/9) in placebo-treated patients. In addition, baseline concentrations of TNF-alpha and its soluble receptors were significantly higher in infliximab-treated responders versus non-responders (p5 mg/L had a 10-fold higher concentration of CRP in the CSF than patients with a plasma CRP.
Conclusions: Further studies are required to determine whether the efficacy of biologic TNF antagonists is dependent on inflammation-induced influences on BBB permeability. Nevertheless, the data suggest that TNF antagonism may improve depressive symptoms in patients with high baseline inflammatory biomarkers.
Disclosure: A. Miller, **Part 1:** Abbott Laboratories, AstraZeneca, Centocor Inc., GlaxoSmithKline, Lundbeck Research USA, F. Hoffmann-La Roche Ltd., Schering-Plough Research Institute and Wyeth/Pfizer Inc., **Part 4:** Centocor Inc., GlaxoSmithKline, and Schering-Plough Research Institute

Panel

49. Broadening the Trajectories of Risk: Specific and Non-specific Markers of Risk of Psychopathology

49.1 Brain-behavior Measures in Psychosis Spectrum Youths of the Philadelphia Neurodevelopmental Cohort

Raquel E. Gur*

University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania

Background: Across medicine, early identification of disorders is prerequisite for effective prevention and intervention. A rapidly growing literature has examined the clinical course of help-seeking individuals with prodromal presentation and gauged conversion rates to schizophrenia. Overall, the inclusion of brain-behavior measures has added to the prediction of clinical course. There is no population- based study with comprehensive assessment of psychosis spectrum clinical features, neurocognition and structural and functional neuroimaging. The goal of the present study was to examine prospectively a sample of youths who have endorsed psychotic symptoms and evaluate neurocognitive functioning compared to healthy people balanced sociodemographically. We also examined brain structure and function in a subsample that underwent neuroimaging. A longitudinal follow-up will permit determination of clinical phenotype and brain-behavior measures.
Methods: The Philadelphia Neurodevelopmental Cohort includes over 9,000 genotyped youths (age 8–21) who presented to pediatric clinics at Children's Hospital of Philadelphia and consented to follow-up. They had a structured clinical interview, adapted from the K-SADS, supplemented with evaluation of psychosis spectrum symptoms (SIPS, PRIME), enabling identification of at risk status. In addition, a computerized neurocognitive battery (CNB) that evaluated performance (accuracy, speed) on several neurobehavioral domains (executive, episodic memory, complex cognition, emotion processing, sensorimotor) was administered to all participants. A subsample (1,500) underwent neuroimaging (3T Siemens) including sMRI, DTI, fMRI and perfusion. Follow-up of psychosis spectrum (PS, $n = 300$) and typically developing (TD, $n = 200$) individuals at 1–2 years interval included extended comprehensive clinical assessment and repeated CNB and neuroimaging.
Results: About 10% of the sample endorsed psychotic symptoms. The psychosis spectrum group (PS) showed deficits in neurocognitive performance across several domains that were more pronounced for accuracy than for speed. The deficits implicate executive-control, episodic memory and social cognition systems. MRI volumetric analysis indicated overall lower intracranial volume (ICV) in the PS group and after accounting for ICV, reduced volume specific to gray matter in several cortical and subcortical regions. Functional MRI showed reduced resting connectivity and impaired activation for a working memory and emotion identification tasks.
Conclusions: The proportion of individuals who endorsed psychosis spectrum features is similar to that observed in other population–based studies. Notably, the pattern of neurocognitive impairment is similar to that observed in help-seeking youths at clinical risk. Furthermore, the neuroimaging measures also suggest aberrant brain structure and function. Both measures of brain-behavior are similar to the pattern described in schizophrenia and the extent of abnormalities in this sample of youths could predict the extent and severity of psychotic features and functioning.
Disclosure: R. Gur, **Part 4:** Investigator initiated grant from Astra-Zeneca.

49.2 Clinical, Neurobiological and Circadian Correlates of the Onset and Course of Major Mood Disorders: From Childhood Risk to Adolescent-onset and Persistence into Adulthood

Ian Hickie*

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

Background: Major mood disorders have their onset in the post-adolescent and early adult periods. A range of these disorders occur against the background of earlier childhood cognitive or behavioural phenotypes or other clear risk factors. As these disorders often pass through a number of stages before development of the full adult phenotype, we have developed a clinical staging model to describe this process (1). Clinically, we attempt to link these stages also to relevant developmental trajectories (anxiety-depression, circadian-depression or developmental impairment-depression) (2). In parallel epidemiologically-based studies we track the interaction between developmental neurobiology, genetic and environment exposures (eg alcohol and substance misuse) and the mental health phenotypic and disability outcomes
Methods: The clinical and neurobiological correlates of the early phases of the major mood and sleep-wake disorders are tracked in association with young people presenting to

novel clinical services (Headspace centres) (3). Of these, over 2,000 participate in longitudinal clinical studies and 700 are participating in relevant neuropsychological, brain imaging. circadian and metabolic risk factor studies (50% male, mean age = 19 years), Concurrently, we conduct a longitudinal study of adolescent twins ($n = 2459$, mean age = 16 years) and include neuropsychological, brain imaging and circadian measures. Over 900 of these twins (average age 26 years) have now completed long-term phenotypic assessments (4).
Results: In clinical samples, there is provisional support for the validity of both the trajectory model that differentiates the precursor risk phenotypes (anxiety, sleep-wake/circadian, developmental impairment) on the basis of family history, childhood risk patterns, neuropsychological performance and patterns of comorbidity. The staging concept also has provisional support for its validity on the basis of longitudinal course, brain imaging studies, circadian disruption and neuropsychological performance (5,6). In the twin cohort study, the most remarkable findings relate to the very high prevalence of hypomanic syndromes before age 26 (approx. 18&% of the cohort, with five symptoms for several days) and the commonality of brief psychotic symptoms (approx. 8%). Further, sleep-wake cycle phenotypes are predicted by shared genetic characteristics and are associated with the 'atypical' as distinct from more classical 'anxious depression' illness-type. The relationships between early adolescent phenotypes and later phenotypes are being explored, with provisional support for the concept that differentiates atypical from typical depression phenotypes being sustained across this developmental period
Conclusions: These clinical and epidemiological studies provide unique data concerning the relationships between childhood risk phenotypes, the onset and course of depression and other major mood and psychotic disorders and their neurobiological correlates. The data support an increased focus on objective measurement of changes in brain structure, neuropsychological function, circadian and other neurobiological markers.
Disclosure: I. Hickie, **Part 1:** Servier, Astrazeneca, Pfizer, Janseen, Eli lilly, **Part 4:** Servier Laboratories.

49.3 Comorbidity of Medical and Psychiatric Disorders in the Neurodevelopmental Genomics Cohort Study

Kathleen Ries Merikangas*

NIMH, Bethesda, Maryland

Background: An abundance of previous research has investigated associations between physical and mental conditions among children and adolescents. Although results vary depending on the condition of interest, work has generally demonstrated that youth affected with physical conditions are at greater risk for mental health problems relative to youth without such conditions. However, the majority of prior work that has examined associations between physical and mental health conditions has employed fairly small sample sizes, focused on one or a limited number of physical and/or mental health problems, or aggregated many heterogeneous physical conditions together in analyses, and/or failed to control for additional mental health and physical conditions, as well as the severity of physical conditions, in estimates of association.This report presents the first comprehensive assessment of comorbidity of the full range of classes of mental disorders with clinically diagnosed medical condition in a large and ethnically diverse sample of U.S. youth.
Methods: The sample includes 9,014 youth ages 8–21 years selected at random after stratification by sex, age and ethnicity from the Children's Hospital of Philadelphia genomics registry who participated in the University of Pennsylvania Neurodevelopmental Genomics Study. Children were assessed directly by clinically trained interviewers and ancillary information was obtained from a parent or caretaker.
Results: Findings indicate that emotional and behavioral symptoms and disorders, particularly ADHD and behavior disorders, are more common in large pediatric samples than in the general population of youth in the U.S. Youth with medical conditions had a 3-fold increased risk of any psychiatric disorder, and severity of the condition also elevated the risk of psychiatric conditions. Findings revealed both specific patterns of associations between specific classes of mental disorders, as well as general associations between medical conditions that involve the CNS and ADHD and behavior disorders. For example, gastrointestinal ($OR_I = 1.32$, 95%CI = 1.13–1.56) and autoimmune/inflammation diseases OR = 1.23, 95%CI = 1.03–1.46) were significantly associated with mood disorders but not with other mental disorders. By contrast, youth with neurologic/CNS diseases were significantly more likely to experience mood disorders (OR = 1.32, 95% CI = 1.13 – 1.54), ADHD (OR = 1.35, 95% CI = 1.17–1.55) and behavior disorders (OR = 1.37, 95% CI = 1.22–1.55) than those without these conditions. Likewise, developmental disorders were also associated with ADHD, behavior disorders and mood disorders. Birth anomalies were specifically associated with anxiety disorders (OR = 1.26, 95% CI = 1.09–1.46).
Conclusions: These findings have important implications for potential etiologic links between medical and psychiatric disorders. Recent findings regarding CNS-gut interactions suggest potential mechanisms for the links between gastrointestinal disorders and inflammatory conditions with mood disorders. Likewise, there is growing evidence for CNS insults and neurodevelopmental anomalies that may predispose to behavior disorders and ADHD. Finally, the results highlight the importance of comprehensive evaluation of mental disorders in pediatric services.
Disclosure: K. Merikangas, Nothing to Disclose.

49.4 The Study of Developmental Trajectories in Autism Spectrum Disorders; Lessons Learned

Peter Szatmari*

Hospital for Sick Children, Dundas, Ontario, Canada

Background: AutismSpectrum Disorder is a heterogeneous condition characterized by impairments in social-communication and repetitive interests. The presentation varies widely among children with ASD and within the same child over time. The study of this heterogeneity has presented

significant problems when describing the developmental trajectories of the disorder. The objective of this presentation is to illustrate two different approaches to the study of heterogeneous developmental trajectories as a way of understanding the complex interplay of phenotypes over time.
Methods: Multi-method and multi-informant instruments were used to characterize key developmental phenotypes over 4 time points, roughly 6 months apart. The phenotypes studied include language, socialization skills, adaptive functioning and a composite measure of autistic symptoms. Two different approaches were taken to study change over time; one, assumed that developmental trajectories were heterogeneous and the other that trajectories were homogeneous but phenotypes might might interact over time. Phenotypes of autistic symptoms and adaptive functioning were analyzed using semi-parametric group based trajectory analysis. Structural equation modeling allowing for reciprocal interaction was used to study the influence of language and social skills over time.
Results: Adaptive functioning and autistic symptoms showed markedly heterogeneous developmental trajectories with differing predictor and outcome associations. There was little developmental 'yoking' of these phenotypes. In contrast, there was remarkable reciprocal interaction between socialization and language over time with social skills having a greater influence on language than vice versa.
Conclusions: These data illustrate that for ASD, different developmental phenotypes show different developmental trajectories in the preschool period. Phenotypes interact much like risk and protective factors. The study of developmental trajectories provides a rich opportunity to study change over time and appreciate the heterogeneity of these disorders both within and between children.
Disclosure: P. Szatmari, Nothing to Disclose.

Panel

50. Building a More Predictive Mouse: Humanized Mouse Models for Neuropsychiatric Disorders

50.1 Development and Characterization of Mice Humanzied for the COMTval158met Polymorphism

Victoria Risbrough*

University of California, San Diego, La Jolla, California

Background: The COMTval158met polymorphism has been one of the most well studied single nucleotide polymorphisms in neuropsychiatry. This polymorphism is a valine (Val)-tomethionine(Met) substitution in the coding sequence for catechol-o-methyltransferase (COMT) and results in a 40% reduction in COMT enzyme activity in the Met/Met carriers. It has been associated with risk for schizophrenia, OCD, ADHD, PTSD and other disorders, however many of these associations have failed to replicate. COMTval158met has also been shown to modulate many cognitive functions in healthy subjects and patient populations, as well as differential treatment response. Because of the difficulty in isolating the effect of one mutation in genetically heterogonous human populations, animal models can be of great utility to dissect the causal relationship between a given gene mutation and function. Mouse COMT however has differential enzyme activity than human COMT, thus is not an ideal control. Thus we created the first 'humanized' COMTval158 mouse line, which carries only either the human Val or Met version of the gene knocked into the mouse COMT locus. This 'humanized' mouse model will allow direct comparison of the two human alleles on brain development, function, and behavior.
Methods: Here we will present the initial characterization of the COMTval158met mouse line. Based on the reported differences in human Met and Val carriers across COMT enzymatic efficacy, cortical volume, working memory, fear processes, sensorimotor gating, and novelty seeking, we examined these functions using homologous measures across mice homozygous for the COMTval or COMTmet alleles. We examined (1) cortical COMT enzyme efficacy and cortical thickness, (2) spatial working memory via spontaneous alternation, (3) fear processes via Pavlovian fear conditioning and extinction of the freezing response, as well as (4) sensorimotor gating and exploratory behavior.
Results: As has been shown in human cortical tissue, COMT enzyme assay on cortical tissue showed that mice homozygous for COMTval exhibited a 30% reduction in enzyme efficacy compared to mice homozygous for COMTmet. COMTval mice exhibited increased cortical thickness compared to COMTmet mice, potentially mimicking reports of increased grey matter and cortical thickness in human COMTval carriers. COMTval158met had a sex specific effect on sensorimotor gating, with male COMTval mice exhibiting lower prepulse inhibition compared to COMTmet mice, similar to human studies. Conversely, female COMTval mice, however, exhibited higher PPI than female COMTmet mice. COMTmet mice also exhibited small increases in exploratory behavior compared to COMTval mice. The COMTval carriers of both sexes exhibited robust reductions in spatial working memory compared to COMTmet carriers. Previous studies in humans show that tolcapone, a COMT inhibitor, has genotype specific effects on working memory. Our studies in COMTval158met mice mimicked these findings, with Tolcapone reversing the COMTval working memory deficits while attenuating working memory in COMTmet mice. Finally, COMTmet mice exhibited reduced contextual fear but increased cued fear and reduced extinction recall, which may mimic the fear processing deficits described in human COMTmet carriers.
Conclusions: These data indicate that the COMTval158-mouse line may mimic many of the specific phenotypes reported across human COMTval158met genotypes in a number of behavioral domains relevant to neuropsychiatric disorders, including sensorimotor gating, working memory, and fear processes. These mice may thus be a useful model for understanding the developmental effects of the COMT158met mutation on cortical morphology and function, its modulation of behaviors relevant to neuropsychiatric symptoms, and most importantly, how these two alleles may modulate treatment response.

Disclosure: V. Risbrough, **Part 4:** Service contracts with Omeros Pharmaceuticals, Sunovion Pharmaceuticals and Johnson and Johnson.

50.2 DISC1-Boymaw Fusion Gene May Contribute to Major Psychiatric Disorders in the Scottish Schizophrenia Family

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Background: The t(1; 11) translocation appears to be the causal genetic lesion with 70% penetrance for schizophrenia and major depression in a Scottish schizophrenia family. Molecular studies identified the disruption of the DISC1 (disrupted-in-schizophrenia) gene by chromosome translocation at chromosome 1q42. Our previous studies, however, found that the translocation also disrupted another gene, Boymaw, on chromosome 11. After translocation, two fusion genes were generated between the DISC1 and Boymaw genes.
Methods: Expression of full-length DISC1, truncated DISC1, DISC1-Boymaw, and Boymaw-DISC1 proteins were examined in different cell lines. To assess their functions on broad cellular activities, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assays were performed. RNA expression and proteomic analyses were also conducted. Humanized DISC1-Boymaw mice were generated to mimic the chromosome translocation in the Scottish family. Molecular and behavioral analyses were also conducted in humanized DISC1-Boymaw mice.
Results: Expression of the DISC1-Boymaw fusion gene reduces both intracellular NADH oxidoreductase activities as well as synthesis of ribosomal RNA in both *in vitro* cell culture and humanized mouse brains. Molecular studies found reduced expression of GAD67, NMDAR1, and PSD95 proteins. Behavioral analyses revealed abnormal information processing of acoustic startle, exaggerated responses to NMDA receptor antagonist ketamine, as well as depressive-like behaviors in the humanized mice.
Conclusions: Our studies suggest that the DISC1-Boymaw fusion gene is a causal gene mutation in the pathogenesis of major psychiatric disorders in the Scottish DISC1 family. We hypothesize that decreased rRNA synthesis, resulting from expression of the DISC1-Boymaw fusion gene, may reduce protein translation of genes critical in NMDA and GABA neurotransmissions as well as synaptogenesis in the pathogenesis of schizophrenia and major depression.
Disclosure: X. Zhou, Nothing to Disclose.

50.3 Modeling the Contributions of Dopamine to ADHD via a Novel, Knock-in Mouse Model

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Background: Attention-Deficit Hyperactivity Disorder (ADHD) has long been suspected to involve alterations in CNS dopamine (DA) signaling. Human genetic studies have demonstrated association of primary diagnoses and/or treatment response with 3′ noncoding variation in the DA transporter (DAT) gene and changes in DAT levels have been reported via PET methods in unmedicated subjects with an ADHD diagnosis. Additionally, the most common medications used to treat ADHD, amphetamine (AMPH) and methylphenidate, are DAT targeting psychostimulants. Our lab has discovered multiple instances of rare coding variants that impact DAT trafficking and/or function when expressed *in vitro* (Mazei *et al*, 2009; Sakrikar *et al*, 2012). One of these variants, DAT A559V, demonstrates a striking spontaneous efflux phenotype, similar to the effect of amphetamine to produce non-vesicular, DAT-mediated DA efflux, and is not responsive to AMPH to trigger DA release. These studies predict that the subjects bearing the DAT A559V variant may exhibit a spontaneous leak of DA at synapses that could trigger hyperactivity and depresses the dynamic range needed for high-fidelity DA signaling related to cue saliency. To assess the *in vivo* impact of the DAT A559V mutation, we have generated transgenic, knock-in (KI) mice where the A559V variant is expressed by the endogenous *Slc6a3* locus.
Methods: DAT A559V mice were generated by homologous recombination in 129S6 and then bred to wild-type C57BL/6J mice to produce germ-line transmission. Heterozygous offspring were intercrossed to yield all three genotypes (homozygous WT DAT, heterozygous DAT A559V and homozygous DAT A559V on a 129S6/C57BL/6J background). Owing to the bias in diagnoses toward boys vs girls with ADHD, and due to our identification of the DAT A559V variant in two male siblings, we focused our studies on analysis of male, adolescent (6–8) week old males. Animals were housed on a reverse (12:12) light cycle to allow for analysis of animals during their active period. Behavioral, biochemical and pharmacological studies were performed under an animal protocol approved by the Vanderbilt Institutional Animal Care and use Committee (IACUC). All behavioral tests were performed in the Vanderbilt Laboratory for Neurobehavior Core Facility, operated by the Vanderbilt Brain Institute.
Results: DAT A559V heterozygous breeders produced offspring at expected Mendelian ratios, with the exception that male (but not female) animals exhibited a significant underrepresentation of heterozygous and homozygous animals. Biochemical analyses of brain samples revealed no significant differences in DA or DOPAC levels in any brain region, nor were DAT, tyrosine hydroxylase or the density of D1 and D2 DA receptors altered. The three genotypes did not differ in baseline physical characteristics and sensorimotor responses as assessed in the Irwin screen. Heterozygous and homozygous animals exhibited no differences from WT animals in spontaneous locomotion in the Open Field Test (OFT) nor were differences detected in center *vs* surround analyses of time spent in different areas of the chamber. However, we detected a significant, gene-dose dependent impact on motor reactivity in response to experimenter approach, assessed as the speed of movement away from a hand entry into the cage detected by automated video recordings. Although animals were not hyperactive in the OFT, the heterozygous and homozygous DAT A559V mice demonstrated a significantly blunted response to i.p. Injections of AMPH. To explore the basis

for the blunted AMPH behavioral phenotype, we performed *in vitro* DA release studies using superfused, acute striatal slices. These studies revealed that both the heterozygous and homozygous DAT A559V mice exhibit reduced K+ as well as AMPH evoked release of DA. Consistent with a chronic state of DA efflux, we found total insensitivity of DA release to stimulation of presynaptic D2 receptors by the D2 agonist quinpirole. Consistent with these *in vitro* studies, microdialysis of the striatum demonstrated a marked elevation of basal DA levels in homozygous DAT A559V KI mice relative to littermate controls and a significantly blunted (10% of control) ability of i.p. AMPH to produce DA release.
Conclusions: Studies with the DAT A559V mice reveal that the variant imposes a basal elevation in extracellular DA that is consistent with a constitutive, spontaneous DA efflux. Similarly, our studies reveal a loss of AMPH responsiveness in these animals, both neurochemically and behaviorally, and the presence of a context-dependent hyperactivity. These studies, the first to derive from a construct-valid model of ADHD-associated genetic variation in DA signaling pathways, demonstrate *in vivo* phenotypes that argue for a significant opportunity in using the DAT A559V model to elucidate molecular, cellular and circuit level plasticities induced by perturbed DA signaling in ADHD.
Disclosure: M. Hahn, Nothing to Disclose.

50.4 The Tryptophan Hydroxylase Arg439His Knockin (Tph2KI) Mouse: A Naturalistic Model of 5-HT Deficiency

Jacob Jacobsen*

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Background: The tryptophan hydroxylase $^{Arg}439^{His}$ knockin (Tph2KI) mouse carries a single-nucleotide polymorphism in *tph2* homologous to a polymorphism originally discovered in humans. In contrast to mouse models of 5-hydroxytryptamine (5-HT) ablation, the regulation and dynamics of 5-HT neurotransmission is preserved yet overall blunted in the Tph2KI mice. Thus, the Tph2KI mouse may offer a more translational model system for the study of 5-HT deficiency, since complete lack of 5-HT is not known to occur in humans. 5-HT deficiency is associated with suicidality, borderline personality disorder and impulsive aggression in humans.
Methods: Tph2KI mice were generated by homologous recombination and breed via heterozygous crosses. Extracellular 5-HT levels were quantified by microdialysis. Dyadic-testing aggression, tail suspension, light-dark box emergence, novelty suppressed feeding and marble burying were executed using standard protocols. Affiliative behaviors were assessed by co-housing two unfamiliar male mice in a novel environment and scoring the trajectory of agonistic and antagonistic interactions.
Results: The Tph2KI mice presents with 'depression-like' behaviors, impaired affiliative behaviors and increased aggression. The Tph2KI mice recapitulate with high fidelity the biochemical anomalies previously assumed to be 'biomarkers' of central 5-HT deficiency, thereby confirming the *bona fides* of these measures, which have been employed in clinical research for decades. Further, the effect of chronic selective-serotonin reuptake inhibitor (SSRI) treatment on extracellular 5-HT is severely blunted, indicating that the Tph2KI mice may represent a model of SSRI treatment-resistance. Commensurately, behavioral (eg novelty suppressed feeding, marble burying) and neurogenic (eg dentate gyrus neuron proliferation) responses to chronic SSRI treatment are abolished or blunted in the Tph2KI mice.
Conclusions: Our investigations exemplify the advantage of animal models where the deficit in question closely parallels that occurring in humans. The Tph2KI mouse is an excellent tool for the study of 5-HT deficiency, and seemingly also for the study of SSRI treatment-resistance. The latter aspect is key since SSRI treatment-resistant depression, -anxiety and -OCD are large, serious and expensive health care problems. To characterize the impact of naturalistic 5-HT deficiency on antidepressant responses, we will in future studies examine the effects of SSRIs and non-5-HTergic antidepressants in the Tph2KI mice, looking at complementary behavioral, biochemical and gene regulation outcomes. This, in turn, may yield information of the neurobiology of antidepressant treatment-resistance, a poorly studied area.
Disclosure: J. Jacobsen, Nothing to Disclose.

Panel

51. Cognition, Biomarkers, and Longitudinal Outcomes in Geriatric Mood Disorders

51.1 Cognitive Control Network: Motivational Disturbances and Treatment Response of Late Life Depression

George S. Alexopoulos*

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Background: Excessive activation of emotional limbic networks has been hypothesized to mediate the depressive syndrome. Aging preferentially affects cognitive structures. For this reason, we focused on the role of the cognitive control network in late life depression and apathy because it modulates emotional limbic networks.
Methods: We conducted a series of studies utilizing systematic behavioral and cognitive assessment, diffusion tensor imaging, and functional connectivity (FC) at rest and after probing with a response inhibition task in non-demented, non-MCI older adults with major depression.
Results: Depressed older patients had decreased FC at rest in the cognitive control network and increased connectivity in the default mode network (double dissociation) compared to elderly controls. These observations parallel our finding of decreased FC between the dorsal anterior cingulate cortex (dACC) and the dorsolateral prefrontal cortex (DLPFC) in response to incongruent trials relative to congruent trials during performance of a cognitive Stroop task. We also observed that abnormal performance in response inhibition tasks, semantic organization, and dysexecutive behavior predicted poor response to SSRIs. Biological indices of these functions, i.e. microstructural (DTI) abnormalities in white matter connecting cognitive control structures and abnormal resting functional con-

nectivity within the cognitive control network, predicted poor response to SSRIs while connectivity within the default mode network was unrelated to treatment response. In our non-demented, non-MCI depressed subjects, apathy was associated with impaired performance in the Iowa Gambling Task, which assesses the ability to maximize earnings by selecting low-risk, low-reward responses over high-risk, high-reward responses and presumably reflects ventromedial PFC function. Apathetic depressed patients had lower resting FC of the nucleus accumbens with the amygdala, caudate, putamen, globus pallidus, and thalamus and increased FC with the dmPFC, the superior frontal cortex, and the insula than non-apathetic patients. Moreover, apathetic patients had lower FC of the dACC with dorsolateral and ventrolateral prefrontal cortices and higher FC with the insula and the OFC than non-apathetic patients. Persistence of apathy, following SSRI treatment, was significantly correlated with smaller posterior subgenual cingulate gray matter volume and lower uncinate fasciculus white matter integrity. These structural abnormalities may interfere with prefrontal-limbic communication needed for motivated behavior and impede adequate treatment response of apathy.
Conclusions: Behavioral, structural and functional abnormalities of the cognitive control network distinguish depressed older patients from normal controls and are associated with poor treatment response of depressive symptoms and apathy.
Disclosure: G. Alexopoulos, **Part 1:** I have a research grant from Forest; have consulted for Hoffman-LaRoche, Janssen, Lilly, Navidea, Pfizer, and Otsuka; and have been on speakers' bureaus for Astra Zeneca, Avanir, Merck, Novartis, and Sunovion., **Part 2:** Astra Zeneca, Forest, Merck, Sunovion, **Part 4:** I have received grant support from Forest.

51.2 Combination of Methylpehnidate with Citalopram is Superior to Either Drug Alone in Improving Clinical and Cognitive Outcomes in Geriatric Depression

Helen Lavretsky*

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Background: Enhanced and accelerated antidepressant treatment response may be particularly beneficial for older patients given low remission rates and high rates of suicidal ideations and cognitive impairment, yet there are few data to inform clinical practice. We evaluated the potential of methylphenidate to accelerate and enhance antidepressant response to citalopram in elderly depressed patients with respect to clinical and cognitive outcomes.
Methods: 133 elderly participants with major depression were treated in a 16-week double-blind placebo controlled trial of methylphenidate (MPH) augmentation of citalopram (CIT) ($N=43$) compared to CIT and placebo (PBO) ($N=45$) and MPH and PBO ($N=45$). We compared differences on remission rates, change scores in depression and measures of apathy, anxiety, health related quality of life, and cognitive measures using multivariate analyses and mixed regression models.
Results: The three groups did not differ significantly in any of the demographic measures at baseline. All 3 groups showed significant changes in the severity of depression as measured by the Hamilton Depression Rating scale (HAM-D) and Montgomery-Asberg Depression rating scale (MADRS)(p
Conclusions: Combined treatment with citalopram and methylphenidatedemonstrated an improved response profile in the mood compared to either drug alone, and appears to be a viable option for accelerating and enhancing antidepressant response in elderly depressed patients that may be limited by individual tolerability of the side-effects.
Disclosure: H. Lavretsky, **Part 1:** Rsearch grants from Forest Research Institute and Alzheimer's Research and Prevention foundation, **Part 2:** None, **Part 3:** None, **Part 4:** Forest Research Institute- research grants.

51.3 Late-life Depression May Increase Risk of Dementia but Does Not Increase Risk of Developing Mild Cognitive Impairment

Meryl Butters*

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Background: Community-based epidemiologic and case-control studies suggest an association between late-life depression and persistent as well as progressive cognitive impairment, and subsequent dementia. If it is true that a history of major depression increases risk of developing cognitive impairment and dementia, then preventive and aggressive early intervention for major depression may reduce future rates of dementia in the population. In a prospective study, we aimed to determine whether cognitively normal individuals with a history of late-life major depression develop Mild Cognitive Impairment (MCI; an intermediate state between normal cognitive function and dementia) at a faster rate than never-depressed individuals.
Methods: A total of 192 cognitively normal participants (114 with late-life depression and 78 never-depressed) age 69 + underwent clinical and neuropsychological assessment annually for up to 15 years. Using all available data we conferred cognitive diagnoses at every assessment time point. Cox proportional-hazards models were used to examine the association between history of depression and time to MCI both with and without adjustment for potential confounders, including age, education, gender and cerebrovascular risk factors.
Results: The mean follow up period was 4.34 (+/-3.23) years and 63/192 participants developed incident MCI. Participants with late-life depression and never-depressed individuals did not significantly differ with respect to the incidence of, and time to MCI. Both groups showed similar incidence: 33.33% (38/114) *vs* 32.05% (25/78) and median time to MCI: 6.33 years *vs* 9.14, respectively (HR = 1.32; 95%CI = 0.79–2.20, $p=0.29$). Multivariate modeling adjusting for age, education, gender and cerebrovascular risk factors, showed that age (HR = 1.12; 95%CI = 1.07–1.18, p
Conclusions: While late-life depression may increase risk of dementia, we found no independent association with

increased risk for MCI. Prospective studies documenting rate of progressive decline from normal cognitive function to MCI and then to dementia are required to definitively determine whether depression increases risk of future dementia.
Disclosure: B. Meryl, Nothing to Disclose.

51.4 Longitudnial BDNF Levels in Both an Elderly Cohort and an Inflammatory Cytokine-exposed Cohort: Risk for Cognitive Deficits

Francis E. Lotrich*

Western Psychiatric Institute and Clinics, Pittsburgh, Pennsylvania

Background: BDNF levels have been cross-sectionally associated with a variety of mental health problems including mood disorders and cognitive deficits. Longitudinal changes have been less well delineated. In medicine, longitudinal changes in biomarkers have been used to track risk for vascular disease and cancer. This method may be of use for elderly at risk for depression and dementia. We thus followed and compared two distinct non-depressed cohorts that were at risk for both depression and worsening cognition.
Methods: The first cohort was a group of non-depressed subjects receiving interferon-alpha (IFN) as therapy for hepatitis C ($n = 127$; median age 50 years), many of whom develop cognitive complaints and/or depression during IFN treatment. The second was a group of non-depressed elderly ($n = 82$), some with depression in remission and normal cognitive function ($n = 39$), some with remitted depression but mild cognitive impairment (MCI) ($n = 22$), and never depressed controls ($n = 21$). In these cohorts, BDNF levels were examined using ELISA. Repeated-measure mixed-effect analyses were used to examined leves and symptoms over time.
Results: In the IFN cohort with baseline serum BDNF $>$ 17 ng/mL, 90% had no cognitive complaints, but this decreased to 56% by month three of IFN injections. In those with 0.4). In the never-depressed elderly control group, serum BDNF went from 18.1 to 14.8 ng/mL by year 2, despite not developing depression. In those with co-existing MCI, BDNF started at 14.0 and decreased to 11.7 ng/mL. However, the remitted MDD patients without MCI went from 16.5 to only 16.3 ng/mL. Thus, over a 2 year period, BDNF levels decreased in the elderly cohort—but not in remitted patients who did not already have nor develop MCI.
Conclusions: In both the inflammatory cytokine-exposed cohort and the elderly cohort, low BDNF at baseline was most notably associated with current cognitive deficits. In prior work, we have observed that the Met/Val allele for BDNF is specifically associated with a subset of cognitively related depression symptoms during IFN therapy. In both cohorts (one exposed to an exogenous inflammatory cytokine, the other to natural aging) BDNF decreases over time – although not in older remitted MDD subjects who remain without cognitive problems. These data suggest that lower BDNF enhances vulnerability for subsequent development of cognitive impairments, rather than depression *per se*.
Disclosure: F. Lotrich, Nothing to Disclose.

Panel

52. Experimental Therapeutics and Drug Development Targeting Inflammation in Developmental Disorders

52.1 Gastrointestinal Symptoms in a Mouse Model of an Environmental Risk Factor for Autism and Schizophrenia

Paul H. Patterson*

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Background: While autism is a neurodevelopmental disorder characterized by language and social deficits, recent studies have highlighted striking dysregulation in the neural, peripheral, and enteric immune systems of autistic individuals. There are also reports that subsets of children with autism spectrum disorder (ASD) display gastrointestinal (GI) abnormalities, including increased intestinal permeability and altered composition of GI microbiota. To explore the potential connections between GI problems and the brain and behavior, we use a mouse model of an ASD risk factor, maternal immune activation (MIA). We also tested the efficacy of probiotic treatment in MIA offspring that display the cardinal ASD behaviors and neuropathology.
Methods: Pregnant mice are injected with the immune-activating dsRNA poly(I:C) or saline on E12.5. Offspring are fed the probiotic bacteria, *Bacteroides fragilis*, at weaning for one week. Young and adult offspring are assessed for (i) intestinal barrier integrity by measuring leakage of FITC-dextran through the intestinal epithelium and tight junction expression, (ii) GI inflammation by cytokine Luminex array and histology, (iii) serum metabolome profiles by GC-MS and LC-MS.
Results: MIA offspring display decreased intestinal barrier integrity and corresponding changes in levels of tight junction proteins. These symptoms are associated with altered expression of colon cytokines and changes in serum metabolite levels. Postnatal *B. fragilis* treatment ameliorates these GI abnormalities, and normalizes certain serum metabolites and many ASD-related behaviors.
Conclusions: These studies highlight the potential relevance of the gut-brain axis for ASD, where manipulation of the intestinal microbiome can influence GI physiology and behavioral performance. The results raise the possibility of testing a probiotic therapy in individuals with autism and co-morbid GI symptoms. Moreover, the altered serum metabolite profiles in the MIA mouse model raise the possibility of testing particular metabolites as candidate biomarkers for subsets of human ASD.
Disclosure: P. Patterson, Nothing to Disclose.

52.2 Immune System as a Target for Therapeutic Intervention in Neurodevelopmental Disorders: Lessons from the Rett Syndrome

Jonathan Kipnis*

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Background: Glial and immune participation in central nervous system function is becoming increasingly recog-

nized as essential. Although Rett syndrome has been considered to be an exclusively neuronal disease, a key role for astroglia in Rett pathology was recently shown. Moreover, our group recently found that microglia (and to some extent peripheral myeloid immune cells) are also critical players in Rett pathology.
Methods: We use mouse models of Rett syndrome.
Results: Our results demonstrate that expression of wild type Mecp2 protein in myeloid cells on otherwise knockout background arrests disease progression. Mice with wild type myeloid cells exhibit normalized breathing patterns, significantly reduced apneas, normalized body weight and brain size, and improved open-field activity. We found an unexpected role of Mecp2 in myeloid cell differentiation, including tissue resident macrophages and microglia and the role of Mecp2 in macrophage activation and function. Our recent results also indicate that microglia and peripheral immunity are involved in pathology and repair of Mecp2-triplication syndrome.
Conclusions: Our data unexpectedly implicate Mecp2 in myeloid cell differentiation and function and in turn the role of myeloid cells in Rett and duplication/triplication pathology, and suggest that these immune cells might offer a feasible target for future therapeutic intervention for this devastating disease.
Disclosure: J. Kipnis, Nothing to Disclose.

52.3 PET Imaging of Microglial Activation in Young Adults with Autism Spectrum Disorder

Kazuhiko Nakamura*

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Background: Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders characterized by pervasive abnormalities in social interaction and communication and by repetitive and restricted behavioral patterns and interests. A growing body of evidence suggests that aberrant immunologic systems underlie the pathophysiologic characteristics of ASD. However, to our knowledge, no information is available on the patterns of distribution of microglial activation in the brain in ASD. To identify brain regions associated with excessively activated microglia in the whole brain, and to examine similarities in the pattern of distribution of activated microglia in subjects with ASD and control subjects.
Methods: Twenty men with ASD (age range, 18–31 years; mean [SD] IQ, 95.9 [16.7]) and 20 age- and IQ-matched healthy men as controls participated in this study. Diagnosis of ASD was made in accordance with the Autism Diagnostic Observation Schedule and the Autism Diagnostic Interview-Revised. Using positron emission tomography and a radiotracer for microglia–[11C](R)-(1-[2-chrorophynyl]-N-methyl-N-[1-methylpropyl]-3 isoquinoline carboxamide) ([11C](R)-PK11195), we determined regional brain [11C](R)-PK11195 binding potential as a representative measure of microglial activation.
Results: The [11C](R)-PK11195 binding potential values were significantly higher in multiple brain regions in young adults with ASD compared with those of controls ($P<0.05$, corrected). Brain regions with increased binding potentials included the cerebellum, midbrain, pons, fusiform gyri, and the anterior cingulate and orbitofrontal cortices. The most prominent increase was observed in the cerebellum. The pattern of distribution of [11C](R)-PK11195 binding potential values in these brain regions of ASD and control subjects was similar, whereas the magnitude of the [11C](R)-PK11195 binding potential in the ASD group was greater than that of controls in all regions.
Conclusions: Our results indicate excessive microglial activation in multiple brain regions in young adult subjects with ASD. The similar distribution pattern of regional microglial activity in the ASD and control groups may indicate augmented but not altered microglial activation in the brain in the subjects with ASD.
Disclosure: K. Nakamura, Nothing to Disclose.

52.4 Translational Experimental Therapeutics of Inflammation and Fever in Autism Spectrum Disorder: Hot Tubs, Locus Coeruleus Modulation and Helminth Therapy

Eric Hollander*

Albert Einstein College of Medicine, Bronx, New York

Background: Approximately one third of patients with Autism Spectrum Disorder (ASD) have a history of clinical improvement (improved social reciprocity, eye gaze, hyperactivity and communication) in response to a fever. One hypothesis explaining the fever response in ASD suggests that elevated maternal stress during pregnancy increases cortisol release resulting in a developmental maturational hypofunctioning of the locus coeruleus norepinephrine system (LC–NE). Another hypothesis suggests that maternal immune activation during pregnancy results in inflammation in the neonate which may be associated with developmental and behavioral abnormailities. We examined these observations and tested these hypotheses with three sets of clinical studies to determine whether improvement with fever in ASD is 1) a direct effect of increased temperature, 2) a result of activating a hypofunctioning LC-NE system, or 3) a result of modulating an overactive immune-inflammatory system via altering the microbiome.
Methods: Study 1: Fifteen children with ASD had clinical measures (Social Responsivenes Scale (SRS), Clinical Global Impression-Improvement Scale (CGI-I), pupillary biomarkers, and gene expression profiles collected during a hot tub study which compared responses during separate days at 102 degrees vs 98 degrees Farenheight. Study 2: Thirty four high functioning adults with ASD participated in a 12 week randomized parallel design trial of the NE reuptake inhibitor milnacipran vs placebo with outcome measures of attention, executive function, and motor inhibition.Study 3: Ten high functioning adults with ASD participated in a randomized crossover trial of 12 weeks of the helminth trichura suis ova (TSO) vs 12 weeks of placebo, with a 4 week washout period in between active phases. Outcome measures including repetitive (YBOCS, RBS-R) and disruptive (ABC-I) behaviors and cytokine profiles.

Results: Study 1: Children with ASD and a history of fever response had improvement on measures of social cognition and of global improvement on the 102 degree hot tub day vs the 98 degree day. Pupillometry and gene expression measures were associated in part with clinical change.Study 2: Adults with ASD on milnacipran vs placebo had improvement on the Connors Scale measures of Inattention and Impulsivity symptoms, on global improvement measures(CGI-I) and on executive function.Study 3: Adults with ASD on 12 weeks of the helminth TSO vs placebo had improvement on repetitive behaviors measured by the YBOCS-compulsion subscale and the RBS-R ritualistic behaviors scale, and on global improvement measures (CGI-I).
Conclusions: We prospectively demonstrated that children with a history of fever response had improvement in clinical measures and biomarkers as a direct result of temperature elevation. It remains unclear whether this response can be attributed to CNS thermolabile enzymes that effect gene-expression. This fever effect could be due to enhancement of a hypofunctioning LC system, since the NE reuptake inhibitor milnacipran resulted in improvement of attention, impulsivity and executive function. Altering the microbiome via administration of the helminth TSO was associated with improvement in rigidity and its resulting disruptive behaviors, which is consistent with the notion that inflammatory mechanisms may play a role in the pathophysiology of ASD and be a target for experimental therapeutics in ASD.
Disclosure: E. Hollander, **Part 1:** research grants: Simons Foundation, Roche, Transcept, Forest, Coronado Biosciences, consultant: Roche, Coronado Biosciences, **Part 4:** research grants: Simons Foundation, Roche, Transcept, Forest, Coronado Biosciences,

Panel

53. Melatonin and Its Receptors: Important Players in Major Depressive Disorder

53.1 A Pilot, Placebo-controlled Study of Buspirone Plus Melatonin in Major Depressive Disorder

Maurizio Fava*

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Background: We recently conducted a pilot of the combination of buspirone and melatonin. This combination had displayed antidepressant activity across *in vitro* neurogenesis-based human neural stem cell (hNSCs) assays and rodent *in vivo* behavioral assays, whereas neither buspirone nor melatonin alone showed any antidepressant-like profile in these assays. After evaluating numerous combination ratios, we determined that low dose buspirone 15 mg combined with melatonin-SR 3 mg yielded optimal antidepressant efficacy in our pre-clinical platform. The low dose of buspirone suggested that antidepressant efficacy might be achieved with only minimal adverse event liability.
Methods: Based on these data, we conducted an exploratory 6-week, multi-center, double-blind, randomized, placebo- and comparator-controlled study of the combination of buspirone and melatonin in subjects with acute Major Depressive Disorder (MDD). The study inlcuded the use of several measures (Clinical Global Impression of Severity and Improvement, Inventory of Depressive Symptomatology) of depressive symptoms.
Results: The combination treatment revealed a significant antidepressant response in subjects with MDD on several measures (Clinical Global Impression of Severity and Improvement, Inventory of Depressive Symptomatology) compared to either placebo or buspirone 15 mg monotherapy.
Conclusions: These preliminary findings have clinical implications and suggest that the combination of buspirone and melatonin may have antidepressant properties in MDD.
Disclosure: M. Fava, **Part 1:** Advisory/Consulting: Abbott Laboratories; Affectis Pharmaceuticals AG; Alkermes, Inc.; Amarin Pharma Inc.; Aspect Medical Systems; AstraZeneca; Auspex Pharmaceuticals; Bayer AG; Best Practice Project Management, Inc.; BioMarin Pharmaceuticals, Inc.; Biovail Corporation; BrainCells Inc; Bristol-Myers Squibb; CeNeRx BioPharma; Cephalon, Inc.; CNS Response, Inc.; Compellis Pharmaceuticals; Cypress Pharmaceutical, Inc.; DiagnoSearch Life Sciences (P) Ltd.; Dinippon Sumitomo Pharma Co. Inc.; Dov Pharmaceuticals, Inc.; Edgemont Pharmaceuticals, Inc.; Eisai Inc.; Eli Lilly and Company; EnVivo Pharmaceuticals, Inc.; ePharmaSolutions; EPIX Pharmaceuticals, Inc.; Euthymics Bioscience, Inc.; Fabre-Kramer Pharmaceuticals, Inc.; Forest Pharmaceuticals, Inc.; GenOmind, LLC; GlaxoSmithKline; Grunenthal GmbH; i3 Innovus/Ingenis; Janssen Pharmaceutica; Jazz Pharmaceuticals, Inc.; Johnson & Johnson Pharmaceutical Research & Development, LLC; Knoll Pharmaceuticals Corp.; Labopharm Inc.; Lorex Pharmaceuticals; Lundbeck Inc.; MedAvante, Inc.; Merck & Co., Inc.; MSI Methylation Sciences, Inc.; Naurex, Inc.; Neuralstem, Inc.; Neuronetics, Inc.; NextWave Pharmaceuticals; Novartis AG; NuPathe;Nutrition 21; Orexigen Therapeutics, Inc.; Organon Pharmaceuticals; Otsuka Pharmaceuticals; Pamlab, LLC.; Pfizer Inc.; PharmaStar; Pharmavite® LLC.; PharmoRx Therapeutics; Precision Human Biolaboratory; Prexa Pharmaceuticals, Inc.; Puretech Ventures; PsychoGenics; Psylin Neurosciences, Inc.; Rexahn Pharmaceuticals, Inc.; Ridge Diagnostics, Inc.; Roche; Sanofi-Aventis US LLC.; Sepracor Inc.; Servier Laboratories; Schering-Plough Corporation; Solvay Pharmaceuticals, Inc.; Somaxon Pharmaceuticals, Inc.; Somerset Pharmaceuticals, Inc.; Sunovion Pharmaceuticals; Supernus Pharmaceuticals, Inc.; Synthelabo; Takeda Pharmaceutical Company Limited; Tal Medical, Inc.; Tetragenex Pharmaceuticals, Inc.; Teva; TransForm Pharmaceuticals, Inc.; Transcept Pharmaceuticals, Inc.; Vanda Pharmaceuticals, Inc.; Speaking/Publishing: Adamed, Co; Advanced Meeting Partners; American Psychiatric Association; American Society of Clinical Psychopharmacology; AstraZeneca; Belvoir Media Group; Boehringer Ingelheim GmbH; Bristol-Myers Squibb; Cephalon, Inc.; CME Institute/Physicians Postgraduate Press, Inc.; Eli Lilly and Company; Forest Pharmaceuticals, Inc.; GlaxoSmithKline; Imedex, LLC; MGH Psychiatry Academy/Primedia; MGH Psychiatry Academy/Reed Elsevier; Novartis AG; Organon Pharmaceuticals; Pfizer Inc.; PharmaStar; United BioSource,Corp.; Wyeth-Ayerst Laboratories; Equity Holdings: Compellis; PsyBrain, Inc.; Royalty/patent, other income: Patent for Sequential Parallel Comparison Design (SPCD), which are licensed by MGH to RCT Logic, LLC; and patent application for a

combination of azapirones and bupropion in Major Depressive Disorder (MDD).; Copyright for the MGH Cognitive & Physical Functioning Questionnaire (CPFQ), Sexual Functioning Inventory (SFI), Antidepressant Treatment Response Questionnaire (ATRQ), Discontinuation-Emergent Signs & Symptoms (DESS), and SAFER; Lippincott, Williams & Wilkins; Wolkers Kluwer; World Scientific Publishing Co. Pte.Ltd. , **Part 2:** Belvoir Media Group for editing a newsletter: 2011-$12,000., **Part 4:** Abbott Laboratories; Alkermes, Inc.; Aspect Medical Systems; AstraZeneca; BioResearch; BrainCells Inc.; Bristol-Myers Squibb; CeNeRx BioPharma; Cephalon, Inc.; Clintara, LLC; Covance; Covidien; Eli Lilly and Company; EnVivo Pharmaceuticals, Inc.; Euthymics Bioscience, Inc.; Forest Pharmaceuticals, Inc.; Ganeden Biotech, Inc.; GlaxoSmithKline; Icon Clinical Research; i3 Innovus/Ingenix; Johnson & Johnson Pharmaceutical Research & Development; Lichtwer Pharma GmbH; Lorex Pharmaceuticals; NARSAD; NCCAM; NIDA; NIMH; Novartis AG; Organon Pharmaceuticals; PamLab, LLC.; Pfizer Inc.; Pharmavite® LLC; Photothera; Roche; RCT Logic, LLC (formerly Clinical Trials Solutions, LLC); Sanofi-Aventis US LLC; Shire; Solvay Pharmaceuticals, Inc.; Synthelabo; Wyeth-Ayerst Laboratories.

53.2 Impact of Hippocampal Neurogenesis on Cognition and Mood

Rene Hen*

Columbia University, New York, New York

Background: Adult hippocampal neurogenesis is a unique form of plasticity that generates new neurons in the dentate gyrus throughout life. Adult-born neurons have been implicated in both cognitive functions and in mediating the behavioral effects of antidepressants. However, it is not known whether stimulation of adult hippocampal neurogenesis is sufficient to improve cognition and mood.
Methods: Here we use a combination of pharmacological and optogenetic approaches in mice to explore the impact of adult hippocampal neurogenesis on cognitive functions such as pattern separation and generalization and on mood and anxiety-related behaviors. Pharmacological manipulations include compounds that stimulate neurogenesis and target melatonin and serotonin receptors such as agomelatine as well as compounds that target apoptotic cell death such as Bax antagonists. Opogenetic approaches allow us to manipulate young adult-born granule cells specifically in the dorsal or the ventral hippocampus.
Results: We show that both pharmacological and optogenetic approaches aimed at stimulating neurogenesis result in improved pattern separation in contextual fear discrimination tasks. Furthermore we show that young adult-born granule cells modulate the activity of mature granule cells in the dentate gyrus. Finally we show that an optogenetic stimulation of mature granule cells in the ventral dentate gyrus results in a decrease in anxiety-related behaviors.
Conclusions: Our findings indicate that hippocampal neurogenesis impacts both pattern separation and anxiety-related behaviors. These results suggest that strategies aimed at stimulating neurogenesis may be useful to treat anxious or depressed patients who display excessive generalization. Compounds such as agomelatine that stimulate neurogenesis preferentially in the ventral dentate gyrus may be particularly effective treatments.
Disclosure: R. Hen, **Part 1:** Serve on Scientific Advisory Boards for Roche Pharmaceuticals, Lundbeck and Servier.

53.3 Interactions between Melatonin and 5-HT Receptors to Enhance Monoaminergic Transmission in the Rat Brain

Pierre Blier*

University of Ottawa, Ottawa, Ontario, Canada

Background: Although ome of the signs and symptoms of major depressive disorder (MDD) can be alleviated by melatonin, it is not an antidepressant. In contrast, the melatonin type1 and 2 receptor agonist and 5-$HT_{2C/2B}$ receptor agonist agomelatine has been established as an antidepressant in placebo-controlled trials and active comparator studies. The present studies were designed to assess the possible contribution of the activation of melatonin receptors and the antagonism of 5-HT_{2C} and 5-HT_{2B} receptors in modyfying monoaminergic transmission in the rat brain using electrophysiological techniques.
Methods: Male Sprague Dawley were given either agomelatine or melatonin (40 mg/kg ip at 17:00), and/or the 5-HT_{2C} antagonist SB-242084 and the 5-HT_{2B} antagonist LY 266097 (both 2 mg/kg ip at 17:00) daily for 2 or 14 days. Recordings of 5-HT, norepinephrine (NE), dopamine (DA), or CA3 hippocampus pyramidal neurons were obtained under chloral hydrate anesthesia the morning after the last injection.
Results: Two days of agomelatonine enhanced the number of spontaneously active DA neurons in the ventral tegmental area without changing their firing rate or pattern. After 14 days, there were still more DA neurons firing spontaneously and their firing rate as well as their bursting activity were markedly enhanced. These effects were blocked by a melatonin antagonist.Only at this time, the firing rate of 5-HT neurons was marked increased; it was reversed by acutely injecting a DA2 antagonist. The enhanced firing of 5-HT neurons led to a net increase in 5-HT_{1A} transmission. The enhancing action of agomelatine on DA and 5-HT neuronal firing could only be reproduced by administering melatonin with the 5-HT_{2C} and the 5-HT_{2C} antagonist. There were no sustained effects on the activity of NE neurons.
Conclusions: In summary, repeated administration of agomelatine resuls in an enhancement of both DA transmission, at least on 5-HT neurons, and on 5-HT on pyramidal neurons of the hippocampus. The clear antidepressant effect of agomelatine may be attributable to a synergy between the potent activation of melatonin receptors combined the effective blockade of 5-HT_{2C} and the 5-HT_{2C} receptors.

Disclosure: P. Blier, **Part 1:** I participated in advisory boards, gave presentations, and/or received research grants (without a salary portion and administered by my institution) from Astra Zeneca, Bristol Myers Squibb, Eli Lilly, Forest, Lundbeck, Merck, Otsuka, Pfizer, Roche, Takeda, and Servier.

53.4 Melatonin-Mediated Potentiation of Physical Activity-induced Neurogenesis in the Dentate Gyrus of the C3H/HeN Mouse

Margarita L. Dubocovich*

University at Buffalo, Buffalo, New York

Background: Major depressive disorders are characterized by a constellation of symptoms that affect mood, neurochemical balance, sleep patterns, circadian and/or seasonal rhythm entrainment, and promotes anxiety, and neuronal atrophy. Melatonin through activation of MT_1 and/or MT_2 melatonin receptors is known to modulate responses alter in depression, however it does not exert clear antidepressant activity in humans. Voluntary running wheel activity induces antidepressant like activity and promotes neurogenesis in the mouse hippocampus (Van Pragg *et al*., 1999). Melatonin increases neurogenesis (Ramirez-Rodriguez *et al*., 2009) and displays antidepressant-like effect in mouse models of mild stress [Detanico *et al*., 2009]. The goal of this study was to test the effect of chronic melatonin treatment on running wheel activity-induced cell proliferation and cell survival in the dentate gyrus of the C3H/HeH mice.
Methods: To assess the role of melatonin treatment on running wheel (RW) induced cell proliferation and survival, single housed C3H/HeN mice (3–4 month old) in a 12/12 hr light/dark cycle with access to fixed wheels (FW) or RW were treated with vehicle (VEH: 0.01% ethanol) or melatonin: (0.02 mg/ml in VEH) via drinking water ad libitum for 12 days. Six injections of bromodeoxyuridine (BrdU 75 mg/kg, ip) are given at 12 hr intervals started on day 9. Brains were fixed and collected on day 12 to assess alterations in cell proliferation or on day 40 to determine cell survival. Coronal brain sections (50 um) from each mouse were stained with BrdU antibody. Cell proliferation and survival were determined by the mean number of BrdU-labeled cells in the inner granular cell layer across six sections of dentate gyrus.
Results: RW activity increased cell proliferation and survival in the dorsal dentate gyrus (DG) (66.6 ± 4.4, $n = 9$, p
Conclusions: Our data indicate a unique role for melatonin in potentiating the survival of new hippocampal cell whose formation is induced by RW activity. This capacity of melatonin is selective for the survival phase of neurogenesis because no effect was observed in the cell proliferation phase. Together these results suggest a potential new paradigm to promote hippocampal neurogenesis in depression by combining melatonin treatment with physical activity. *Supported by MH42922.*
Disclosure: M. Dubocovich, **Part 1:** Takeda Pharmaceuticals North America Inc.

Panel

54. Molecular Regulation and Clinical Applications of Phosphodiesterase 4, the Major Enzyme for Degrading cAMP

54.1 Binding of 11C-(R)-rolipram to Phosphodiesterase 4 is Downregulated in Major Depressive Disorder and Normalized with Antidepressant Treatment

Robert Innis*

NIMH, Bethesda, Maryland

Background: Rat and human postmortem studies suggest that the cAMP cascade is downregulated in major depressive disorder (MDD) and that antidepressant treatment normalizes this downregulation. The efficacy of experimental antidepressants such as rolipram may depend on the regulatory state of its target, PDE4. To better understand how negative mood and drug therapy affect cAMP metabolism, we used ligand-based PET imaging to assess PDE4–^{11}C-(*R*)-Rolipram binding in healthy controls and unmedicated MDD patients. We also compared Rolipram binding before and after selective serotonin-reuptake inhibitor (SSRI) therapy in MDD patients.
Methods: Thirty five healthy controls (36 ± 11 years; 11 F/24 M) and 35 unmedicated patients (37 ± 11 years; 11 F/24 M) were studied. Seventeen of the MDD patients had repeat scans $\sim$8 weeks after SSRI treatment. Eleven healthy controls had repeat scan without SSRI. Total distribution volume normalized to plasma free fraction, V_T/f_P, of ^{11}C-(*R*)-rolipram, was measured in 10 brain regions using an unconstrained two-compartment model and metabolite-corrected arterial input function.
Results: Unmedicated patients showed lower ^{11}C-(*R*)-rolipram binding than controls in all regions with an average change of 17% ($p = 0.005$). Seventeen patients who had two scans showed an increase of $+15 \pm 40$% after SSRI treatment across brain regions ($p = 0.004$ when age was used as a covariate), while 11 controls showed similar binding in two scans with changes of only -2 ± 13%. The change in rolipram binding after SSRI varied markedly among patients. Those patients with lower rolipram binding before SSRI showed significantly greater increase after therapy ($p < 0.02$ in all regions) indicating normalization of rolipram binding by treatment. Older patients also showed greater increase in rolipram binding after SSRI (p 0.001). Eight male patients who were older than 30 years of age showed a significant correlation between symptom improvement and the SSRI-induced increase in rolipram binding in putamen, medial temporal, and occipital cortices ($p < 0.04$).
Conclusions: The cAMP cascade, as indirectly measured with binding of ^{11}C-(*R*)-rolipram, is downregulated in unmedicated patients with MDD, and antidepressant treatment normalizes this downregulation. These studies suggest that inhibition of PDE4, perhaps via subtype selective agents, might again be assessed for efficacy in MDD.
Disclosure: R. Innis, **Part 1:** Eli Lilly has provide funds to NIMH to support my research., **Part 4:** Eli Lilly has provide funds to NIMH to support my research.

54.2 Control of Mood by Selective Potentiation of cAMP Signaling in Ventral Striatum

James A. Bibb*

The University of Texas Southwestern University, Dallas, Texas

Background: Current antidepressant medications have limited efficacy and long therapeutic delays. Therefore, a better understanding of the molecular mechanisms and neuronal circuitry underlying the pathophysiology of depression is needed to identify new targets and develop more effective treatments. We discovered regulation of PDE4 by Cdk5 and used cell-type specific knockout and novel small interfering peptides to explore the circuitry and validity of these novel signaling mechanisms as potential therapeutic targets
Methods: *In vitro* biochemistry was used to study PDE4 phosphorylation by PKA, MAPK, and Cdk5. Phosphorylation state-specific antibodies were used to characterize these *in vivo*. Conditional Cdk5 knockout (KO) throughout the brain or only in D1- or D2 dopamine receptor-positive neurons was used to characterize Cdk5's role in regulating mood in mesocorticolimbic circuitry. Behavioral models of acute and chronic stress were used as indications of the antidepressant effects of small interfering peptides designed to selectively target PDE4 activation.
Results: Here, we report that cyclin-dependent protein kinase 5 (Cdk5) controls cAMP degradation via phosphodiesterase-4 (PDE4). We implicate this novel molecular mechanism in depression-like behavior. We show that phosphorylation of PDE4 by Cdk5 contributes to its activation, thereby controlling cAMP signaling. Consequently, loss of Cdk5 in mouse striatal neurons disrupted this regulatory mechanism and caused elevated cAMP levels, increased cAMP-dependent protein kinase (PKA) activity, and antidepressant-like behavior. Finally, small drug-like interfering peptides that selectively target this mechanism acted as antidepressants when infused into ventral striatum.
Conclusions: Our results demonstrate that cAMP signaling in medium spiny neurons of ventral striatum controls mood and suggest that disruption of Cdk5/PKA-dependent PDE4 activation may provide an effective treatment strategy for depression and other psychiatric disorders.
Disclosure: J. Bibb, Nothing to Disclose.

54.3 PDE4 in Huntington's Disease: Pathology of Cross-seeding of Huntingtin and Amyloidogenic DISC1

Koko Ishizuka*

Johns Hopkins University, Baltimore, Maryland

Background: Huntington's disease (HD) is characterized by a triad of motor, cognitive, and psychiatric symptoms. Identification of the causal gene, *Huntingtin (Htt)*, has helped to build etiology-relevant mouse models of HD and facilitate understanding of molecular pathways that mediate the disease pathology. With these pathologies as hallmarks, animal models are now being used to screen compounds that may ameliorate them. An underemphasized research area for HD thus far is study of mediators responsible for psychiatric symptoms. Recent publications have indicated that deficits of PDE4 exist in HD and a PDE4 inhibitor ameliorates the pathology of HD in an animal model: nonetheless, the underlying mechanism is unclear.
Methods: PDE4 enzymatic activity was measured by a fluorescence polarization assay *in vitro* and *in vivo*. Biochemical, biophysical, and immunohistochemical approaches were applied to cell/mouse models and patient autopsied brains of HD to study protein interactions of PDE4, DISC1, and Htt as a mechanism underlying the augmented PDE4 enzymatic activity in HD.
Results: We found a ternary protein complex of PDE4B, DISC1, and Htt. In HD, amyloidogenic DISC1 was selectively sequestrated into Htt aggregates, which in turn released PDE4B from the ternary protein complex and augmented cellular activity of PDE4.
Conclusions: We provide a novel mechanism of how the dysfunction of PDE4 occurs in HD. This mechanism also provides insight of how PDE4 activity is regulated in a general context. The information may help identifying a novel therapeutic strategy for psychiatric symptoms in HD.
Disclosure: K. Ishizuka, Nothing to Disclose.

54.4 Structural and Pharmacological Studies of PDE4 Subtype Selective Allosteric Inhibitors

Mark Gurney*

Tetra Discovery Partners, Grand Rapids, Michigan

Background: The PDE4 enzymes are regulated by the opening and closing of helical protein domains over the active site, thereby controlling the access of the cAMP substrate. Long splice isoforms of the PDE4 enzymes contain three important regulatory domains known as UCR1, UCR2 and CR3. UCR1 and UCR2 comprise a negative regulatory module that holds the PDE4 enzyme in a partially inactive conformation. cAMP-dependent phosphorylation of UCR1 by protein kinase A (PKA) releases the enzyme from inhibition and causes an 8 fold increase in enzymatic activity. An amino acid sequence polymorphism in UCR2 that is unique to primates allows the design of PDE4D selective allosteric inhibitors that are able to capture UCR2 in the closed conformation across the active site. Correspondingly, an amino acid sequence difference in CR3 allows the design of PDE4B selective allosteric inhibitors that close CR3 across the active site. The design of PDE4D and PDE4B selective allosteric inhibitors that distribute to brain has allowed us to explore the rich neuropharmacology of these important enzymes.
Methods: Long splice isoforms of PDE4B1 and PDE4D7 have been expressed with and without a PKA-activating mutation in UCR1 (PDE4B1 S133D or PDE4D7 S54D). Biochemical assays are performed with purified PDE4 proteins using a linked enzyme reaction (Burgin 2010, Nat Biotech *28:63–70*) and 4 μM cAMP. Rodent cognition tests (novel object recognition) and assessments of antidepressant-like activity (forced swim and tail suspension) were performed using standard protocols.
Results: In the absence of phosphorylation, the UCR1/UCR2 negative regulatory module holds the PDE4D7 dimer (or higher order multi-mer) in a trans-capped mostly inhibited

basal state, and also prevents binding of the allosteric inhibitor. This allows the design of allosteric inhibitors that are more than 500 fold selective for the PKA activated form of the enzyme. Binding of the inhibitor returns PDE4D7 to the basal state, thus, the compound behaves as a partial inhibitor. In contrast, capping of the active site by CR3 (Control Region 3) occurs in cis-, so compounds targeting CR3 completely inhibit enzymatic activity. Structural studies show how compounds can be tuned to bind UCR2 or CR3. PDE4D inhibitors have potent pro-cognitive benefit in mice, rats and non-human primates. Contrastingly, PDE4B inhibitors have antidepressant-like activity in standard antidepressant models while lacking pro-cognitive benefit in adult rats and mice.
Conclusions: The prototypical PDE4 inhibitor rolipram has convincing pro-cognitive and antidepressant benefit in multiple rodent and monkey models, but lacks tolerability in humans due to nausea. Rolipram is the prototypical example of a UCR2-directed allosteric inhibitor, but has low selectivity for PDE4D versus PDE4B (4.7x) and for the PKA-activated versus basal forms of PDE4D7 (13.4x). Thus, at the concentration needed to inhibit the PKA-activated form of PDE4D7, there is substantial inhibition by rolipram of the basal, non-phosphorylated enzyme which appears to be associated with lack of tolerability. By optimizing compounds for inhibition of the PKA-activated conformer, basal PDE4D activity is not inhibited, thereby improving tolerability. Our data indicate that PDE4D and PDE4B selectively regulate distinct signaling pathways within neurons and within the brain.
Disclosure: M. Gurney, **Part 1:** Dr. Gurney is an employee of Tetra Discovery Partners., **Part 2:** Dr. Gurney is an employee of Tetra Discovery Partners., **Part 3:** Dr. Gurney is an employee of Tetra Discovery Partners.

Panel

55. Naltrexone Revisited: New Findings Beyond Mu, Beyond Dopamine and Beyond AddictionMicroglial Activation Alters Reward Circuitry in Chronic Pain States

Catherine Cahill*

Anesthesiology & Perioperative Care, Irvine, California

Background: Opioids are among the most potent analgesics available, and are the cornerstone for severe acute and chronic cancer pain management. However, their long-term use in management of chronic non-malignant pain is now being challenged, where concerns about safety and efficacy and addiction potential are debated. In addition to their powerful analgesic properties, opioids also possess incentive-motivational properties that contribute to their pain relieving attributes. Alterations in the affective-reward system in a chronic pain state are a logical hypothesis for their sub-optimal analgesic effects in treating neuropathic pain. In chronic pain, low doses of opioid antagonists such as naloxone and naltrexone can have analgesic effects. The impact of how chronic pain modifies opioid reward and analgesia remain poorly explored.
Methods: In this study, we examine how the reinforcing properties of opioids change in a chronic pain state, and examine the effect these changes have on the analgesic efficacy of these drugs. We use an animal model of chronic pain whereby the left sciatic nerve was loosely constricted with a polyethylene cuff, a chronic constriction injury (CCI). The reinforcing properties of opioids were then tested using the conditioned place preference test (CPP).
Results: CCI led to significant microglial activation in the ventral tegmental area (VTA), the brain region containing the dopaminergic cell bodies of the mesolimbic dopamine system. Microglial activation was correlated with significant increase in expression of BDNF, and blocking microglial activation reversed BDNF levels to normal levels. CCI also lead to significant dysregulation of chloride homeostasis in GABAergic interneurons of the VTA, an effect that could be reversed by a TrkB antagonist. Under basal conditions, morphine place preference was similar in both sham and neuropathic animals. However, concomitant treatment with a dopamine antagonist blocked morphine CPP in neuropathic, but not sham animals.
Conclusions: These results point to a significant dysregulation in reward systems of the brain in chronic pain conditions, and show that motivation for morphine preference may be altered. These results implicate microglial activation in mediating some of these changes.
Disclosure: C. Cahill, Nothing to Disclose.

55.1 Naltrexone Effects on GABAergic Neuroactive Steroids: Associations to Subjective Responses and Pharmacogenetics

Lara Ray*

University of California, Los Angeles, California

Background: Naltrexone pharmacotherapy for alcoholism may be most effective for individuals that harbor a functional polymorphism of the mu-opioid receptor gene (OPRM1). The mechanism of improved treatment response remains unclear. GABAergic neuroactive steroids have been shown to promote GABAergic transmission, enhance ethanol sensitivity, modulate opioid inhibition of the HPA axis and protect against inflammatory neuronal damage. We tested whether naltrexone effects on the GABAergic neuroactive steroids may differ in those with the OPRM1 polymorphism.
Methods: Non-treatment seeking heavy drinkers received naltrexone (50 mg) or placebo for three consecutive days followed by an intravenous infusion of ethanol. GABAergic steroids were measured in serum, by radioimmunoassay using an antiserum that detects the GABAergic steroids allopregnanolone and 3alpha-hydroxypregn-4-ene-20-one. Assessments were conducted at baseline (i.e., pre-alcohol) and at target breath alcohol concentration (i.e., 0.06 g/dl).
Results: Naltrexone treatment increased allopregnanolone-like activity by 48% compared to placebo in subjects with the A118G SNP of the OPRM1 gene. No effect of naltrexone was found in those who were homozygous for the major allele. The interaction between naltrexone and genotype was statistically significant [$F(1,27) = 6.34$, $p < 0.001$], but this effect was not modulated by naltrexone administration or genotype.
Conclusions: These results suggest a neurosteroid contribution to the therapeutic efficacy of naltrexone in carriers of

the G-allele of the OPRM1 gene. Additional analyses are underway to test associations between neurosteroid activation and subjective responses to alcohol as well as the contribution of kappa (OPRK1) and delta (OPRD1) opioid receptor gene polymorphisms to neurosteroid response. GABAergic neurosteroids may be useful adjunctive therapy for alcoholism in those that lack the OPRM1 polymorphism associated with therapeutic efficacy. Further, neuroactive steroids may help elucidate the mechanisms of action of naltrexone and ultimately inform treatment development for alcoholism.
Disclosure: L. Ray, **Part 1:** I am a paid consultant for GSK.

55.2 Naltrexone Pharmacotherapy for Adverse Metabolic Outcomes of Second Generation Antipsychotic Agents

Igor Elman*

Harvard Medical School, Ashland, Massachusetts

Background: In schizophrenia, obesity is twice as prevalent as in the general public afflicting over 50% of the patients and shortening their lifespan by about 15 years. Although excessive consumption of fast food and pharmacotherapy with second-generation antipsychotic agents (SGAs) has been implicated in the schizophrenia/obesity comorbidity, the pathophysiology of this link remains unclear. The mechanism proposed here is based on the central opioidergic system owing to opioids' role in: (a) enhancing rewarding features of food; (b) boosting orexigenic and suppressing anorexigenic neuropeptides; (c) reducing peripheral insulin secretion and (d) desensitizing insulin receptors. Opioidergic mechanisms of obesity may be particularly pertinent for schizophrenic patients because elevation of endogenous opiate concentrations in their cerebral spinal fluid and in plasma is a relatively consistent clinical finding while some SGAs (eg, olanzapine) purportedly exhibit pro-opioidergic brain effects. If excess of central opioid activity, which is consequential to schizophrenia neuropathology with or without SGA pharmacotherapy creates metabolic problems for the patients it is reasonable to expect amelioration of the symptoms through the blockade of opioid receptors. The purpose of this presentation is to discuss a potential heuristic value of such a blockade for SGA-treated patients' metabolic status.
Methods: Translational evidence to be presented in support of the above contention includes a preclinical and two clinical studies. First, four groups of Wistar Han IGS rats were treated for 28 days with olanzapine, a combination of olanzapine and an opioid receptor antagonist, naltrexone, naltrexone alone or vehicle, and their food consumption and body weight were measured daily for the first nine days and every other day thereafter. Second, a potential mechanism of naltrexone action was explored in 15 patients with heroin dependence who underwent the standard sweet taste test before- and 7 days after the injection of depot naltrexone. Third, schizophrenic or schizoaffective patients on a stable dose of olanzapine were randomized in a double-blind fashion to receive naltrexone ($n = 14$) or placebo ($n = 16$).
Results: Rats treated with olanzapine and naltrexone were similar to the vehicle-treated animals with respect to food intake and body weight gain, whereas olanzapine treatment alone induced overeating and obesity ($p < 0.001$; group-by-time interaction). Data from heroin dependent human subjects demonstrated a reduction in the hedonic and motivational ratings of sweet solutions (p
Conclusions: These data suggest that naltrexone addition results in clinically meaningful attenuation of olanzapine-induced metabolic side effects. Potential mechanisms of naltrexone action may involve diminution of rewarding features of food in conjunction with favorable effects on insulin sensitivity. If confirmed, our results may contribute to the identification of an inexpensive and effective treatment that specifically targets the underlying pathophysiologic effects of SGAs and provides a substantial clinical benefit to the at risk population. Our findings also support further inquiry into the pathophysiologic significance of opioidergic function derangements in schizophrenia along with various therapeutic strategies aimed at alleviating such deficits.
Disclosure: I. Elman, Nothing to Disclose.

55.3 Neurocognitive Effects of Naltrexone

Charlotte A. Boettiger*
University of North Carolina, Chapel Hill, North Carolina

Background: Studies of naltrexone's behavioral effects have largely focused on how it impacts the intake and subjective effects of substances of abuse (primarily alcohol), and reward processing. However some evidence indicates that naltrexone (NTX) also modulates aspects of executive function. This talk will review the data from several laboratory studies in human subjects demonstrating NTX's acute effects on aspects of cognitive control. In addition, we will present new, unpublished data demonstrating that chronic NTX produces similar cognitive effects in treatment seeking alcoholics.
Methods: Study 1: Abstinent alcoholics and healthy controls were given a single acute dose of NTX (50 mg) in a double-blind, randomized, placebo-controlled cross-over design, and computer-based cognitive tasks were used to quantify the effects of NTX on decisions between small immediate rewards ('*Now*') and larger delayed rewards ('*Later*'), and a measure of attentional bias to attractive cues. Study 2: As for Study 1, but in the context of moderate alcohol intake (0.03 BrAC), and using only healthy controls.Study 3: As for Study 1, but in the context of an fMRI scan session; cognitive tasks were completed during scanning. We also quantified NTX's effects on task-related brain activity. Study 4: In the context of a 12 week, double-blind, randomized, placebo-controlled clinical trial for NTX, treatment seeking alcoholics were given daily NTX (50 mg), and effects of NTX on impulsive decision-making, and attentional bias to visual alcohol cues were quantified using computer-based cognitive tasks; for a subset of participants, the *Now/Later* task was completed during fMRI scanning, and thus we also quantified NTX's effects on brain activity.
Results: addition, we find that in people with a positive family history of alcoholism, acute NTX effects on *Now* vs *Later* bias depend on a personality measure (Locus of Control; LOC) linked to frontal dopamine (DA) signaling, with more external LOC scores predicting less impulsive choices on NTX. In other studies, we have found that indices of tonic frontal DA signaling predict impulsive choice according to a U-shaped relationship between

impulsive choice and frontal DA levels, leading us to speculate that NTX modulates impulsive choice by altering tonic DA transmission in the frontal cortex. We hypothesize that those with a family history of alcoholism experience relatively greater effects of kappa opioid receptor blockade, resulting in more consistent elevation of frontal DA levels in response to NTX in this population. This frontal DA elevation then reduces *Now* bias in those with an external LOC, and increases *Now* bias in those with an internal LOC. Finally, we show that acute NTX modulates activity in the orbitofrontal cortex. We now show that chronic NTX similarly affects decision-making and orbitofrontal activity, and also reduces attentional bias to alcohol cues in treatment seeking alcoholics.
Conclusions: These data support further investigation of the cognitive effects of NTX, particularly in the domain of executive functions subserved by the frontal lobes. Moreover, the ability of NTX to modulate executive function points to possible therapeutic potential in disorders beyond addiction that are also characterized by executive impairment. Finally, the consilience of acute and chronic effects of NTX highlight the utility of acute studies for economically probing the neurocognitive effects of NTX.
Disclosure: C. Boettiger, **Part 1:** I own shares in the following companies: Becton Dickinson Co., Bio Rad Laboratories Inc. CL A, Sigma Aldrich Corp., Thermo Fisher Scientific Inc., **Part 2:** Becton Dickinson Co., Sigma Aldrich Corp.

Panel

56. Novel Molecules and Mechanisms in Vulnerability and Resilience Throughout Life

56.1 Epigenetic Pathways During Early Postnatal Life: How does a Neuron 'Know' to Modulate Its Epigenetic Machinery in Response to Early-life Experience?

Tallie Z. Baram*

University of California, Irvine, California

Background: Exciting information is arising about epigenetic mechanisms and their role in long-lasting changes of neuronal gene expression. Whereas these mechanisms are active throughout life, recent findings point to a critical window of early postnatal development during which neuronal gene expression may be persistently re-programmed via epigenetic modifications. However, it remains unclear how modulation of the epigenetic machinery is triggered and executed. Here we focus on an important example of early-life programming: the effect of sensory input from the mother on expression patterns of key stress-related genes in the developing brain. We describe recent work that integrates organism-wide signals with intercellular and intracellular events that, in turn impact epigenetic regulation. We focus on the lasting effects of enriched early life experience on *Crh* gene expression in the hypothalamus, and describe the operational brain networks that convey sensory input to CRH expressing cells, highlighting the resulting 're-wiring' of synaptic connectivity to these neurons. We will then move from inter-cellular to intracellular mechanisms, delineating recent and emerging information about the induction and maintenance of life-long *Crh* repression provoked by early-life experience, and the responsible molecular mediators. Elucidating such pathways is critical for understanding the enduring links between experience and gene expression. In the context of the responses to stress, such mechanisms should contribute to vulnerability or resilience to number of stress-related disorders.
Methods: n/a
Results: Much work has centered on the enduring effect of maternal-derived sensory signals on gene expression. However, how these signals propagate within the brain and arrive at the target neurons is less well understood. We describe neuronal pathways activated by specific patterns of maternal behavior, that carry patterns of maternal care to stress-responsive hypothalamic neurons. How does activation of this neuronal network influence neurons to modulate cellular processes? We find that a week of early-life augmented maternal care reduces the number and function of excitatory synapses onto CRH-expressing hypothalamic neurons. This reduced excitatory input triggers intracellular cascades culminating in epigenetic chromatin changes, as evident from recent data showing that reduced glutamatergic neurotransmission suffices to repress *Crh* expression in hypothalamic slices *in vitro*. The responsible mechanisms involve the transcriptional repressor NRSF/REST.
Conclusions: As shown above, the resulting life-long repression of *Crh* contributes to attenuated response to stress throughout the life-time. However, neuronal programming likely involves epigenetic, coordinate changes in the expression of large gene networks that, together, underlie the life-long phenotype of resilience to stress-related disorders induced by enriched early-life experience in animal models- and possibly in humans.
Disclosure: T. Baram, **Part 1:** Baram's institution (UCI) received travel & consultanship; Questcor, **Part 2:** stock ownership, TEVA.

56.2 How Neocortical Tet-mediated DNA Hydroxymethylation Regulates Memory

Timothy W. Bredy*

The University of Queensland, Brisbane, Australia

Background: Anxiety disorders are characterized by an impaired ability to inhibit the fear response, which is a hallmark signature of phobia and post-traumatic stress disorder (PTSD). How does the brain acquire strongly emotional memories and how are they maintained across the lifespan? Perhaps more importantly, why do these memories become debilitating in phobia and PTSD, and how can they be minimized through the inhibitory learning process known as extinction? Previous work from our lab and others has advanced the understanding of experience-dependent effects on brain function by demonstrating that epigenetic mechanisms, including histone modifications and DNA methylation, are necessary for neural plasticity associated with fear-related learning and long-term memory
Methods: Using a combination of robust behavioral paradigms and sophisticated molecular techniques, including a newly developed genome-wide sequencing approach,

quantitative PCR, *in vitro* primary cortical neuron preparations, and *in vivo* lentiviral-mediated gene transfer we have addressed, for the first time, whether there is a causal relationship between active DNA demethylation and the formation of memory for fear extinction.
Results: Genome-wide analysis of 5-hmC revealed learning-dependent redistribution of 5hmc across the genome, emphasizing inter- and intra- genic regions of coding genes related to neural plasticity and fear extinction. This process is dependent on the Ten-eleven translocation (Tet) family of enzymes, which mediate the conversion of 5-methylcytosine to 5-hydroxymethylcytosine; a critical component of the active DNA demethylation pathway.
Conclusions: Our data suggest that active DNA demethylation within the adult prefrontal cortex is more extensively involved in experience-dependent plasticity than currently realized, and that this epigenetic mechanism may be particularly important for the extinction of conditioned fear.
Disclosure: T. Bredy, Nothing to Disclose.

56.3 miRNA Programming in Neurodevelopment: Epigenetic Targets in a Dynamic Landscape

Tracy Bale*

University of Pennsylvania, Philadelphia, Pennsylvania

Background: Neurodevelopmental disorders including autism and schizophrenia have been highly associated with parental factors including maternal stress as well as paternal age. The epigenetic mechanisms through which these influences may contribute to disease development are not well understood; though likely involve very complex interactions between parental germ cell lifetime exposures, the maternal milieu and the fetal genetic background. We have identified a sensitive period of early gestation where maternal stress produces sex-dependent epigenetic programming effects on offspring stress pathway neurodevelopment. In addition, in a paternal stress model, we have similarly found that offspring from males chronically stressed over puberty or as adults have offspring with significant alterations in their HPA stress axis. In order to determine potential epigenetic mechanisms by which these parental stress experiences can be transmitted to and affect offspring brain development, we have examined the miRNA landscape in the neonatal brain and paternal germ cells.
Methods: In our maternal stress model, we examined the PN2 brain for changes in the miRNA environment by ABI Taqman array following stress. In addition, as stress produces robust sex differences, comparisons were also made to neonates administered an aromatase inhibitor (formestane) at PN1 to prevent testosterone conversion to estradiol in the brain. Identification of gene targets of these miRNAs was done using HITS-CLIP analyses. Mature sperm from our paternal stress model was also examined for miRNA expression differences that would predict changes in regulating maternal stored mRNAs at fertilization.
Results: We found dramatic changes in the neonatal brain miRNA environment in response to either formestane or stress. Large-scale bioinformatics analyses specifically identified the miR-200 family in the developing hypothalamus and olfactory bulb as being regulated by estradiol and affected by maternal stress only in males. These changes correlated with an elevated HPA stress axis in adult offspring. HITS-CLIP analyses found intriguing gene targets involved in neuronal activation and chromatin remodeling. In the sperm from paternally stressed males, we detected 9 specific miRNAs that were significantly elevated and predicted a significant reduction in the HPA stress axis of adult offspring.
Conclusions: These results may provide valuable insight into novel epigenetic mechanisms contributing to sex-biased disease vulnerability to maternal or paternal stress exposure impacting the developing brain via effects on the dynamic miRNA environment. Identification of the gene targets from these studies may provide important new therapeutic targets for treatment of stress-related neuropsychiatric disease.
Disclosure: T. Bale, Nothing to Disclose.

56.4 Neuron-specific Nucleosome Remodeling: A Missing Link in Our Understanding of Epigenetic Mechanisms Underlying Intellectual Disability Disorders

Marcelo A. Wood*

University of California, Irvine, California

Background: Intellectual disorders are characterized by impairments in cognition, social behaviors, and communication. Recent human exome sequencing studies have identified subunits of the polymorphic Brg1-associated factor (BAF) complexes (mammalian SWI/SNF chromatin remodeling complex) that are frequently mutated in sporadic mental retardation and sporadic autism. Moreover, *de novo* mutations in various subunits of the neuron-specific BAF (nBAF) nucleosome remodeling complex have been implicated in Coffin-Siris and Nicolaides-Baraitser syndromes, both of which are associated with intellectual disability. Together, these studies suggest that nBAF function is necessary for normal cognitive function. Nucleosome remodeling complexes modify chromatin structure and regulate gene expression by repositioning nucleosomes at the promoters of genes.Why disturbances to chromatin remodeling via mutations in BAF complexes result in cognitive dysfunction is unknown. Although an important topic in other fields (eg cancer), nucleosome remodeling has received little attention in neuroscience. The nBAF complex has a unique subunit, called BAF53b, which is neuron-specific and only found in the nBAF complex, making BAF53b an ideal target for investigating the contributions of nBAF to synaptic physiology and behavior. We tested the hypothesis that nBAF, after playing a key role in neuronal fate decisions during development, continues to regulate gene expression and does so in a manner critical to adult plasticity and memory.
Methods: To investigate the role of BAF53b in learning and memory, we used several different lines of genetically modified BAF53b mutant mice. Behavioral studies were carried out to assess learning and memory. Electrophysiological studies were carried out to examine long-term potentiation, a form of synaptic plasticity. Spine morphology and synapse function were also examined. Finally, RNA sequencing was performed to examine gene expression.

Results: There were no effects on anxiety, locomotion, or short-term memory observed in any of the genetically modified BAF53b mutant mice. Mutant mice exhibited severe long-term memory impairments and hippocampal slices from genetically modified BAF53b mutant mice exhibited significant impairments in the stabilization of long-term potentiation. To understand what might be causing the effects on long-term memory and long-term potentiation, we examined spine and synapse function. Spine morphology and synapse function were found to be significantly altered, and correlated with changes in gene expression caused by mutation of BAF53b.
Conclusions: Together, these findings demonstrate that nucleosome remodeling is a major epigenetic mechanism underlying learning and memory. They further identify a mechanism by which mutations in the nBAF complex lead to intellectual disability disorders in humans.
Disclosure: M. Wood, Nothing to Disclose.

Panel

57. 'Strategies for the Development of Novel Therapies for Schizophrenia: From Clinic To Laboratory (And Back Again)'

57.1 Applying Lessons from DISC1 to Convert Gene Discoveries into Drug Discoveries

Nicholas Brandon*

AstraZeneca, Lexington Massachusetts

Background: Over ten years ago we initiated an effort into exploiting the then recently described gene 'Disrupted in Schizophrenia 1′ (DISC1) for drug discovery purposes. With hindsight, powered by the explosion of new genomic tools and findings, it is clear that though our approach was very naïve we were able to make some critical neurobiological findings which have guided drug hunting efforts around DISC1 and will allow us to set up a strategy for future targets like ZNF804A. Upon the cloning of DISC1 by Miller, Porteous and colleagues in 2000 we were presented with an 854 amino-acid protein of unknown function. Analysis of the sequence showed the presence of a number of domains likely to mediate protein-protein interactions. Thus our simple approach to understand this proteins function, and we hoped to provide new drug targets for Psychiatric illness, was to identify and characterize DISC1 interacting partners. I will summarize our efforts over the last decade on DISC1, where the 'DISC1 Interactome' has proved a very fertile resource for understanding a role for DISC1 in neuronal and glial functions. The importance of these findings for schizophrenia etiology and treatment though still needs to be confirmed. Our experience with DISC1 provides a 'Road Map for Target Discovery' where a risk variant is in a gene of unknown function. Matching this descriptor is ZNF804A, which has emerged as a robust schizophrenia gene from GWAS studies, but with little in the way of function known. It is likely a DNA or RNA binding protein and so understanding its impact on the expression of other genes is likely to be important.
Methods: Yeast 2 –hybrid screening on a semi-industrial scale was the core method employed to identify DISC1 interactors. Characterization of critical partners employed standard biochemical methods and most importantly multiple collaborations between industry and academia. Our studies have focused on the role of DISC1 at the synapse, with biochemical and electrophysiological analysis of synapse form and neuronal activity. I will describe the discovery of the DISC1 interactor and kinase TNIK as a key regulator of synapse function and more recent observations of the impact of DISC1 on the function of ionotropic glutamate receptors, which may involve another well known DISC1 partner, PDE4. Simulating the drug discovery pathway for ZNF804A will be performed through analysis of the literature, where some early publications have looked at the impact of ZNF804A on gene expression.
Results: The DISC1 interactome clearly predicted that DISC1 is likely to be involved in a number of cellular processes, but in particular was likely to have a role at glutamatergic synapses. We selected TNIK to scope out DISC1 s role in synapses as it had previously been found in post-synaptic densities in proteomic studies. The role of TNIK in the stability of synapses will be reviewed (see Wang *et al*, 2010). More recent data has shown that DISC1 regulates the expression of NMDAR subunits in particular NR2A in both primary neurons and *in vivo* through cAMP dependent mechanisms. Thus DISC1 clearly plays multiple roles at synapses, from regulating a critical synaptic kinase to regulating the levels of NMDAR subunits. These processes, if dysregulated, clearly could play important roles in the etiology of mental illness. Analysis of the ZNF804A transcriptome opens up a number of complex avenues for drug discovery research, which will be discussed.
Conclusions: Studies of DISC1, a risk factor for mental illness, which owes its position in the field to its celebrated discovery in a densely impacted pedigree in Scotland, have further exemplified the complexity of mental illness. We were naïve to assume that it would provide easy targets for drug hunting. Within this complexity, in TNIK and PDE4 we were able to highlight druggable targets. The validation and development of either of these will need to overcome the issues of target validation, safety, toxicity and human efficacy which are the challenges faced by all drug discovery programs. We have recently described the evolving ecosystem of psychiatric research in 2013 (Rizzo *et al*, 2013) and will put these targets into this context. This will provide a path forward for new and novel approaches, including those emanating from ZNF804A.
Disclosure: N. Brandon, **Part 1:** I am a full time employee of AstraZeneca., **Part 2:** I am a full time employee of AstraZeneca., **Part 3:** I am a full time employee of AstraZeneca.

57.2 Integrating the Genome, Epigenome and Transcriptome in the Human Brain: Accounting for Biological and Technical Heterogeneity

Andrew Jaffe*

Lieber Institute for Brain Development, Baltimore, Maryland

Background: Genome-wide DNA methylation and mRNA expression generated from post-mortem human brain tissue may be the most proximate or 'ultimate' intermediate

phenotypes in the study of schizophrenia, and combined with underlying genetic variation data, can help to elucidate the molecular mechanism of this disorder. Advanced statistical approaches can enhance biological signal in these postmortem human brain studies by accounting for technical variation and changes in cellular composition, to better functionally integrate epigenetic and transcriptome datasets, which typically consist of millions of measurements per sample.
Methods: Microarray-based gene expression, DNA methylation, and genetic variation were measured in the dorsolateral prefrontal cortex (DLPFC) in 410 post-mortem human brain samples, consisting of 229 non-psychiatric adult controls and 181 patients with schizophrenia. We estimated the relative abundance of neurons using our genome-wide DNA methylation combined with publicly-available NeuN-sorted data (Kaminsky 2013) using a recently published regression method (Houseman 2012). We isolated the genomic data in and around *DISC1* and *ZNF804A*, two genes identified in large clinical genetics studies, and identified relationships between DNA methylation, gene expression, genetic variation, and illness. Lastly, we performed extensive RNA sequencing on a subset of the samples ($N = 214$, 107 case-control pairs) to characterize the transcriptome at base-pair resolution.
Results: Cell composition estimates were strongly associated with DNAm at individuals CpGs in both *DISC1* and *ZNF804A* (7/65 and 1/17 sites with $p < 1 \times 10^{-20}$ respectively), suggesting cell-type specific epigenetic regulation of these important clinical risk genes. However, composition estimates were not associated with array-based or sequencing-based gene expression measurements, suggesting additional levels of epigenetic regulation. Both genes were relatively lowly expressed in adult brain samples, and were not differentially expressed between schizophrenia patients and controls. However, DNAm in an exon of *DISC1* was strongly associated with presence of schizophrenia, independent of composition and 'batch' effects ($p = 4.8 \times 10^{-15}$). Lastly, an intronic single nucleotide polymorphism (SNP) in *DISC1* was associated with gene expression of a specific exon ($p = 7.2 \times 10^{-6}$).
Conclusions: Epigenetic mechanisms including DNA methylation may functionally link genetic variation in clinical risk genes with gene expression levels in neuropsychiatric illness, including schizophrenia. Care should be taken when interpreting epigenetic and transcriptional measurements from post-mortem human brain samples, especially when samples contain mixtures of cell types.
Disclosure: A. Jaffe, Nothing to Disclose.

57.3 Psychiatric GWAS Consortium Triples Schizophrenia GWAS Sample-size to 31,000 Cases and 37,000 Controls

Stephan Ripke*

Massachusetts General Hospital, Boston, Massachusetts

Background: The PGC (Psychiatric GWAS Consortium) is an international group of researchers, one of whose aims is to maximize the utility of extant psychiatric GWAS through mega-analysis. In a previous study, our first wave of genome-wide schizophrenia association analysis identified multiple loci involved in this genetically complex and clinically heterogeneous disorder (NG, Sept 2011). While around 20,000 individuals were necessary to achieve this result detailed analysis of the data suggested that there are many more genes to discover, and that this should be possible by further increase of sample size.
Methods: Here we present an update of this international endeavor, which now comprises more than 31,000 cases and 37,000 controls. The presented data is imputed into 1KG (Aug, 2012) and analyzed using standard logistic regression with MDS components as covariates.
Results: The number of distinct genome-wide significant regions in this newest round of meta-analysis did increase to more than 80 passing the commonly used p-value threshold of 5.0E-08. Common suspects in psychiatric genetics like MIR137, DRD2 and ZNF804A are found in these regions of genome-wide significant regions. We will present details about this meta-analysis as well as comparisons to other successful GWAS on human traits.
Conclusions: These results are in line with former predictions and developments in other big complex disease GWAS like Crohn's disease. They provide new insights into the biology of Schizophrenia.
Disclosure: S. Ripke, Nothing to Disclose.

57.4 Understanding ZNF804A: Allelic Variation, Alternative Transcripts, Brain Development and Schizophrenia

Thomas M. Hyde*

Lieber Institute for Brain Development, Baltimore, Maryland

Background: Multiple genome-wide association studies (GWAS) have identified *ZNF804A* as a risk gene for schizophrenia and bipolar disorder [O'Donovan MC, *et al.* 2008; Riley B, *et al.* 2010; Steinberg S, *et al.* 2011; Williams HJ, *et al.* 2011; Zhang F, *et al.* 2011]. Allelic variation at rs1344706, the most robust GWAS-positive SNP in *ZNF804A*, also has been associated with expression of full-length *ZNF804A* in human brain [Riley B, *et al.* 2010]. Studies of its mouse homologue, *ZNF804A*, suggest that this gene plays an important role in early brain development, including neurite outgrowth, and axonal and dendritic arborization [Hill MJ, *et al.* 2011]. A recent study of schizophrenia-derived induced pluripotent stem cells showed the expression of *ZNF804A* dramatically increased during the transition from a pluripotent stem cell to a neuronal precursor [Pedrosa E, *et al.* 2011]. The authors concluded that abnormally high expression of *ZNF804A* may occur in schizophrenia patients in early brain development.
Methods: Best estimates suggested that 90% of multi-exon genes, including *ZNF804A*, undergo alternative splicing. Using RNA sequencing, 5′ cDNA RACE and end-to-end PCR in the postmortem human brain, we have defined the transcriptome of *ZNF804A*. Next, we characterized the developmental expression pattern of the full length and truncated *ZNF804A* transcripts in 672 human samples from dorsolateral prefrontal cortex ranging in age from 14

gestational weeks to 97 years using q-rtPCR. Finally, we studied transcript expression in three diagnostic groups (patients with schizophrenia ($n=169$), bipolar disorder ($n=57$), and major depression ($n=133$)) as well as normal controls ($n=198$).
Results: In addition tp confirming the previously known full-length transcript from *ZNF804A*, we also identified a novel truncated transcript that was composed of a novel exon 3 and exon 4. The trajectory of expression of the truncated transcript and the full-length transcript were similar across the lifespan. Both rose during the second trimester in the fetus and then fell after birth. The expression of the two *ZNF804A* transcripts showed heterogeneity in the DLPFC of patients with schizophrenia and affective disorders. The expression of full length *ZNF804A* was significantly decreased in the DLPFC of patients with bipolar disorder ($p=0.0001$). The truncated transcript was down regulated in the patients with schizophrenia ($p=0.04$), but up regulated in the patients with bipolar disorder ($p=0.03$) and MDD ($p=3.59\times$ E-14). In a previous study from our group, there was no difference in the expression of *ZNF804A* in the patients with schizophrenia, but that assay measured both transcripts together. Finally, a polymorphism in *ZNF804A* at rs1344706 was associated with the expression of truncated transcript in patients with bipolar disorder ($p=0.02$), as well as in fetal controls ($p=0.03$).
Conclusions: Both the full length and truncated transcripts from *ZNF804A* may be in important in brain development as they are most highly expressed in the second trimester fetus. Moreover, there appears to be differential regulation of expression of these transcripts in schizophrenia and affective disorders. Finally, alternations in the expression of the alternate transcript may be the mechanism by which allelic variation at rs1344706 increase the risk for bipolar disorder. Interrogation of the splicing variants of *ZNF804A* in the human brain will help define the mechanisms by which allelic variation in this gene leads to increased risk of neuropsychiatric illness.
Disclosure: T. Hyde, Nothing to Disclose.

Panel

58. The Insula Salience Network: Alterations in Its Connectivity in Developmental, Anxiety, Mood and Personality Disorders

58.1 Borderline Personality Disorder Patients Show Reduced Insula-amygdala Functional Connectivity and Fail to Habituate When Viewing Repeated Negative Emotional Pictures

Harold W. Koenigsberg*

Icahn School of Medicine at Mount Sinai, Bronx, New York

Background: Extreme emotional reactivity to psychosocial cues is a defining feature of borderline personality disorder (BPD), yet the neural-behavioral mechanisms underlying this affective instability are poorly understood. One possible contributor would be a diminished ability to engage the adaptive implicit regulatory mechanism of emotional habituation. We hypothesized that BPD subjects would not behaviorally habituate to negative pictures as well as healthy controls and that connectivity to the insula—a critical node in processing subjective emotional experience—would be altered in BPD patients.
Methods: 23 BPD patients, 28 healthy volunteers (HC) and 27 avoidant personality disorder patients (AvPD) were shown emotionally negative pictures as fMRI images were obtained. Two thirds of the pictures were shown two times, separated by 5 minutes. Immediately after viewing each picture, subjects rated their emotional reaction to the picture. Group comparisons of the novel *vs* repeat image contrasts were carried out. Changes in functional connectivity to a left insula seed region when viewing novel *vs* repeat pictures were examined using a psychophysiological interaction analysis.
Results: Consistent withpsychological habituation, HC subjects rated repeat negative pictures significantly less negatively than novel negative pictures ($p=0.02$), but BPD and AvPD subjects did not. Increasingly negative subjective ratings of picture valence correlated with increasing left middle-posterior insula activity in all three groups, and thus this region was used as the seed for subsequent functional connectivity analyses. Connectivity change to this insula seed region with repeated negative picture viewing differed between groups. HC subjects demonstrated a greater increase in insula connectivity to both left and right amygdalae compared to BPD subjects ($p<0.01$, two-tailed). Greater increases in insula-amygdala connectivity were associated with greater behavioral habituation for both HC and BPD subjects ($r=0.490$, $p=0.015$ and $r=0.525$, $p=0.024$, respectively). AvPD subjects differed from both groups in showing a negative correlation between change in insula-amygdala connectivity and behavioral habituation.
Conclusions: This study highlights the role of insula-amygdala connectivity in the implicit regulation of negative affect via habituation and provides evidence that anomalies in this connectivity may be associated with impaired behavioral habituation in borderline patients.
Disclosure: H. Koenigsberg, Nothing to Disclose.

58.2 Elevated Posterior Insula–ventral Striatal Connectivity to Reward in Youth with Bipolar Spectrum Disorders Relative to Youth with Other Behavioral and Emotional Dyregulation Disorders: A Potential Neural Marker of Heightened Reward-related Perceptual Salience in Bipolar Youth

Mary L. Phillips*

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: The posterior insula has a critical role in general salience and environmental monitoring. In parallel, heightened reward sensitivity is reported in adults with bipolar spectrum disorders (BPSD: bipolar I, bipolar II, bipolar NOS, cyclothymic disorder). Altered functional coupling (eg, functional connectivity, FC) between the posterior insula and reward circuitry may thus reflect a pathophysiologic process for heightened reward sensitivity in BPSD. Yet, no study has examined this in adults or youth.

We aimed to determine whether distinct patterns of FC between insula and prefrontal cortical-amygdala-ventral striatal reward circuitry were associated with BPSD in youth.
Methods: 85 youth were recruited from the Longitudinal Assessment of Mania Study (LAMS), assessing youth with several behavioral and emotional dysregulation diagnoses (BPSD, depressive disorders, ADHD, disruptive behavior disorders (DBD), anxiety disorders), from three sites: Case Western Reserve University ($n = 25$); Cincinnati Children's Hospital ($n = 31$); and University of Pittsburgh Medical Center ($n = 29$). 33 youth had a BPSD diagnosis (comorbid diagnoses included the other disorders above). 52 youth were without a BPSD diagnosis, but had one or more of the following disorders: anxiety disorders, ADHD, DBD, and depressive disorders. All participants completed a monetary reward, number guessing task with win, loss and no change (control) outcomes during neuroimaging. Analyses were in 2 stages. Psychophysiological Interaction analysis first examined FC within a large bilateral region of interest (ROI) mask that included key reward circuitry regions: bilateral ventral striatum (VS), insula, amygdala, and orbitofrontal, ventrolateral prefrontal, middle prefrontal and anterior cingulate cortices, in all LAMS youth to win (*vs* control) and loss (*vs* control). Multiple regression analyses then examined relationships among FC patterns in stage 1 and different diagnostic categories, covarying for demographic, medication, and scanning site variables.
Results: All youth showed significant FC (p
Conclusions: Youth with a BPSD diagnosis show elevated FC, relative to youth without such as diagnosis, between a key reward processing region, VS, and left posterior insula to win during a reward paradigm. Given the role of the posterior insula in general salience and environmental monitoring, these findings suggest that monetary reward may be perceived as more salient by youth with BPSD than by youth with other disorders. These findings parallel previous reports of heightened reward sensitivity in adults with bipolar disorder, and provide a potential neural marker of elevated reward-related perceptual salience in youth with BPSD.
Disclosure: M. Phillips, **Part 2:** I have been a consultant for Cardiff University, Department of Psychological Medicine, UK. This relationship is due to end in 2013.

58.3 Insula—Conceptualizing Its Architecture, Function and Connectivity, with Applications to Understanding Large-scale Brain Networks in Psychopathology and Autism

Vinod Menon*

Stanford University School of Medicine, Stanford, California

Background: The insula is a brain structure implicated in disparate cognitive, affective, and regulatory functions, including interoceptive awareness, emotional responses, and empathic processes. While classically considered a limbic region, recent evidence from network analysis suggests a critical role for the insula, particularly the anterior division, in high-level cognitive control and attentional processes.
Methods: I will first review new data that are leading to a reconceptualization of insula architecture, connectivity and function. The crucial insight from these studies I will present is that the anterior insula is an integral hub in mediating dynamic interactions between other large-scale brain networks involved in externally oriented attention and internally oriented or self-related cognition. The model I present postulates that the insula is sensitive to salient events, and that its core function is to mark such events for additional processing and initiate appropriate control signals.
Results: The anterior insula and the anterior cingulate cortex form a 'salience network' that functions to segregate the most relevant among internal and extrapersonal stimuli in order to guide behavior. Within the framework of our network model, the disparate functions ascribed to the insula can be conceptualized by a few basic mechanisms: (1) bottom-up detection of salient events, (2) switching between other large-scale networks to facilitate access to attention and working memory resources when a salient event is detected, (3) interaction of the anterior and posterior insula to modulate autonomic reactivity to salient stimuli, and (4) strong functional coupling with the anterior cingulate cortex that facilitates rapid access to the motor system. In this manner, with the insula as its integral hub, the salience network assists target brain regions in the generation of appropriate behavioral responses to salient stimuli. I will present findings from a recent study which shows how that insula function is impaired in children with autism and demonstrate that insula connectivity has good sensitivity and predictive ability as a potential biomarker of autism (Uddin *et al.* 2013, *JAMA Psychiatry*).
Conclusions: I conclude by proposing a systems framework which provides a parsimonious account of insula function in neurotypical adults, and suggest that it can be used to conceptualize why insula dysfunction is a prominent feature of several psychiatric disorders. A unifying triple network model of psychopathology is proposed, with a key role for the insula (Menon, 2011, *Trends in Cognitive Sciences*).
Disclosure: V. Menon, Nothing to Disclose.

58.4 Insula-amygdala Function and Connectivity in Trauma-related Disorders: Relationship to Childhood Maltreatment

Murray B. Stein*

University of California, San Diego, La Jolla, California

Background: Intimate-partner violence (IPV) is one of the most common causes of posttraumatic stress disorder (PTSD) among women. PTSD neuroimaging studies have identified functional differences in the amygdala and insular cortex during emotion processing. Childhood maltreatment (CM) is a strong risk factor for development of posttraumatic stress disorder (PTSD) upon adult exposure to extreme adverse events. However, the neural underpinnings of this relationship are not well understood. In this presentation, human functional MRI data will be presented from studies in women with intimate partner violence-related PTSD that underscore the importance of insula-amygdala functional connectivity in PTSD and consider a role for CM in these findings.

Methods: Dr. Stein will provide a brief overview of preclinical lesion and clinical morphometric and functional neuroimaging studies suggesting that altered amygdala- and/or hippocampal connectivity to insular cortex may underlie aspects of the clinical syndrome of PTSD and related conditions. He will then present fMRI data from studies in women with IPV-related conducted in collaboration with Drs. Martin Paulus, Alan Simmons, Robin Aupperle., and Gregory Fonzo. These data include 12 women with IPV-PTSD and 12 nontraumatized comparison women who underwent blood oxygenation level-dependent (BOLD) fMRI while completing (1) an emotional face-matching task, and (2) an inhibitory 'Go-No Go' task. In addition (3) multivariate regressions examined the relationship of childhood maltreatment to patterns of activation, connectivity, and gray matter volumes.
Results: (1) For the emotional face-matching task, IPV-PTSD subjects relative to comparison subjects displayed significantly increased activation of the anterior insula and amygdala and decreased connectivity among the anterior insula, amygdala, and ACC while matching to fearful versus happy target faces. (2) Data for the Go-No Go Task are currently being analyzed and will be presented at the meeting. (3) Childood maltreatment (CM) severity was positively correlated with limbic-prefrontal connectivity while processing fear faces but negatively correlated with amygdalo-insular connectivity while processing fear and angry faces.
Conclusions: Women with IPV-PTSD display hyperactivity and disconnection among affective and limbic sensory systems while processing threat-related emotion. These results further suggest CM exposure may account for variability in limbic/prefrontal brain function and prefrontal structure in adulthood PTSD and offer one potential mechanism through which CM confers risk to future development of PTSD.
Disclosure: M. Stein, **Part 1:** Care Management Technologies (Consultant), Up-to-Date (Co-Editor-in-Chief, Psychiatry), Depression and Anxiety [Wiley] (Associate Editor), **Part 2:** University of California San Diego, Va San Diego Healthcare System, Up-To-Date, **Part 3:** Up-To-Date, **Part 4:** Janssen (Co-Investigator on Research Contract)

Panel

59. Understanding Neurodevelopmental Risk Factors Leading to Anxiety and Depression to Inform Novel Early Interventions in Vulnerable Children

59.1 Glucocorticoid Receptor Activation Induced Epigenetic Changes and Their Moderation by Genetic Variants as Potential Mediators of Risk and Resilience to Early Trauma-associated Psychiatric Disorders

Elisabeth B. Binder*

Max-Planck Institute of Psychiatry, Atlanta, Georgia

Background: We could recently show that exposure to adverse life events has a very different long term impact on peripheral blood gene expression and DNA methylation in patients with post traumatic stress disorder depending on the developmental period (Mehta *et al*., PNAS in press) suggesting that distinct pathomechanisms can underly the same psychiatric disorder following trauma in childhood *vs* adulthood.
Methods: To explore the importance of developmental windows for longterm gene expression and epigenetic changes following adverse life events in more depth, we treated a human fetal hippocampal progenitor cell line with the glucocorticoid receptor agonist dexamethasone during the proliferation and differentiation phase as well as after maturation and compared the developmentally and treatment-related changes in genome-wide gene expression and DNA methylation profiles with data from peripheral blood cells in a highly traumatized human cohort. We also include data from expression and methylation quantitative trait locus analysis in peripheral blood cells moderated by exposure to early trauma ($N = 380$) and gene $\times$ environment interaction data in a cohort of 3000 individuals for a better functional annotation of the identified CpGs.
Results: We observed distinct gene expression and DNA methylation changes in the hippocampal progenitor cells when activating the glucocorticoid receptor in the two distinct developmental stages. In fact for the gene FKBP5, identical changes in DNA methylation in functional glucocorticoid response elements were observed in the neuronal cell lines following treatment during the proliferation and differention phase and following exposure to child abuse in DNA from peripheral blood in adults but following treatment in mature neurons. We are currently testing whether these findings from a candidate gene level extend to genome wide data. When analyzing expression quantitative trait loci moderated by early trauma, we found that the SNPs ($N = 497$) interacting with child abuse to predict gene expression levels after correction for multiple testing, also interacted with child abuse to predict DNA methylation pattern in the same loci in over 97%. In addition, the sequences surrounding these early trauma sensitive eSNPs were enriched for glucorticoid response elements. Finally, eSNPs interacting with early trauma to predict gene expression differences, also showed interactions predicting depressive and anxiety symptoms in the large sample.
Conclusions: Our findings suggest that glucocorticoid receptor activation induced DNA methylation changes and their moderation by genetic variants may contribute to the risk and resilience profile to psychiatric disorders following exposure to early trauma. A better understanding of the downstream mechanisms activated by these changes and their time sequence may help guide prevention and early intervention strategies.
Disclosure: E. Binder, Nothing to Disclose.

59.2 Neurobiology of Trauma and Infant Attachment: Short-term Benefits and Long-term Costs

Regina M. Sullivan*

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Background: Children learn to attach to the caregiver, even when experiencing abuse. We explore unique attributes of

the learning attachment system in infant rodents to better understand how the brain supports this learning. Our previous work showed that the amygdala's learning plasticity is suppressed during attachment learning with an abusive caregiver. Here we present data indicating that, although the amygdala is not supporting fear learning, it is responding to the trauma and organizing to produce later-life pathology associated with malfunctioning amygdala.
Methods: Infant rats pups were either reared with a maltreating mother for natural attachment learning or classically conditioned in an experimentally controlled attachment learning paradigm that included pain (odor-0.5 mA shock) or no pain (odor-tactile stimulation). These procedures produce a maternal odor that pups require to interact with the mother. Controls included rearing with a typical mother and controls that do not learn the maternal odor (presentations of rewards unpaired with the odor). Social behaviors and neural activity (microarray, microdialysis, electrophysiology, 2-DG autoradiography) were assessed in pups and older animals to explore short-term and enduring effects.
Results: The short-term effects of pups experiencing pain within attachment (abusive mother, learning the maternal odor with pain) seem minimal since pups showed attachment to the caregiver and the amygdala did not participate in behaviour—similarly to that expressed by controls. While this attachment system ensures infants attach to their caregiver regardless of the quality of care received, the long-term effects indicate these early-life experiences with maltreatment have costs. Amygdala and behavioural abnormalities emerge around weaning and continue into adulthood. However, presentation of the early life maternal odor (natural and learned) appears to normalize both the behaviour and amygdala.
Conclusions: Together, these data suggest that the attachment system in the brain is robust and supports attachment learning in myriad contexts, including pain. While there are short-term benefits, the long-term effects appear to go beyond normal programming and initiate a pathway to pathology and non-adaptive behaviors. Furthermore, learning in early life appears to be retained with the early life odors retaining strong ability to modify and normalize non-adaptive amygdala related behaviors into adulthood.
Disclosure: R. Sullivan, Nothing to Disclose.

59.3 Primate Anxious Temperament and Amygdala Metabolism are Environmentally Sensitive and Associated with Amygdalar Gene Expression

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Background: Children with extreme dispositional anxiety are at increased risk to develop anxiety, depression, and substance abuse. We have extensively validated a rhesus monkey model of this anxious temperament (AT), and using this model we have outlined the neural correlates of AT with FDG-PET imaging. The results identified the central nucleus of the amygdala (Ce) to be a critical contributor to early-life AT.
Methods: We exposed 592 young rhesus monkeys from a multi-generational pedigree (mean Age: 1.9 years; range: 0.74 to 4.2 years) to a human intruder who made no eye contact (NEC) with the animal. AT and brain metabolism were assessed during NEC using expert raters and high-resolution FDG-PET imaging. Brain volume was assessed on a separate day with structural MRI. We used SOLAR to estimate the heritability of AT and NEC-related brain metabolism in this large sample of young monkeys. To understand the molecular mechanisms that contribute to AT, we extracted RNA from the Ce of a subset of 48 young rhesus monkeys previously phenotyped for AT, NEC-related brain metabolism and brain structure. We measured transcriptome-wide gene expression in all 48 samples using microarray technology. Moreover, we further assessed gene structure and transcript expression using deep RNA sequencing in 24 of these samples. Regressions were performed between gene expression and AT, as well as AT-related metabolism and brain structure.
Results: Consistent with prior research, AT was significantly heritable. Although some regions, such as hippocampus were highly heritable (peak voxel $>60\%$ heritable), Ce metabolism was found to be considerably less heritable (peak voxel approximately 20% heritable). While Ce metabolism did not meet whole-brain bonferroni multiple comparison correction, heritability in regions of Ce survived FDR multiple comparison correction (q
Conclusions: Taken together, these data highlight the utility of the monkey AT model for identifying potential therapeutic targets and support the hypothesis that early-life experience and learning can impact AT and its neural substrates via neuroplasticity-related gene expression in the Ce.
Disclosure: A. Fox, Nothing to Disclose.

59.4 The Pervasive and Persistent Neurobiological Consequences of Child Abuse and Neglect; Clinical Implications

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Background: There is now unequivocal evidence that early life adverse events including childhood sexual, physical and emotional abuse, as well as emotional neglect, interacts with genetic polymorphisms and epigenetic mechanisms to dramatically increase vulnerability to mood (depression and bipolar disorder), psychotic (schizophrenia) and anxiety (PTSD) disorders. In order to attain one of our major goals, namely to develop early interventions that prevent the now increasingly well documented clinical consequences of early life trauma, we need to better understand the cascade of neurobiological sequelae and the molecular mechanisms underlying such changes.
Methods: This presentation will highlight recent structural and functional brain imaging alterations using magnetic resonance imaging in adult victims of child abuse and neglect. In addition persistent neuroendocrine alterations, particularly of the HPA axis, that have been observed in patients with depression and early life trauma will be described. Finally novel candidate genes have been identi-

fied that appear to mediate, in part, the vulnerability of patients exposed to early adverse events, to depression and PTSD as adults. Finally neuropsychological testing has revealed relatively specific cognitive alterations in patients as a function of the nature of the abuse they endured.

Results: In the structural imaging studies, region specific alterations in cerebrocortical areas including thinning of the somatosensory cortex encoding for genital sensation was observed in women who were sexually abused during childhood. Alterations in the anterior cingulate were observed in patients exposed to emotional abuse in childood. Functional brain imaging studies revealed significant alterations in response to provocative stimuli in patient with early life trauma histories. In neuroendocrine studies, persistent alterations in HPA activity has been observed using provocative tests in men and women with a history of child abuse and neglect. Some of the genes comprising the HPA axis and related systems have been found to mediate the depressogenic and anxiogenic effects of early life trauma and new data on the oxytocin system will be presented. Long lasting neurocognitive alterations in response to physical, sexual and emotional abuse and neglect were documented using the CANTAB battery.

Conclusions: With the rapid accumulation of these findings of persistent CNS alterations in individuals exposed to untoward early life events, we can begin to turn our attention to both prevention and treatment strategies. It is now well established in several studies that in depressed patients, those with a positive child abuse history, exhibit a diminished response to antidepressants compared to equally depressed patients without such a history. The unique neurobiological consequences of child abuse and neglect produce a distinctive endophenotype that likely requires a novel treatment strategy based on the emerging neurobiological findings.

Disclosure: C. Nemeroff, **Part 1:** Skyland Trail, Cenerx, Novadel Pharma, Takeda, Revaax Pharma, Xhale, Allergan, Lilly, Roche, Shire, SK Pharma, PharmaNeuroboost, **Part 2:** Cenerx, Novadel Pharma, PharmaNeuroboost, Xhale, **Part 3:** Xhale, PharmaNeuroboost, Cenerx, Novadel Pharma