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Panel, Mini-panel, and Study Group
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Panel
1. Impact of Common and Rare Genetic Variants on Brain Phenotypes

1.1 Exploring the Genetic Contributions to fMRI-based Schizophrenia Intermediate Phenotypes: From Classical Candidate Variant Approaches to the Hypothesis-free Identification of Genes and Pathways

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Background: Schizophrenia is a highly debilitating brain disorder with a heritability of up to 80% and a complex genetic architecture. A popular research strategy for the dissection of schizophrenia pathophysiology is imaging genetics, an approach founded on the idea that some neuroimaging phenotypes may bear a closer relationship to the genetic mechanisms of the disorder than the clinical phenotype itself. Here, much interest has been devoted to functional MRI (fMRI)-based phenotypes related to the genetic risk for the disorder; in particular, functional connectivity of the DLPFC and hippocampus during working memory and ventral striatal activation during reward processing. Beyond the classical candidate variant approach, the imaging genetics methods repertoire has recently been extended to include more complex strategies to aid the hypothesis-free identification of variants, genes, and pathways associated with these risk-related neuroimaging phenotypes.
Methods: In a series of studies in healthy individuals and unaffected first-degree relatives of schizophrenia patients we have established and confirmed the link of these phenotypes to the genetic liability for schizophrenia. We have further explored the genetic contributions to these phenotypes using a broader array of imaging genetics methods including single-variant approaches exploring the effects of candidate genes and genome-wide supported psychosis risk variants. Recently, we have utilized more complex strategies in order to examine numerous genetic variants simultaneously using reliability-optimized neuroimaging risk phenotypes, gene fine mapping approaches, and gene set enrichment analyses.
Results: For DLPFC - hippocampus functional connectivity our analyses replicate prior associations of this phenotype with the genetic risk for the illness, highlight associations with genetic loci supported by prior meta-analysis and genome-wide association studies (e.g., NRG1, ZNF804A, CACNAB2, extended MHC genomic region), and provide evidence for the role of genes and biological pathways involved in neurodevelopmental and plasticity processes. For ventral striatal activation during reward processing our data provide the first evidence for a systems-level intermediate phenotype signaling increased genetic risk for schizophrenia, which demonstrates association with a genome-wide supported psychosis risk variant in ITIH3/4 as well as the enrichment of gene sets and pathways involved in dopamine neurotransmission.
Conclusions: Our findings support the utility of fMRI-based neuroimaging phenotypes for the examination of genes and pathways associated with an increased genetic liability for schizophrenia. They further underscore the value of different imaging genetics analysis strategies, the reliability-based definition of neuroimaging risk phenotypes, the independent replication of findings, and the use of comparable data processing methods and analysis strategies across centers.
Disclosure: Nothing to Disclose.

1.2 Impact of Highly Deleterious Functional Genetic Variants on Subcortical Brain Volume

David Glahn

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Background: There is growing evidence that the same genetic factors that influence brain structure and function also confer risk for child- or adolescent onset mental illnesses like schizophrenia, bipolar disorder, major depression and autism. If so, genes associated with neuroanatomic variation in healthy populations are reasonable candidate genes for mental illnesses. Subcortical brain regions act jointly with cortical areas to coordinate movement, learning and memory, emotional responses and reinforcement and have been shown to be sensitive to genetic liability to a host of mental illnesses. Recently, the ENIGMA2 consortium used genome-wide association to search for genetic loci influencing subcortical regions in over 29,000 subjects, reporting a number of genome wide significant SNPs for the putamen, caudate nucleus, and hippocampal volume. While this effort represents a major advance for imaging genomics research, the common variants localized in this study are not explicitly functional and thus do not directly point to specific genes. Like most GWAS studies, localized SNPs indicate loci of variable size depending on local linkage disequilibrium and follow-up studies are needed to definitively identify genes. In addition to common variants, rare variants derived from either whole genome or exome sequencing appear to play a roll in risk for mental illness and in neuroanatomic variation. Identification of a rare functional variant with a large absolute effect size, though present in a handful of affected individuals, can be sufficient to verify that a given gene is involved in trait variance. However, tens of millions of potential variants are provided in whole genome sequencing studies and methods for controlling statistical bias resulting from performing that many statistical tests are often prohibitive. One approach is to use bioinformatic data to select nonsynonymous variants shown to be highly deleterious for protein expression a

priori. This approach dramatically reduces the number of variants tested and provides strong evidence that a specific gene influences the selected trait.
Methods: Here, we examined the association between 1981 rare highly deleterious nonsynonymous (HD-NS) variants and subcortical brain volumes parcellated with FreeSurfer in over 800 Mexican-American from randomly selected extended pedigrees.
Results: Six HD-NS variants in six separate genes were identified influencing bilateral putamen (MOG gene, p = 6.27x 10-6; SCN10A gene, p = 2.50x10-5), pallidum (OR5AR1 gene, p = 1.50x10-5, SERPINA3 gene, p = 2.60x10-5), and caudate (CEP128 gene, p = 3.30x10-5, CEP55 gene, p = 3.70x10-5) volumes. Of these, a variant on chromosome 3, position 38739265, in the SCN10A gene was associated with a 2.24 standard deviation reduction in putamen volume in the 8 subjects with this rare variant. The sodium channel, voltage gated, type X, alpha subunit protein is encoded by SCN10A and is expressed in the human putamen. This protein is involved in the onset of pain associated with peripheral neuropathy and pain perception and evaluation has been consistently linked to putamen function in humans.
Conclusions: Our results demonstrate that rare variants clearly influence subcortical brain anatomy.
Disclosure: Nothing to Disclose.

1.3 Common Genetic Variants Influence Subcortical Brain Volumes: Data from the Philadelphia Neurodevelopmental Cohort

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Background: Subcortical nuclei are critical for many emotional and cognitive processes, and are frequently implicated in the pathogenesis of major mental illness. While prior work has identified common genetic variants that impact subcortical structures in adult populations, it is unknown whether genetic variants impact subcortical structures during youth. Examining brain structure in adolescence is motivated by the fact that many major mental illnesses including psychosis emerge during this critical period. Additionally, samples from young subjects are less likely to be influenced by confounds such as medication and disease chronicity. Here we capitalized upon the data of the Philadelphia Neurodevelopmental Cohort (PNC) to examine associations between common genetic variants and subcortical volumes, and additionally investigate if variants identified were related to individual differences in cognitive performance.
Methods: The sample comprised 1,445 youth ages 8-22 imaged as part of the PNC. All images were acquired at the same site, using the same scanner (Siemens 3T Tim Trio, 32 channel head coil). Subcortical segmentation was performed using FSL's FIRST utility, providing estimates of thalamus, caudate, putamen, hippocampus, and amygdala volumes that adjusted for intra-cranial volume. Detailed manual quality assurance was performed at every stage of image processing; outliers were flagged for re-evaluation. A second cohort of subjects included 750 participants from the Brazilian High Risk Cohort. The PNC samples were genotyped using either the Illumina HumanHap550, HH610 or Omni Express Bead Chips, the Brazilian cohort was genotyped on the Illumina HumanCore Array. Genetic data was imputed to the 1000 Genomes Phase I integrated variant set. Volumetric data from subcortical nuclei were tested for association as quantitative traits in linear models adjusting for age and sex. Fixed effect meta-analyses of the PNC Caucasian and African American and the PNC and Brazilian data were performed for each region and hemisphere separately. Significant variants were tested for association with cognitive performance, which was assessed in all PNC subjects using a computerized neurocognitive battery.
Results: Four loci reached genome wide significance (threshold set at Pval $\leq$ 5x10-8) following meta-analysis. After multiple testing correction, variants at 4 loci remained significantly associated with caudate, hippocampus and thalamus volumes. Variants at an intergenic locus upstream of ANO3 on chr11p14.2 (rs11029069 P-val 3.639x10-8) were associated with caudate volumes and variants at an intergenic locus upstream of ADCY2 on chr5p31 (rs7703067 P-val 2.1x10-8) were associated with hippocampus volumes. Variants at two loci, an intergenic locus on chr8p22 upstream of FGF20 (rs148338392; p-val 2.98 x10-8) and a locus on chr10p14 that contains 4 genes including ITIH2 and ATP5C1 (rs11255401 p-val 4.9x10-8) were associated with thalamus volumes. Variants at the caudate-associated locus (chr11p14.2; top SNP rs11029069) were associated with spatial memory accuracy, whereas variants at the thalamus locus on chr10p14 (top SNP rs11255401) demonstrated an association with the CNB motor speed.
Conclusions: Genome-wide association testing revealed multiple significant associations between subcortical volumes and common genetic variants. Three of the four loci identified in the meta-analyses were intergenic and the fourth contained multiple genes. All loci were associated with genes that are relevant for brain structure and development. For example, at the caudate locus on 11p14.3 was flanked by ANO3, which ranks 2nd in differential expression in the Caudate nucleus with a five-fold enrichment between striatum and frontal cortex. The hippocampus locus on 5p31 was flanked by ADCY2, the most differentially expressed gene in the hippocampus, and recently associated with bipolar disorder in a large-scale meta-analysis. In summary, data provides novel evidence that common genetic variants of clear neural relevance are related to subcortical volumes during the critical period of adolescence and impact cognitive performance.
Disclosure: Nothing to Disclose.

1.4 Dosage Effects of 22q11.2 Genes on Brain Structure and Function

Carrie Bearden

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Background: Recently, the largest copy number variant (CNV) analysis of schizophrenia to date revealed that duplications at 22q11.2 are substantially less common in

schizophrenia cases than in the general population, indicating that duplications within this locus may represent a putative protective mutation for schizophrenia. However, duplications at the 22q11.2 locus are increasingly associated with autism risk. This intriguing distinction suggests that investigating the effects of these reciprocal imbalances on underlying neural processes may offer a potential window into how these CNV's disrupt the brain and ultimately, contribute to disease pathogenesis.
Methods: Here we used high-resolution structural magnetic resonance imaging (MRI) and network analyses of blood-based gene expression data to investigate the hypothesis that specific dosage effects of genes within the 22q11.2 locus may regulate neuroanatomic traits in a reciprocal manner. We investigated regional neuroanatomic variation in fifty children and adolescents with 22q11.2 deletions or duplications, relative to typically developing, age-matched controls. Next, we conducted whole-genome transcriptional profiling and used systems biology methods (Weighted Gene Coexpression Network Analysis; WGCNA) to identify networks of co-expressed genes associated with these neuroanatomic traits.
Results: Similar to children with idiopathic autism spectrum disorder (ASD), patients with 22q11.2 duplications showed robust volumetric increases in the medial orbitofrontal cortex (mOFC), cingulate gyrus, precuneus/posterior cingulate (PCC), insula and the fusiform gyri, brain regions critically implicated in social cognition, attention and affective processing. In contrast, 22q11.2 deletion patients showed significant decreases in these same brain regions relative to typically developing controls. WGCNA identified 3 gene expression modules that were enriched for brain-expressed genes, which were significantly associated with reciprocal variation in these brain regions. Moreover, genes co-expressed in these modules were down-regulated in 22q11.2 deletion patients with more severe psychotic symptoms.
Conclusions: This "genetics first" approach reveals that dosage-sensitive genes in the 22q11.2 locus give rise to mirrored phenotypes in brain regions critical for social processes. Further, gene co-expression modules were significantly related to psychotic symptomatology and brain structural variation in patients with 22q11.2 mutations. This approach has the potential to accelerate the shift towards personalized therapeutic approaches, and to contribute new insights into the pathogenesis of neuropsychiatric disorders associated with these CNV's, particularly schizophrenia and autism spectrum disorder.
Disclosure: Nothing to Disclose.

Panel
2. Rhythm Disruptions and Mood Disorders: Looking Beyond the SCN

2.1 Atypical Photoreceptors Influence Mood-related Behavior in Mice

Samer Hattar

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Background: The evolution of life on earth depended on light causing organisms to adapt their behaviors to the solar cycle. It is not surprising that light has profound effects on many behaviors that vary from simple phototropism all the way to complex image vision. In humans, light is used for the conscious perception of images and object tracking and for the subconscious regulation of the pupillary light reflex, circadian rhythms, sleep, mood and alertness. It was merely a decade or so ago that rods and cones were thought to comprise the only photoreceptors in the mammalian eye. However, a third type of atypical retinal photoreceptors, the intrinsically photosensitive retinal ganglion cells (ipRGCs), which express the photopigment melanopsin were recently discovered.
Methods: We use extensive gene-targeted mouse line, genetic labeling methods, innovative light cycles, EEGs, optogenetics and behavioral assays.
Results: Our laboratory has revealed extensive brain regions that are innervated by the melanopsin expressing cells, including areas important for sleep and mood regulation. In addition, we used innovative light cycles to show that ipRGC stimulation at irregular times during the activity-rest cycle causes mood and learning deficits independent of sleep deprivation or circadian arrhythmicity. We are currently employing optogenetic techniques to specifically activate melanopsin fibers in defined brain targets, which will allow us to understand how light modulates sleep and mood. For example, we have generated an animal line that lacks the majority of innervations in the brain from ipRGCs, but maintains robust innervation in the suprachiasmatic nucleus (SCN), the central circadian pacemaker. These assays will allow us to test the sufficiency of the SCN for controlling sleep and mood. Recently, we have generated an animal model that will allow us to only eliminate the SCN projections while maintaining innervations to all other brain regions. The use of both of these animals will lead to further understanding of how different brain regions innervated by ipRGCs control behavior.
Conclusions: These studies point to a direct effect of light exposure on mood and learning via ipRRC stimulation of SCN independent circuits.
Disclosure: Nothing to Disclose.

2.2 Daytime Spikes in VTA Dopaminergic Activity Underlie Rapid Mood-cycling in a Mouse Model of Bipolar Disorder

Colleen McClung

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Background: Disruptions in circadian rhythms and dopaminergic activity are involved in the pathophysiology of bipolar disorder, though their interaction remains unclear. Moreover, mood stabilizing compounds like lithium can alter dopaminergic activity and stabilize molecular rhythms.
Methods: Here we used a variety of behavioral assays to assess changes in activity, reward and mood-related behavior. We also performed a number of molecular assays to examine changes in gene and protein expression and transcription factor binding. We also employed optogenetics with a stabilized step function opsin (SSFO) to

control neuronal activity. Finally we performed in vivo recordings with EEGs and/or optical stimulation.
Results: Here we find that mice with a mutation in the Clock gene (Clockdelta19) exhibit rapid mood-cycling, with a profound manic-like phenotype emerging during the day following a period of euthymia at night. Mood cycling coincided with abnormal daytime spikes in ventral tegmental area (VTA) dopaminergic activity, tyrosine hydroxylase (TH) levels, and dopamine synthesis. To determine the significance of daytime increases in VTA dopamine activity to manic behaviors, we developed a novel optogenetic stimulation paradigm that produces a sustained increase in dopamine neuronal activity and find that this induces a manic-like behavioral state. Time-dependent dampening of TH activity during the day reversed manic-related behaviour in Clock mutant mice. Finally, we show that CLOCK acts as a negative regulator of TH transcription by competing with the transcription factor, CREB specifically during the daytime at an overlapping site in the TH promoter.
Conclusions: Taken together, these findings reveal a novel molecular mechanism underlying cyclic changes in mood-related behaviour. They also underscore the importance in diurnal changes in dopaminergic circuitry in the regulation of mood.
Disclosure: Nothing to Disclose.

2.3 Circadian Clocks in Fibroblast and Mouse Models of Mood Disorders

David Welsh

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Background: There has long been evidence of circadian rhythm disruption in mood disorders patients, lately including associations with clock gene polymorphisms and weaker rhythms of gene expression in the depressed brain. Our goal is to define cellular circadian clock dysfunctions in fibroblasts from mood disorders patients, whether such dysfunctions extend to neurons from mood-regulating brain regions in mouse models, and whether manipulating circadian clocks in such neurons affects vulnerability to depression-like behavior.
Methods: We study circadian rhythms in fibroblasts from depressed and bipolar patients, and in brain slices from mice exhibiting either depression-like behavior (induced by a learned helplessness procedure) or manic-like behavior (induced by knockdown of the dopamine transporter; DAT-KD). To monitor circadian clock function, we measure rhythmic expression of the circadian clock gene Per2 over time in the same cells using bioluminescent reporters. To manipulate clock function in specific mouse brain areas, we perform stereotaxic injection of siRNAs directed against the essential clock gene Bmal1.
Results: In fibroblasts from patients with bipolar disorder ($n = 19$), compared to control cells ($n = 19$), PER2 rhythms have a longer circadian period and respond less strongly to amplitude-enhancing effects of the mood stabilizer lithium. We are currently performing similar studies in cells from patients with major depression ($n = 33$). In mouse studies, two mood-regulating brain regions of helpless mice, the nucleus accumbens (NAc) and periaqueductal gray (PAG), show reduced PER2 rhythmicity compared to controls. In these brain regions, reduced rhythmicity arises from fewer cells expressing PER2 as well as a broader phase distribution of single-cell PER2 rhythms. We are now beginning to test the effects of manipulating clock genes in these brain regions on depression-like behavior. We are also studying circadian rhythms in the DAT-KD model of mania.
Conclusions: In cells from bipolar patients, circadian clocks are slower and less responsive to lithium. In NAc and PAG brain regions of helpless mice, circadian clocks are less rhythmic. This may reflect a causal role for weakened circadian clocks in increasing vulnerability to mood disorders, which we are now testing in ongoing experiments.
Disclosure: Nothing to Disclose.

2.4 Role of the Anterior Cingulate in the Pathophysiology of Mood Disorders: Circadian Abnormalities

William Bunney

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Background: Depression has been associated with circadian abnormalities including sleep, hormones, temperature and mood which are regulated by core clock genes. The anterior cingulate (ACC) serves as a modulator of affective and cognitive function. Structural and functional brain imaging studies consistently implicate the ACC as a critical structure in the pathophysiology of major depressive disorder (MDD). However, there has been no data to show that clock gene function in the ACC of depressed patients is dysregulated.
Methods: Microarray analysis (12,000 transcripts) were run in the ACC in controls and MDDs matched for time-of-death around the 24hr clock. To discover the cyclic genes, we fit the expression values for each gene by a sinusoidal function of time using the method of least squares and fixing the period at 24hrs. The statistical significance of the findings was evaluated by permutation, randomly assigning time-of-death data across subjects 1000 times.
Results: The 10 top-ranked circadian genes with the most robust rhythms showed consistent disruption in 24 hr expression patterns in the ACC in MDD patients compared to matched controls.
Conclusions: First direct evidence that core clock genes showed highly significant disruption in the ACC in MDD patients.
Disclosure: Nothing to Disclose.

Panel
3. Drug Repurposing and Emerging Adjunctive Treatments for Schizophrenia

3.1 Adjunctive Minocycline in Clozapine Treated Schizophrenia Patients with Persistent Symptoms

Deanna Kelly

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Background: Clozapine is the most effective antipsychotic treatment for chronic and treatment refractory

patients with schizophrenia. It is the only antipsychotic that has been FDA-approved for treatment-resistant schizophrenia and it provides effective treatment even when patients do not respond to other second-generation antipsychotics. However, there is no evidence base for treatment selection in patients who are partially responsive to clozapine treatment. Clinical trials in this population with adjunct antidepressants, antipsychotics and other medications have at best shown minor effects. Minocycline is a synthetic, FDA approved derivative of tetracycline with a more tolerable side effect profile than other agents in this class. This medication crosses the blood brain barrier and has pharmacologic effects at the glutamatergic system (GluR1 AMPA subtype) as well as anti-inflammatory actions, antioxidant and antiapoptotic effects. Accumulating preclinical and clinical data suggest minocycline may be effective for treatment of schizophrenia, particularly in first episode psychosis and for negative symptoms.
Methods: We have completed a 10 week, randomized double blind placebo controlled study of adjunct minocycline (100 mg BID) compared to placebo in 50 participants stabilized but only partially responsive to clozapine. We examined the effects of minocycline on cognitive function (MATRICS Consensus Cognitive Battery, MCCB), positive and anxiety/depressive symptoms (Brief Psychiatric Rating Scale, BPRS), avolition (Scale for the Assessment of Negative Symptoms, SANS) and the Calgary Depression Scale (CDS). Primary endpoints were predetermined as cognitive function and positive symptoms.
Results: 29 participants were assigned to minocycline and 23 to placebo. Two participants assigned to minocycline discontinued early, all others completed. Patients did not differ on demographic variables, clozapine dose or clozapine blood level at baseline. On the MCCB there was no significant improvement on the composite score but a domain x treatment interaction ($F = 2.78$, $df = 6,41.6$, $p = 0.03$) showed an improvement in working memory among minocycline patients (minocycline-placebo difference = 4.81 ± SE 1.82, $p = 0.023$ (effect size 0.41). The BPRS total score ($p = 0.075$, effect size 0.55) and BPRS psychosis factor ($p = 0.098$, effect size = 0.39) tended to improve more with minocycline versus placebo. There was no significant treatment effect on the SANS total score, but there was a significant improvement in avolition in patients assigned to minocycline compared to placebo patients (minocycline-placebo difference = -0.22, $p = 0.012$, effect size = 0.34). There was also a significant improvement with minocycline on the anxiety/depression subfactor on the BPRS ($p = 0.028$, effect size = 0.49). Minocycline was well tolerated with significantly fewer headaches and constipation compared to placebo.
Conclusions: We found modest improvements in working memory and avolition with minocycline in a treatment resistant and chronically ill population. Larger studies are needed to confirm these findings and determine whether there are any effects on psychosis or anxiety/depressive symptoms.
Disclosure: Nothing to Disclose.

3.2 Positive Symptoms Respond to Add-on Aspirin in Schizophrenia Patients with High Sera CRPLevels: A Post-hoc Analysis of an RCT

Mark Weiser

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Background: Investigators have hypothesized that schizophrenia might have an inflammatory component. We tested this hypothesis with a post hoc analysis of data from a previously performed RCT which administered add-on aspirin or placebo to patients with schizophrenia receiving anti-psychotics. We hypothesized that patients with high levels of CRP, perhaps reflecting high levels of inflammation, would have a better response to aspirin compared to patients with lower levels of CRP.
Methods: The original study was a multi-center, $N = 400$ trial was designed with one placebo arm to be employed as a comparator for 3 active arms. Inclusion criteria were 4 (moderate) or above on CGI-S and 4 (moderate) score or above on two of the following four PANSS items: delusions, hallucinatory behaviors, conceptual disorganization or suspiciousness/ persecution, and/or a total PANSS negative symptoms score above 18. Before entering the trial and throughout the trial all subjects received anti-psychotics at doses within PORT recommendations. Upon entering the trial they were randomized to aspirin 1000 mg/d + pantoprazole 40 mg/d, minocycline 200 mg/d, pramipexole 1.5 mg, or placebo. Duration of the study was 16 weeks. Primary outcome measure was changes in total PANSS scores, secondary outcome measures included PANSS subscales.
Results: Mean age of patients was 42, 50% were females, mean duration of illness was 13 years, mean PANSS total score at baseline was 92. The ANOVA for overall change for all comparison of 3 drugs and placebo for the primary outcome of the total PANSS scores was significant, $p = 0.03$. Individual comparisons between each drug and placebo showed trends for significance (Effect size, ES = 0.28, $p = 0.056$) for aspirin, and were non-significant for minocycline ($ES = 0.14$, $p = 0.33$) and for pramipexole ($ES = 0.01$, $p = 0.95$). For positive symptoms the overall ANOVA was not significant, $p = 0.084$. Individual comparisons between each drug and placebo showed a trend for significance for aspirin ($ES = 0.24$, $p = 0.08$), and were non-significant for minocycline ($ES = 0.04$, $p = 0.77$) and pramipexole ($ES = 0.11$, $p = 0.45$). The sample was then divided into thirds according to CRP level at baseline. Patients with high ($CRP > 3850$ng/ml) were significantly more likely to have improvements in their mean PANSS positive scores ($ES = 0.61$, $p = 0.03$), whereas patients with intermediate CRP scores ($1300 < CRP \leq 3850$ ng/ml, $ES = 0.07$, $p = 0.78$) or low CRP scores ($CRP \leq 1300$ ng/ml, ES = -0.35, $p = 0.19$) did not. These results were not observed on the effects of aspirin on negative symptoms, general psychopathology or total PANSS, nor were they observed in the patients receiving minocycline or pramipexole.
Conclusions: The results of this post-hoc analysis might cautiously be interpreted as indicating that a subgroup of patients with relatively high levels of CRP, a non-specific

marker of inflammation, have significant improvements in positive symptoms upon inhibition of COX-1 or COX-2, or other biological effects, both inflammatory and non-inflammatory of aspirin. The effect of aspirin on this small subgroup of responders might be the reason that previous studies found a small, consistently replicated over-all effect of aspirin in schizophrenia which was too small to be of clinical significance. This issue should be further tested by 1) performing similar post-hoc analyses on previous RCTs which administered aspirin or other anti-inflammatory agents in schizophrenia. Future studies might screen patients for CRP and randomize those with high CRP levels to add-on treatment with aspirin or placebo.
Disclosure: Nothing to Disclose.

3.3 Effects of (6S)-5-Methyl-5,6,7,8-Tetrahydropteroyl-L-Glutamic Acid Supplementation on Cortical Thickness in Schizophrenia

Joshua Roffman

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Background: Altered one-carbon metabolism has been implicated in genetic, epidemiologic, and brain imaging studies of schizophrenia. Supplementation with folic acid, which supplies one-carbon moieties for methylation reactions, has been associated with modest improvements in schizophrenia symptoms. However, treatment response differs based on the presence of low-functioning variants in folate-related genes. Similarly, genetic variation in MTHFR, a key enzyme in the one-carbon pathway, has been associated with increased schizophrenia risk and altered MRI phenotypes. Here, we determined whether (6S)-5-methyl-5,6,7,8-tetrahydropteroyl-L-glutamic acid (5-MTG), the fully reduced and bioactive form of folate that circumvents MTHFR, influenced cortical thickness as part of a placebo controlled trial in schizophrenia patients.
Methods: 50 outpatients with chronic schizophrenia completed a single site, parallel-group, randomized, double-blind, placebo controlled clinical trial of 12 weeks of treatment with 15 mg 5-MTG. 35 patients with high quality pre- and post-treatment MRI scans (Siemens TIM Trio) were included in the analysis. Cortical thickness was determined across the entire cortical mantle using FreeSurfer. Clusters showing significant time x treatment interactions were subject to 10,000 Monte Carlo simulations to control for multiple comparisons across the cortical surface.
Results: When compared with the placebo group, patients who received 5-MTG exhibited increased pre- to post-treatment thickness within the left medial prefrontal cortex (time x treatment interaction $p < 0.05$, corrected). In contrast, patients receiving 5-MTG showed pre- to post-treatment thickness decreases within the right inferior and middle temporal gyrus ($p < 0.05$, corrected). In planned post-hoc analyses of the active treatment group based on MTHFR genotype, medial prefrontal thickness changes were driven by patients homozygous for the MTHFR 677C allele (0.17 mm in C/C versus 0.03 mm in T carriers, $p = 0.044$), while lateral temporal thickness changes were driven by 677T carriers (-0.02 mm in C/C versus -0.13 mm in T carriers, $p = .015$).
Conclusions: 5-MTG supplementation was associated with opposing cortical thickness changes within medial prefrontal and lateral temporal regions, suggestive of remodeling within the default network. Overall, these changes occurred across both genotype groups, as would be expected given that 5-MTG bypasses MTHFR. However, surprisingly, genotype effects differed between regions, suggesting that local cortical effects of 5-MTG reflect disparate mechanisms. Additional planned analyses, including resting state functional connectivity and task-related activation, may further clarify this pattern.
Disclosure: Part 4: Pamlab.

3.4 Folate Supplementation for Antipsychotic Cardiovascular Complications and the Impact of Cardiovascular Disease on Neurocognition in Schizophrenia

Vicki Ellingrod

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Background: Previous work done by our group has shown that the occurrence of metabolic syndrome in schizophrenia patients is related to dietary folate, its pharmacogenetically regulated metabolism, and atypical antipsychotic (AAP) exposure. Therefore, we examined how folate supplementation would affect metabolic measures and endothelial functioning (RHI) in schizophrenia subjects meeting NCEP-ATP-III metabolic syndrome criteria who were treated with antipsychotics. Additionally we examine the impact of cardiovascular disease (measured using RHI) on neurocognition in schizophrenia patients.
Methods: Subjects included in this analysis were enrolled in one study which consisted of two phases. For phase I, subjects who had a diagnosis of schizophrenia, and had been receiving treatment with antipsychotics were included. Subjects who had a substance abuse diagnosis or were unwilling or unable to participate were excluded. For phase I on this study, subjects were seen in a cross-sectional fashion and assessed and screened for endothelial functioning (RHI assessment using the Endo-PAT2000), a fasting metabolic laboratory panel was obtained, and DNA was gentoyped for the methylenetetrahydrofolate reductase (MTHFR) 677C/T and catechol-O-methyltransferase (COMT) 158 Val/Met variants. Based on these results, subjects were grouped according to their MTHFR 677 T allele and COMT 158Val allele status, and those whose RHI < 1.67 met criteria for endothelial dysfunction. Additionally subjects were given the classification of meeting metabolic syndrome criteria using the NCEP-ATP-III guidelines utilizing our laboratory data. Neurocognitive performance was determined using the Brief Assessment of Cognition in Schizophrenia (BACS). Subjects meeting NCEP-ATP-III criteria for metabolic syndrome were invited to participate in the Phase II and were given 5mg/day open label folate for 3 months. Baseline and 3 month measurements included RHI, BMI, fasting metabolic laboratory measures, C-reactive protein, homocysteine, IL-6, and leptin. Additionally we measured physical activity using a standardized assessment.
Results: A total of 95 participants were included in the Phase I analysis. Subjects had a mean age of 45 years and 56% male. Additionally the subjects' average duration of illness was 19 years, 44% were Caucasian, and 38% met metabolic syndrome criteria. All analyses will controlled for race, gender, and

education since there were genetic differences in these baseline demographics and both genotypes were in Hardy Weinberg disequilibriurm. Overall the BACS composite scores were predicted by level of education as well as, MTHFR genotype and an interaction between MTHFR and COMT genotypes ($p = 0.02$). Additionally an interaction between genotype groups and endothelial dysfunction was found ($p = 0.03$). For subjects not meeting endothelial dysfunction (RHI $\geq$ 1.67), education best predicted BACS composite scores ($p < 0.0001$), while for those meeting endothelial dysfunction criteria (RHI < 1.67), poorer overall neurocognition was seen which was associated with the MTHFR 677 T and COMT 158 Val alleles ($p = 0.03$). For Phase II a total of 35 subjects with a mean age of 50 ± 9 years and 70% Caucasian were enrolled. After 3 months supplementation, RHI improved by 20% ($p = 0.02$), mean homocysteine decreased 14% ($p = 0.006$), and IL-6 decreased 13% ($p = 0.09$). Subjects exercised 15% less during the study ($p = 0.05$). At baseline 61% met endothelial dysfunction criteria (RHI < 1.67), which decreased to 27% ($p = 0.0006$) at endpoint. The MTHFR 677C/C + COMT 158Met/Met subjects had a 44% RHI improvement versus 10% improvement for MTHFR 677T/COMT Val allele carriers ($p = 0.06$). The MTHFR 677C/C + COMT 158Met/Met group also showed significant reduction in those meeting endothelial dysfunction (83% baseline and 16% endpoint), compared to the MTHFR T + COMT Val allele carriers (54% baseline and 31% endpoint[$p = 0.001$]).

Conclusions: Cardiovascular disease (CVD) and endothelial dysfunction, in particular, is a specific concern in this population and may have a greater impact of neurocognition in schizophrenia than previously thought. Assessment for overall CVD risk or endothelial functioning is rarely done in clinical practice, but may be an exceedingly important assessment as it is treatable condition. Use of folate to reduce antipsychotic-associated metabolic risks may result in significant reductions in the number of subjects meeting endothelial dysfunction. This is remarkable given that ALL subjects met metabolic syndrome criteria. This may prove as a useful avenue to reducing CVD risk as well as potentially improving neurocognition in some patients with schizophrenia; however those with the MTHFR T or COMT Met alleles may not see this benefit although this needs further follow up.

Disclosure: Nothing to Disclose.

Panel
4. Trans-species Models Examining Estradiol Effects on Emotion and Cognition Across Development

4.1 Estradiol Replacement in Ovariectomized Rats Increases Resilience in the Learned Helplessness Model of Depression and Protects Hippocampal Function

Lori McMahon

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Background: Declining ovarian hormones during postpartum, peri-menopause transition, or menopause may be linked to increased susceptibility to stress and the development of depression in women, which often is accompanied by hippocampal learning and memory impairment. Stress-induced elevations in glucocorticoids decrease dendritic spine density and LTP at hippocampal synapses, while proestrous levels of estradiol increase spine density and LTP. We tested the hypothesis that estradiol opposes the detrimental effects stress and decreases acquisition of helplessness and hippocampal dysfunction related to depression. We are also testing the hypothesis that estradiol increases resilience through increasing transmission mediated specifically by GluN2B-mediated NMDAR currents on CA1 pyramidal cells and GABAergic interneurons and whether hippocampus-dependent learning and memory deficits ocurr in helpless but not in resilient rats experiencing the same stress.

Methods: The learned helplessness preclinical model was used to determine whether estradiol replacement decreases acquisition of helplessness in ovariectomized female rats and whether the depession-like phenotype is accompanied by hippocampus dependent learning and memory deficits. Brain slice electrophysiology and golgi staining is used to investigate whether helplessness is linked with deficits in dendritic spine density and LTP. Whole- cell patch clamp recordings of GABAergic interneurons is used to determine how acquisition of helpless alters the strength of glutamate transmission at synapses onto select GABAergic interneurons located in area CA1 and whether estradiol increases GluN2B-mediated NMDAR currents. Novel object recognition and placement are used to assess learning and memory deficits in rats reaching criteria for helplessness versus those that are considered resilient. An unpaired t test was used to determine significance at $P < 0.05$.

Results: Fifty-five percent of vehicle-treated ovariectomized rats reached criteria for helplessness, while only twenty-two percent were helpless when treated with proestrous-like levels of estradiol. Importantly, helplessness was associated with decreased spine density and a deficit in LTP, while LTP and spine density in rats experiencing the same inescapable shock but demonstrate resilience during escape are equivalent to non-shocked controls. Moreover, estradiol replacement reversed previously established helpless behavior in 40% of rats tested. In ongoing studies, we are assessing whether rats which have acquired the helpless phenotype have hippocampus dependent learning deficits and how the helpless phenotype impacts excitation/inhibition balance in hippocampal synaptic circuits.

Conclusions: Proestrous levels of estradiol can protect against acquisition of helplessness and prevent hippocampal synaptic dysfunction and learning deficits. These results may be relevant to the potential use of estradiol replacement in some women to enhance resilience to stress and for treating cognitive deficits in depression when estrogen levels decline.

Disclosure: Nothing to Disclose.

4.2 Sex Differences in Fear Extinction and Its Relevance to Anxiety Disorders

Kelimer Lebron-Milad

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Background: Over the last decades there has been a lot progress elucidating the neural circuits of fear extinction,

which are important and relevant to the pathophysiology of anxiety disorders. We have used cross-species (female rodents and women) translational approach to show that fear extinction memory is modulated by endogenous and exogenous manipulations of estradiol. We are now examining the mechanisms by which estrogen strengthens extinction memory consolidation.

Methods: We conducted parallel human and rodent studies using fear conditioning and extinction protocol. In female rats, we investigated how acute and chronic SSRI treatment might influence fear extinction memory differently depending on the phase of the estrus cycle. Moreover, we are currently examining how estradiol is modulating the consolidation of extinction recall and the cellular and molecular mechanisms by measuring c-Fos and several markers of neural activity such as MAP kinases. In women and using fMRI, we examined how the dynamic fluctuations of estradiol across the menstrual cycle and the use of hormonal contraceptives may influence the BOLD signal during fear conditioning and extinction at the trial-by-trial level.

Results: In female rats, while the acute anxiogenic effects of SSRI treatment did not differ at different phases of the cycle, chronic SSRI treatment was most effective in facilitating extinction memory consolidation at the metestrus phase of the cycle (low estrogen). Preliminary immunohistochemistry data showed that estradiol induces greater c-fos expression in the infralimbic cortex relative to prelimbic cortex following extinction training and recall, while no significant differences in c-Fos expression were noted in the amygdala. In women, activation induced by conditioned cues during fear acquisition tracked the expression of skin conductance responses at the trial-by-trial level in different brain regions including the dorsal anterior cingulate cortex, amygdala, and hypothalamus. The magnitude of the correlation level and the timing of the correlation (during early phase vs. late phase of learning) were influenced by the natural variance of estrodiol in women.

Conclusions: These findings highlight the complex role estrodiol (and possibly other sex hormones) are playing to mediate memory consolidation related to fear extinction. Implications for women's health and anxiety disorders will be discussed in light of these novel findings.

Disclosure: Nothing to Disclose.

4.3 Estradiol Level Changes Alter Brain and Subjective Response to Psychosocial Stress and Negative Emotional Processing

Paul Newhouse

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Background: One of the most replicated epidemiologic findings in psychiatry is the higher incidence of affective/anxiety disorders in women with rates above puberty and below menopause approximately twice that of men. Among the strongest candidates for an important role in this gender difference are sex hormones, particularly estradiol, interacting with genetic vulnerability and life stress. To investigate what role(s) sex hormones play in mood and cognition regulation, we have conducted experimental studies that are simultaneously manipulating sex hormone levels, neurotransmitter function, and cognitive/emotional stimuli to parse what role the presence or absence of these hormones may have with regard to both normal functioning and psychopathology, especially in women. Studies performed in human models utilize translational and multimodal approaches fusing imaging, neuropharmacologic and neurohormonal manipulations, and sophisticated psychological and cognitive approaches.

Methods: We have recently examined the effects of differing estradiol (E2) levels across the menstrual cycle and after menopause on brain activity and negative mood response to negative emotional images and psychosocial stress. In the pre-menopause stress study, 28 normally cycling women (18-45, mean 30.4) were examined during the low E2 early follicular phase (day 1-2 of menstrual cycle) or the high E2 periovulatory phase (day 12-14) using the the Montreal Imaging Stress Task (MIST) during fMRI. We have also examined 13 normal cycling women (mean age 24 ± 2.9) at two different times in the menstrual cycle to examine the effects of varying E2 levels on negative emotional stimulus evaluation. Women viewed negative and neutral pictures on the computer screen and were asked to make a judgment about whether each picture was pleasant, unpleasant, or neutral on Day 1 of their menstrual cycles, (low E2), and at Day 12 (periovulatory), high E2. After menopause, we repeated the emotional stimulus study in 20 healthy normal PMW randomly assigned to 3 months of oral E2 or placebo. Finally we examined the mood and cognitive effects of chronic E2 administration in postmenopausal women after acute psychosocial stress in 22 women (64.3 ± 10.6) placed on either placebo or 17β-estradiol. Subjects performed the Trier Social Stress Test (TSST), followed by mood and anxiety ratings and cognitive testing.

Results: In the menstrual cycle stress study, women in the low E2 early follicular phase showed less left hippocampal activity during psychosocial stress than women in the high E2 periovulatory phase Women in the low E2 phase had more subjective distress (change in subjective stress due to the MIST) than women in the high E2 phase. Comparing the brain activity response to psychosocial stress in women who show differing subjective distress (low distress vs high distress), less bilateral hippocampal and greater subgenual cingulate activity during psychosocial stress was seen in women who had high subjective distress to the MIST ($p < .01$). In the emotional images study, during exposure to negative images in the high-E2 phase, greater activity was seen during negative picture blocks in the right insula (BA 13), and precuneus (BA 7) compared to the Low-E2 phase. In addition, greater connectivity was seen to the same areas plus also the subgenual cingulate (BA 25), suggesting E2-induced increases in connectivity among emotion-regulating structures. By contrast, after menopause, the effects of E2 appear to reverse. In postmenopausal women, high E2-treated women show reduced brain activity during negative picture blocks in many of the same limbic structures that had shown increases in activity during high E2 menstrual phase in younger women, including the left dorsal anterior cingulate (BA 32). Connectivity was no longer enhanced to more dorsal emotional regulatory structures such as was seen in the cycling women, but only to the subgenual

cingulate (BA 25). Finally acute stress in E2-treated post menopausal women E2-treated subjects produced significant ($p < .01$) increases in negative mood, hostility, anxiety, and impairment of cognitive performance compared to placebo-treated women, which is in contrast to results seen high E2 levels in cycling women.
Conclusions: These data indicate the subjective distress to acute psychoscial stress is associated with reduced hippocampal activity and increased activity in subgenual cingulate (a brain area important in negative mood) and that premenopausally, the greater the E2 level, the less hippocampal deactivation occurs with psychosocial stress. Thus, during the normal menstrual cycle, endogenous E2 reduces the brain activity changes and negative mood response to psychosocial stress. Also, before menopause, higher E2 levels appear to enhance the more dorsal structures that regulate activity associated with negative emotional information. However, postmenopause, high E2 levels appear to reduce activity in the same structures and only enhance connectivity to areas associated with depressed mood. Finally, exogenous E2 also enhances the negative effects of psychosocial stress on mood and cognition. These results indicate that estradiol can modulate the brain activity and mood response to negative emotional information and psychosocial stress and that this is related to subjective mood response. However, at menopause, the relationship appears to change and what explains this apparent reversal is as yet unclear. Significant effects of varying E2 levels on emotional processing and psychological stress that change across the reproductive lifecycle of women may lead to better understanding of the increased vulnerability to mood disorders, and posttraumatic stress disorder.
Disclosure: Nothing to Disclose.

4.4 What Doesn't Kill You Might Make You Stronger: The Relationship Between Early Life Adversity and Risk for Depression and Cognitive Decline at Menopause

Cynthia Epperson

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Background: The menopause transition is associated with an increased risk of first onset major depression, an age-independent decline in verbal memory and new onset executive function difficulties. We present here compelling evidence that adverse childhood experiences (ACEs) contribute significantly to this vulnerability at menopause even in women who have been resilient until this transition.
Methods: Utilizing both proton magnetic resonance spectroscopy (1H-MRS) and functional magnetic resonance imaging (fMRI) at 7 Tesla we examined the impact of ACEs on hippocampal and prefrontal cortex (PFC) neurochemistry and activation during working memory task performance among menopausal women both pre and post estradiol or psychostimulant administration. For the estradiol paradigm, women also underwent tryptophan depletion (TD) to examine the individual and interactive effects of estradiol and serotonin on brain activation. Women participating in the psychostimulant study were hypogonadal after a natural or surgical menopause and underwent both fMRI and 1H-MRS to examine the impact of lisdexamfetamine (LDX) on both dorsolateral PFC activation and glutamate levels. Outcomes were compared for women with no history of ACEs and those with >2 ACEs.
Results: In whole brain analyses of 18 women, left hippocampal activation during N-Back Task performance is accentuated under conditions of TD in those women with >2 ACEs. Similarly, ACE history is associated with greater whole brain activation, particularly in task relevant regions during N-Back Task performance, an association that is completely absent after LDX treatment. Notably, subjective improvement in executive functions and objective measures of delayed verbal memory were correlated with reductions in DLPFC glutamate with LDX treatment.
Conclusions: ACE history impacts brain activation and neurochemistry in a manner that may contribute to mood and cognitive changes at menopause.
Disclosure: Part 1: Novartis and Shire– research grant support, **Part 4:** Novartis- products.

Panel
5. Stress Resilience Molecules and Mechanisms

5.1 Endocannabinoids, Stress and Psychiatric Disorders

Sachin Patel

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Background: The stress reducing effects of the CB1 cannabinoid receptor agonist, delta-9-tetrahydrocannabinol, have long been appreciated by users of cannabis sativa. CB1 cannabinoid receptors are expressed in brain regions known to be stress-responsive, including the hypothalamus, hippocampus, amygdala and prefrontal cortex. Brain concentrations of the endogenous ligands of CB1 receptors, N-arachidonylethanolamine (AEA or anandamide) and 2-arachidonoylglycerol (2-AG), are altered by stress mediators, including glucocorticoids, CRF and norepinephrine. Both endocannabinoids are also present in the circulation and are responsive to stress and altered in individuals with psychopathologies.
Methods: The results of two studies will be reported. In the first, plasma or serum samples were isolated from venous blood draws taken from human subjects. Lipids were extracted using solid phase chromatography and AEA, 2-AG and related lipids were quantified using isotope dilution, liquid chromatography mass spectrometry. In the second study, Sprague Dawley rat dams and their litters were assigned to one of two groups; one group was housed from days 1-10 in a cage containing limited bedding while the control group had three times as much bedding and paper towels. On day 10, all of the pups were sacrificed; brains were dissected and mRNA quantified using real time PCR techniques.
Results: Data from healthy controls demonstrate that concentrations of AEA are rapidly increased in the serum of males following exposure to the Trier social stress test. The response in females is less robust. Interestingly,

baseline AEA concentrations are inversely correlated with measures of anxiety, leading to the hypothesis that hypoactive AEA-mediated signaling could contribute to the symptoms of anxiety. This hypothesis is supported by other preclinical and human subjects studies. To further explore this hypothesis, endocannabinoid concentrations were measured in serum from individuals directly or indirectly exposed to the 9/11 trauma. Although AEA concentrations were not different between those diagnosed with PTSD and controls; AEA concentrations were negatively correlated with intrusive symptoms.
Conclusions: Circulating concentrations of AEA are tonically regulated by the psychological state, including anxiety and the presence of intrusive aversive memories following trauma. In addition, serum of AEA are acutely elevated by exposure to a stress. These and other findings lead to the hypothesis that serum concentrations of AEA are in equilibrium with concentrations in the brain and, therefore, function as a biomarker of fear processing.
Disclosure: Nothing to Disclose.

5.2 Endogenous Opioids: Restraining Stress with a Cost

Rita Valentino

The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

Background: The locus coeruleus (LC)-norepinephrine (NE) system is a major stress response system that mediates certain cognitive aspects of the stress response including initiating arousal, shifting attention and promoting cognitive flexibility. LC activation during stress is mediated in part by the stress-related neuropeptide, corticotropin-releasing factor (CRF). Along with CRF release, stressors release endogenous opioids that have opposing inhibitory effects on LC neurons and may serve to restrain the acute response of the LC-NE system to stress and facilitate recovery after stressor termination. In contrast to acute stress, repeated or chronic stress results in maladaptations that are associated with stress-related pathology. Because social stress is a prevalent stressor for humans that has been implicated in psychiatric disease, we studied the effects of repeated social stress on co-regulation of the LC-NE system by CRF and opioids and its consequences.
Methods: Single unit LC neuronal activity was recorded from locus coeruleus neurons of adult male unanesthetized rats before and after repeated exposure to social stress using the resident-intruder model or control manipulation. The effects of repeated social stress on cognitive performance and on the relationship between LC discharge and cognitive performance was also determined by recording LC activity during the performance of an attentional set shifting task. Finally, the potential for sex differences in opioid modulation of the LC was examined in electrophysiological studies using adult female rats.
Results: Although both CRF and endogenous opioids are released to regulate LC activity during acute stress, CRF excitation is the prominent influence. Repeated social stress shifts this such that LC neurons come under a prominent endogenous opioid inhibition, in part, as a result of CRF receptor downregulation. Administration of the opiate antagonist, naloxone selectively increased discharge rates of stressed rats providing evidence that LC neurons were under the influence of opioids. Notably, LC discharge rates of stressed rats were increased by naloxone to levels well above those of control rats, resembling a cellular correlate of opioid withdrawal. Consistent with this, naloxone also elicited signs resembling mild opioid withdrawal in stressed rats. Rats exposed to repeated social stress performed better in certain components of the attentional set shifting task. LC recordings during task performance revealed that repeated social stress renders LC neurons reward-responsive irrespective of predictability of reward. Finally, initial studies comparing the efficacy of the m-opioid receptor (MOR) agonist, DAMGO, on LC neuronal activity in male and female rats suggests a decreased efficacy in females. Consistent with this, MOR protein levels are decreased in rat LC.
Conclusions: Stress engages CRF and opioid afferents to the LC that have excitatory and inhibitory influences on LC neuronal activity, respectively. During acute stress CRF excitation predominates. If this persisted, it would be expressed as arousal symptoms of stress-related psychiatric disorders. The opposing opioid influence may restrain or protect against this. With repeated social stress, the opioid inhibition predominates. Although this may protect against hyperarousal symptoms, the development of a cellular state of opioid dependence and increased neuronal responses to reward may predispose to substance abuse. The decreased sensitivity of LC neurons of female rats to opioids taken with the previously described increased sensitivity to CRF may explain their vulnerability to the hyperarousal-like features of stress-related psychiatric diseases.
Disclosure: Nothing to Disclose.

5.3 The Role of the Urocortins/CRFR2 System in the Regulation of the Central Stress Response

Alon Chen

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Background: Maintenance of homeostasis in the presence of real or perceived challenges requires numerous adaptive responses involving changes in the central nervous, immune and neuroendocrine systems. Both activation and termination of the behavioral, autonomic and adrenocortical stress responses are critical for adaptation and survival. Accumulating genetic and pharmacological studies support an important role for the brain Urocortins / corticotropin releasing factor receptor type 2 (CRFR2) system in mediating behavioral and physiological responses to diverse stressors. This system may be particularly important in situations where an organism must mobilize not only the hypothalamic-pituitary-adrenal system, but also the central nervous system in response to environmental challenge. Clearly, dysfunction in such a fundamental brain-activating system may be the key to a variety of pathophysiological conditions involving abnormal responses to stressors such as anxiety, affective and eating disorders. While CRFR2 and the triple Urocortin mutant mice show increased anxiety-like behaviors following

challenge, the anxiety-related effects of administration of CRFR2 agonists and antagonists into the cerebral ventricles or into specific brain regions have been less consistent, with some evidence for brain site or ligand specificity.
Methods: The inconsistency between studies regarding the effects of pharmacological manipulation of CRFR2, coupled with the existence of multiple potential endogenous ligands (Urocortin-1, Urocortin -2, Urocortin -3), has made the attribution of precise stress-related functions to individual Urocortin family peptides problematic. Conventional gene knockout models, generated by homologous recombination for different family members, have provided important information toward elucidating the function of these genes. However, these mice showed significant changes in the expression levels of the other family members in the CNS, likely due to developmental compensatory mechanisms, which may have contributed towards the observed (or non-observed) stress-related phenotypes. Therefore, to avoid both the developmental compensatory changes and to genetically target our gene of interest at specific brain nuclei, we use novel transgenic mice models and viral and optogenetic tools that we have recently generated and validated, which allow us to manipulate both the levels and site of expression of our gene of interest, in adult mice.
Results: A brief summary of our recently published findings concerning the Urocortin / CRFR2 system will be followed by new and unpublished set of data. We observed a prolonged up-regulation of CRFR2 expression in the posterior bed nucleus of stria terminalis (pBNST) following exposure to stressful challenges. CRFR2 was specifically expressed in GABAergic neurons within the pBNST, indicating their potential role in mediating stress coping. To investigate the specific contribution of these cells to stress-related behaviors and neuroendocrine functions, we established and characterized a novel transgenic mouse model expressing the Cre-recombinase enzyme specifically in CRFR2 neurons. Validated CRFR2-Cre mouse line was crossbred with conditional channelrhodopsin or halorhodopsin mouse lines to allow optogenetic control of CRFR2-positive neurons. Mice subjected to bilateral pBNST-CRFR2 neurons optogenetic activation or inhibition were evaluated using a battery of anxiety-like behavior tests and HPA axis activity. Interestingly, light-induced activation of the pBNST CRFR2 neurons decreased anxiety-like behavior, attenuated the neuroendocrine stress response, and ameliorated the long-term behavioral effects of stress exposure, as well as the memory for the stressful event. Intriguingly, the light-induced suppression of the same neuronal population induced the exact opposite behavioral and neuroendocrine effects.
Conclusions: We demonstrate that CRFR2 mRNA levels in BNST are specifically and substantially increased for at least 48 hours after a stressful challenge while site-specific knockdown of CRFR2 in the pBNST or CRFR2 optogenetic inhibition promotes an anxiogenic phenotype following stressful challenge. We conclude that the pBNST CRFR2 subpopulation exerts an anxiolytic role during the post-stress period. This is in agreement with the proposed role of CRFR2 in promoting successful recovery from stress and the role of the BNST in sustained fear states, and furthermore suggests that this process may be dependent on CRFR2 activity in the pBNST site.

Disclosure: Part 1: Consultant for miCure Therapeutics, **Part 2:** Consultant for miCure Therapeutics, **Part 4:** Consultant for miCure Therapeutics.

5.4 Neurobiological Mechanisms of Exercise-evoked Stress Resistance

Monika Fleshner

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Background: Exposure to acute and excessive stressors produces changes in the brain/behavior and other physiological systems. Resistance to suffering negative mental and physical consequences of stress can be modulated by many factors, including physical activity status. Rats that voluntarily run on wheels for 6 weeks compared to sedentary rats, display a stress resistant phenotype. Using this model, we have characterized changes in the brain that contribute to stress resistance.
Methods: Adult, male F344 rats were allowed to live with either locked or mobile running wheels in their home cages. After 6 weeks, rats are exposed to an acute stressor (100, 1.5mA, 5-s, intermittent tailshocks) previously reported to produce anxiety and depression-like behaviors, changes in immune function, sensitization of central 5HT systems, and disruptions in sleep.
Results: Rats that run on wheels prior to exposure to stress do not develop anxiety and depression-like behaviors. In addition, physically active rats fail to show stressor-evoked changes in serotonin circuitry activation, serotonin receptors, expression of CX3CR3, and sleep EEG.
Conclusions: Regular, moderate, physical activity promotes stress resistance; whereas a sedentary lifestyle promotes stress vulnerability. This could be due to adaptations in stress activation and negative feedback systems that are optimized with use during daily exercise and become less tightly regulated in sedentary organisms.
Disclosure: Nothing to Disclose.

Panel
6. Abnormal Calcium Regulation in Bipolar Disorder: Genetics, Cellular Phenotype, Biomarkers, Molecular Pathways, and Novel Therapeutic Targets

6.1 The Bcl-2 Gene Polymorphism rs956572AA, Endoplasmic Reticulum-mediated Intracellular Calcium Release and Signaling Cascades in Subjects with Bipolar Disorder: Lithium Effects and Identification of Potential Therapeutic Targets

Rodrigo Machado-Vieira

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Background: Bipolar disorder (BPD) is characterized by altered intracellular calcium homeostasis and signaling cascades dysfunction. Underlying mechanisms involve dysfunctions in endoplasmic reticulum (ER) and mitochondrial calcium handling, potentially mediated by B-cell

lymphoma 2 (Bcl-2), and AKT-mTOR pathways, which have been implicated in the pathophysiology of BPD. Here, we examined the effects of the Bcl2 gene single nucleotide polymorphism (SNP) rs956572 on intracellular calcium dynamics in patients with BPD. Also, we examined expression of diverse intracellular proteins and networks related to neuroplasticity and cellular resilience.
Methods: Live cell fluorescence imaging and electron probe microanalysis were used to measure intracellular and intra-organelle free and total calcium in lymphoblasts from 18 subjects with BPD carrying the AA, AG, or GG variants of the rs956572 SNP. Analyses were carried out under basal conditions and in the presence of agents that affect calcium dynamics. Also, Bcl2 and AKT/mTOR pathway expression were assessed lymphocytes from 25 BPD patients after a 6-week treatment with lithium in bipolar depression.
Results: Compared with GG homozygotes, the variant AA-which has decreased Bcl-2 messenger RNA and protein, showed higher basal cytosolic calcium and stimulated (IP3R agonist) cytosolic calcium elevations reversed by chronic lithium treatment. Also, lithium also significantly increased AKT1 expression and altered mTOR expression after a 6-week trial with therapeutic dose. mTOR expression in BPD (baseline) showed a robust positive correlation with AKT1 ($p < 0.001$, $r = 0.672$) and Bcl2 ($p = 0.03$, $r = 0.418$, Pearson). After lithium treatment, the strong positive correlation between mTOR and AKT1 was still present ($p < 0.001$, $r = 0.739$) as well as between mTOR and BAD/Bcl2 ratio ($p = 0.025$, $r = -0.445$). Similar findings were observed when evaluating expression change from baseline to endpoint (mTOR correlated with AKT1 ($p = 0.001$, $r = 0.624$) and Bcl2 ($p < 0.001$, $r = 0.601$) expression), supporting a role for lithium effects at these targets. Changes in AKT1 expression were also positively associated with changes in Bcl2 ($p = 0.039$, $r = 0.415$). Regarding association with clinical improvement, baseline Bcl2 expression predicted overall improvement of depressive symptoms ($p = 0.026$, $r = 0.454$).
Conclusions: These results demonstrate that, in patients with BPD, abnormal Bcl-2 gene expression in the AA variant contributes to dysfunctional Ca(2 +) homeostasis through a specific ER inositol 1,4,5-trisphosphate receptor-dependent mechanism. Also, this is the first study showing lower AKT1 and mTOR expression in leukocytes from unmedicated subjects with short-term BPD, as well as a direct modulation by lithium treatment in vivo. Also, these findings support the presence of integrated intracellular pathways in human lymphocytes in a similar way to the observed in the brain.
Disclosure: Nothing to Disclose.

6.2 Lithium Rescues the Hyperactivity of Hippocampal Neurons Derived from the Induced Pluripotent Stem Cells of Bipolar Disorder Patients

Jun Yao

Tsinghua University, Beijing, China

Background: Bipolar Disorder (BD) is a complex neuropsychiatric disorder that affects between 1-4% of the population in the United States. Previous neuropathological studies have revealed a series of alterations in the brain and neurons of BD patients or animal models, including abnormalities in neuronal activity, the PKA/PKC signaling pathways, and multiple types of neurotransmission. However, the roles and causation of these changes in BD are too complex to exactly determine the pathology of the disease. This has been proved by the fact that none of the current BD animal models can recapitulate both manic and depressive phenotypes or cycling of BD simultaneously. Therefore, developing an accurate and powerful biological model has been a challenge for research into BD. In 1998, generation of pluripotent human embryonic stem cells (hESCs) from human embryos at early developmental stage endowed scientists with the ability to study human diseases via differentiating hESCs into functional cells. Later, the development of induced pluripotent stem cell (iPSC) technology, which is based on revolutionary reprogramming of adult somatic cells, provides almost an unlimited number of stem cells for disease research. Since its naissance, the iPSC models of a number of inherited neuronal disorders have been established. Many of these models succeeded in recapitulating the neuropathological phenotypes or genetic deficiencies detected from patients or analogue animals, indicating that the cell lines reprogrammed from the somatic cells of humans carry the natural genetic background of the patients. Hence, this approach is particularly useful for study of complex multigenic diseases such as BD.
Methods: In the present study, we developed a human induced pluripotent stem cell (iPSC) model of BD through reprogramming fibroblasts of six BD patients, three of whom showed good lithium response in a prospective clinical trial, and three that did not respond. Fibroblasts of four healthy subjects were used as the control. Several assays, including virus clearance, random differentiation and pluripotency marker expression, were performed to verify the quality of the iPSCs. In the next step, we developed these iPSCs into hippocampal dentate gyrus (DG) granule cells through a neural progenitor cell (NPC) pattern. Immunostaining analysis was performed to analyze the ratio of excitatory glutamatergic neurons, inhibitory GABAergic neurons and Prox1-positive DG granule cells, as well as the densities of glutamatergic and GABAergic synapses. Furthermore, to identify the cellular phenotypes of BD, we carried out patch clamp recording and somatic Ca2 + imaging to investigate the neuronal activity and neural network excitability of BD neurons. The Li treatment was used to determine the relevance of the cellular phenotype to the clinical symptoms of BD. In addition, we also carried out mitochondrial function assays such as the JC-1 assay to investigate the size and function of mitochondria in BD neurons.
Results: The iPSCs of the healthy control and the BD patients could successfully develop into hippocampal dentate gyrus (DG) granule cells through a neural progenitor cell (NPC) pattern. More than 80% of the DAPI-positive cells were glutamatergic neurons, most of which were Prox1-positive DG granule cells. Patch clamp recording analysis revealed hyperactivity in the neurons of BD patients compared to those of healthy people. We next found that the hyperactivity of the neurons derived from the BD Li responsive patients, but not those derived from the Li non-responsive patients, was significantly rescued by

chronic Li+ treatment. Furthermore, Ca2+ imaging experiments demonstrated that the hyperactivity of single neurons induced over-excitation in the neural network. Finally, we analyzed the mitochondrial functions in the neurons of BD patients, and found that the BD neurons showed greater functioning efficiency and smaller mitochondrial size.
Conclusions: Together, our results indicated that the neurons derived from BD patients exhibited hyperactivity phenotype at the cellular level, and Li could selectively rescue this neuronal abnormality in the Li responsive BD neurons. In addition, BD neurons showed mitochondrial dysfunctions.
Disclosure: Nothing to Disclose.

6.3 Calcium Signaling in Induced Pluripotent Stem Cell Models of Bipolar Disorder

Melvin McInnis

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Background: Bipolar Disorder (BP), schizophrenia (SZ), autism spectrum disorders, and major depressive disorder (MD) belong to a group of related, severe, yet poorly understood neuropsychiatric conditions. The WHO ranks them among the top causes of lifelong disability; the social, personal and vocational consequences are often disastrous. Although phenotypically distinct categories, these conditions appear to arise early in development and may share common genetic modifications and pathways. Alterations in calcium signaling appears to be one shared susceptibility factor, as polymorphisms in the CACNA1C gene which encodes the α subunit of the L-type voltage-gated calcium channel Cav1.2, are thought to contribute broadly to cortical dysfunction in SZ, BP and MD. In fact, alterations in calcium signaling are the strongest and most replicated risk in BP.
Methods: Induced pluripotent stem cell (iPSC) lines from 3 BP and 3 unaffected control subjects were derived from fibroblasts, characterized, then differentiated into neurons. Calcium imaging was performed after 8 and 12 weeks of differentiation using Fluo-4 AM to track intracellular calcium using laser confocal microscopy + lithium pre-treatment. RNAs extracted from undifferentiated iPSC and following neuronal differentiation were used to probe Affy U133 Plus 2 microarrays. Because a SNP in CACNA1C (rs1006737) has been widely implicated in psychiatric disorders, iPSC are being derived from carriers of the AA risk allele, and we are using genome editing to correct the G→A polymorphism, developing isogenic lines for additional study.
Results: Both calcium transient and wave amplitudes were significantly higher in BP compared with control neurons. In addition, lithium chloride – the mainstay treatment for BP – significantly reduced calcium signaling activity in both groups, bringing wave amplitudes to control levels. Microarray analysis identified striking changes in gene expression as iPSC from BP patients differentiate into neurons; the "Calcium signaling" pathway was altered; transcripts for membrane bound receptors and ion channels were significantly increased compared to controls. Overall, these data suggest that alterations in membrane organization and/or ion channelopathies may contribute to BP. Investigations using the edited lines are in progress.
Conclusions: iPSC provide a tractable model system to study neuropsychiatric disease, including bipolar disorder. Consistent with our microarray analysis, we observed alterations in calcium signaling in neurons derived from BP iPSC compared with controls. Derivation of iPSC from BP carrying the SNP in CACNA1C with genome editing will allow us to test a widely held hypothesis that alterations in calcium signaling during development produce widespread but subtle alterations in differentiation and plasticity throughout the nervous system that may influence susceptibility to bipolar disorder.
Disclosure: Nothing to Disclose.

6.4 Function of Risk Genes for Mental Disorders in Neural Development: A DISC1 Story

Guo-li Ming

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Background: Schizophrenia and affective disorders are chronic and generally disabling brain disorders with a prominent genetic basis and with neurodevelopmental origin. A number of susceptibility genes have been identified, including DISC1, neuregulin, COMT, FEZ1. How dysfunction of these genes leads to aberrant neural development and contribute to the pathology of the disorder is largely unknown. DISC1 is by far the best-characterized risk genes for schizophrenia and other major mental disorders, and almost nothing is known about its function in human neural development.
Methods: To understand how mutation of DISC1 gene in patients impact the development of human neurons, we generated iPSC lines from multiple patients from one family with a DISC1 mutation and we have fully characterized these lines. In addition, we have generated multiple isogenic lines. We are also able to differentiate the iPSCs into cortical neurons.
Results: We found that mutation of DISC1 leads to both morphological and synaptic deficits of developing human neurons and it plays a causal role for these deficits.
Conclusions: DISC1 plays an important role in synaptic function during human neural development, which may underlie the pathogenesis of major mental disorders.
Disclosure: Nothing to Disclose.

Panel
7. Hypodopaminergia: Does It Have a Role in Drug Addiction?

7.1 Imaging Vesicular Monoamine Transporter, Type 2 (VMAT2) in Cocaine Dependence

Raj Narendran

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Background: A possible mechanism for lower amphetamine-induced DA release in cocaine abusers (CDS) relative

to matched healthy controls (HC) is that fewer DA storage vesicles are available in the pre-synaptic terminals for release. Consistent with this, postmortem studies in CDS have reported a reduction in striatal VMAT2, the membrane protein that regulates the size of the vesicular DA pool. Lower VMAT2 in CDS likely reflects a compensatory down regulation of pre-synaptic DA storage vesicles (an adaptation) and/or a loss of DA terminals (toxicity). Here, we use PET imaging to evaluate the in vivo status of VMAT2 in chronic cocaine, and whether there is microglial activation to suggest inflammation-mediated toxicity.
Methods: 1. To demonstrate lower striatal VMAT2 availability in recently abstinent CDS compared to matched HC with [11C]DTBZ and PET (between-subject design) 2. To demonstrate lower striatal VMAT2 following chronic (16-months) cocaine self-administration in primates with [11C]DTBZ and PET (within subject design) 3. To demonstrate an increase in stratal VMAT2 availability following six months of supplementation with omega-3 polyunsaturated fatty acids (n-3 PUFA) in HC. Such a finding would support the use of n-3 PUFA to enhance VMAT2 in CDS 4. To demonstrate an increase in striatal 18 K-Da translocator protein expression (a marker of activated microglia) in CDS compared to HC with [11C]PBR28 and PET. Such a finding would inflammation-mediated toxicity to DA neurons in CDS.
Results: 1. [11C]DTBZ binding potential (BPND) was lower by 10 to 16% in the striatal subidvisions in CDS 2. Chronic cocaine self-administration led to a 26 ± 8% reduction in [11C]DTBZ BPND 3. No significant change in striatal [11C]DTBZ BPND was observed after n-3 PUFA supplementation 4. No significant difference in striatal [11C]PBR28 binding was observed in CDS compared to HC.
Conclusions: Chronic cocaine abuse lowers VMAT2 availability in humans and non-human primates. This is not due to inflammation-mediated toxicity to DA neurons. This deficit is unlikely to be corrected by n-3 PUFA supplementation.
Disclosure: Nothing to Disclose.

7.2 Cocaine Self-administration Induces Tolerance to Cocaine and Reduces Dopamine Signaling

Sara Jones

Wake Forest University Health Sciences, Winston Salem, North Carolina

Background: Tolerance to the neurochemical and psychoactive effects of cocaine after repeated use is a hallmark of cocaine addiction in humans. However, the neurochemical mechanisms of tolerance are not fully defined. We evaluated the behavioral and neurochemical consequences of extended access cocaine self-administration, which resulted in escalation of intake.
Methods: Rats self-administered cocaine (1.5 mg/kg/infusion) on a fixed ratio 1 schedule of reinforcement for either 5 or 14 days. Amphetamine was administered to rats via minipumps (5 mg/kg/day) or i.p. injections. Microdialysis was used to measure tonic dopamine levels and their response to an i.v. infusion of cocaine (1.5 mg/kg). Fast scan cyclic voltammetry in brain slices containing the nucleus accumbens was used to quantify electrically evoked release, subsequent uptake through the dopamine transporter and cocaine-induced inhibition of uptake.
Results: Cocaine self-administration reduced tonic extracellular levels of dopamine and decreased the amount of dopamine release evoked by electrical stimulation. In addition, the potency of cocaine to inhibit dopamine uptake, elevate dopamine levels and increase locomotion was reduced. Further, there was cross-tolerance to other dopamine transporter blockers, but not releasers. Cocaine self-administration leads to the formation of high molecular weight dopamine transporter complexes, which may contribute to tolerance, and administration of amphetamine reverses cocaine's effects, setting dopamine signaling back to normal. After cocaine self-administration and subsequent administration of amphetamine by either minipump or i.p. injection, tonic and phasic dopamine release was increased back to control levels or above. Further, amphetamine minipumps prevented escalation of cocaine intake during self-administration, potentially through prevention of tolerance.
Conclusions: Thus, repeated long-access exposure to high doses of cocaine down-regulated dopamine function and induced profound tolerance to cocaine effects on dopamine in the nucleus accumbens, and this hypodopaminergia may contribute to negative affective states during abstinence from cocaine, increasing the probability of relapse to drug taking behaviors. Amphetamine or other releasers may provide a promising avenue to explore potential pharmacotherapies to reverse the deleterious effects of cocaine exposure.
Disclosure: Nothing to Disclose.

7.3 Phasic Dopamine Release to Drug Cues over the Progression of Cocaine Self-administration

Paul Phillips

University of Washington, Seattle, Washington

Background: Altered dopamine transmission is implicated in most contemporary theories of drug abuse. However, the manner and even the direction of these changes are quite controversial. We tracked dopamine release in rats over histories of cocaine self-administration in that modeled recreational and excessive drug use.
Methods: Dopamine release was monitored in the nucleus accumbens core and dorsolateral striatum of rat performing cocaine self-administration, using fast-scan cyclic voltammetry at chronically implanted electrodes. We compared dopamine release to response-contingent and non-contingent presentation of drug cues in short-access (one hour per session) and long-access (six hours per session) cocaine self-administration.
Results: Phasic dopamine release was observed following individual behavioral response to obtain cocaine during the response-contingent presentation of a drug-associated conditioned stimulus. This neurochemical signal was present in the nucleus accumbens core from the earliest sessions recorded but did not emerge in the dorsolateral striatum until the second week of cocaine self-administration. When animals underwent long-access cocaine self-administration (six hour per session), dopamine release to

the contingent drug cue diminished in both striatal regions ($P < 0.05$). This loss of dopamine signaling was observed in the dorsolateral striatum of all animals ($P < 0.05$). However, dopamine release in the nucleus accumbens was only diminished in the subset of animals that exhibited escalation of drug consumption across days of long-access self-administration ($P < 0.001$). Systemic administration of L-DOPA (30 mg/kg) with benserazide (2 mg/kg) restored dopamine release in the nucleus accumbens ($P < 0.05$) and returned drug consumption to pre-escalation levels ($P < 0.01$). In contrast to the decline in dopamine release to the response-contingent presentation of drug cues, dopamine release to non-contingent (experimenter-delivered) presentation of the same cues increased over the course of cocaine self-administration ($P < 0.05$). This increase occurred for non-contingent drug cues presented under intoxication at the end of a self-administration session, or following twenty-four hours of withdrawal from drug, indicating that the different neurochemical patterns for do not reflect a contrast in physiological status between intoxication and withdrawal, but the manner by which the cues were presented.
Conclusions: Over the progression of drug use there are divergent changes in dopamine release to contingent and non-contingent drug cues controlling different aspects of drug-related behavior. Phasic dopamine release to contingent cues diminishes during cocaine self-administration in animals that escalate their drug use. This reduction in dopamine drives the increased drug consumption. In contrast, dopamine release to non-contingent drugs increases potentially mediating heightened craving elicited by drug cues.
Disclosure: Part 1: My spouse is an employee of Amgen, Inc and we own stock in that company. **Part 2:** My spouse is an employee of Amgen, Inc and we own stock in that company, **Part 3:** My spouse is an employee of Amgen, Inc., **Part 5:** My spouse is an employee of Amgen, Inc.

7.4 Stimulant Induced Dopamine Increases are Markedly Blunted in Active Cocaine Abusers

Nora Volkow

National Institute on Drug Abuse, National Institutes of Health, Bethesda, Maryland

Background: Dopamine signaling in nucleus accumbens is essential for cocaine reward. Interestingly, imaging studies have reported blunted dopamine increases in striatum (assessed as reduced binding of [11C]raclopride to D2/D3 receptors) in detoxified cocaine abusers.
Methods: In this paper, we evaluated whether the blunted dopamine response reflected the effects of detoxification and the lack of cocaine-cues during stimulant exposure. For this purpose we studied 62 participants (43 non-detoxified cocaine abusers and 19 controls) using PET and [11C]raclopride (radioligand sensitive to endogenous dopamine) to measure dopamine increases induced by intravenous methylphenidate and in 24 of the cocaine abusers, we also compared dopamine increases when methylphenidate was administered concomitantly with a cocaine cue-video versus a neutral-video.
Results: In controls, methylphenidate increased dopamine in dorsal (effect size 1.4; $p < 0.001$) and ventral striatum (location of accumbens) (effect size 0.89; $p < 0.001$), but in cocaine abusers methylphenidate's effects did not differ from placebo and were similar when cocaine cues were present or not. In cocaine abusers despite the markedly attenuated dopaminergic effects, methylphenidate-induced changes in ventral striatum were associated with intense drug craving.
Conclusions: Our findings are consistent with markedly reduced signaling through D2 receptors during intoxication in active cocaine abusers regardless of cues exposure, which might contribute to compulsive drug use.
Disclosure: Nothing to Disclose.

Panel
8. Latest Development in Convulsive Therapy for Depression and Schizophrenia: A Revival Story

8.1 Focal Electrically-administered Seizure Therapy for Depression

Ziad Nahas

American University of Beirut, Beirut, Lebanon

Background: Electroconvulsive therapy (ECT) remains the most effective acute treatment for severe major depression, but with significant risk of adverse cognitive effects. Unidirectional electrical stimulation with a novel electrode placement and geometry (Focal Electrically-Administered Seizure Therapy (FEAST)) has been proposed as a means to initiate seizures in prefrontal cortex prior to secondary generalization. As such, it may have fewer cognitive side effects than traditional ECT. We report on its first human clinical application.
Methods: Three consecutive trials following the same methods will be presented. To date, over 45 patients with major depression have been enrolled whereby open-label FEAST was administered with a modified spECTrum 5000Q device and a traditional ECT dosing regimen until patients clinically responded. Clinical and cognitive assessments were obtained at baseline, and end of course. Time to orientation recovery, a predictor of long-term amnestic effects, was assessed at each treatment. Nonresponders to FEAST were transitioned to conventional ECT. A subset of patients underwent three 64 channel EEG recordings during the titration session, treatment session and treatment session with reversed polarity.
Results: The preliminary results from the 2 ongoing trials are in line with first FEAST report. After the course of FEAST (median 10 sessions), there was a $46.1 \pm 35.5\%$ improvement in Hamilton Rating Scale for Depression (HRSD24) scores compared to baseline (33.1 ± 6.8, 16.8 ± 10.9; $p < 0.0001$). Patients also appear to achieve full re-orientation (4 of 5 items) in 5.5 ± 6.4 minutes (median = 3.6), timed from when their eyes first opened after treatment. Most interesting, 64 channel EEG recordings are demonstrating a unique seizure induction pattern supporting initiation of seizure in the right prefrontal lobe. Coherence patterns in the theta and delta range are 4 times

fold ones in FEAST versus reversed polarity treatment sessions. In addition, comparative data from bilateral ECT and right unilateral ECT 64 channel EEG data will also be presented.
Conclusions: FEAST appears to produce clinically meaningful antidepressant improvement, with relatively short time to reorientation. Our unique work with high resolution inter-ictal and post-ictal EEG provide support for the focally of the induced seizure prior to its propagation. Collectively, the research demonstrates that FEAST is feasible, safe, well-tolerated and, if efficacy can be optimized, has potential to replace traditional ECT. This in turn will have dramatic impact of the field of convulsive therapy.
Disclosure: Part 1: Eli Lilly supported on lecture, **Part 4:** MECT, Pfizer.

8.2 Frontal MST for Treatment Resistant Depression

Daniel Blumberger

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

Background: Magnetic seizure therapy (MST) has been investigated in preclinical and clinical studies in major depression. Initial studies have primarily investigated stimulation delivered to the vertex. This stimulation site is further away from important areas involved in the pathophysiology of depression. We hypothesized that MST administered over the frontal cortices would elicit effective seizures and clinical efficacy with minimal cognitive adverse effects.
Methods: Patients with a moderate to severe major depressive episode in the context of major depressive disorder or bipolar disorder were enrolled. Patients were included if they had a 24 item Hamilton Rating Scale for Depression (HRSD-24) score greater than 21. Treatment resistance was quantified using the Antidepressant Treatment History Form (ATHF). Prior to the initial treatment session the prefrontal cortices were localized using F3 and F4 on an EEG cap using the 10-20 system. All patients were treated with 100% machine intensity and treatment occurred twice or three times per week for up to 24 treatments. Three consecutive series of patients were treated using 100Hz, 50Hz and 25 Hz stimulation. Methohexital sodium with or without Remifentanyl and succinylcholine were used for anaesthesia and muscle relaxation. The primary efficacy endpoint was remission, defined as <10 on the HRSD-24 and a 60% reduction from baseline at two consecutive assessments. Comprehensive cognitive and memory assessments occurred at baseline and endpoint.
Results: 68 subjects have been enrolled in this open label pilot study at the time of writing. Treatment courses ranged from 7 to 24 treatments. Remission rate were 40% across the three frequency series. Seizure quality and length was best in the 25Hz group. One missed seizure occurred in one subject. Headache and nausea were the most common adverse effects. Two patients experienced the emergence of mania and hypomania during the course of treatment. No significant worsening on any of the cognitive measures were observed at the endpoint of treatment in remitters and non-remitters.
Conclusions: Prefrontal coil placement for MST may be an effective method of achieving robust clinical efficacy in treatment-resistant depression while sparing cognition. These pilot results warrant comparing prefrontal MST to ECT in a randomized clinical trial.
Disclosure: Part 1: Research support from Tonika/Magventure and Brainsway Limited, **Part 4:** Tonika/Magventure: in-kind equipment support for an investigator initiated study, Brainsway Ltd: in-kind equipment and financial support for an investigator initiated study administered through the Canadian Institute Health Research University Industry Partnered granting mechanism.

8.3 Electrophysiological Markers of Brain Health in Understanding the Mechanisms of Action of ECT in Depression

Faranak Farzan

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

Background: Electroconvulsive therapy (ECT) remains to be one of the most effective treatment options in treatment resistant major depressive disorder (MDD). However, the mechanisms of action of ECT remain poorly understood. Several lines of evidence exist in support of aberrant neural connectivity and impairments in inhibitory mechanisms in MDD. Furthermore, independent neurophysiological studies in ECT suggest that ECT may modify neural connectivity and may result in potentiation of inhibitory mechanisms. We propose a new unifying hypothesis regarding the mechanisms of action of ECT, the "connectivity resetting hypothesis". In this hypothesis, we propose that ECT exerts its therapeutic efficacy through resetting aberrant neural connectivity, mediated through activating the thalamocortical pathways and potentiation of the central inhibitory mechanisms.
Methods: To evaluate this hypothesis, we have embarked on evaluating changes in the brain connectivity and inhibitory mechanisms using methods of resting state Electroencephalography (EEG) and Transcranial Magnetic Stimulation combined with Electroencephalography (TMS-EEG).
Results: We have collected data in 30 patients with MDD before and after receiving a course of ECT treatment. We have also collected normative data in 30 healthy subjects. We are currently evaluating: (1) whether changes in EEG markers of inhibition and EEG markers of connectivity is associated with ECT-related improvements in MDD symptoms, (2) whether ECT-induced potentiation of inhibitory mechanisms mediates the association between changes in the brain connectivity and symptoms improvement, and (3) whether the EEG makers of inhibition and EEG markers of connectivity at baseline prior to ECT treatment can predict response to treatment.
Conclusions: Our study evaluates a new hypothesis that we have proposed based on numerous but independent lines of evidence in support of impairments of connectivity and inhibitory impairments in patients with MDD, as well as ECT induced modification of inhibitory and connectivity mechanisms. The results of our study will provide further evidence for validity of this hypothesis and will provide

direction for future studies examining the therapeutic mechanisms of ECT in MDD.
Disclosure: Nothing to Disclose.

Mini-Panel
9. Inhibitory Neuron Development in Developmental Psychopathology: Animal Models of Gabaergic Neuron Genetic Regulation, Responses to Prenatal Stress and Postnatal Parvalbumin Elimination

9.1 Cortical Interneurons in Neuropsychiatric Disorders and their Transcriptional Regulation

John Rubenstein

University of California at San Francisco, San Francisco, California

Background: Cortical interneurons are clearly implicated in multiple neuropsychiatric disorders.
Methods: We are using a combination of transgenic, fate mapping and in vivo transplantation techniques to elucidate the role of interneurons in cortical circuitry.
Results: In my talk I will describe our current understanding of transcriptional regulation of the development and function of these cells.
Conclusions: I will describe our current efforts to elucidate the transcriptional circuitry that regulates specific processes such as cell fate, migration and differentiation.
Disclosure: Nothing to Disclose.

9.2 Prenatal Stress Disrupts the Postnatal Development of GABAergic Populations and Correspondingly Increases Behavioral Inhibition

Hanna Stevens

Yale University School of Medicine, New Haven, Connecticut

Background: Prenatal stress (PS) is associated with neuropsychiatric disorders and delays embryonic GABAergic cell migration.
Methods: We used GAD67-GFP knock-in mice in normal housing–non-stressed (NS)—or a standard repetitive restraint model of prenatal stress (PS) from embryonic day 12 (E12) until birth. BrdU injection at E13 allowed for tracking of birth-dated subsets of interneuron populations. At 2 months of age, mice were run on tests of anxiety: open field, elevated plus maze and marble-burying. Brain tissue was collected at multiple postnatal time points and analyzed by immunocytochemistry and stereology of dorsal and ventral forebrain interneurons.
Results: PS mice showed alterations in the normal developmental trajectory of postnatal GABAergic populations. Normal developmental overproduction of GAD67GFP+ cells in medial frontal cortex and hippocampus postnatally were heightened following prenatal stress despite inhibitory neuron deficits in these same regions at birth. No deficit was present in caudate putamen at birth following PS but the developmental postnatal increase in GAD67GFP+ cells in this region was also enhanced following early stress. These altered developmental trajectories resulted after five months in prenatally-stressed animals with reduced GABAergic populations in cortex and hippocampus but increased populations in caudate putamen. GABAergic neurons of the parvalbumin subtype showed a similar change in trajectory after prenatal stress. These cell population findings, particularly in caudate putamen, correlated with behavioral changes in these mice. PS mice compared to NS mice were behaviorally inhibited with significantly reduced locomotor activity in the open field, more time in the closed arms of the elevated plus maze and more marbles unburied.
Conclusions: GABAergic cell populations have been implicated in many disorders for which prenatal stress is a risk factor. Altered postnatal development may be one mechanism by which inhibitory neuron pathophysiology arises in the forebrain of patients with these disorders.
Disclosure: Nothing to Disclose.

9.3 Experimental Ablation of Striatal Parvalbumin-expressing Fast Spiking Interneurons

Christopher Pittenger

Yale University School of Medicine, New Haven, Connecticut

Background: Tourette syndrome (TS) is characterized by vocal and motor tics, and by abnormalities in sensorimotor gating. Tics are repetitive, rapid, purposeless, semi-voluntary movements that are potentiated by stress and sleep deprivation. Parvalbumin-expressing fast-spiking interneurons (FSIs) in the striatum are disrupted in post-mortem tissue of patients with severe TS. Similarly, a spontaneous hamster mutation that exhibits movement abnormalities, the dystonic Syrian hamster, has a disruption in these interneurons. However, it is unclear whether disruption of striatal FSIs is sufficient for the production of tic-like phenomenology and other symptoms of TS, is a contributor but is not sufficient for the production of symptoms, or is an epiphenomenon of other pathophysiological events.
Methods: We developed a novel method for the experimental ablation of striatal FSIs in otherwise normal adult mice, using a combination of transgenic mice, recombinant adeno-associated virus, and systemic toxin administration. Ablation was high efficient and specific. Mice with FSI ablation were compared to control animals in a battery of TS-relevant behavioral assays.
Results: FSI ablation did not produce spontaneous movement abnormalities or difficulties with motor learning. Prepulse inhibition, a measure of sensorimotor gating that is disrupted in TS and in other TS models, was normal. However, after acute stress, mice developed repetitive grooming-like movements that are similar to those seen in other models of TS.
Conclusions: FSI ablation is not by itself sufficient to produce spontaneous TS-like phenomenology but does lead to the production of tic-like movements after acute stress. We hypothesize that FSI ablation destabilizes the network activity of the corticostriatal circuitry. Co-occurrence with other pathogenic insults, such as pathology of other

interneuronal populations, may be necessary for the emergence of spontaneous tics.
Disclosure: Nothing to Disclose.

Mini-Panel
10. Preclinical Alzheimer's Disease: Industry, NIA, and Academic Perspectives

10.1 Challenges in Conducting and Interpreting Results of Preclinical Alzheimer Disease Trials: One Academic Perspective

Lon Schneider

Keck School of Medicine, University of Southern California, Los Angeles, California

Background: Disease-modifying interventions targeted toward Alzheimer's pathology before the symptoms arise and that would slow or stop progression of illness would be a tremendous accomplishment. Clinical research is increasingly targeted toward earlier stage patients. Although, conceptually, early intervention offers substantial promise for patients in that its effects would border on prevention or immunization, there is no proof of concept that interventions in cognitively-unimpaired or preclinical Alzheimer disease is feasible. This earlier focus creates challenges in all areas of clinical trials design: recruitment, validation of diagnosis, use of biomarkers for enrichment, the therapeutic intervention, and outcomes.
Methods: We present an overview of the current paradigm, discuss the anticipated challenges as research focuses earlier, and debate future requirements. In particular we examine the issues involved in the recruitment of large numbers of preclinical AD participants; review new, early AD diagnostic criteria and inclusion criteria for participants; the range of accuracies for preclinical AD diagnoses, implications for targeted designs; the several potential interventions; and the types of outcomes. Finally, we consider the various inferences that can be made either if a trial is positive or shows null outcomes.
Results: Criteria for early stage AD, before dementia onset, are not sufficiently validated; MCI is often misspecified and preclinical AD is likely to so. Although some biomarkers may help to exclude AD diagnoses they are used mainly to enrich the proportion of trials samples likely to develop AD dementia over a short period. However, preclinical AD as a diagnosis reduces to either an 'at-risk' condition or a symptomatic condition with Alzheimer pathology. And when supported with positive Abeta biomarker enrichment overlaps with AD dementia but does not define it. As these trials also have a short follow-up period of 5 years or less, first, very few participants will progress; and, second, a drug would have to exert its disease-modifying effect during that period. Finally, outcomes are limited to the potential to detect subtle cognitive change due to treatment, and such change may not be due to the progression of illness.
Conclusions: The current trials are optimistic in relying on early diagnosis, at-risk samples, biomarkers, long-term interventions, and single composite cognitive outcomes that have not been well-worked out to determine efficacy. The idea that prevention or preclinical AD trials will more likely lead to successful AD drugs than trials for symptomatic patients are without evidence support. Prevention interventions might be better discussed as risk reduction and directed toward conditions that may be risks for AD or dementia such as inactivity, depression, high blood pressure, low education, and smoking.
Disclosure: Part 1: Abbvie, ACImmune, Allon, Biogen Idec, Cerespir, Forum, FujiFilm, GenLilly, Medavante, Merck, Novartis, Orion, Roche, Servier, Takeda, Zinfandel, **Part 2:** Merck, Takeda, **Part 4:** Forum, Genentech, Lilly, Lundbeck, Merck, Novartis, TauRx.

10.2 An Industry Perspective on Intervention Trials in Preclinical Alzheimer's Disease

Michael Egan

Merck, North Wales, Pennsylvania

Background: Secondary prevention trials in subjects with preclinical AD, such as A4, DIAN, and others, are critically important in testing specific mechanisms as well as the general approach of early intervention. Will or should this become the preferred approach for testing new drugs with novel mechanisms of action, such as BACE inhibition and tau-focused interventions? From an industry perspective, preclinical AD trials are attractive but have challenges.
Methods: Review of literature on clinical trials of various stages of AD.
Results: Several primary and secondary prevention trials using a variety of drugs have been conducted. None have shown evidence of disease modification at either early or later stages of AD. Early intervention trials completed to date tested drugs that were approved for AD dementia or other conditions, or vitamin supplements. Testing novel, unapproved compounds raises several questions, such as what safety data are needed, when preclinical trials should be initiated in a development program, and whether drug target impacts consideration of this timing. Another consideration is whether or not Preclincal AD is early enough and biologically distinct enough from later stages to justify the investment. Clinical trial considerations include what endpoints are suitable (e.g. qualitative vs quantitative) for detecting a drug effect in a reasonable period of time and are approvable by regulators. Biomarkers such as genetic profile, PET amyloid and tau imaging, MRI structural imaging, and CSF measures could be used to identify appropriate patients. Drug effects on biomarkers may also be important for providing supportive data for efficacy and for regulatory approval. Logistical concerns include whether such a trial can be conducted globally, whether biomarker assessments can be adequately implemented, and whether dropout rates can be sufficiently limited.
Conclusions: Trials in Preclinical AD populations may potentially offer a more robust test of some mechanisms. From an industry perspective, significant scientific and operational challenges exist which may be informed by currently planned early intervention trials.

Disclosure: Part 1: I am a full time employee of Merck, **Part 2:** I am a full time employee of Merck. **Part 5:** I am a full time employee of Merck.

10.3 National Institute on Aging Research on Pre-symptomatic/Pre-clinical Alzheimer's Disease

Neil Buckholtz

National Institute on Aging, Bethesda, Maryland

Background: The National Institute on Aging (NIA) is the lead Institute for support of Alzheimer's disease (AD) research at the National Institutes of Health. In 2011 the NIA, in collaboration with the Alzheimer's Association, developed revised diagnostic guidelines for AD. Recognizing the changing focus to earlier stages of AD, a guideline for mild cognitive impairment (MCI) due to AD was also developed, as well as a research diagnostic guideline for pre-clinical AD. This recent focus on a pre-clinical or pre-symptomatic phase of AD in which individuals may be cognitively intact but harbor indications of AD pathophysiology has important implications for testing new early intervention therapeutic strategies for modifying disease progression or delaying disease onset.
Methods: In order to understand the progression of AD brain pathophysiology from normal cognitive aging to AD, studies have utilized a variety of biomarkers in living humans. Specifically, NIA has supported the Alzheimer's Disease Neuroimaging Initiative (ADNI) since 2004. ADNI is a longitudinal study of older individuals ranging from those who are cognitively normal, to early and late stages of MCI, to early AD. The goal of ADNI is to understand which biomarkers or combinations of biomarkers can best assess disease progression so that these can be incorporated into clinical trials for enriching trials with subjects at highest risk for progression and also for assessing response to theraeutic interventions. ADNI collects information from many modalities including cognitive/clinical, genetic/genomic, MRI, FDG-PET, amyloid-PET, blood, and CSF. These biomarkers have been incorporated into pre-clinical trials of individuals at risk for AD because of genetic mutations for early-onset disease, a genetic risk factor for late-onset disease, and brain amyloid.
Results: These pre-clinical/pre-symptomatic trials are just beginning. The interventions being tested are anti-amyloid agents, primarily monoclonal antibodies against beta-amyloid. A new public-private-partnership at NIH, the Accelerating Medicines Partnership (AMP), is focused on three diseases, one of which is AD. Through AMP support, additional novel biomarkers will be added beyond those already in the trials.
Conclusions: Through the use of a variety of neuroimaging and fluid biomarkers, it is now possible to follow AD pathophysiology in the living human brain from the earliest stages of pre-clinical AD through MCI into early AD. This is transforming how clinical trials for AD are being done by focusing on older people who are cognitively normal but at high risk of developing AD. Our hope is that by intervening with appropriate therapy at this early stage of disease, it will be possible to slow disease progression and ultimately delay disease onset.
Disclosure: Nothing to Disclose.

Mini-Panel
11. Drug Memories: Is It All about Craving?

11.1 Alcohol-associated Contexts Alter Cognitive Function, Alcohol Subjective Experiences, and Increase Alcohol Drinking

Emma Childs

University of Chicago, Chicago, Illinois

Background: Psychoactive drugs produce potent effects which become associated, over multiple pairings, with the places where drug-taking occurs. These learned associations are thought to produce robust, long-lasting changes in motivational and emotional systems that contribute to compulsive drug use, however there is little clinical evidence of how these associations are formed and how they come to profoundly control behavior. Recently, we have developed a new model to establish conditioned associations between drugs and contexts in human volunteers. Now, we have applied the model to study the emotional, cognitive and behavioral responses, including drug-taking, elicited by drug-paired contexts.
Methods: Healthy moderate drinkers ($N = 112$) underwent place conditioning sessions, 3 with alcohol-containing drinks and 3 with non-alcoholic drinks in randomized order. One group (paired) always received alcohol (0.8g/kg) in one room and no alcohol in another room, while a second group (unpaired) received alcohol and no alcohol in each room. Following conditioning, participants' behavioral preference for the testing rooms was assessed during a drug-free room exposure test. Participants' then completed cognitive testing (Study 1) and alcohol self-administration (Study 2) in either the alcohol- or no alcohol-paired room.
Results: In comparison to the unpaired group, the paired group spent significantly more time in the alcohol-paired room at the post-conditioning exposure test ($p < 0.05$). Paired group participants tested in the alcohol-paired room exhibited poorer working memory performance ($p < 0.05$) yet faster go reaction times ($p < 0.05$) than paired group participants tested in the no alcohol-paired room. When tested in the alcohol-paired room, paired group participants reported less negative subjective responses to alcohol and chose to consume significantly more alcohol drinks ($p < 0.05$) than paired group participants tested in the no alcohol-paired room.
Conclusions: Our findings demonstrate that moderate drinkers come to exhibit a behavioral preference for an environment previously paired with alcohol administration and, most notably, that they will choose to consume more alcohol in the alcohol-paired environment than in one paired with administration of non-alcoholic drinks. Our results also indicate that altered cognitive processing or subjective responses to alcohol in the conditioned environment may contribute to a loss of control over alcohol drinking in that environment. The implications of these findings include that approaches to enhance cognitive resources and improve executive functioning in the conditioned environment may help drinkers to control their alcohol use.
Disclosure: Nothing to Disclose.

11.2 Cognitive and Brain Mechanisms of Alcohol and Stress Effects on the Salience of Alcohol Related Stimuli and on Inhibitory Control

Theodora Duka

University of Sussex, Brighton, United Kingdom

Background: As addiction progresses stimuli that are regularly associated with the drug become salient whilst deficits in inhibitory control contribute to a loss of self-directed behaviour leading to relapse during abstinence. Another factor that contributes to relapse is acute stressful experience. Acute alcohol as well as acute stress have been shown to impair behavioral inhibition and increase emotional responses to alcohol-related stimuli. We have examined in three studies the psychological processes and the neuronal mechanisms of alcohol and stress effects on behavioral inhibition and reward-seeking behaviors to understand the mechanisms underlying the development of alcohol addiction and the risk for relapse during abstinence.
Methods: Twenty-six healthy social drinkers received either 0.4g/kg of alcohol or placebo (study 1), and underwent functional magnetic resonance imaging (fMRI) while performing an attentional task, the Eriksen Flanker task, concurrently with background, task-unrelated alcohol-associated or neutral pictures. Participants responded to the direction of a central "target" arrow and ignored adjacent congruent (low cognitive load) or incongruent (high cognitive load) "flanking" arrows. The effects of stress on the same task was examined in a population of 64 social drinkers (32 heavy and 32 light alcohol drinkers; study 2). In study 3, 40 social-drinkers received either 0.4g/kg of alcohol or placebo and underwent functional magnetic resonance imaging (fMRI) while performing in a Stop-Signal task (SST) and in a forced choice instrumental reward-seeking procedure, in the presence of stimuli serving as S+ (always predicted a reward) and S- (always predicted absence of reward).
Results: Alcohol ingestion increased the time it took to respond to trials of the task in the presence of background alcohol-associated pictures, relative to neutral pictures ($p<0.05$), and the activity evoked by alcohol-associated pictures within a region encompassing the bed nucleus of the stria terminalis (BNST). Stress also increased the time it took to respond to trials of the task in the presence of background alcohol-associated pictures ($p<0.05$) and increased craving albeit in the group of heavy drinkers. Alcohol ingestion impaired relative to placebo inhibitory control in the SST ($p<0.05$) and increased rates of responding in the presence of S- ($p<0.05$). Conjunction analyses ($p<0.005$,$k=13$) revealed a significant linear reduction in activation in Inferior frontal cortex.
Conclusions: Alcohol increases attentional bias to alcohol-cues via an increased activation of subcortical areas implicated in arousal and/or salience attribution and decreases inhibitory control via a decrease in activation of prefrontal cortical areas. Stress acutely impairs inhibitory control and increases interference by alcohol related stimuli but only in heavy alcohol drinkers.
Disclosure: Nothing to Disclose.

11.3 Dissecting What Drives Dopamine in Drinking: PET Studies of Human Ventral Striatal Effects Related to Alcohol Intoxication and Alcohol's Conditioned Associations

David Kareken

Indiana University School of Medicine, Indianapolis, Indiana

Background: Striatal dopamine has long been thought of as a key neurotransmitter that encodes the rewarding properties of both natural rewards and drugs of abuse. Beyond the reward itself, dopaminergic transmission is also thought to reflect that associated with, and predictive of, the reward's reinforcing properties. This talk will present and integrate findings from three human imaging studies of dopamine, showing how ventral striatal dopamine relates to alcohol's drug effects, as well as its attendant non-pharmacologic sensory properties and surrounding expectancies.
Methods: Three samples of heavy drinking subjects underwent imaging with positron emission tomography (PET) and the ligand [11C] raclopride (RAC) to measure dopamine receptor availability as a function of different behavioral states. In Experiment 1, 49 male subjects (mean age = 25, SD = 4) underwent two randomized scans during alcohol-related cue exposure "in extinction," where alcoholic drink flavors were examined as isolated from their intoxicating effects. In one scan, subjects tasted a habitually preferred beer flavor in quantities devoid of any significant alcohol content. In the second, scan subjects tasted a flavor intensity-matched sports drink control. In Experiment 2, 26 heavy drinking male subjects (mean age = 23, SD = 3) performed a novel operant self-administration task across three scans, with resultant delivery of: (i) the flavors of alcohol (beer) or a sports drink control, and (ii) alcohol intoxication (15 min rising limb, followed by 60 mg% breath alcohol clamp) or a vehicle control. In this experiment, alcohol intoxication (and the saline vehicle) was administered intravenously, allowing for the independent manipulation and dissociation of effects due to: (i) alcohol's conditioned flavor and (ii) alcohol's pharmacologic properties. In the third experiment (n = 8 subjects, mean age = 24, SD = 4), alcohol's conditioned sensory properties (smell, sight) were used to predict the onset of intravenous alcohol intoxication. Voxelwise data were processed with SPM8, using relative changes in RAC's binding potential (BP) to infer striatal dopamine release ($p< 0.05$ threshold, corrected for family-wise error in striatal regions of interest).
Results: In Experiment 1, beer flavor significantly reduced RAC BP in the right ventral striatum relative to the control flavor, suggesting that the alcohol-associated flavor induced DA release. BP reductions were strongest in those with first-degree alcoholic relatives. In Experiment 2, beer flavor (controlling for ethanol exposure) was again associated with right ventral striatal DA release. However, ethanol exposure (controlling for flavor) resulted in left ventral striatal DA release. Bilateral ventral striatal DA release was evident only when both flavor and ethanol exposure differed from their respective controls ([beer flavor +

ethanol] vs. [control flavor + saline]). In Experiment 3, sensory cue effects that predicted impending ethanol caused DA changes in the right ventral striatum, while unanticipated ethanol intoxication caused DA changes in the left striatum.
Conclusions: These data suggest that the DA response to stimuli that are habitually paired with ethanol intoxication (flavor) may be greater in those with familial risk. The data also suggest that the human ventral striatal response to these well-learned stimulus associations between alcohol and its conditioned sensory properties may be lateralized, with cue effects most present on the right, and drug effects most present on the left. Understanding how the constituent components of the drinking experience drive striatal dopamine should permit a more precise investigation of the neural vulnerabilities that underlie alcoholism risk.
Disclosure: Nothing to Disclose.

Panel
12. Early Precursors, Core Features and Intermediate Phenotypes of Bipolar Disorder

12.1 Incidence of Psychopathology in Offspring of Parents with Bipolar and Unipolar Mood Disorders: 10-Year Follow-Up

Martin Preisig

Centre Hospitalier Universitaire Vaudois, Prilly, Switzerland

Background: Recent family studies of mood disorders suggest independent familial aggregation of bipolar disorder (BPD) and major depressive disorder (MDD) as well as their major components manic and depressive episodes. Using a prospective high-risk study design, our aim was to 1) establish the incidence of mood disorders and episodes in offspring of parents with mood disorders and 2) determine the incidence of mood disorders and episodes in offspring in function of the onset of parental mood disorders.
Methods: Clinical information was collected on 81 treated probands with BPD, 64 with MDD and 62 medical controls as well as their 365 children who were 6–17 years old on inclusion (mean age: 10 years). Offspring were interviewed every 3 years (mean duration of follow-up was 9.9 years). Assessments of parents and offspring were based on direct diagnostic interviews using the Diagnostic Interview for Genetic Studies and the Kiddie-Schedule for Affective Disorders and Schizophrenia. Parental age of onset was dichotomized at the age of 21 years. The impact of parental mood disorders on the onset of psychopathology in offspring was assessed using shared gamma frailty models for survival data. Models were adjusted for the effects of demographic characteristics in probands and offspring, comorbid disorders in probands as well as mood disorders in co-parents.
Results: Only offspring of parents with BPD revealed an increased risk of bipolar disorders or manic episodes. Similarly only the offspring of parents with MDD were at an elevated risk of MDD or major depressive episodes. The increased risk of BPD in offspring of parents with BPD was entirely attributable to families where the parent had a BPD with an onset earlier than 21 years. Offspring of parents with early onset BPD showed a 12-times increased risk of developing mania/hypomania.
Conclusions: Our results further support the specific and independent familial aggregation of BPD and MDD and their major components. Moreover, they suggest the specificity of the familial aggregation of early onset BPD, which could be a promising target for future genetic and other biological studies.
Disclosure: Nothing to Disclose.

12.2 Core Features and Intermediate Phenotypes of Bipolar Disorder in the NIMH Family Study of Affective Spectrum Disorder

Kathleen Merikangas

National Institute of Mental Health, Bethesda, Maryland

Background: Although there has been substantial progress in identifying intermediate phenotypes for schizophrenia and psychotic disorders, there have been fewer efforts to conduct comprehensive evaluation of the core features and biologic markers for bipolar disorder. We recently showed independent familial aggregation of mania and major depression, with substantially greater heritability for mania than for major depression.
Methods: The study includes 500 probands from the local community with a range of manifestations of mood and anxiety disorders and 1209 relatives. The endophenotype component of the study has been completed on 495 participants from the study who have been directly evaluated at the NIH Clinical Center for more in depth assessments of reactivity, rhythms, as well as numerous other measures including cognitive function, olfaction, structural neuroimaging, and temperament. This presentation focuses on the two broad domains of reactivity assessed by autonomic nervous system function (tilt challenge with catecholamine and blood pressure response over 10 minutes post tilt), potentiated startle and heart rate variability (24 hour holter monitor); and rhythms as indexed through objective measures of activity and electronic diary dimensional measures of mood, energy, stress, and sleep 4 times per day across two weeks, and sleep patterns and disorders.
Results: Findings to date indicate that probands with bipolar disorder tend to be characterized by disturbances in activity and sleep with greater variability and circadian shifts that are clearly differentiated from those with major depression or anxiety disorders. By contrast, probands with anxiety disorders tend to have disturbances of reactivity, particularly anxiety potentiated startle and increased autonomic reactivity.
Conclusions: These findings provide the first evidence that disturbances in circadian rhythms and activity may comprise an intermediate phenotype for bipolar disorder where as enhanced reactivity may underlie anxiety disorders and major depression.
Disclosure: Nothing to Disclose.

12.3 Genetic Dissection of Bipolar Disorder Using Intermediate Phenotype Approach in Extended Pedigrees

Scott Fears

UCLA Semel Institute for Neuroscience and Human Behavior, Los Angeles, California

Background: Bipolar disorder (BP) is an etiologically complex disorder with polygenic risk. Recent successes in genome wide association studies have started to identify risk loci, but the pathogenic processes influencing the emergence of the disorder are poorly understood. Several genetic strategies may address the challenges associated with genetic dissection of complex psychiatric disorders. An intermediate phenotype approach focused on quantitative brain and behavioral traits associated with bipolar disorder may provide more power to identify genetic loci correlated with specific biomarkers of the disorder. Additionally, implementation of family based methods in genetically isolated populations with reduced genetic heterogeneity may facilitate the discovery of high-impact variants.
Methods: We acquired a broad range of temperament, neurocognitive and neuroimaging phenotypes from ~750 participants from multi-generational pedigrees with heavy loading for BP living in genetically isolated regions of Costa Rica and Colombia. Quantitative genetic analysis was used to estimate heritability, association with BP, and to perform linkage analysis. Additionally, full genome sequence was obtained from ~500 family members and used for high-resolution analysis of chromosomal regions linked to the quantitative traits.
Results: Seventy-five percent of the brain and behavioral traits were significantly heritable, ~30% were associated with BP, and ~25% were both heritable and associated with BP. The analysis of neuroimaging data showed that family members with BP had reduced total cerebral volume and increased ventricular volume. Additionally, BP was associated with reduced cortical thickness in most of the prefrontal and temporal cortex and decreased white matter integrity of the corpus callosum. Analysis of the temperament data showed increased scores on dimensional scales of irritability, delusion-proneness and creativity in BP family members and the neurocognitive analysis showed impairments in working memory, processing speed, inhibitory control declarative memory and verbal fluency. Linkage analysis of the quantitative measures identified more than a dozen suggestive loci (LOD > 3.2) including a region on chromosome 6q25 for amygdala volume (LOD = 5.1). Analysis of the full genome sequence under the linkage peaks has revealed promising candidate genes influencing the brain and behavioral traits.
Conclusions: This is the most extensive genetic investigation of intermediate phenotypes for BP to date. The brain and behavioral quantitative traits associated with BP are consistent with expectations from case-control studies and the gene mapping results have identified promising loci influencing highly relevant neurobiological traits.
Disclosure: Nothing to Disclose.

Mini-Panel
13. Using Big Neuroimaging Datasets to Understand Neuropsychiatric Disease Across the Lifespan

13.1 Title Multimodal Neuroimaging with MR Indicate Complementary Age-related Effects on Structure and Function: Results from Philadelphia Neurodevelopmental Cohort of 1500 Children Age 8-21

Ruben Gur

University of Pennsylvania, Philadelphia, Pennsylvania

Background: Neuroimaging studies of large samples across the lifespan have examined single modalities, focusing primarily on structural volumetric measures. These studies documented increase in white matter volume between childhood and young adulthood, considered to reflect myelination, and reduced gray matter volume, considered as evidence for pruning. Few studies examined physiological parameters such as cerebral blood flow, resting-state connectivity or task-activation effects, and fewer still associated these effects with cognitive performance. We obtained multimodal neuroimaging data on a large sample of genotyped and behaviorally phenotyped youths and examined age related effects.
Methods: Multimodal neuroimaging using the same 3Tesla scanner (Siemens TIM Trio, Erlangen, Germany) was performed on a sample of 1600 youths age 8-21 from the Philadelphia Neurodevelopmental Cohort (PNC). The neuroimaging procedures have been detailed (Satterthwaite et al., NeuroImage, 2013) and yield both structural (T1-weighted 1x1x1mm resolution, diffusion tensor imaging with 64 directions) and functional (arterial spin labeling measures of cerebral blood from, resting-state blood oxygenation-level dependent, and activation with two tasks) parameters. Follow-up data at 18 months are available on 500 youths, 200 who reported significant psychotic symptoms, 100 who reported mild symptoms and 200 typically developing youths. The MR data were processed using standard procedures and regional values were obtained using a multi-atlas deformation based approach. We used a Mixed Model approach for analyzing between-group differences and CORANOVA for examining differences among correlated correlations.
Results: The volumetric measures confirmed earlier findings of increased WM and reduced GM between the ages of 8 to 21, and extended them to show sex differences and greater regional specificity to the effects. We were able to evaluate the effects of puberty by comparing same-aged participants who have and who have not matured sexually, and show that puberty affects these changes independent of age. With the DTI data we demonstrate the evolution of WM connectivity, which in males becomes dominated by within-hemispheric connections while in females inter-hemispheric connection dominate in the older cohorts. Physiologic measures likewise showed age-related effects, with CBF declining in both males and females until pubescence, after which CBF in males shows continued age related decline while in females there is reversal in this trend. Resting state connectivity and task activated

BOLD changes indicate increased connectivity in the default mode and working memory network, and increased coordination of task-related activation reflected both in more complete recruitment of the executive network and deactivation of the default mode network for a working memory task. The age related differences in brain structure and function are related to cognitive performance. A subsample that reports psychotic symptoms shows aberrations across anatomic and functional measures. The 18 months follow-up data indicated that individuals who continue to report psychotic symptoms have poorer indicator of brain structure and function compared to typically developing youths and those who reported psychotic symptoms at intake and no longer have them.
Conclusions: Transition from childhood to adulthood is a critical period in behavioral maturation and is associated with pronounced changes in brain structure and function. Documenting these changes in a cross-sectional design requires large samples, especially when multimodal imaging is obtained for the purpose of correlating brain measures to behavior within the genomics context. The Philadelphia Neurodevelopmental Cohort affords such an evaluation because it includes a large sample of youths with multimodal neuroimaging and phenotypic characterization of behavior, including cognitive performance. We found age related effects in both anatomy and physiology, and these diverge in males and females. The differences are consistent with a process of honing the brain toward more efficient information processing capacity. The age related honing includes improved myelination and connectivity as well as pruning of brain tissue associated with reduced overall perfusion but more coordinated task-specific activation. Sex differences indicate earlier maturation in females but also divergent effects of puberty. Associations of brain parameters with performance can help link behavior to underlying brain maturation. The finding of persistent aberrations in participants who report psychotic symptoms indicates that these brain and behavior parameters can serve as endophenotypic biomarkers.
Disclosure: Part 1: Brain Resource Center, **Part 2:** Brain Resource Center, **Part 4:** AstraZeneca.

13.2 Cognition, Connectomics and RDoCs

Deanna Barch

Washington University, Saint Louis, Missouri

Background: Historically much of the work on understanding the neural mechanisms of psychiatric disorders has focused on particular brain regions that may play specific roles in cognitive, emotional or social function. Although this is highly important and has led to novel insights, it is also become increasingly clear that the vast majority of deficits associated with facets of psychopathology reflect deficits in neural systems that involve the coordinated action of multiple brain regions. Further, developments in graph theory and other approaches to characterizing the function of networks of brain regions provide new metrics for understanding relationships to individual differences in behavior. This presentation will use data from a multi-site study of psychosis and from the Human Connectome Project to illustrate key brain-network-behavior relationships relevant to psychopathology, with a particularly focus on cognition, motivation and emotional processing.
Methods: The first data set was a five site imaging study conducted by the CNTRaC consortium. Healthy controls and individuals with schizophrenia performed three different tasks in 3T MR scanners (goal maintenance, relational encoding and retrieval, and visual integration). We used general linear modeling to remove the deterministic effects of task structure and then applied graph theory methods to examine the connectivity of the frontal-parietal and cingulo-opercular networks. We examined both local and global efficiency of these networks and the integrity of specific hub regions (e.g., anterior insula, anterior cingulate, dorsolateral prefrontal) using participation coefficient metrics. Participants completed a battery of cognitive tasks, and we examined relationships to both specific tasks and to a summary metric of cognitive performance that captures shared variance across tasks. The second data set was resting state data from the Human Connectome Project examining similar connectivity metrics in relationship to fluid intelligence, cognitive control, reward processing and emotional function.
Results: We found that both the global and local efficiency of the frontal-parietal and cingulo-opercular networks were strongly predictive of shared variance across cognitive domains among individuals with psychosis. In addition, the participation coefficient (a measure of information transfer among networks) for the right anterior insula was also predictive of this common cognitive factor, as well as of performance on both goal maintenance and episodic encoding tasks individually. Additional results will focus on examining similar metrics and their relationships to cognition in the human connectome project, as well as the integrity of the salience, default mode and amygdala networks in relationship to reward processing, social function, and emotional reactivity and regulation.
Conclusions: These data illustrate the importance of examining brain network integrity in addition to the function of individual brain regions, and also highlight the ways in which large scale data sets can move forward our understanding of functional brain organization in relationships to psychopathology.
Disclosure: Part 1: I am a consultant for Pfizer, Roche, Amgen and P1Vital.

13.3 Effects of Gene-Gene Interactions Within an Early Risk Pathway for Alzheimer's Disease

Aristotle Voineskos

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

Background: Big datasets provide adequate power to examine gene-gene interaction effects on neuroimaging and other risk phenotypes that might improve prediction of

disease risk and disease heterogeneity that might not otherwise be possible using smaller datasets. We have shown that variants within two replicated Alzheimer's (AD) risk genes (apolipoprotein E (APOE) and sortilin related receptor (SORL1)) are individually associated with structural brain changes across the lifespan. There is long-standing evidence for APOE as a genetic risk factor for AD, and recent genome-wide association studies (GWAS) support SORL1 as a genetic risk factor for this disorder. These genes are biologically related: SORL1 is a receptor for APOE helping direct amyloid clearance, and may also alter risk for AD through cerebrovascular mechanisms via a low density lipiprotein binding domain. Therefore in two large neuroimaging samples and one large postmortem sample, we evaluated the statistical interaction of known SORL1 and APOE risk variants on AD-related neural phenotypes.

Methods: From the Centre for Addiction and Mental Health, 135 healthy subjects underwent imaging-genetics procedures. Cortical thickness was calculated using the CIVET pipeline, and white matter FA was calculated from diffusion tensor imaging (DTI) scans. From the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset, cortical thickness was similarly calculated in $n = 193$ normal controls, $n = 294$ late mild cognitive impairment (MCI) subjects, and 133 people with AD. From the Rush University Religious Orders Study and Memory and Aging Project (ROS/MAP), 884 postmortem brains (310 healthy, 226 MCI, 348 AD) underwent neuropathological examination; amyloid and cerebral infarcts were quantified as present or absent. Subjects were genotyped for SORL1 rs689021 and APOE ε4, and interactions were modeled using regression with appropriate covariates for cortical thickness in vivo, and amyloid, and cerebral infarct neuropathology.

Results: In the CAMH sample, SORL1 by APOE interaction predicted average cortical thickness ($p = 0.04$), with greater prominence in specific lobar regions, and hippocampal volume. In the ADNI sample this interaction was present across all participants ($p < 0.05$ in frontal and temporal lobes). In the ROS/MAP sample, SORL1 by APOE interaction predicted risk for cerebral infarcts ($p = 0.0009$), but not amyloid, although there were main effects on amyloid from both SORL1 and APOE genotype. Individuals with a specific combination of APOE and SORL1 genotype had both the lowest average thickness in the CAMH sample and highest proportion of cerebral infarcts in the ROS/MAP sample.

Conclusions: Our results suggest that common genetic variation in SORL1 and APOE may interact to predict AD using both in vivo neuroimaging data and postmortem neuropathological data. Main effects of these variants appear to also influence brain structure and postmortem pathology, but their interaction provides a unique picture of risk for AD over and above main effects. Large datasets are required to provide adequate power to model such interactions. Understanding how effects of one genotype are modified by another is critical for dissecting the complexity of the AD risk profile. Furtheremore, such approaches move us closer toward molecular diagnoses for individuals, and personalizing treatment.

Disclosure: Nothing to Disclose.

Tuesday, December 09, 2014

Study Group

14. Proponents and Opponents of Legalization of Marijuana: Evidence of Benefits and Costs in Three Areas (Psychosis, Cognition, and Motivation)

Susan Weiss*, James Swanson, Anne Evins, Lynn DeLisi, Madeline Meier, Raul Gonzalez, Michael Bloomfield, H Valerie Curran

National Institute on Drug Abuse, Bethesda, Maryland

A political debate about benefits and costs of exposure to marijuana has accompanied initiatives by many states to legalize its use. In 2012, two states (Colorado and Washington) passed laws to legalize marijuana for recreational use, and 20 others currently have medical marijuana laws – making a scientific debate on its risks vs. benefits both relevant and timely. We propose a Study Group to engage in a scientific debate on the effects of marijuana on the human brain, including the impact of varying cannabis constituents. To narrow the scope, we define three critical areas where differences of opinion exist in the scientific community (and in the ACNP membership): Psychosis, Cognition, and Motivation. We identified scientists who have contributed to the scientific literature and who will serve as proponents or opponents to these propositions:.

1. Exposure to marijuana increases risk for psychosis (schizophrenia).
2. Exposure to marijuana decreases cognitive capacity (lower IQ).
3. Exposure to marijuana decreases motivation (apathy).

Psychosis: Proponent will be Anne Eden Evins from Harvard Medical School, who is a lead author on this subject (Evins et al, 2012). She concluded that marijuana is one of the many causes of schizophrenia (even though psychosis does not occur in most marijuana users). Opponent will be Lynn DeLisi from the Brockton VA (VA Boston Healthcare System)(Proal et al, 2014) who proposes that increased familial risk underlies the increased risk for schizophrenia in cannabis users and not cannabis use by itself.

Cognition: Proponent will be Madeline Meier from Arizona State University, who reported that chronic marijuana use initiated in adolescence reduces IQ significantly in adulthood (Meier et al, 2012). Opponent will be Raul Gonzalez from Florida International University, who has shown that deficits associated with marijuana use are not general, but specific to some areas of cognition, and that at least a subset of persistent neurocognitive impairments likely stem from a pre-existing vulnerability to cannabis addiction rather than a consequence of use (Gonzales et al, 2012).

Motivation: Proponent will be Michael Bloomfield from Imperial College London, who conducted a PET study (Bloomfield et al, 2014) and found that the reduction in striatal dopamine synthesis capacity associated with chronic cannabis use may underlie reduced reward sensitivity and amotivation. Opponent will be Valerie Curran (University College, London), who has suggested marijuana increases divergent thinking (Shafer et al, 2012) and hyper-priming (Morgan et al, 2010).

We anticipate a lively debate on these issues that are highly relevant to psychiatry and to society more broadly as policy changes are occurring rapidly and often without sufficient attention to the potential public health impacts.
Disclosure: S. Weiss, Nothing to Disclose; J. Swanson, **Part 1:** Consulting and Advisory Board, Speaker's Bureau, Clinical Trials, and Research Grants in the past for J&J, Janssen, McNeil, Alza, Novartis, COBA, UCB, Medeva, Shire, Richwood, Celgene, Cephalon, Gliatech, Lilly, Purdue, and Watson; Previous Legal Testimony and Current Patent Issues for J&J, Alza, Janssen; Previous Research Grants from NIMH, NIDA, and NICHD; Recent Travel Support to meeting of the European Network for Hyperkinetic Disorders and the Pediatric Academic Societies to present Invited Lectures; A. Evins, Nothing to Disclose; L. DeLisi, Nothing to Disclose; M. Meier, **Part 1:** SAMHSA consultant-gave a talk on cannabis and IQ; R. Gonzalez, Nothing to Disclose; M. Bloomfield, Nothing to Disclose; H Valerie Curran, Nothing to Disclose.

Panel
15. Developmental and Molecular Mechanisms in Frontal Systems in Suicide

15.1 Multimodality MRI Evidence for Altered Frontal System Trajectories in Association with Suicide Attempts in Adolescents: Common and Distinct Features Across Bipolar and Major Depressive Disorders

Hilary Blumberg

Yale University School of Medicine, New Haven, Connecticut

Background: Adolescence is a critical period in the development of suicide behaviors. The majority of attempts occur within the context of mood disorders. Thus, elucidating neural circuitry development associated with suicide risk in adolescents with mood disorders is important to understanding the development of risk, and providing leads for early identification, intervention and prevention strategies. It is not known whether risk circuitry is the same in bipolar disorder (BD) and major depressive disorder (MDD). Therefore, neural circuitry associated with attempts was examined within and across the disorders.
Methods: High resolution structural MRI, functional MRI and diffusion tensor imaging were performed in adolescents: 114 with mood disorders, including 51 with suicide attempts, and a matched healthy control group. Neural circuitry associated with suicide attempts was investigated within and across the disorders. Longitudinal data was analyzed for neural circuitry features predicting future attempts and trajectory patterns associated with attempts. Associations between imaging findings and suicide-related behaviors, and childhood maltreatment, were examined.
Results: Within BD and MDD, frontal system abnormalities were associated with attempts. White matter findings were especially prominent. However, there were differences noted between the attempters with BD or MDD in the distribution of white matter abnormalities and associated gray matter, as well as functional and behavioral, abnormalities. For example, attempters with BD showed prominent ventral frontal system connectivity abnormalities, including white matter structural integrity decreases in uncinate and ventrolateral frontal regions and functional connectivity from the amygdala to ventral prefrontal cortex, that were significantly lower than in the adolescents with MDD ($p<0.005$). In contrast, attempters with MDD showed decreases in insula and lateral and medial dorsal frontal regions, including reductions in gray matter and responses to emotional face stimuli ($p<0.005$). Longitudinal data showed associations between reductions in frontal system gray and white matter and future suicide attempts, as well as greater reductions in the gray matter volume and white matter integrity over time in the adolescent attempters. Differing behavioral associations of the regional findings suggest the presence of separable phenotypes within attempters, including a subgroup with more externalizing symptoms, emotional dysregulation and impulsive behaviors, and one with more internalizing symptoms, depression, and suicide ideation. Findings were also associated with childhood maltreatment subtypes. For example, history of emotional abuse was associated with ventral white matter abnormalities.
Conclusions: The results support abnormalities in gray and white matter structural, and functional, trajectories in frontal systems in adolescent suicide attempters with mood disorders. These include findings associated with prediction of future attempts and that progress over time during adolescence. While there are some features common across attempters with BD and MDD, distributions of findings also differed across diagnoses, and were associated with differing patterns of symptoms and behaviors, suggesting subtypes within attempters that may be associated with different paths towards attempts. Exposure to childhood maltreatment subtypes may be one factor that may contribute.
Disclosure: Nothing to Disclose.

15.2 Higher Dorsal Raphe Nucleus 5HT1A Binding Potential Predicts Lethality of Future Suicidal Behavior

Maria Oquendo

Columbia University, New York, New York

Background: The 5-HT1A autoreceptor regulates serotonin neuron firing and, thereby, serotonin release. Elevated 5-HT1A autoreceptor binding results in less serotonin firing and upregulation of terminal field receptors observed in MDD and suicide attempters may be a compensatory effect. We have reported that 5-HT1A receptor binding potential is associated with higher lethality in past suicidal behavior and with suicide intent of the most lethal past attempt. In this study, we examine 5-HT1A binding potential using PET and [11C]-WAY100635, a 5-HT1A receptor antagonist. We sought to determine its relationship to the lethality of future suicidal behavior and to specific features of suicidal ideation.
Methods: Depressed subjects ($n=134$) had PET scanning at baseline with [11C]-WAY100635 and were followed for 2 years. 13 subjects made a suicide attempt and 2 died by

suicide. Lethality of attempts was measured on the Beck Lethality scale with ranges from 0 (no damage) to 8 (death). Suicidal ideation was assessed using the Beck Scale for Suicidal Ideation. Analyses were weighted by the inverse estimated variance of ROI-specific binding potential (BPf). Weights were windsorized to reduce the influence of extreme outliers.

Results: Higher 5HT1A BPF in the dorsal raphe nucleus was associated with higher future attempt lethality ($p = 0.03$). When 3 subjects with 0-lethality attempt were removed, there was a widespread association between attempt lethality and 5HT1A BPF, including dorsal raphe nucleus ($p = 0.0002$), parietal ($p = 0.02$), parahippocampal ($p = 0.03$) and temporal cortex ($p = 0.04$), anterior cingulate ($p = 0.03$), dorsolateral ($p = 0.01$), insular ($p = 0.04$), occipital ($p = 0.02$), and orbital prefrontal cortex ($p = 0.03$). However, after adjustment for multiple testing, only dorsal raphe nucleus stayed significant. Chronic, persistent suicidal ideation was positively associated with 5HT1A BPF across all the above-mentioned regions ($0.02 < p < 0.003$) as well.

Conclusions: Consistent with our post-mortem findings, higher baseline 5HT1A BPF in dorsal raphe nucleus predicts the medical damage of future suicide attempt. Whether these effects are mediated through aggression or more persistent, chronic, suicidal ideation remains unknown. Nonetheless, if such leads to clarifying the pathophysiology of highly lethal suicidal behavior and their mediators are confirmed, intervention strategies may be more easily identified.

Disclosure: Part 1: Spouse works for Bristol-Myers-Squibb and is paid in salary and stock options, **Part 2:** Spouse works for Bristol-Myers-Squibb and is paid in salary and stock options, **Part 3:** Spouse works for Bristol-Myers-Squibb and is paid in salary and stock options. Royalties from the commercial use of the C-SSRS.

15.3 miRNA Networks in dlPFC of Suicide Subejcts: Role in Pathophysiology and Therapeutics

Yogesh Dwivedi

University of Alabama at Birmingham, Birmingham, Alabama

Background: miRNAs are small (~ 22 nt) non-coding RNA transcripts, which by binding to the 3′ UTR of specific mRNA targets, regulate their translation and/or stability. miRNAs have been implicated in synaptic plasticity, genetic susceptibility to stress and coping to stress response. The present study will examine the contribution of miRNAs in the reorganization of gene expression networks in dorsolateral prefrontal cortex (dlPFC) and to suicide risk in depression and whether manipulating miRNA biogenesis machinery can ameliorate depressive behavior in animal model system.

Methods: miRNA expression was studied in dorsolateral prefrontal cortex of antidepressant-free depressed suicide subjects ($n = 20$) and healthy normal controls ($n = 20$) by small RNA sequencing. Role of miRNAs in synaptic plasticity was studied by examining miRNA sequencing in synaptonerosomes. Sequences were mapped to human pre-miRNA and mature miRNA databases provided in the miRBase, ncRNA, piRNA, repeats and genome databases using BLAST. To identify differentially expressed miRNAs, raw small RNA sequence counts in each sample were normalized by endogenous miRNAs and synthetic miRNA added as spike-in control (ath-miR-159a, not expressed in humans). To examine whether modulation of miRNA can ameliorate depression, rats were treated with fluoroquinolone compound enoxacin (10 and 25 mg/kg for 7 days), which binds to HIV-1 TAR RNA binding protein (TRBP) and stabilizing the dicer-TRBP complex to raise miRNA levels. Stress-induced behavioral depression was examined by determining escape latency. Expression of brain enriched miRNA was determined in the frontal cortex. Multiple test corrections (Benjamini and Hochberg) and false discovery rate (FDR) were used to identify miRNAs that are most influential in distinguishing between various groups. Data were also analyzed by paired non-parametric Wilcoxon sign-rank tests and quantile analysis.

Results: We found a global downregulation of miRNAs in suicide subjects (21 miRNAs significantly downregulated; 29 were decreased by $>35\%$ but were not significant). Many of them were synaptically enriched and encoded at nearby chromosomal loci, shared motifs within the 5'-seeds, and shared putative mRNA targets. In addition, we found a dramatic reorganization of miRNAs in a coordinated and cohesive fashion in suicide subjects which was not present in the control group. Synaptically enriched miRNAs were highly associated with this reorganization. Target analyses showed genes that are implicated in synaptic plasticity and neurogenesis. Treatment of both 10 or 25 mg/kg of enoxacin to rats for one week increased the expression of miRNAs in rat frontal cortex and decreased the proportion of rats exhibiting learned helpless behavior following inescapable shock.

Conclusions: Our findings show that miRNAs contribute substantially to a reorganization of gene expression network that occurs in suicide. Affected miRNAs are likely to participate in pathogenesis of suicide via altering the expression of mRNAs that they regulate. We also found that manipulating TRBP/dicer complex prevents stress-induced depressive behavior in rats, which suggests the possibility of new therapeutic approach to human neuropsychiatric diseases including.

Disclosure: Nothing to Disclose.

15.4 Suicide, Childhood Maltreatment and Methylation Changes in the Anterior Cingulate Cortex

Gustavo Turecki

McGill University, Montreal, Canada

Background: Suicidal behaviors are complex phenomena that are thought to result from the interaction of distal and proximal factors. Among distal risk factors, a history of childhood maltreatment (CM) is one of the factors with strongest effects. Epigenetic marks associate with genomic responses to environmental stimuli, and animal studies suggest that stable epigenetic factors mediate the long-term behavioral consequences of variation in early-life environment. The anterior cingulate cortex (ACC) is

implicated in the encoding of CM, and in the regulation of mood states.
Methods: We characterized genome-wide DNA methylation patterns in the ACC using reduced representation bisulfite sequencing (RRBS), and its correlation with the expression of related genes, as measured by RNA-Seq. Using brain post-mortem tissues available through the Douglas-Bell Canada Brain Bank, we compared 27 depressed suicide completers with a history of severe CM, with 26 psychiatrically normal individuals with no history of CM. Information on psychiatric diagnoses and history of CM were obtained for all subjects through psychological autopsies.
Results: A total of 67% of sequences were aligned, with an average of 21.25M (0.9M) and 21.90M (0.8M) reads were sequenced, respectively, for suicides with histories of CM and controls. The analysis was conducted using 800bp windows. Over one hundred genomic regions were differentially methylated between groups at genome-wide significant level (FDR corrected). Many of these differentially methylated sequences were clustered in genomic regions associated with functional implications.
Conclusions: These results provide us with interesting leads into biological processes differentially regulated by the early-life environment, some of which may help us better understand behavioral dysregulation frequently present in individuals with histories of CM, and may suggest possible new avenues for intervention.
Disclosure: Nothing to Disclose.

Panel
16. Beyond AKT1: Emerging Role of the AKT Signaling Network in Neurodevelopment, Cognition and Developmental Psychiatric Disorders

16.1 Dissecting the Role of AKT2 and AKT3 in Neurodevelopment and Schizophrenia

Amanda Law

University of Colorado School of Medicine, Aurora, Colorado

Background: The AKT family consists of three highly homologous serine/threonine protein kinases (AKT1; 14q32; AKT2, 19q13.2 and AKT-3, 1q44) that play essential roles in cell development, growth, and survival. Genetic variation in AKT1 is associated with risk for schizophrenia and prefrontal cortical (PFC) physiology in humans and recent schizophrenia genome-wide association studies report significant association to AKT3 [1q44]. In addition, rare mutations and copy number variations in human AKT3 are associated with congenital brain abnormalities, including micro- and macrocephaly, suggesting a key role for AKT3 in early human neurodevelopment. Whilst rodent studies implicate AKT1 in hippocampal and PFC development, data is absent on the neurological role of AKT2 and AKT3 and the temporal dynamics of AKT isotype expression during human brain development is unknown. Here we examined AKT1, 2 and AKT3 mRNA expression across human brain development and aging and in mice examined the consequences of germline genetic deletion of Akt2 or Akt3 on prefrontal cortical and hippocampal circuit development and behaviors relevant to schizophrenia.
Methods: AKT1, AKT2 and AKT3 expression was measured using real-time QPCR in human postmortem PFC derived from fetal (14–39 weeks gestation; $N = 41$) and postnatal subjects (0–83 years; $N = 245$). C57/B6, Akt2 [wt, +/-, -/-] or Akt3 [wt, +/-, -/-] mutant mice were tested in a comprehensive behavioral battery relevant to domains of cognition and schizophrenia. Single cell slice electrophysiology was used to assess long-term potentiation (LTP) and excitatory and inhibitory neurotransmission.
Results: AKT1 and AKT2 expression was temporally regulated across human brain development, being significantly higher in fetal, neonate and infant PFC, declining to adult levels by adolescence and becoming stable across age (ANOVA; $p < 0.001$). In contrast, AKT3 expression was tightly regulated across human brain development and showed no association to pre- or postnatal developmental stage. In mice, Akt2 hemi- or homozygous deletion impaired discrimination memory ($p < 0.0001$); PPI ($p < 0.01$), contextual fear conditioning ($P = 0.003$) and preference for social novelty ($p = 0.002$). Furthermore, Akt2 mutants exhibited selective anxiety-like phenotypes. Genetic deletion of Akt2 impaired excitatory and inhibitory circuit development in the hippocampus and mPFC and diminished hippocampal LTP. In contrast, Akt3 mutants showed selective deficits of mPFC function related to discrimination memory and associative learning (ANOVA $p < 0.0001$).
Conclusions: Our data demonstrate a novel role for AKT2 and AKT3 in brain development, cognitive function and behaviors relevant to schizophrenia and provide a platform for understanding mechanisms of genetic risk for schizophrenia at the AKT loci. Study of AKT3 gene splicing in human brain and its relevance to schizophrenia risk genetic variation are currently underway.
Disclosure: Part 1: DHHS, NIH, Government owned and filed. Patent Pending 'Novel Drugs for the Treatment of Schizophrenia'. Inventor.

16.2 AKT Transcript Variation in the Context of Psychiatric Genetics

Daniel Weinberger

Lieber Institute for Brain Development, Baltimore, Maryland

Background: AKT has been prominently implicated in signaling pathways related to risk for psychiatric disorders, particularly those involving DRD2, ERBB4 and DISC1/GSK3b. We and others have previously shown that a coding variant in AKT1 (rs 1130233) is associated with risk for schizophrenia, but only in an interactive context with either other genes (Tan et al, Mol Psychiatry 2011) or with environmental risk factors (Nicodemus et al, Mol Psych 2008, Di Forti et al, Biol Psych 2012). AKT3 has been recently implicated as a schizophrenia susceptibility gene achieving association at the genome wide significance level in the latest PGC2 GWAS. We have now explored the presence of transcript and epigenetic variation in relation to schizophrenia

and to risk associated SNPs in human brain tissue from DLPFC of normal subjects and individuals with the diagnosis of schizophrenia.
Methods: We have now explored the presence of transcript and epigenetic variation in relation to schizophrenia and to risk associated SNPs in human brain tissue from DLPFC of normal subjects and individuals with the diagnosis of schizophrenia. Methods: PolyA capture RNA sequencing (HiSeq 2000 80-100M paired end 100 base pair reads) was performed in 409 subjects (including 175 controls/135 patients >20 years of age) controlling for RIN and age and NeuN+ composition proportions, and Infinium HumanMethylation450 Bead Chip analyses were performed in 200 RIN matched samples (100 each adult patients/controls). Transcripts, exons and junctions in AKT1 and AKT3 were explored for illness associated differential expression and eQTLs and mQTLs.
Results: At the gene level, AKT3 is significantly more abundant during fetal life, with an extended 3'UTR containing transcript being especially fetally abundant. Patients show significantly reduced expression of AKT3 at the gene level ($p = 9.733\text{-}07$, Caucasians only $p = 7.77e\text{-}06$). A significant association with the unique last 3' junction suggests that the fetal transcript is the disease-associated transcript. The PGC2 risk associated SNP did not show association at the gene, exon or junction level. At the gene level, AKT1 also is more abundantly expressed during fetal life, but this also is because of specific transcripts variants that are differentially regulated. Gene level expression of AKT1 was slightly increased in patients ($P = .001$). Interestingly, rs1130233 is one nucleotide 3' to a CpG which is differentially upmethylated in schizophrenia ($p < .03$) and predicts transcription skipping of exon 9 ($N = 57$, Rho = -0.28, $p = 0.03$).
Conclusions: Schizophrenia association with AKT1 and AKT3 is related to fetally abundant transcript variants and to epigenetic factors.
Disclosure: Nothing to Disclose.

16.3 The Role of Genomic Risk Variation in AKT1 in the First Steps of Human Brain Development

Ronald McKay

Lieber Institute for Brain Development, Baltimore, Maryland

Background: Significant investments are being made worldwide in basic and translational research on stem cell biology in an effort to reveal the next generation of molecular targets for neurologic and psychiatric dosorders. These in vitro models have yielded novel insight into signaling pathways that restore CNS function in vivo and are strongly informing novel regenerative therapeutic strategies. The powerful new idea revealed by these studies is that the molecular pathways that regulate stem cell function in development also play key roles throughout life in both homeostasis and disease. Factors associated with risk for neurodevelopmental disorders such as AKT1, ERBB family members, and NRG1 are critical regulators of stem cell survival, growth, and proliferation.
Methods: Here we will show lineage bias and reversible differences in susceptibility to differentiation exist in distinct spatial regions of human pluripotent stem cell colonies.
Results: Surprisingly, in this system, the expression of core pluripotency regulators OCT4, NANOG and SOX2 are regulated in a spatially constrained way. OTX2 and SOX21, transcription factors essential for brain development, are dynamically regulated in pluripotent cells playing a crucial role in the early specification of neuroectoderm. Neuregulin 1 -ERBB2/3 signaling suppresses neuroectoderm specification through AKT activation.
Conclusions: This work shows that NRG1-AKT signaling regulates the balance between spatial domains biased towards mesendodermal or neurectodermal fates under self-renewing conditions. Genome specific regulation of these early mechanisms opens the way to a systematic definition of the developmental landscape of genetic risk for brain disorders.
Disclosure: Nothing to Disclose.

16.4 Aberrant AKT Signaling Disrupts Central DA Homeostasis and Amphetamine-induced Behaviors

Aurelio Galli

Vanderbilt University School of Medicine, Nashville, Tennessee

Background: Aberrant Akt signaling has been linked to the etiology of dopamine (DA)-associated neuropsychiatric disorders, including schizophrenia. Data from our laboratory demonstrate that the phosphorylation state of the metabolic kinase Akt regulates central DA homeostasis. This suggests that dysfunction of Akt might support disrupted central DA neurotransmission. Akt is a key mediator of signal transduction downstream of growth factors, cytokines, external stimuli, and insulin. The activity of Akt is regulated by phosphorylation of two key residues: Thr308 by PDK-1 and Ser473 by the mTORC2 (mammalian target of rapamycin complex 2) complex that comprise the protein RICTOR.
Methods: To study how disrupted function of Akt signaling influences central DA neurotransmission, we first ablated phosphorylation of Akt at Ser473 by targeted whole brain deletion of the RICTOR protein.
Results: This mouse model exhibits exaggerated hypersensitivity to psychomotor effects of amphetamine (AMPH) and causes aberrant expression and/or function of major markers of DA homeostasis including tyrosine hydroxylase, DA transporter (DAT), D2 DA receptor (D2R), and its downstream kinase ERK1/2. Importantly, we demonstrate that the exaggerated responses to AMPH in this model are dependent on D2R signaling as they are reduced by D2R pharmacological blockade. Combining viral interventions and conditional gene knock out techniques, we determined that the observed exaggerated AMPH phenotypes are caused by impaired Akt function in the dorsal striatum.
Conclusions: Our data suggest that Akt phosphorylation influences AMPH-induced behaviors in a brain region-specific and D2R signaling-dependent manner.
Disclosure: Nothing to Disclose.

Panel
17. Psychosis Prodrome: Toward the Validation of Biomarkers for Clinical Trials

17.1 Biomarkers of Risk for and Progression to Psychosis in the North American Prodrome Longitudinal Study (NAPLS)

Tyrone Cannon

Yale University, New Haven, Connecticut

Background: This talk will present findings on clinical risk prediction algorithms as well as biomarkers assessed longitudinally in youth at clinical high-risk for psychosis.
Methods: The study cohort consists of 765 participants from the second stage of the North American Prodrome Longitudinal Study (NAPLS). The primary outcome was conversion to psychosis over 2-years from initial evaluation. Participants were evaluated with structural MRI, electrophysiology (mismatch negativity, auditory P300), and cortisol assays at baseline and at 12-months or at conversion to psychosis. Smaller subgroups were evaluated with functional MRI (resting state, verbal working memory, associative learning, emotion processing) and plasma analytes (indexing inflammatory and oxidative stress markers) at the baseline assessment.
Results: Multivariate models incorporating risk factors from clinical, demographic, neurocognitive, and psychosocial assessments achieved high levels of predictive accuracy when applied to individuals who meet criteria for a prodromal risk syndrome. A risk calculator was created that can be used to scale the risk for newly ascertained cases based on this set of predictors. With respect to biomarkers, at risk individuals who converted to psychosis showed elevated levels of cortisol and pro-inflammatory cytokines, as well as lower MNM and P300 amplitude and disrupted resting state functional connectivity at baseline, compared to those who do not. Further, converters showed a steeper rate of gray matter reduction, most prominent in prefrontal cortex, that in turn was predicted by higher levels of cortisol and inflammatory markers as well as by lower MNM amplitude at baseline. Each biomarker was a significant predictor of psychosis on its own, and several improved prediction over and above the level achieved by the clinical, demographic, and cognitive algorithm.
Conclusions: This work supports that view that the emergence of psychosis is marked by a dynamic and potentially reversible process that results in a reduced structural and functional connectivity in circuits involved in cognitive control, learning and memory, emotional regulation, and auditory-verbal processing.
Disclosure: Part 1: I am a consultant to the Los Angeles County Department of Mental Health on the implementation of early detection and intervention services for youth at risk for psychosis.

17.2 Early Prospective Assessment of Cognition and Brain Function in Psychosis Spectrum

Raquel Gur

University of Pennsylvania, Philadelphia, Pennsylvania

Background: Much of the work to-date on Psychosis Prodrome has focused on help-seeking individuals in late adolescence and early adulthood. These studies identified neurocognitive deficits and brain structural abnormalities in such samples that are similar in nature to those observed in psychosis. Such data indicate that abnormalities could be present and detectable early in the psychotic process. However, the prodromal study design targets individuals with sufficiently severe symptoms to elicit help-seeking and may miss individuals at earlier stages of illness or who are at a younger age. Prospective sample of youths who are characterized clinically and neurocognitively could yield information on the extent and nature of cognitive deficits prior the stage at which symptoms have triggered help seeking. Large samples are needed for such studies below of the low base-rate of psychosis in the general population.
Methods: The Philadelphia Neurodevelopmental Cohort is a population-based study that provides information on clinical phenotypes and neurocognitive measures in 9,500 youths age 8-21. A subsample underwent multi-modal neuroimaging enabling the establishment of precursors and biomarkers of psychosis risk. The overall procedures for clinical and neurocognitive assessment as well as for neuroimaging have been published (Gur et al, JAMA Psychiatry 2014; Satterthwaite et al, Neuroimage 2013). A subsample of 500 participants (200 who report significant psychotic symptoms, 100 who report mild psychotic symptoms and 200 typically developing youths) received an 18 months follow-up that included more extensive clinical assessment, repeated neurocognitive assessment and multimodal neuroimaging. We applied a mixed model analysis to evaluate group differences that are age related or symptom related (presence of psychotic symptoms), regression analysis with 10-fold cross-validation to examine predictive value of neurocognitive performance and CORANOVA to test fro differences among correlated correlations.
Results: There was age-related increase in accuracy and speed of neurocognitive performance, which was most pronounce fro the Executive domain of Attention, as well as for Complex Reasoning, Social Cognition and Motor Speed and was least pronounced for Episodic Memory. Individuals who reported significant psychotic symptoms showed deficits across domains and a delay of between 6 months and 2 years in neurocognitive performance, especially for the domains of Complex Cognition and Social Cognition. This group also showed abnormalities in brain structure and function at baseline. Structural abnormalities included reduced cortical volume and diminished white matter connectivity. Functional abnormalities included failure to activate DLPFC and associated regions in response to a working memory task and over activation of amygdala to threat related emotional expression during an emotion identification task. These abnormalities are associated of severity of symptoms. The follow-up results indicate that individuals who continue to report significant psychotic symptoms have greater abnormalities in the neurocognitive performance especially for Memory and Social Cognition, compared to individuals who no longer reported psychotic symptoms and typically developing youths. The symptomatic group also showed less improvement in neurocognitive performance and had more abnormalities in brain structure and function.
Conclusions: Individuals with significant psychotic symptoms can be identified and followed in a population-based community sample. Such youths can be evaluated at a

young age n demonstrate deficits in neurocogniitve performance that are comparable to deficits to deficits documented in psychosis. These individuals also have brain abnormalities that indicate maturational aberrations. The follow-up study supports the role of these deficits in defining the psychotic process and the presence of these abnormalities portends symptom progression. Linking the neurocognitive deficits and brain abnormalities can lead to a better understanding of the pathophysiology and evolution of psychosis.
Disclosure: Nothing to Disclose.

17.3 Using Pattern Recognition to Identify and Validate Biomarkers for the Psychosis Prodrome

Nikolaos Koutsouleris

Ludwig-Maximilian-University, Munich, Germany

Background: Multivariate analysis tools have increasingly developed into powerful methodological frameworks enabling the identification of biomarkers for the psychosis prodrome based on behavioural, neurocognitive and neuroimaging data. Although the feasibility of predicting psychosis has been demonstrated in single-center studies, further validation of these candidate markers is required in independent and large-scale datasets following industry-standard regulatory frameworks.
Methods: The talk will (1) review the current state-of-the-art in the identification of single- and multi-domain markers of emerging psychosis, (2) introduce machine learning tools and multivariate statistics as the core methodological backbone of the biomarker identification process, (3) present new data on the generalizability and differential diagnostic specificity of neurodiagnostic signatures of psychosis, and (4) provide perspectives how to further validate candidate markers using FDA's biomarker regulatory processes.
Results: The current literature suggests that an individualized prediction of psychosis is feasible using behavioral and neuroimaging data at accuracies ranging from 70% to 90%. Furthermore, the talk will present new results on neuroimaging data pooled across independent sites, which support a psychosis prediction accuracy of $>80\%$.
Conclusions: If the out-of-center generalization capacity of these markers could be demonstrated and replicated during the validation process this would translate into a significant increase of predictive certainty, thus allowing for a risk-based stratification of high-risk individuals. if further validated, such single-subject risk profiling methods could (1) facilitate risk-stratified clinical trials, (2) provide novel targets for drug development and, (3) tools for the individualized neuromonitoring of preventive treatments.
Disclosure: Nothing to Disclose.

17.4 Cognitive Decline as a Biomarker in the Early Stages of Schizophrenia

Rene Kahn

University Medical Center Utrecht, Utrecht, Netherlands

Background: There is consistent evidence for cognitive changes prior to the onset of psychosis in schizophrenia. Also, progressive brain changes during the course of the disease have consistently been shown, but it has not been studied whether the two phenomena are related. This is important, since brain loss over time is only observed in a subgroup of patients; this group may well be characterized by (relative) worsening in cognitive function.
Methods: A total of 78 first-episode schizophrenia patients and 113 age-matched healthy controls were tested three times each with a three year interval (i.e. 3 and 6 years after baseline assessment) I.Q and 1.5T sMRI were obtained at each time point. Surface-based analysis with FreeSurfer software (5.1.0) was applied to measure global and local cortical thickness change in each subject. Yearly cortical thickness change was defined as the percent thickness change per year during the scan interval.
Results: Data are currently available for the 3 year follow-up, but 6 years follow-up data will be ready for presentation at the conference. Cortical thickness, but not surface, decreased in the patients versus controls (at 3 year follow-up) and this loss was significantly associated with decreases in IQ ($r=.385$, $p=0.015$). This finding was particularly pronounced in the earliest phases of the illness ($r=.588$, $p<0.001$) and was not confounded by medication use.
Conclusions: Cortical thinning in schizophrenia is related to worsening in global cognitive function and this is most prominent in the early stages of the illness. Thus, a subgroup of schizophrenia patients is characterized by both cognitive decline and loss in brain volume. This may signify a (genetically distinct) group of patients, for instance with a higher load of (rare or common) genetic abnormalities.
Disclosure: Nothing to Disclose.

Panel
18. Genetic and Epigenetic Contributions to Reproductive-related Mood Disorders

18.1 Application of Latent Class Analysis to Investigate the Heterogeneity of Postpartum Depression in an International Perinatal Psychiatry Consortium

Samantha Meltzer-Brody

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

Background: Postpartum Depression (PPD) confers substantial morbidity and mortality. However, the distinguishing characteristics that define PPD are a matter of controversy. PPD is categorized as a subtype of MDD in DSM-5, and the diagnosis requires that DSM criteria are fulfilled and that the onset is during pregnancy or the 4 –week post-birth period. Additionally, the phenotypic presentation of PPD may differ from MDD occurring outside of the perinatal period. This study is an empirical investigation of PPD heterogeneity to identify clinical subtypes. Data were aggregated from the international perinatal psychiatry consortium, PACT (Postpartum Depression: Action Towards Causes and Treatment). PACT members are from 24 institutions in 7 countries, and 27,776 subject records with phenotypic data were available for analysis.

Methods: We applied latent class analyses (LCA) in a 2-tiered approach to assess the empirical validity of heterogeneity and subtypes of PPD. Tier 1 examined PPD heterogeneity in subjects with complete data on the Edinburgh Postnatal Depression Scale (EPDS) (N = 6556), including PPD cases and controls. Tier 2 subjects included only PPD cases (N = 4245) as assessed by formal clinical interview. In the Tier 2 analyses, indicator variables were hypothesized distinguishing clinical features of PPD having commonality among sites. These indicator variables included depression severity, EPDS total, EPDS anxiety subscale, timing of symptom onset, pregnancy complications, obstetrical complications during labor and delivery, suicidality, and psychiatric history/comorbidity of anxiety and depression.
Results: A 3 class solution yielded the best fit in both Tier 1 and Tier 2. In both Tiers, the most striking characteristics were severity, timing of onset, comorbid anxiety, and suicidal ideation. The class with the most severe PPD symptoms had significantly worse mood (mean EPDS = 20.3), greater anxiety, symptom onset that began during pregnancy, more obstetrical complications during labor and delivery, and endorsed suicidal ideation. The other PPD class (mean EPDS = 12.3) had less severe symptoms; the majority (54%) endorsed symptom onset in the first month postpartum and had more pregnancy complications.
Conclusions: PACT represents an important next step toward large scale collaborative research efforts needed to disentangle the pathophysiology of PPD. Examination of PPD heterogeneity to identify more precise phenotypes is a critical first step toward future biological and genetic investigations.
Disclosure: Nothing to Disclose.

18.2 Female-specific Development of Depressive-like Behaviors and Hippocampal Transcript Levels in a Genetic Rat Model

Eva Redei

Northwestern University School of Medicine, Chicago, Illinois

Background: The incidence of major depressive disorder (MDD) and the prevalence of co-morbid anxiety are much higher in women than in men, specifically during their reproductive years. These gender biases appear after puberty and their etiology is mostly unknown. Although many studies investigated the effect of gonadal hormones, particularly estrogen, on depression and anxiety-like behaviors in animal models, none have evaluated the appearance of these behaviors in a genetic model of depression. We have previously generated two fully inbred sub-strains from the Wistar Kyoto (WKY) rat strain, an accepted model of adult and adolescent depression. Adult WKY More Immobile (WMI) rats of both sexes consistently show increased depression-like behavior in the forced swim test (FST) compared to their genetically close control WKY Less Immobile (WLI) strain.
Methods: Pre-adolescent and adult WMI and WLI males and females were tested in the FST and the open field tests for depression and anxiety-like behaviors, respectively. Hippocampal transcript levels of genes, previously identified from genome-wide transcriptional analyses for their sex-specific differences in the adult WMIs and WLIs, were determined by quantitative RT-PCR.
Results: Depression-like behavior is present in WMI males both pre-puberty and in adulthood. In contrast, the high depression- and anxiety-like behaviors of the female WMIs appear only after puberty. These sex- and age-specific behavioral patterns are paralleled by hippocampal expression differences of genes.
Conclusions: The contribution of specific genes, likely regulated by estrogen, to depression- and anxiety-like behaviors in the female genetic model of depression could be enlightening towards the understanding of the gender bias in depression. The strength of the model, that the male and female WMIs are genetically identical in their somatic chromosomes, allows for powerful genetic and molecular mechanistic studies in the future.
Disclosure: Nothing to Disclose.

18.3 Attachment Insecurity and DNA Methylation in Risk for Postpartum Depression

Thalia Robakis

Stanford University, Stanford, California

Background: Postpartum depression (PPD) is common and debilitating. Predisposing factors and clinical phenotypes in PPD differ from those of depression in other life periods (Cooper and Murray 1995; Altemus et al. 2012); most sufferers have no history of mood disorder (Dietz et al. 2007, Banti et al. 2011). Thus, distinct psychobiological pathways may underlie PPD. Identification of personality factors associated with PPD susceptibility may assist in the discovery of PPD-specific pathways. While several aspects of personality have been implicated in PPD risk (Bifulco et al. 2004, Dennis et al. 2004, Phillips et al. 2010), potential biological correlates of personality-based susceptibilities have not been investigated.
Methods: SCID-based psychiatric evaluation and validated scales measuring attachment security (ASQ, Feeney et al. 1994), neuroticism/interpersonal sensitivity (VPSQ, Dennies et al. 2004), and negative cognitive style (DAS-24, Power et al. 1994) were administered to 101 healthy pregnant women. Buccal swabs were obtained from a subset of women for analysis of DNA methylation patterns by reduced-representation bisulfite sequencing (Gu et al. 2011). Depressive symptoms were evaluated by EPDS (Cox et al. 1987) and CESD (Radloff 1977) monthly for six months postpartum.
Results: All personality measures were potent predictors of postnatal depression (Pearson correlations with mean postnatal EPDS/CESD over three months postpartum: VPSQ 0.500/0.396, DAS-24 0.495/0.492, ASQ 0.526/0.579). This exceeded the predictive value of mood disorder history (Pearson 0.292/0.316 for EPDS/CESD) and neared the predictive value of antenatal depression (Pearson 0.550/0.561 for EPDS/CESD). Only the ASQ was robust across adjustment for intercorrelations between behavioral measures and other confounders. Correlation of DNA methylation at key loci with ASQ scores to be discussed.

Conclusions: Attachment insecurity is a major factor predisposing to postpartum depression. Methylation at key genetic loci may be related to attachment insecurity, representing an important biological mechanism linking early life stress, personality characteristics, and psychiatric diatheses.
Disclosure: Nothing to Disclose.

18.4 Estradiol for Postpartum Depression: Translational Challenges

Katherine Wisner

Northwestern University School of Medicine, Chicago, Illinois

Background: Estradiol (E2) is associated with enhancement of monoaminergic systems and mitigation of oxidative stress, glutamateric excitotoxicity, and β-amyloid tocixity. Consistent with its beneficial effects on neural circuits, E2 has antianxiety and antidepressant effects (via ERβ) in animal models. To study the potential therapeutic impact of E2 in postpartum depression, an NIMH funded study was conducted to compare E2 (delivered transdermally) vs. placebo (PL), with the antidepressant sertraline (SERT) as a comparator.
Methods: The protocol was a double blind, 8 week trial with dose escalation every 2 weeks to 200 mcg/d E2, 200 mg/d SERT or PL, with a 28 week continuation for responders. Exclusion criteria related to E2 use included: breast, uterine, or ovarian cancer; heavy smoking; hypertension, personal history or first degree relative with thromboembolic event; vascular or heart disease, liver disease, elevated lipids. Substance use was an exclusion factor and urine drug screens were obtained. Subjects ($N = 85$) were randomized. Serum concentrations of E2 (and E1) and SERT were obtained to assess both compliance and exploration of relationship to response.
Results: Baseline characteristics across the 3 groups did not differ. Consistent with the vulnerability of postpartum women to mania, 5 of 85 randomized women developed hypomania, and 4 of the 5 women were assigned to SERT and 1 to PL (Fisher's Exact $p = 0.123$). No women assigned to E2 developed mania. No thromboembolic or other adverse events occurred. The response/remission rates did not differ across the 3 groups on the primary outcome measure, the SIGH-ADS-29. At the end of the 8-week acute phase, the mean E2 levels in the E2-treated group was higher (75 pg/ml) than in the SERT (61 pg/ml) and PL groups (45 pg/m). The E1 levels were: 58, 40 and 33 pg/ml, respectively. These concentrations were much lower than we predicted in planning this study. We based our dosing protocol on E2 concentrations that non-postpartum women develop when treated with transdermal estradiol. We evaluated patch adherence problem reports, which did not differ between the treatment groups. The E2-treated group failed to reach the mid-cycle levels in reproductive-aged women and were not clinically significantly higher; however, the E2 concentration associated with response remains to be elucidated. No difference in serum E2 levels in breastfed infants across the 3 groups occurred, and serum concentrations were typically <2.5 pg/ml. Infant weight gain and mother-reported infant feeding satisfaction were comparable across groups.
Conclusions: Although E2 treatment is theoretically promising, feasible and safely delivered by an Ob/Gyn-Psychiatry team, the current evidence base for efficacy is weak. The dose and/or serum concentration of transdermal E2 has not been clarified, and pharmacokinetic studies are needed to direct dosing in postpartum women, which may be affected by residual increased drug metabolism induced during pregnancy. Additionally, our team is exploring the impact of obesity on transdermal E2 absorption, since we have published data to demonstrate that the majority of women with depression have pre-pregnancy BMIs in the overweight/obese range. E2 treatment did not reduce breastmilk supply in the dosage range used and infant serum concentrations did not differ across breastfed infants (typically <2.5 pg/ml).
Disclosure: Part 1: The Department of Psychiatry at Northwestern University receives contractual fees for Dr. Wisner's consultation to Quinn Emanuel Urquhart & Sullivan, LLP (New York City), who represent Pfizer Pharmaceutical Company.

Panel
19. Alcohol Craving: The Gut and Liver in the Brain

19.1 Endotoxins, Alcohol Consumption and Neuroimmune Signaling: A Vicious Cycle

Robert Adron Harris

University of Texas, Austin, Texas

Background: Molecular and behavioral studies suggest a role for the innate immune system in mediating the acute and chronic effects of alcohol and support a neuroimmune hypothesis of alcohol addiction. Changes in expression of neuroimmune genes and microglial transcripts occur in postmortem brain from alcoholics and animals exposed to alcohol, and null mutant animals lacking certain innate immune genes show decreased alcohol-mediated responses. Many of the differentially expressed genes are part of the toll like receptor (TLR) signaling pathway and culminate in an increased expression of pro-inflammatory immune genes. We asked if mouse models of excessive alcohol consumption show similarities to human alcoholics in expression of neuroimmune genes and if activation of the immune system could promote excessive alcohol consumption.
Methods: We profiled brain gene expression using RNA sequencing and oligonucleotide microarrays in brain regions from human alcoholics and from mouse models of excessive alcohol consumption. We activated TLR signaling in mice by injection of lipopolysaccharide (LPS) and measured gene expression, alcohol consumption and firing of brain dopamine neurons. We also used pharmacological and genetic blockade of neuroimmune signaling to reduce alcohol consumption.
Results: We found that activation of innate immune signaling by injection of LPS produced changes in brain gene expression which are similar to the effects of chronic alcohol consumption, reduced firing of brain dopamine neurons and produced a long lasting increase in alcohol consumption. No changes in taste (sweet or bitter)

perception, palatability (sucrose intake) or olfactory recognition were found after LPS-pre-treatment. Reduction of TLR signaling and other proinflammatory pathways reduced ethanol intake. Local brain signaling was shown to be important as inhibition of IKKβ – a key element of TLR-pathways – in the nucleus accumbens using local injection of viral CRE in mice with a floxed IKKβ genes.
Conclusions: Taken together, these results provide the most compelling evidence to date for immune signaling in regulation of alcohol consumption and that excessive alcohol consumption activates neuroimmune signaling. This is the basis for our proposal that alcohol consumption and craving is driven by a vicious cycle of neuroimmune activation. Compounds known to inhibit inflammation, microglial activation, and neuroimmune gene expression have shown promising results in reducing alcohol-mediated behaviors in our animal models, indicating that neuroimmune signaling pathways offer unexplored targets in the treatment of alcohol abuse.
Disclosure: Nothing to Disclose.

19.2 Altered Gut-Brain Signaling in an Animal Model of Roux-En-Y Gastric Bypass Surgery: Implications for Alcohol Consumption and Reward

Andras Hajnal

Pennsylvania State University College of Medicine, Hershey, Pennsylvania

Background: Roux-en-Y gastric bypass (RYGB) is a very effective surgical method to treat obese patients. Rodent models of obesity have shown that RYGB produced reduced preference and motivation for food and thus may reduce both palatability and rewarding effects of foods. In contrast, concerns have been raised by clinical reports of an increased risk for alcohol use following RYGB surgery. Our recent studies in dietary obese rats corroborated this notion and further investigated the hypothesis that increased alcohol preference and intake is driven by the enhancement in alcohol rewarding effects rather than altered alcohol absorption following RYGB. As a potential underlying mechanism, we investigated involvement of ghrelin and activation of brain reward areas.
Methods: High fat diet-induced obese Sprague Dawley male rats underwent RYGB and were habituated along with their sham-operated (SHAM) obese controls and with lean, normal diet-fed rats (ND) to increasing concentrations of alcohol (2-8%) in a two-bottle choice paradigm (Experiment 1) or a progressive ratio-10 (PR10) schedule of reinforcement operant oral self-administration task (Experiment 2). In addition, the effects of the ghrelin-1a-receptor antagonist D-Lys3-GHRP-6 (50, 100 nmol/kg, IP) were tested on PR10 responding for 4% alcohol. To control for differential alcohol absorption, in a third experiment, rats self-administered alcohol (1%) intravenously (IV). A forth experiment tested whether RYGB also alters motivation to IV self-administer morphine on a fixed ratio-5 (FR-5) and PR-2 schedule. Finally, a fifth experiment examined RYGB effects on the anticipatory responses to palatable versus regular food. Rodents were conditioned to chambers paired with bacon or chow, and then tested for conditioned place preference (CPP) for the bacon-paired chamber, relative to the chow-paired chamber. After CPP, rats were placed in either the bacon- or chow-paired chamber without the food stimulus, and brain-glucose metabolism (BGluM) was measured using positron emission tomography (μPET).
Results: In Experiment 1, RYGB rats drank twice as much alcohol (2-4%) as SHAM and 50% more than ND lean controls. Obese SHAM drank on average significantly less (50%) alcohol than lean ND controls. In Experiment 2, compared to SHAM, RYGB rats made significantly more active spout responses to earn reward, and achieved higher breakpoints on PR-10 task. Pretreatment with a single peripheral injection of D-Lys3-GHRP-6 at either dose was ineffective in altering appetitive or consummatory responses to 4% alcohol in the SHAM. In contrast, RYGB rats demonstrated reduced operant performance to earn alcohol reward on the test day and reduced consummatory responses for two subsequent days following the drug. In Experiment 3, compared to SHAM, RYGB rats made significantly more operant lick responses to earn IV alcohol infusion (+30-50%) on a FR-5 schedule, and achieved higher breakpoints (+25-40%) during a PR-2 task. In Experiment 4, compared to SHAM, RYGB rats completed more cycles on a fixed ratio-2 task to obtain IV infusions of morphine (0.225 mg/kg/infusion) in daily sessions at an accelerated rate starting on Day 6 to reach a two-fold increase by Day 12. Finally, μPET imaging results show that RYGB may lead to changes in brain activity and in particular, brain regions that may underlie changes in reward and taste related behavioral responding.
Conclusions: Our results provide evidence that RYGB increases the rewarding effects of alcohol and that ghrelin receptor is involved in the mechanism. The findings of the IV self-administration study indicate that the enhanced alcohol reward after surgery is not just due to changes in alcohol absorption but may reflect direct effects in the sensitivity to alcohol's rewarding effects in the brain. Furthermore, the greater cue-induced activation of the reward system together with increased morphine self-administration suggests an augmented risk of compulsive drug use after RYGB. Future research is warranted to determine underlying mechanisms and identify individual risk factors preoperatively.
Disclosure: Nothing to Disclose.

19.3 Gut Permeability, Gut Microbiota and Inflammation in Alcoholism: Clinical Data

Philippe de Timary

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Background: Recent data have shown that clinical symptoms of alcohol dependence such as depression and alcohol-craving are related to increases in gut permeability and in inflammation, and that both psychological and biological dimensions at least partially recover after detoxification. These observations suggest that the gut could influence the behavior. However, the nature of the intestinal processes involved in the change in gut permeability and in inflammation and their relation to psychological symptoms are still unknown.

Methods: Two studies were conducted: 1- 13 non-cirrhotic alcohol-dependent (AD) subjects hospitalized for a detoxification program with 15 healthy controls matched for age, sex and BMI. Gut permeability was measured using 51Cr-EDTA. Fecal samples were collected to analyze the gut microbiota composition (using pyrosequencing and qPCR) and metabolomic analysis (GC/MS) was used to assess the gut microbiota function. 2- 63 non-cirrhotic AD subjects were compared to 14 healthy subjects. The inflammatory pathways that were stimulated by gut-derived bacterial products were also analyzed in peripheral blood mononuclear cells (PBMC) at the mRNA level by quantitative PCR, western blotting and DNA binding assays were used for transcription factor analysis. Toll-like receptors activation was assessed by cell cultures. In both studies, psychological symptoms of patients were assessed by self reported questionnaires (depression (BDI), anxiety (STAI) and alcohol craving (OCDS)). The analyses were performed twice, at the first day of alcohol withdrawal and after 18 days of abstinence.
Results: Gut permeability was higher in some AD subjects and was associated with specific alterations in the gut microbiota composition and function. The leaky gut allowed the translocation of gut-derived bacterial toxins such as lipopolysaccharides (LPS) and peptidoglycans (PGN) to the systemic circulation. Correlation analyses revealed that the gut permeability was strongly related to psychological symptoms of alcohol-dependence, at both times of withdrawal. The bacterial toxins simulated their Toll-like receptors in PBMCs and activated specific inflammatory pathways that were found to correlate with alcohol craving. The alcohol withdrawal induced a decrease in gut permeability and in LPS-associated inflammatory pathways. However, 18 days of abstinence did not restore the gut microbiota composition, except in some specific species.
Conclusions: These observations suggest that alterations at the level of the gut microbiota influence the gut permeability and activate specific inflammation pathways that are related to psychological symptoms of alcohol-dependence. Altogether these observations are consistent with a role of inflammation as one mediator of a gut-brain communication in AD patients.
Disclosure: Part 1: I have been consultant or presented conferences for Lundbeck company and Astra Zeneca company, but these activities are totally unrelated to the topic that is exposed in the talk.

19.4 GLP-1 and Ghrelin as New Targets for Alcoholism Treatment? A Translational Overview

Lorenzo Leggio

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Background: Recent research suggests that the gut-liver-brain axis may play a role in alcohol use disorder (AUD). Two specific pathways for which recent preclinical studies suggest a role in AUD are the glucagon-like peptide-1 (GLP1) and ghrelin. A few preclinical studies suggest that GLP1 receptor (GLP1R) activation attenuates alcohol reinforcing properties; and studies with rodents show that ghrelin administration increases alcohol reward, whereas antagonism of the ghrelin receptor results in reduced alcohol reward and consumption.
Methods: We investigated whether single nucleotide polymorphisms (SNPs) in the GLP1R gene are associated with AUD. Case-control analysis ($n = 908$) was performed in AUD subjects and controls using logistic regression while controlling for self-reported ancestry. The SAGE GWAS sample ($n = 3803$) was used for confirmation purposes. To obtain functional validation, secondary analyses were carried out on data from a human laboratory study of intravenous alcohol self-administration (IV-ASA). As for ghrelin, we conducted a double-blind placebo-controlled proof-of-concept study. Non-treatment seeking heavy drinking alcoholic individuals ($n = 45$) were randomized to receive IV ghrelin 1mcg/kg, 3 mcg/kg or placebo, followed by a cue-reactivity procedure, during which participants were exposed to neutral (juice) and alcohol cues. The primary outcome was the increase in alcohol craving.
Results: As for the set of genetic analyses, 5 GLP1R SNPs were significantly associated with AUD in the case-control study. A trend-level association between a previously reported functional SNP (rs6923761; Ser168Gly) and AUD ($p < .01$) was also observed. In the SAGE samples, the association between 168Ser allele and AUD was further confirmed in males ($p < .05$), but not in females. In the human laboratory experiment, the 168Ser-allele was associated with peak breath alcohol concentrations ($p < .05$) achieved during the IV-ASA. As for the ghrelin IV study, repeated measures of ANCOVA revealed a group effect across ghrelin doses in increasing alcohol craving ($p < .05$). A dose-specific examination revealed a significant effect of ghrelin 3 mcg/kg vs. placebo in increasing alcohol craving ($p < .05$) with a large effect size ($d = .94$). By contrast, no significant ghrelin effect was found in increasing either urge to drink juice or food craving (p: n.s.).
Conclusions: The GLP1 genetic experiments provide novel and convergent findings suggesting that GLP1 signaling may be of importance in AUD, making the GLP1R an attractive target for personalized pharmacotherapy. The IV ghrelin study provides novel pharmacological evidence that ghrelin may play a role in the neurobiology of alcohol craving, thus demonstrating a novel pharmacological target for treatment. Consistent with recent preclinical evidence, our group provides human evidence supporting that GLP1 and ghrelin pathways may represent new intriguing pharmacological targets to treat AUD patients.
Disclosure: Nothing to Disclose.

Panel
20. Neural Circuitry Contributing to Mood, Impulsivity, and Decision Making in Bipolar and Other Inhibitory Disorders: Studies from Imaging and Genetics, to Pharmacology and Model Organisms

20.1 Effect of DAT Genotype on Striatal Function during Response Inhibition to Emotional Stimuli in Bipolar Disorder

Amit Anand

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Background: Both neurobiological and genetics research suggest that abnormalities of the brain neurotransmitter

dopamine (DA) may be central to the etiology of bipolar disorder (BD). Activity of the dopamine transporter protein (DAT) is primarily responsible for termination of dopamine's pharmacological action by re-uptake of DA into the presynaptic neuron. Therefore, changes of DAT expression in BD may lead to abnormalities in DA transmission in BD. The DAT gene (SLC6A3) is associated with a variable number of tandem repeats (VNTR) polymorphism (Costa et al, 2011). The most common forms are 10-repeat and 9-repeat allele gene. The 9 allele is thought to be dominant so most studies have looked at differences between 10-repeat homozygotes (10R) vs. 9-repeat carriers or homozygotes (9R)(Costa et al, 2011). In this study, we investigated the effect of DAT gene polymorphism on fMRI measures of striatal activation in a group of unmedicated BD subjects as well as healthy controls.

Methods: 80 medication-free BD subjects - 40 10R (Age: 33 + 12 yrs, 22F) and 37 9R (Age: 33 + 10 yrs, 26F) and 39 healthy subjects - 24 10R (Age: 31 + 9 yrs, 13F) and 15 9R (Age: 30 + 11 yrs, 9F) underwent fMRI imaging while performing response inhibition tasks . All subjects were administered the Young mania rating scale (YMRS) and the 17-item Hamilton Depression Rating Scale (HDRS). BD subjects were also genotyped for DAT polymorphism. Using a motor (letters) and emotion (happy and sad faces) Go-NoGo task, striatal activation during response inhibition (Hummer et al, 2013) was measured bilaterally in the caudate, putamen and pallidium. Mixed models regression analysis was conducted to investigate effects of DAT genotype on the imaging measures while controlling for effects of diagnosis, mood state, age and gender.

Results: Happy Face Go-NoGo task (i.e. inhibition of motor response to happy faces) showed a significant effect of DAT genotype on left-pallidial activation ($10R > 9R$; $df = 1,68$; $p = 0.001$) and right pallidial activation ($10R > 9R$; $df = 1,68$; $p = 0.03$) in the BP group while no such effect was seen in the healthy group. No significant effect of DAT genotype was seen on striatal activation during inhibition to sad faces or during inhibition to letters.

Conclusions: The 10R DAT genotype had increased pallidial activation in the BD group while no effect was seen in the healthy group. The pallidium is a major component of the cortico-striatal-pallidio-thalamic mood circuit. The effect of DAT gene polymorphism on pallidial function during inhibition of response to positive stimuli could provide important clues to the etiology of bipolar disorder.

References: Costa A, Riedel M, Müller U, Möller H-J, Ettinger U (2011). Relationship between SLC6A3 genotype and striatal dopamine transporter availability: A meta-analysis of human single photon emission computed tomography studies. Synapse 65(10): 998-1005. Hummer TA, Hulvershorn LA, Karne HS, Gunn AD, Wang Y, Anand A (2013). Emotional Response Inhibition in Bipolar Disorder: A Functional Magnetic Resonance Imaging Study of Trait- and State-Related Abnormalities. Biological Psychiatry 73(2): 136-143.

Disclosure: Nothing to Disclose.

20.2 Impulsivity and Substance Use in Bipolar Disorder: Genetic Contributions and Treatment Implications

Katherine Burdick

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Background: Patients with bipolar disorder (BD) show elevated impulsivity that are independent of clinical state. Family-based data suggest that this trait may be genetically mediated. Pramipexole is a D2/D3 agonist which has been implicated in the emergence of impulsivity and risk-seeking behaviors such as pathological gambling in multiple case reports and cross-sectional studies in patients with Parkinson's disease (PD). A purported mechanism for this effect is related to pramipexole's high selective affinity for D3 receptors, which are primarily expressed in the mesocorticolimbic dopamine (DA) pathway – a circuitry that is active during impulsive decision-making. Indeed, several studies that have used pramipexole in single-dose challenge paradigms have confirmed its actions on reward-related neural networks, primarily at low doses and in healthy individuals (Riba et al, 2008; Ye et al, 2011). Higher doses of pramipexole, including those in the range used to treat PD and in the range used in our cognitive enhancement trial in BD (Burdick et al. 2012), act as specific agonists both presynaptically and postsynaptically to enhance DA activity (Mierau et al. 1995). These higher doses of pramipexole are the ones that have been linked to impulse-control disorders and anti-anhedonic (antidepressant) effects across several major psychiatric disorders.

Methods: In a series of studies, we evaluated trait-based ratings of impulsivity in BD patients, their unaffected siblings, and unrelated healthy controls to test how this trait is related to genetic risk for the illness. Next, we conducted a controlled cognitive trial of a dopamine agonist in euthymic BD patients and measured outcome on an impulsivity-based gambling task after 8 weeks. Finally, we conducted secondary pharmacogenetic analyses evaluating the effect of variation in the dopamine transporter gene (DAT) on treatment outcome in the pramipexole study.

Results: In our sample, we find elevated ratings of impulsivity in both BD probands ($F = 69.1$; $df = 1,192$; $p = 1.7$ x 10 -17) and their unaffected siblings ($p < 0.05$). Moreover, in an 8-week trial in BD patients, pramipexole (1.5 mg/day) directly affects performance on the Iowa Gambling Task such that after active treatment, euthymic BD patients made more high-risk/high-reward choices as a result of an increased attention to feedback associated with monetary wins vs. losses (Burdick et al. 2013 Neuropsychopharmacology). These results stand somewhat in contrast to the our data showing beneficial effects of the agent on measures of attention and working memory in the same cohort (Burdick et al. 2012 J Clin Psychiatry). We also found a significant effect of the functional DAT Intron 8 VNTR polymorphism on IGT indices in the BD cohort after pramipexole treatment.

Conclusions: Impulsivity is a core feature of BD which is associated with genetic risk for the illness. Patients with BD show changes in impulsivity-related decision-making after exposure to a dopamine agonist which has been previously

shown to induce impulse control disorders in Parkinson's disease. The effect of pramipexole on reward processing is related to variation in the DAT gene, further supporting an association of impulsivity with genetic risk in BD. Treatment implications for BD will be discussed.
Disclosure: Part 1: Advisory board DSP Pharma; Speaker in CME event sponsored by Takeda.

20.3 Biomarkers of Novelty Seeking and Exploration in Bipolar Disorder and Substance Use

Arpi Minassian

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Background: Novelty seeking, defined as approaching and exploring new situations, is a hallmark phenotype of the mania of bipolar disorder (BD) and substance use disorders. Dopamine (DA) plays a vital role in novelty seeking and reward; dysregulation of this reward system is implicated in BD as well as addiction. The specific mechanism of DA dysregulation in BD has not been reliably established. Unlike other characteristic symptoms of BD, e.g., elevated mood, novelty seeking can be quantified across species, lending to its utility as a surrogate marker of bipolar mania in animal models. Furthermore, "reverse translation" of a classic animal exploratory paradigm to a human analog enables cross-species studies of psychiatric diseases such as BD and other conditions with altered DA function. Such work helps refine animal models of these diseases and illuminate their neurobiology.
Methods: A series of studies were conducted using the human Behavioral Pattern Monitor (hBPM), a translation of the animal "open field" test. Comparisons were conducted among subjects in manic vs. non-manic (euthymic, depressed) phases of BD and subjects with schizophrenia (SCZ). Individuals with a history of methamphetamine (METH) dependence were studied, as were healthy volunteers administered placebo or a single dose of amphetamine (10 or 20 mg; double-blind). Subjects were placed in the hBPM, a room they were unfamiliar with, and given no instructions except to wait. Multivariate quantification of novelty seeking includes measurement of motor activity in the novel environment with an ambulatory monitoring device worn by participants, measurement of activity patterns in areas containing novel objects that invite exploration, and number and duration of interactions with objects. Manic patients were genotyped for two DA-related genetic polymorphisms, catechol-O-methyltransferase (COMT) Val158Met, and the DA transporter (DAT) polymorphism rs27048 A/G.
Results: Manic BD subjects demonstrated a characteristic pattern of heightened exploration in the hBPM that distinguished them from SCZ patients and healthy subjects, namely increased motor activity [$F(2,52) = 7.6$, $p < 0.001$], increased novel object interactions [$F(2,52) = 18.5$, $p < 0.001$], and straighter spatial movement trajectories [$F(2,52) = 5.1$, $p < 0.01$]. Furthermore, the same pattern was present in attenuated form in non-manic BD subjects. METH-dependent individuals had increased novelty seeking as measured by object interactions [$F(1,32) = 11.8$, $p < .01$], similar to what has been observed in the rodent BPM with a mouse model of chronic METH administration.
Conclusions: The novelty-seeking signature of BD is unique from other DA-related disorders such as SCZ and stimulant dependence. These findings and the observed preliminary relationships between novelty-seeking phenotypes and DA-related genes (below) demonstrate the utility of translational studies in probing the distinct versus overlapping biological mechanisms underlying perturbed reward processing in BD and other disorders of disinhibition.
Disclosure: Nothing to Disclose.

20.4 Reducing Dopamine Transporter Expression Reproduces Patterns of Inattention and Risk Taking Seen in Manic Bipolar Patients

Jared Young

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Background: Polymorphisms of the dopamine transporter (DAT) are associated with bipolar disorder (BD), ADHD, and addictions. While the precise effect of such polymorphisms remains unclear but they likely reduce DAT function by lowering cell surface expression. Indeed, lower DAT levels are observed in unmedicated BD patients, athough their functional significance has yet to be determined. Using the reverse-translated behavioral pattern monitor (BPM), we demonstrated that reducing DAT levels in mice recreates the abnormal exploratory pattern of manic BD patients which is partially attenuated by the BD treatment valproate. Of primary importance, however, is treating the cognitive deficits and risk-taking behavior of these patients. We hypothesized that mice with reduced DAT levels would exhibit impaired attention in the 5-choice continuous performance test (5C-CPT) and risk preference in the Iowa Gambling Task (IGT) in patterns consistent with BD mania patients in the same tests.
Methods: 16 adult participants who met SCID (Structured Clinical Interview for DSM-IV) criteria for BD were recruited from inpatient and outpatient psychiatric clinics at UCSD Medical Center. 9 participants met criteria for a manic episode and 7 were classified as hypomanic. 17 healthy comparison (HC) participants were also recruited. BD and HC groups were matched demographically. We tested the performance of these subjects on the IGT and a subset in the 5C-CPT. Male DAT wildtype (WT; $n = 28$) and knockdown (KD; $n = 31$) mice were bred using heterozygous breeding pairs. At 3 months, they were trained in 5-hole operant chambers to holepoke for rewards. Once trained, their performance in the mouse IGT and 5C-CPT was assessed.
Results: BD patients exhibited poorer risk-related learning than HC [$F(1,31) = 6.7$, $p < 0.05$]. In a rodent testing-derived measure, all subjects showed increased preference to stay with a safe choice over time [$F(2,62) = 5.3$, $p < 0.05$], but the BD subjects tended to make fewer safe choices than HCs [$F(1,31) = 4.0$, $p = 0.054$], shifting away from the safe side more than HC [$F(1,31) = 5.0$, $p < 0.05$]. BD patients exhibited poorer attention in the 5C-CPT as measured by d' [$F(1,22) = 6.4$, $p < 0.05$], driven by a lower hit rate and tendency toward a higher false alarm rate. DAT KD mice

exhibited poorer learning in the IGT compared with WT mice [$F(2,36) = 5.0$, $p < 0.05$], with KD mice making significantly fewer safe choices than WTs ($p < 0.05$). Consistent with BD patients, KD mice stayed less at the safe choices than WTs over time [$F(4,106) = 8.8$, $p < 0.001$]. Finally, consistent with manic BD patients, KD mice exhibited poorer attention in the 5C-CPT measured by d' ($t = -2.2$, $p < 0.05$), driven by lower hit rate and higher false alarm rate.

Conclusions: Reducing DAT function impairs attention and increases risk taking, recreating the behavioral abnormalities seen in manic BD patients using identical tasks. Thus, reduced DAT functioning likely plays a role in BD mania-related behavior. These mice represent a unique opportunity to test novel anti-mania agents that could treat poor cognition of sufferers as well as overactivity.

Disclosure: Part 1: Consultancy fees from Amgen, **Part 4:** Grants from Lundbeck Ltd & Omeros Corporation.

Study Group
21. Developing Methods for Cross-species Research on Impairing Irritability in Children

Ellen Leibenluft*, Shelli Avenevoli, Ned Kalin, Thomas Insel, Kerry Ressler, Trevor Robbins, Jacqueline Crawley, Sheena Josselyn, Joel Nigg

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Severe, impairing irritability is one of the most common presenting complaints in child psychiatry clinics. Relatively little is known about its pathophysiology, although there has been a recent upsurge in research interest, fueled in part by controversy about the role that irritability should play in child psychiatric diagnosis. From a clinical perspective, irritability can be defined as an abnormal mood state (persistently angry, grumpy, and grouchy), usually accompanied by developmentally inappropriate outbursts of intense anger. From a neuroscience perspective, irritability can be defined as 1) aberrant responses to frustration, where frustration is the emotion evoked by blocked goal attainment, and/or 2) aberrant approach responses to threat. Irritability is well-suited to research from an RDoC perspective, in that it is a dimensional trait in the population that, at its extreme, is a clinically important trans-diagnostic symptom. Anger outbursts in particular could be modeled across species e.g., by using behavioral paradigms that involve blocked goal attainment, or by exposing organisms to environmental contexts that elicit an approach response to threat. However, little such cross-species work has been done, and there has been little work in relevant paradigm development and standardization. The development of such methods would greatly facilitate translational work on irritability, thus advancing the development of novel treatments aimed at this extremely impairing and common symptom in children. This study group will bring together researchers with expertise in behavioral phenotyping of model animals and those interested in irritability to discuss the feasibility of, and approaches to, developing methods for cross-species research on irritability. Dr. Leibenluft, Kalin, and Insel will give brief introductory remarks to frame and focus the discussion, leaving the vast bulk of the session's time for discussion amongst the group members and with the audience. Dr. Leibenluft will describe the relevant human phenotype and information-processing deficits that have been identified in irritable children, including aberrant responses to reward-based frustration paradigms and deficits in cognitive control. Dr. Kalin will discuss his translational work on behavioral inhibition and anxiety in humans and other primates. Anxiety, like irritability, is associated with aberrant responses to threat, and irritability and anxiety often co-occur in children. Dr. Insel will discuss pitfalls and opportunities of cross-species work in the RDoC era. Other members of the group have relevant expertise that will enrich the subsequent discussion. Dr. Ressler conducts work in anxiety in rodents and humans; Dr. Robbins has done considerable translational work on cognitive control; Dr. Crawley is expert in rodent behavioral phenotyping, with applications to autism; Dr. Josslyn conducts research in humans and rodents on learning and memory; and Dr. Nigg conducts pathophysiological work on children with impairing irritability.

Disclosure: E. Leibenluft, Nothing to Disclose; S. Avenevoli, Nothing to Disclose; N. Kalin, **Part 1:** Corcept Therapeutics, Neuronetics, Cenerx Biopharma, CME outfitters, Elsevier, **Part 2:** Elsevier; T. Insel, Nothing to Disclose; K. Ressler, Nothing to Disclose; T. Robbins, **Part 1:** Consultancy; Cambridge Cognition, Eli Lilly; Lundbeck, Merck,Sharpe and Dohme, Chempartners, Teva, Shire Pharmaceuticals Royalties for CANTAB (Cambridge Cognition) Research Grants: GSK, Lilly, Lundbeck Education talks; E Lilly, Lundbeck Editorial honoraria; Springer Verklag, Elsevier, Society for Neuroscience, **Part 2:** Cambridge Cognition consultancy; Lilly consultancy., **Part 3:** Cambridge Cognition, **Part 4:** Lilly, Lundbeck, GSK; J. Crawley, Nothing to Disclose; S. Josselyn, Nothing to Disclose; J. Nigg, Nothing to Disclose.

Study Group
22. Industry and Academic Science: Can Academia Work More Effectively and Ethically with Industry to Get New Therapies to the Market?

Jerrold Rosenbaum*, Richard Keefe, Jeffrey Lieberman, Steven Romano, Ross McKinney, Harry Orf

Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

Recent past events have had a chilling effect on industry and academic collaboration in treatment discovery. Several academic medical institutions, however, are engaged in consideration about how academic and industry colleagues can work more productively together in a manner that advances science and therapeutics and upholds ethical principles. Academic medical institutions are motivated by the recognition of the need for increasing collaboration in the service of translating science into therapeutics and the recognition of need for diversification of sources of support for research. In light of renewed or increasing interest to establish, enhance and in some cases to reestablish connections with industry in the service of supporting innovative science and advancing therapeutics discovery,

the Ethics Committee is proposing a Study Session, to address such questions as:.
1) Would society benefit from growing these collaborations and in what way?.
2) What are the ethical issues that complicate these efforts?.
3) How should institutions manage the inherent potential and real conflicts of interest in an ethical manner that preserves academic freedom and protects against biased research, yet facilitates collaboration?.
4) Are there new models or examples of innovation and entrepreneurship involving such collaborations and how do they address traditional concerns about academic-industry engagement?.
5) How can academic scientists have access to proprietary experimental medications/compounds that become important, if not critical, tools for assessing etiological mechanisms and potential treatments? How do we maintain a relationship that allows access to such research tools, while at the same time not becoming beholden to those that provide them.
6) How might these interactions be communicated to the public in a transparent manner that improves rather than worsens the public's view of industry/academic collaboration for drug development?.
Disclosure: J. Rosenbaum, **Part 1:** PsyBrain; Medavante, **Part 2:** Medavante; PsyBrain; R. Keefe, **Part 1:** Abbvie, Akebia, Amgen, Asubio, AviNeuro/ChemRar, Biogen Idec, BiolineRx, Biomarin, Boehringer-Ingelheim, Eli Lilly, EnVivo/FORUM, GW Pharmaceuticals, Lundbeck, Merck, Minerva Neuroscience Inc., Mitsubishi, Novartis, Otsuka, Pfizer, Roche, Shire, Takeda, Targacept, **Part 2:** Abbvie, Akebia, Amgen, AviNeuro/ChemRar, Biogen Idec, BiolineRx, Biomarin, Boehringer-Ingelheim, Eli Lilly, EnVivo/FORUM, GW Pharmaceuticals, Lundbeck, Merck, Mitsubishi, Novartis, Otsuka, Pfizer, Roche, Shire, Takeda, Targacept, **Part 3:** NeuroCog Trials, Inc., **Part 4:** GlaxoSmithKline, PsychoGenics, Novartis, Brain Plasticity Institute; J. Lieberman, **Part 4:** Biomarin, EnVivo, Genentech, Novartis, Psychogenics, Sunovion, Lilly; S. Romano, **Part 1:** Full time employee of Pfizer, Inc., **Part 2:** Full time employee of Pfizer, Inc., **Part 3** Full time employee of Pfizer, Inc., **Part 5:** Pfizer, Inc.; R. McKinney, **Part 1:** Gilead Sciences; Janssen Pharmaceuticals, **Part 2:** Janssen Pharmaceuticals (not yet, but projected); H. Orf, Nothing to Disclose.

Panel
23. Local and Global Sleep Regulation, Cellular Functions of Sleep and Neuropsychiatric Disorders

23.1 Local and Global Sleep Regulation: Spatio-temporal Dynamics and Functional Significance

Vladyslav Vyazovskiy

University of Oxford, Oxford, United Kingdom

Background: Whereas it was once thought that sleep is a whole brain phenomenon, an emerging current view is that sleep develops locally at the level of individual neurons or specific neuronal networks in an activity-dependent manner. The mechanisms and functional significance of "local" and "global" sleep remain unknown. Sleep has been proposed to play an important role in macromolecules biosynthesis, prevention of oxidative stress, energy homeostasis, synaptic function and neural plasticity. It is likely that understanding spatio-temporal dynamics of the local and global cortical activity during sleep will provide important insights into cellular and network functions of sleep.
Methods: Continuous recordings of cortical MUA and LFP were performed with microwire arrays across a repertoire of spontaneous (sleep, waking) and induced (sleep deprivation and local cortical electrical stimulation) states in freely behaving laboratory rats and mice. Individual slow waves in NREM sleep were detected after filtering the LFP signals between 0.5-4 Hz. To characterise spatio-temporal structure of cortical activity during REM sleep, instantaneous LFP amplitude was calculated after filtering the signals between 6-9 Hz (theta-activity). The dynamics of cortical MUA and LFPs was analysed during the first minutes after spontaneous awakenings during the light and the dark period.
Results: The cortical activity during spontaneous waking, NREM and REM sleep was characterised by highly complex dynamics, visible at many different spatial and temporal scales. During sleep deprivation, the number of neuronal silent periods increased gradually. Upon falling asleep, slow waves initially were mostly local, and encompassed progressively larger cortical territories in the course of sleep episodes. Cortical electrical microstimulation revealed instances of local cortical unresponsiveness, which occurred immediately after spontaneous slow waves. After the transition to REM sleep, rich local and global dynamics was apparent at the level of neuronal activity and LFP theta-activity, which showed a conspicuous pattern of spatially synchronised bursts recurring at slow $\sim$0.5-1 Hz frequency. In the first minutes upon arousal, firing activity differed substantially between individual neurons and was determined to a large extent by immediate preceding history, such as whether an awakening occurred from NREM or REM sleep.
Conclusions: The data highlight highly complex spatio-temporal dynamics of cortical activity during sleep at many distinct scales – from the occurrence of individual slow waves within local neuronal populations to global state shifts encompassing the whole brain. Notably, local and global dynamics of cortical activity during sleep were determined by the levels of neuronal activity during preceding waking. It is proposed that high spiking and synaptic activity during waking are associated with intense metabolic rates, which leads to mitochondrial production of reactive oxygen species, which can cause damage to DNA, proteins, lipids and other biomolecules ('oxidative stress'). We hypothesize that brief periods of reduced synaptic and spiking activity within specific cortical networks, which correspond to local or global slow waves, enable prophylactic cellular maintenance, which prevents irreversible damage to cellular components. It is concluded that disrupted or insufficient sleep does not fulfill this function efficiently, which exacerbates psychiatric or neurodegenerative disorders, typically associated with aberrant sleep patterns.
Disclosure: Nothing to Disclose.

23.2 Homeostatic Sleep Pressure is the Primary Factor for Activation of Cortical nNOS/NK1 Neurons

Thomas Kilduff

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Background: Cortical interneurons, immunoreactive for neuronal nitric oxide synthase (nNOS) and the receptor NK1, express the functional activity marker Fos selectively during sleep. The proportion of Fos+ nNOS/NK1 neurons increases during sleep deprivation and correlates with several measures of sleep pressure during the period preceding sacrifice. Since time spent in non-rapid eye movement sleep (NREM) and the magnitude of sleep pressure are highly correlated under physiological conditions, it is unclear whether these cells are activated to the same degree throughout NREM sleep or whether the extent of their activation is related to the sleep pressure that accrued during the prior waking period. In both cases, the proportion of Fos+ cortical nNOS/NK1 neurons would correlate with measures of sleep pressure. To distinguish between these possibilities, we used hypnotic medications to control the amount of NREM sleep in rats while we varied the amount of sleep pressure.
Methods: To ensure that our results were not drug-specific, we utilized hypnotics with different mechanisms of action: the dual hypocretin/orexin receptor antagonist almorexant (ALM) and the GABA(A) receptor modulator zolpidem (ZOL). Drug administration was preceded by 6 h of sleep deprivation (high sleep pressure) or undisturbed conditions (low sleep pressure). Rats were assigned to six groups: (1) VEH with low sleep pressure (n = 6); (2) VEH with high sleep pressure (n = 7); (3) ZOL with low sleep pressure (n = 6); (4) ZOL with high sleep pressure (n = 7); (5) ALM with low sleep pressure (n = 6); and (6) ALM with high sleep pressure (n = 7). Dosing occurred at ZT12, 100 mg/kg p.o. in 2 ml/kg for both drugs. Perfusion occurred at ZT14 for VEH and ZOL groups and at ZT14.5 for ALM groups due to its longer latency to sleep onset (Black et al, 2013; Morairty et al, 2012; Morairty et al, 2014). Brains were processed for double-label immunohistochemistry with Fos as a functional marker and nNOS as a phenotypic marker. Single-labeled nNOS and double-labeled Fos+/nNOS cells were counted in one hemisection each at 1.4 mm anterior, 0.5 mm posterior, and 3.0 mm posterior to bregma. The percentage of nNOS neurons expressing Fos was calculated as in our previous studies.
Results: Using hypnotic treatment, we were able to dissociate time spent asleep during the 90 min before sacrifice from the sleep pressure resultant from the prior sleep/wake history. We found that the proportion of Fos+ cortical nNOS/NK1 neurons was minimal when sleep pressure was low, irrespective of the amount of time spent in NREM sleep. In contrast, a large proportion of cortical nNOS/NK1 neurons was Fos+ when an equivalent amount of sleep was preceded by sleep deprivation. Moreover, when we measured NREM time, average NREM bout duration, NREM delta power, NREM delta energy, the slope of slow waves during NREM sleep and the proportion of Fos+/nNOS cells, we found that the proportion of Fos+/nNOS cells was the best predictor of prior sleep pressure.
Conclusions: These results demonstrate that the extent of activation of cortical nNOS/NK1 neurons is determined by prior sleep history and associated sleep pressure. When sleep pressure is low, cortical nNOS neurons are largely inactive even in the presence of high amounts of NREM sleep. We conclude that, while sleep is necessary for cortical nNOS/NK1 neuron activation, the proportion of cells activated is dependent upon the sleep pressure that accumulates during the preceding wakefulness. This implies that cortical nNOS/NK1 neurons are "monitoring" the sleep pressure that accumulates during wakefulness. Current studies are directed toward identifying the neural circuitry that underlies that this property of cortical nNOS/NK1 neurons.
Disclosure: Part 4: Research support from F. Hoffmann-LaRoche.

23.3 Non-REM Sleep EEG Evidence for Dysfunction of Sleep Homeostasis in Insomnia

Andrew Krystal

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Background: There is a compelling body of research indicating that the degree of initial elevation in non-REM sleep EEG slow-wave amplitude (Initial SWA) and its rate of decline over the night (SWA Slope) reflect the degree of homeostatic sleep drive that has accumulated prior to sleep. In this context, restoration indicates the degree to which the homeostatic sleep drive, which would otherwise become manifest in a host of impairments, is dissipated by sleep. It is clear that this model explains the deficits experienced by those who undergo sleep deprivation and the process of their recovery. However, it is not clear whether this model applies to the process of restoration when sleep deprivation does not occur. We sought to test whether this model might be relevant to patients with insomnia and might explain why they appear to fail to achieve adequate restoration from sleep, in terms of objective and self-reported cognitive impairment, yet many do not appear to have diminished total sleep time that would indicate that they are sleep deprived. We tested the hypothesis that impairments in cognitive function seen in insomnia patients would be the consequence of impairments in the restorative process as manifested in alterations in initial SWA and Delta Slope and that this would be independent of their sleep time.
Methods: We obtained polysomnographic (PSG) data and carried out cognitive testing the next day with the Switching Attention Test (SAT) in 79 primary insomnia (PI) patients and 84 healthy controls. We compared SAT performance in the PI and control groups and evaluated the relationship of Initial SWA and SWA Slope derived from the PSG data in the insomnia patients, across all subjects and within the healthy controls while controlling for total sleep time (TST).
Results: We found that the PI patients were statistically significantly more impaired on the SAT than the controls

($p<0.01$). Further, the insomnia patients had significantly lower Initial SWA and lower Delta Slope ($p<0.05$) indicating that they had diminished homeostatic sleep drive despite have equivalent total sleep time as the control group. Further, we found that lower initial SWA and Delta Slope were significantly correlated with SAT performance across all subject, in just the insomnia patients, and in just the healthy controls ($r=0.35$ to 0.40, $p<0.0005$) after controlling for TST.

Conclusions: Given that Initial SWA and Delta Slope are markers for sleep homeostasis, these findings suggest that there is diminished homeostatic sleep drive in insomnia patients, despite equivalent amount of sleep as healthy controls. The fact that this dysfunction in sleep homeostasis in insomnia patients is associated with impairment in daytime cognitive function suggests that the diminished build up of sleep drive and/or lessened dissipation of sleep drive during sleep undermines the restorative process of sleep in insomnia. The fact that the association of diminished homeostatic sleep drive and diminished cognitive performance is also seen in the control subjects suggests that the relationship between SWA dynamics and sleep restoration is not limited to experimental sleep deprivation paradigms or insomnia patients but is a fundamental property of sleep in general and linked to the process of restoration which is disrupted to a greater degree in insomnia patients than the rest of the population. Further work will be needed to elucidate the drivers of dysfunction in sleep homeostasis in insomnia patients and in the broader general population.

Disclosure: Part 1: Teva, Sunovion, Astellas, Abbott, Neosync, Brainsway, Janssen, ANS St. Jude, Novartis, Astellas, AstraZeneca, Attentiv, BMS, Eisai, Eli Lilly, GlaxoSmithKline, Jazz, Janssen, Merck, Neurocrine, Otsuka, Lundbeck, Roche, Sanofi-Aventis, Somnus, Sunovion, Somaxon, Takeda, Transcept, Vantia, **Part 2:** Novartis, Somaxon, Attentiv, **Part 3:** Attentiv, **Part 4:** Teva, Sunovion, Astellas, Abbott, Neosync, Brainsway, Janssen, ANS St. Jude, Novartis, Astellas, Eisai, Eli Lilly, Takeda.

23.4 Local and Global Changes in Sleep EEEG Activity in Aging, Neurodegenerative Disorders and Sleep Disorders: Evidence for Cellular Stress?

Ruth Benca

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Background: Impairments in synaptic plasticity and cellular resilience are thought to contribute to the pathophysiology of many neuropsychiatric disorders. In addition, sleep-disordered breathing (SDB) conditions, which are prevalent in children and adults, are associated with several psychiatric and neurodegenerative disorders and likely contribute to cellular stress, resulting from sleep loss and/or hypoxemia. Abnormalities in EEG activity during sleep, both local and global, may be early indicators of increased cellular stress. This presentation combines data from several studies using high density EEG (hdEEG) to examine brain activity during sleep in conditions associated with cellular stress, including normal and pathological aging, and sleep disordered breathing in children and adults.

Methods: Subjects: Samples of otherwise healthy subjects, with and without SDB, ranging in age from children to elderly; and samples of older adults with amnestic mild cognitive impairment (aMCI), with and without SDB. Sleep: All-night sleep recordings with 256 channel hdEEG were analyzed for global and regional differences in sleep parameters between clinical and control groups using analysis of variance, t-tests, and statistical non-parametric mapping, as a correction for multiple comparisons, where appropriate.

Results: Across normal aging, there are declines in SWA across the entire cortex, as well as frontal declines in theta and sigma frequencies. EEG power in a left centro-parietal region is relatively preserved. In children and adults with SDB, a circumscribed power reduction overlaying the posterior parietal cortex was observed, particularly which included SWA but was present to some extent across many frequency bands during NREM sleep. Sleep topography in aMCI and the effects of SDB in aMCI will also be discussed.

Conclusions: These data suggest that normal aging and SDB are both associated with neural impairment, although the patterns of impairment differ in these conditions. Abnormalities in sleep EEG, particularly SWA, are suggestive of increased cellular stress. Furthermore, brain regions impacted by SDB appear to be similar to areas that preferentially accumulate beta amyloid, which may contribute to the exacerbation of neurocognitive disorders observed in patients with SDB.

Disclosure: Part 1: Consultant for Merck, **Part 4:** Grant support from Merck.

Panel
24. Characterizing Reward Circuitry Dysfunction Across the Mood Disorders Spectrum: Relevance and Predictive Value in Clinical Practice

24.1 Depression-Related Increases and Decreases in Appetite Reveal Dissociable Patterns of Aberrant Activity in Reward and Interoceptive Neurocircuitry

W. Kyle Simmons

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Background: Changes in appetite and weight are cardinal but variable diagnostic markers of major depressive disorder (MDD): While some eat more when they get depressed, others lose their appetite. Given this variable presentation of appetite-related symptoms in MDD, it is significant that the orbitofrontal cortex (OFC), amygdala, insula, and striatal-pallidal neurocircuit have all been implicated in aspects of appetitive responses to food (Simmons, Martin, & Barsalou, Cerebral Cortex, 2005; Simmons et al., Nature Neuroscience, 2013; Simmons et al., Brain Struct. & Funct. 2014), and also exhibit histopathological and functional differences in MDD patients that are thought to underlie depression (Price & Drevets, Neuropsychopharmacology, 2010). Remarkably, although increases and decreases in appetite are antipodal criteria in the diagnosis of MDD, and core regions implicated in the pathophysiology of depression are

implicated in food motivation and hedonics, there are no published studies comparing the neural responses to food stimuli of patients whose depression manifests with increased appetite versus those for whom depression results in appetite loss.
Methods: Forty-eight participants underwent functional Magnetic Resonance Imaging (fMRI) while viewing photographs of food and non-food objects. The participants were drawn from three groups: 16 unmedicated depressed subjects with increased appetite during the current depressive episode, 16 unmedicated depressed subjects with decreased appetite during the current depressive episode, and 16 healthy control subjects with no history of Axis I psychiatric diagnosis. All three groups were matched for age and body mass index (BMI). The two depressed groups were matched for depression severity and anxiety, with both groups reporting clinically significant anhedonia.
Results: Across multiple analysis approaches to the data, depression-related increases and decreases in appetite were associated with distinct response profiles in the brain's reward and interoceptive circuitry. Within regions previously implicated in the mesocorticolimbic reward system, including the OFC, ventral striatum, ventral pallidum, and putamen, depressed participants with increased appetites exhibited significantly greater activity to food stimuli than both those reporting appetite decreases and healthy control subjects. Surprisingly, appetite loss in depression was not associated with abnormal activity in these regions, as these subjects' fMRI responses did not differ from those of healthy control subjects. In contrast, depressed subjects experiencing appetite loss exhibited significant hypoactivation within a region of the mid-insular cortex previously implicated in interoceptive and homeostatic signaling. Importantly, no differences were observed in this region between non-depressed subjects and those with depression-related increases in appetite.
Conclusions: Depression-related increases in appetite are associated with hyperactivation of mesocorticolimbic reward neurocircuitry, while depression-related appetite loss is associated with hypoactivation of insular regions that support interoceptive awareness of the body's physiological state (Avery et al., Biological Psychiatry, 2014). The present findings may thus motivate the use of different therapeutic interventions targeting the dissociable neural systems underlying depressed patients' variable appetitive responses to food.
Disclosure: Nothing to Disclose.

24.2 Trans-diagnostic Patterns of Reward Circuitry Function Are Associated with Anhedonia and Predict Future Clinical Outcome

Mary Phillips

University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Background: Anhedonia, the inability to experience pleasure, is a core symptom dimension of mood disorders, and is associated with abnormal functioning in reward circuitry. Increasingly sophisticated reinforcement learning models have been used to examine inter-individual variation in reward circuitry function, but the extent to which anhedonia disrupts such circuitry functioning during reinforcement learning is yet to be clarified. Furthermore, the extent to which measures of reward circuitry function predict future clinical course in mood-disordered individuals remains unknown.
Methods: Study 1: 77 unmedicated individuals with major depressive disorder (MDD) and 31 healthy control individuals (HC), recruited from the Establishing Moderators and Biosignatures of Antidepressant Response in Clinical Care study (EMBARC), underwent functional magnetic resonance imaging (fMRI) during a card-guessing reward task. Study 2: 73 mood dysregulated youth recruited from the Longitudinal Assessment of Manic study (LAMS) participated in a similar fMRI reward paradigm, and also underwent diffusion imaging of brain white matter.
Results: Study 1: We examined reward expectancy (RE:expected outcome value) and prediction error (PE:discrepancy between expected and actual outcome) ventral striatal and whole brain activity, the relationship between these two measures, and the moderating effect of anhedonia on this relationship. HC, but not individuals with MDD, showed a significant inverse relationship between RE- and PE-related right ventral striatal reactivity ($p < 0.05$). Across participants, greater anhedonia severity was associated with a reduced inverse RE-PE relationship, even after controlling for other MDD symptoms. These findings were confirmed in wholebrain analyses. Study 2: Multiple regression analyses revealed that greater activity and functional connectivity in prefrontal cortical-subcortical reward circuitry predicted worsening of behavioral and mood dysregulation (as measured by the Parent General Behavior Inventory-10 Item Mania Scale, PGBI-10M) at a mean of 15.8 months' follow-up post scan. Measures of white matter in key anterior tracts in reward circuitry also predicted worsening clinical outcome. Neuroimaging measures accounted for 11% of the variance in follow-up PGBI-10M. Novel relevance vector regression machine learning analyses revealed that the pattern of reward circuitry functional connectivity to reward significantly predicted PGBI-10M outcome in individual youth, even after controlling for medication ($r = 0.37$; $p = 0.004$).
Conclusions: Study 1: Findings indicate an inverse relationship between RE- and PE-related ventral striatal reactivity in HC, supporting the temporal difference model that predicts a shift in ventral striatal responding from rewards to reward cues, and suggest less adaptive reward contingency learning with greater anhedonia severity. Study 2: two types of analyses indicate that reward circuitry function (and structure) can predict future clinical outcome in mood disordered individuals. Our findings help elucidate neural mechanisms of anhedonia, and neural circuitry predictors of future clinical outcome, that may act as novel targets for future therapeutic interventions.
Disclosure: Nothing to Disclose.

24.3 A PET Investigation of Dopamine Transporter Binding in Depression Using [11C]Altropane

Diego Pizzagalli

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Background: Depressed individuals, particularly those with anhedonic symptoms, show decreased reward responsiveness (Pizzagalli et al., 2008; Vrieze et al., 2013) as well as a dysregulation of dopamine (DA) (Pizzagalli, 2014), a neurotransmitter critical for goal-directed behavior and reinforcement learning. Further evidence of DA dysfunction in depression can be seen in post-mortem studies that have found decreased dopamine transporter (DAT) binding in MDD, thought to be a result of decreased levels of mesolimbic dopamine (e.g., Klimek et al., 2002). DAT is most highly expressed in regions of the basal ganglia, including the putamen, caudate, pallidum and nucleus accumbens (Piccini, 2003). This is particularly interesting given studies that have found decreased reward responsiveness in the caudate and nucleus accumbens in MDD (Pizzagalli et al., 2009). Findings from studies examining the nature of DAT in MDD have, however, been inconsistent, with some reporting decreased (e.g., Meyer et al., 2001; Sarchiapone et al., 2006) and others increased (e.g., Brunswick et al., 2003; Yang et al., 2008) DAT in MDD. These divergent findings may partially be explained by methodology, given that tracers with similar DAT and serotonin transporter (SERT) affinities were used in these studies. The current study aimed to investigate dopamine transporter binding in MDD using the PET tracer [11C]altropane, which has high selectivity for DAT (vs. SERT) sites (Fischman et al., 2001). We hypothesized that participants with MDD will show reduced altropane binding potential (BP) relative to healthy control participants.
Methods: Dynamic PET scans were acquired on 30 subjects (19-45 yrs; 18 controls, 12 MDD) with an ECAT EXACT HR + and initiated with bolus injection of [11C]Altropane. Regional binding potential (BPND) estimates (Innis et al., 2007) were generated using MRTM2 (Ichise et al., 2003) in the caudate (CAU), putamen (PUT), pallidum (PAL), and nucleus accumbens (NAcc). Linear regressions were performed to determine effects of age on DAT binding. Finally, a Group x Hemisphere x Region ANCOVA on BPND values was conducted (covariate: age).
Results: In controls, DAT BPND decreased with age in the CAU ($R2 = 0.36$, $p < 0.01$), PUT ($R2 = 0.41$, $p < 0.01$), and NAcc ($R2 = 0.34$, $p = 0.01$). No age effects were observed in MDD (all $ps > 0.38$). Critically, the ANCOVA revealed a significant Group × Hemisphere interaction ($F(1,27) = 4.72$, $p = 0.039$), indicating that groups differed in their left vs. right basal ganglia BPND. Follow-up analyses indicated, that healthy controls–but not MDD subjects ($p = 0.33$)–showed significantly higher BP in the left relative to right basal ganglia ($t(17) = 2.17$, $p = 0.045$). MDD was thus characterized by aberrant age-related reduction in DAT expression; moreover, relative to controls, the MDD group showed significantly lower left > right DAT binding asymmetry.
Conclusions: Collectively, these findings are consistent with our a priori hypothesis derived from post-mortem studies that MDD is characterized by decreased DAT. We propose that decreased altropane binding potential might reflect a compensatory downregulation of DAT due to blunted DA transmission.
Disclosure: Part 1: BrainTracer, Pfizer, Servier.

24.4 Deep Brain Stimulation for Treatment Resistant Major Depression – Involving the Dysfunctional Human Reward System

Thomas Schlaepfer

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Background: A core symptom of major depression is anhedonia (decreased drive and reward for pleasurable activities) and reduced motivation. The human reward system consists of the neural pathways involved in eliciting rewarding experiences in animals and humans; its structures, the striatum (particularly the ventral striatum or nucleus accumbens (NAcc) and the medial forebrain bundle (MFB), are important in emotional memory, and might mediate those symptoms. Antidepressant effects of Deep Brain Stimulation (DBS) to the NAcc and two additional targets have been systematically assessed and results in response in 50–60% of patients studied, albeit only at relatively high stimulation intensities. Using probabilistic diffusion tensor imaging (DTI) we were able to demonstrate that all stimulation sites stimulate fibers of the MFB and hypothesized that DBS to the MFB closer to its origin in the ventral tegmental area might be associated with higher antidepressant efficacy at lower stimulation intensities since the extent of antianhedonic effect might be related to the recruited amount of fibers of the MFB. Recently we demonstrated unexpectedly rapid effects of this procedure.
Methods: Study 1: Eight patients suffering from extremely treatment resistant forms of major depression underwent implantation of DBS electrodes at the MFB after individualized targeting using probabilistic DTI. Study 2: 16 patients with the same criteria as those in study 1 were randomized in to either receiving MFB-DTI immediately after implantation or after two months of sham stimulation.
Results: Study 1: As previously published, seven of eight patients showed rapid improvement within 5 days of stimulation at intensities of about 30% of the ones used in previous studies. Here we report on outcomes after one year of therapy. The whole group responded with a 57.9% improvement in depressive symptomatology as rated with the MADRS. Six patients were classified as responders. Study 2: At the time of submission (May 2014) 14 of the 16 patients were implanted. So far, all subjects showed immediate improvement in depressive symptomatology after onset of real DBS stimulation, in parallel to the ones included in study 1. At the time of submission, 12 patients were classified as responders. In the symposium, six months stimulation data of all patients will be presented.
Conclusions: Taken together, results from both studies point to the fact that DBS to the MFB is associated with high antidepressant efficacy in very treatment resistant patients with a rapid onset. The MFB seems to be a germane structure within a network of centers processing affective stimuli. The unexpected acute onset of antidepressant action challenges current hypotheses on network dysfunctions putatively involved in major depression.

Disclosure: Part 4: Grant support for two IIT's by Medtronic Inc.

Panel
25. Is the Associative Striatum a Locus of Vulnerability for Transition to Psychosis?

25.1 The Role of the Associative Striatum in the Development of Schizophrenia and the Response to Treatment

Oliver Howes

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Background: The hypothesis that the dopamine dysfunction in schizophrenia is localized to mesolimbic pathways has been very influential despite the lack of direct evidence from patient studies. We therefore sought to investigate the localization of dopamine dysfunction in schizophrenia and in people at clinical risk of the disorder and the link to symptoms and the development of schizophrenia and treatment response.
Methods: We used PET to study striatal dopamine synthesis capacity in patients with schizophrenia ($n = 36$) and subjects at high clinical risk of psychosis ($n = 54$; all meeting the CAARMS criteria for an at risk mental state [ARMS]) who show prodromal signs of schizophrenia and matched controls ($n = 41$). Additionally, in an entirely new cohort of schizophrenia patients ($n = 18$) we examined the relationship between dopamine function in different striatal sub-divisions and response to treatment. The high risk subjects received assessment of verbal fluency and clinical follow-up to determine who developed psychosis. The striatal regions of interest were the whole striatum (S), and its limbic (LS), associative (AST) and sensorimotor (SMST) subdivisions. Additionally we investigated dopamine synthesis capacity in the nigral origin of the dopaminergic projections to the dorsal striatum in schizophrenia in complementary PET and post-mortem studies. A sub-sample of the ARMS subjects and controls also received fMRI imaging using a task that activates the frontal cortex to investigate fronto-striatal interactions.
Results: Striatal dopamine synthesis capacity was significantly elevated in the associative striatum in the ARMS subjects ($p < 0.05$), and was significantly related to symptoms and cognitive performance ($p < 0.05$), and to fMRI activation during the cognitive task ($p < 0.0.05$). In contrast dopamine synthesis capacity was not significantly elevated in the limbic striatum ($p > 0.1$). The elevation in dopamine synthesis capacity was specific to the ARMS subjects who want on to develop psychosis. In schizophrenia dopamine synthesis capacity was significantly elevated in associative ($p = 0.001$), sensorimotor ($p = 0.001$) and limbic striatum ($p = 0.017$), although this was relatively less marked in the limbic striatum. Furthermore the uptake of labeled-DOPA indexed using PET in vivo and tyrosine hydroxylase staining ex vivo were both elevated in the substantia nigra. In the new cohort of patients with schizophrenia, clinical response to antipsychotic treatment was related to dopaminergic function ($r = 0.68$, $p < 0.05$), but this relationship varied by striatal sub-division.
Conclusions: These findings indicate that i) dysfunction in associative striatal dopamine function, rather than limbic dysfunction, predates the onset of psychosis, ii) in schizophrenia dorsal striatal dysfunction is more marked than limbic alterations; iii) dopamine synthesis capacity is also altered in the nigral origin of dopaminergic projections to the dorsal striatum in schizophrenia; iv) response to antipsychotic treatment is related to dopaminergic function but the relationship is not uniform across the striatum. These findings link dorsal striatal dopamine dysfunction to the development of psychosis and do not support the mesolimbic hypothesis.
Disclosure: Part 1: Membership of speaker bureaux and independent investigator-led research grants from manufacturers of antipsychotic drugs.
Part 4: Membership of speaker bureaux and independent investigator-led research grants from manufacturers of antipsychotic drugs.

25.2 Abnormalities of Cortico-Striatal-Thalamo-Cortical Circuits in Individuals at Clinical High Risk (CHR) for Psychosis

Tiziano Colibazzi

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Background: The development of psychotic illness is preceded by a phase characterized by attenuated or brief psychotic symptoms as well as functional decline. This prodromal period is of particular interest because the absence of a variety of confounding factors such as chronicity, medication use or institutionalization allows one to separate more clearly state- and trait-related effects. Over the last six years, we have been collecting baseline and longitudinal imaging data in multiple modalities (anatomical, diffusion, resting state, task-based fMRI as well as MRS data) in a cohort now consisting of 61 subjects deemed at Clinical High Risk (CHR) for psychosis as well as in age- and gender matched healthy controls. Nine individuals in this cohort have developed full psychotic illness. Recently published anatomical, fMRI and PET studies of UHR cohorts have suggested the presence of abnormalities in fronto-striatal networks which predate the onset of psychotic illness. Using our multimodal dataset, we have investigated whether abnormalities of cortico-striatal circuits are present before the onset of psychotic illness.
Methods: 61 adolescents and young adults at Clinical High Risk (CHR) for psychosis and age and gender-matched healthy controls were enrolled from the prodromal clinic (COPE clinic) at the New York State Psychiatric Institute (NYSPI) Patients were identified as being at clinical high risk for psychosis according to the Structured Interview for Psychosis-Risk Syndromes (SIPS). We acquired data on a 3 Tesla GE scanner in different MRI modalities in the same cohort (anatomical, resting state fMRI, task-based fMRI, DTI, MRS and ASL). A brief description of methods for each modality follows. Anatomical analyses were carried out in Free Surfer (for the cortical mantle). We compared cortical thickness between the patients and the controls while

covarying for age and gender. The significance p value was corrected for multiple comparisons using false discovery rate (FDR). Diffusion images were acquired on the same scanner using 25 gradient directions plus 3 baseline images without applying a diffusion gradient, a b-value of 1000 s/m2and a TR of 17000ms. Reconstructed diffusion tensor images were normalized into standard space and Fractional Anisotropy (FA) and Mean Diffusivity (MD) values were calculated at each voxel. FA and MD were entered in a linear regression as dependent variable with age and gender entered as covariates. Resting State (RS) BOLD sequences were acquired before and after each participant carried out a functional task (Simon task). Each sequence lasted 5 minutes. Analyses of Functional Connectivity (FC) were performed by using 6 seed regions (dorsal caudate, ventral superior caudate, ventral inferior caudate, dorsal rostral putamen, dorsal caudal putamen and ventral rostral putamen). Measures of Degree of Centrality (DC) and Regional Homogeneity (ReHo) were also obtained. For the task-based portion of our study, we used the Simon task, which probes processes supporting cognitive control. In this paradigm, part of a larger group of tasks assessing self-regulation, a conflict is generated when the task-relevant, less automatic response to the direction of an arrow conflicts with the more automatic and prepotent response to the side of the screen where the arrow appears. In total, the task consisted of 3 runs of 55 stimuli, with congruent and incongruent stimuli present in equal number. Using the general linear model (GLM) as implemented in SPM8, we modeled the data for each participant with 6 independent functions and a constant for each run. We implemented a Bayesian posterior inference approach for the second-level analysis of the contrast images generated from the first-level GLM-based analysis.
Results: First, analyses of baseline functional data during performance of the Simon task revealed in CHR subjects elevated functional activity during trials not associated with conflict, even exceeding neural activation related to the processing of cognitive conflict. This abnormality was present throughout the fronto-striatal system and in particular, in the DLPFC, the ventral and dorsal striatum, the IFG and the dACC. Second, analyses of functional connectivity showed increased FC, in CHR participants, between the ventro-rostral putamen and the thalamus as well as increased DC of the thalamus. Third, diffusion imaging maps showed, in patients, decreased FA as well as increased MD in the white matter connecting the associative striatum with the prefrontal cortex. Finally, analyses of cortical thickness showed cortical thinning in CHR participants in the superior temporal sulcus, the middle frontal gyrus, the postcentral gyrus, the dACC, the PCC and in the cortex between the occipital cortex and temporal-parietal areas.
Conclusions: The baseline data from our CHR sample reveal consistent abnormalities of cortico-striatal networks in CHR individuals. Our task-based findings point to a general level of overactivity of the fronto-striatal circuits that does not vary in the face of increasing cognitive conflict. These task-based abnormalities accompany disturbances in patterns of functional connectivity involving the thalamus, the associative striatum and several association cortices, which simultaneously show significant amounts of cortical thinning. The existence of a perturbed cortico-striatal connectivity in these CHR individuals is supported by the finding of disrupted white matter structure in fibers that connect the striatum to the prefrontal areas. Taken together, our study suggests widespread disturbances in the functional and anatomical architecture of cortico-striatal loops in individuals at clinical risk for psychosis.
Disclosure: Nothing to Disclose.

25.3 Striatal GABAergic and Glutamatergic Dysregulations as Potential Predictors of Conversion to Psychosis in Individuals at Ultra-high Risk

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Background: The current hypothesis for the origin of schizophrenia proposes that the disorder stems from neurodevelopmental deficits that result in a disturbance of glutamatergic neurotransmission, especially for N-methyl-D aspartate receptor-mediated signaling. The deteriorating course of the disease maybe partially explained by cortical neuronal toxic effects secondary to enhanced glutamatergic exposure, and dopaminergic dysregulation may be the final common pathway that results from altered glutamatergic neurotransmission. Recently, we and others reported the results of proton magnetic resonance spectroscopy (1H MRS) studies that found elevated glutamate levels in the associative striatum of subjects at ultra-high risk for psychosis, as well as in three different cohorts of antipsychotic naive subjects during their first episode of psychosis. Subsequently, by longitudinally following these UHR subjects over 2 years, we demonstrated that the subjects with higher glutamate levels at baseline later transitioned to psychosis, suggesting the involvement of this excitatory amino acid neurotransmitter in the early or prodromal phases of schizophrenia.
Methods: Twenty-three antipsychotic naive subjects at ultra-high risk for psychosis and 24 healthy controls subjects, matched for age, sex, handedness, cigarette smoking, and parental education underwent proton magnetic resonance spectroscopy scans at 3T. Levels of gamma-aminobutyric acid (GABA) and of the combined resonance of glutamate and glutamine (Glx) were obtained using the standard J-editing technique and expressed as peak area ratios relative to the simultaneously acquired water signal.
Results: Significantly increased levels of GABA ($p < 0.001$) and Glx ($p = 0.007$) were found in the dorsal caudate of the subjects at ultra-high risk for psychosis compared to the healthy controls. No group differences were found for any of the other metabolites (N-acetylaspartate, total choline or total creatine).
Conclusions: This study presents the first evidence of abnormal elevations, in subjects at ultra-high risk for psychosis, of GABA and Glx in a brain region that have been implicated in the pathophysiology of psychosis, warranting longitudinal studies to assess whether these neurotransmitter abnormalities can serve as noninvasive biomarkers of conversion risk to psychosis, as well as of illness progression and treatment response.

Disclosure: Part 1: Camilo de la Fuente- Sandoval has served as consultant and/or speaker for AztraZeneca, Eli Lilly and Janssen (Johnson & Johnson), **Part 4:** Camilo de la Fuente-Sandoval has received grant support from Janssen (Johnson & Johnson).

25.4 Reward Sensitivity in Adolescents and Other Unexpected Properties of the Dorsal Striatum

Nicholas Simon

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: Recent studies in individuals at high risk to develop schizophrenia consistently point to dysregulated striatal-prefrontal cortex interactions that are, in part, reflected in elevated presynaptic striatal dopamine availability. These findings indicate that a dopamine abnormality (i) predates the onset of schizophrenia in individuals with prodromal symptoms, (ii) is predominantly localized in the associative/dorsal striatum, and (iii) is correlated with the severity of symptoms and neurocognitive dysfunction. Therefore, a functional understanding of striatal neurons and dopaminergic neurons that project to regions of striatum during adolescence is necessary to understand how early aberrations in the dopaminergic system are involved with the etiology of schizophrenia.
Methods: To determine how different regions of striatum process rewards and reward-related behavior during adolescence, we used single unit electrophysiology to compare neuronal activity in dorsal striatum (DS) and ventral striatum (VS) between adolescent and adult rats. We then used microdialysis to measure amphetamine-evoked dopamine release in DS and VS between age groups. We also used western blots to quantify TH content in adolescent and adult DS and VS. We then extended our experimental methodology to dopaminergic neurons in ventral tegmental area (VTA), recording from single units in adolescent and adult VTA during reward-related behavior. Finally, we assessed how phasic activation of dopamine neurons in VTA modulates neural activity throughout the brain using optogenetics in concert with anesthetized fMRI.
Results: Dorsal striatal neurons were hyper-responsive to rewards in adolescent compared to adult rats. In addition, we observed reduced amphetamine-evoked dopamine release in adolescent DS (VS) striatum compared to adults, and also that dopamine synthesis capacity as measured by tyrosine hydroxylase levels was significantly attenuated in the DS of adolescent rats. These data suggest that increased DS activity and blunted dopamine release is a component of normal development during adolescence, which may be disrupted in individuals at high risk to develop schizophrenia. Dopamine neurons in VTA were hypo-responsive to reward anticipation and delivery in adolescence. Furthermore, optogenetic stimulation of dopamine neurons in the VTA caused preferential activation of dorsal striatum compared to ventral striatum as assessed by fMRI. This surprising finding suggests that dysfunction of VTA dopamine neurons may profoundly influence dorsal striatum activity.
Conclusions: Collectively, these data show developmental differences in dorsal/associative striatum and dopamine neuron activity, which may be relevant to increased vulnerability to transition to psychosis. Additionally, these data provide an interesting link between VTA dopamine and dorsal striatal activation, which suggests that pathological aberrations in dorsal striatum may arise from VTA dysfunction.
Disclosure: Nothing to Disclose.

Panel
26. Understanding the Effects of Stress at the Intersection of Appetitive and Aversive Functions in Disease: Integrating Across Genes, Brain, and Behavior

26.1 Intersection of Stress, Reward, and Anhedonia in the Rodent Brain

William Carlezon

Harvard Medical School, Belmont, Massachusetts

Background: Stress can precipitate psychiatric conditions including anxiety, depression, and drug addiction. We have been using laboratory animals to examine the effects of stress on behavioral signs and symptoms that cut across these conditions, with the premise that understanding how stress changes the brain and behavior will enable transformational advances in treatments. Our current studies focus on chronic social defeat stress (CSDS), an increasingly utilized model that exploits the ethological relevance of territorial aggression. It is established that CSDS produces some core symptoms of anxiety and depressive disorders, including social avoidance and anhedonia (reduced sensitivity to rewarding stimuli), as assessed in tests quantifying social interaction and preference for sucrose and other natural rewards. However, interpretation of these reward tests can be contentious, and they can be difficult to use repeatedly to track changes over time. As such, we used the intracranial self-stimulation test (ICSS) in mice to confirm that CSDS produces anhedonia, and to characterize the time course at which anhedonia develops and recovers. We also examined the effects of manipulations known to reduce sensitivity to stress and its consequences. Specifically, we examined whether a pharmacological agent (ketamine) or genetic mutations (conditional overexpression of the transcription factor ΔFosB, or ablation of kappa-opioid receptors [KORs]) could mitigate CSDS effects on ICSS. Most recently, we have also been examining CSDS effects on other domains that are often dysregulated in psychiatric illness, including communication (as measured by ultrasonic vocalizations) and sleep/activity patterns (as measured by EEG/EMG).
Methods: Mice were implanted with lateral hypothalamic (LH) electrodes and ICSS thresholds were measured following each of 10 daily CSDS sessions, and during a 5-day recovery period. We also examined if acute administration of ketamine (2.5–20 mg/kg, intraperitoneal) reverses CSDS-induced effects on reward or, in separate mice, social interaction. Parallel studies were conducted in mice that overexpress ΔFosB in striatum or lack KORs on dopamine-expressing neurons. Studies on USVs and sleep patterns in response to CSDS have thus far only been conducted in

wild-type mice. We used a bat sensor to quantify USVs and a microchip transmitter/receiver to quantify sleep/activity patterns before, during, and after CSDS.
Results: Data were analyzed by ANOVAs and significant effects were analyzed using Bonferroni tests. CSDS significantly increased ICSS thresholds, indicating decreases in the rewarding impact of LH stimulation (anhedonia). Although thresholds recovered following termination of the CSDS procedure, they remained significantly elevated for at least 5 days. The effects of CSDS were absent in ΔFosB-mutant mice, consistent with pro-resilient actions of this transcription factor, and the onset of anhedonia was delayed in KOR-mutant mice, consistent with the anti stress-like effects of KOR antagonists. High but not low doses of ketamine administered after completion of the CSDS regimen significantly attenuated social avoidance in defeated mice, although it did not block CSDS-induced anhedonia in the ICSS test. CSDS also reduced USVs and produced increases in non-REM sleep, although these effects were most pronounced in a subgroup of mice, with others seemingly unaffected.
Conclusions: Our findings demonstrate that CSDS triggers persistent anhedonia, and confirm that sensitivity to certain stress effects can be altered by pharmacological treatments or genetic manipulations. Future work will examine how these same manipulations affect communication and sleep/activity patterns, and whether putative resilience in some mice is consistent across multiple domains. This work is germane as the field moves toward focusing on behavioral signs that cut across multiple types of psychiatric illness, and develops models that better approximate these domains as they appear in humans while having improved predictive validity for medication development.
Disclosure: Part 1: Dr. Carlezon is editor of Neuropsychopharmacology Spouse is an employee at EMD Serono, **Part 2:** Dr. Carlezon is editor of Neuropsychopharmacology Spouse is an employee at EMD Serono, **Part 3:** Dr. Carlezon is editor of Neuropsychopharmacology Spouse is an employee at EMD Serono.

26.2 Orbitofrontal Cortical Regulation of Actions and Habits

Shannon Gourley

Emory University School of Medicine, Atlanta, Georgia

Background: An important aspect of goal-directed action selection is differentiating between actions that are more, or less, likely to be reinforced. With repeated performance, stressors, or psychostimulant exposure, however, actions can assume stimulus-elicited — or "habitual" — qualities that are resistant to change. The orbitofrontal prefrontal cortex (oPFC) has been increasingly implicated in these processes, but molecular mechanisms remain opaque.
Methods: We use site-selective gene silencing, Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), pharmacological approaches, and anatomical disconnection techniques to isolate the molecular and neuroanatomical mechanisms by which the oPFC regulates actions and habits.
Results: I will present evidence that site-selective destabilization of the actin cytoskeleton or deletion of Brain-derived neurotrophic factor (Bdnf) within the oPFC impairs action selection strategies in mice, resulting in maladaptive habits. Next, I will discuss novel evidence that the mouse oPFC, like the primate's, projects topographically to the basolateral amygdala, entorhinal cortex, and striatum. Experiments using functional trkB inhibition, DREADDs, and asymmetric gene knockdown indicate that oPFC-derived BDNF acts in downstream targets to regulate action selection strategies. Additionally, our pharmacological studies indicate that BDNF-trkB binding is essential to the consolidation, specifically, of new information regarding the relationship between actions and their outcomes. I will conclude this discussion with new evidence that overlapping mechanisms regulate fear extinction processes.
Conclusions: These findings, particularly regarding common mechanisms of instrumental conditioning and fear extinction, may have critical implications for the treatment of psychopathologies characterized by maladaptive habits and inflexible, ruminative thought patterns.
Disclosure: Nothing to Disclose.

26.3 Stress and Its Influences on Anxiety and Addiction in the Human Brain

Rajita Sinha

Yale University, New Haven, Connecticut

Background: High uncontrollable stress and trauma are major risk factors for the most common neuropsychiatric illnesses of anxiety disorders and addictions. Growing evidence from the neuroscience of stress and adversity suggests alterations in the prefrontal, limbic and striatal systems are involved in the pathophysiology of these illnesses. However, neuroimaging approaches that identify both common and specific, differentiable neural patterns associated with these illnesses are rare.
Methods: Novel neuroimaging results using both structural and functional magnetic resonance imaging (MRI and fMRI) data from three separate studies with at-risk and addicted samples will be shown. Functional MRI responses were assessed using our well established personalized script-driven guided imagery methods for provoking stress, alcohol cue and neutral relaxing states and also a novel brief continuous stress provocation procedure to induce biological and subjective stress and active/avoidant coping.
Results: Consistent and complementary results from the three studies were found to show differential patterns of amygdala, hippocampus and striatal responses to stress, anxiety and reward was predictive of specific risk for anxiety and for addiction, and blunted ventromedial prefrontal cortex (VmPFC) responses (all $p < .05$ whole brain corrected) was predictive of stress-related vulnerability for both anxiety and addiction risk. Furthermore, new findings indicated dynamic recovery of the VmPFC during acute stress represented active healthy coping while hyperactive and inflexible lateral obitofrontal cortex, insula and striatal responses and blunted VmPFC was predictive of avoidant coping and high anxiety symptoms.

Conclusions: Using novel human neuroimaging methods, these results suggest that specific and differentiated patterns of neural responses involving dysfunction of medial prefrontal cortical circuits are involved in the pathophysiology of anxiety and in those that are predictive of addiction risk and relapse. Findings will be discussed in the context of translational data on the inflexibility of the VmPFC in reward prediction and fear extinction, and potential novel therapeutics to target such dysfunction in the prevention and treatment of anxiety and addictive disorders. (Supported by UL1-DE019586, R01-AA013892; PL1-DA24859).
Disclosure: Nothing to Disclose.

26.4 Is Dysregulated Fear Habitual? - Human Genetic and Neuroscience Approaches to PTSD & Addiction Comorbidity

Kerry Ressler

Yerkes Research Center, Emory University, Howard Hughes Medical Institute, Atlanta, Georgia

Background: Exposure to traumatic experiences, especially those occurring in childhood, has been linked to substance use disorders (SUDs), including abuse and dependence. Childhood and adult trauma, as well as SUDs, are also highly associated with Posttraumatic Stress Disorder (PTSD) and other mood-related psychopathology. Here we present data examining these relationships between trauma, substance use, and PTSD, as well as markers of resilience, in a sample of urban primary care patients. Further we examine genetic polymorphisms that appear to be associated with increased risk for both disorders, and neural circuit activity associated with risk for both addiction and unregulated fear responding. We hypothesize that similar genetic and neural biological processes associated with increased habitual and decreased goal-directed behaviors are associated with both SUDs and PTSD and their comorbidity in clinical populations.
Methods: We aimed to examine associations between risk and resilience characteristics and lifetime alcohol and illicit drug use along with PTSD in >2000 inner-city adults with high rates of childhood abuse and adult trauma exposure. In this cross-sectional study, resilience was assessed with the Connor-Davidson Resilience Scale, childhood abuse with the Childhood Trauma Questionnaire, lifetime alcohol and illicit drug use with the Alcohol Use Disorder Identification Test and Drug Abuse Screening Test, and PTSD symptoms with both the PTSD Symptom Scale and the Clinician Administered PTSD scale. Functional neuroimaging was performed with a 3T MRI scanner, at resting state, viewing neutral vs. fearful faces, and in a behavioral inhibition task. Acquisition and extinction of fear were measured with a fear potentiated startle paradigm with neutral cues of computer-based shapes and an aversive cue of an unexpected air blast. Associations between risk & resilience and substance use were examined with linear regression models, adjusting for trauma load, age, and sex. Separate analyses examined the relationships of addiction and PTSD in regression with the fMRI tasks.
Results: In this highly traumatized population, high rates of lifetime dependence on various substances were found (39% alcohol, 34.1% cocaine, 6.2% heroin/opiates, and 44.8% marijuana). The level of substance use, particularly cocaine, strongly correlated with levels of childhood physical, sexual, and emotional abuse as well as current PTSD symptoms. In particular, there was a significant additive effect of number of types of childhood trauma experienced with history of cocaine dependence in predicting current PTSD symptoms, and this effect was independent of exposure to adult trauma. When examining markers of resilience, we found that resilience characteristics mitigated tendency for lifetime alcohol use problems both as a main effect ($\beta = -0.11$; $p = 0.0014$) and an interaction with severity of childhood abuse ($\beta = -0.06$; $p = 0.0115$) after trauma severity, age, and sex were controlled for. Similarly, resilience reduced lifetime illicit drug use both as a main effect ($\beta = -0.03$; $p = 0.0008$) and as an interaction with severity of childhood abuse ($\beta = -0.01$; $p = 0.0256$) after trauma load, age, and sex were adjusted for. We have previously shown, in fMRI, stronger activation in the ventromedial prefrontal cortex (vmPFC) in traumatized subjects without PTSD compared to those with PTSD in the NoGo > Go contrast condition. Activation in the vmPFC was negatively correlated with fear-potentiated startle responses during safety signal learning ($p = .02$) and fear extinction ($p = .0002$). We will present new data consistent with a model in which resilience, decreased substance use, and decreased PTSD are all associated with increased prefrontal regulation of behavioral inhibition and increased prefrontal-amygdala functional connectivity.
Conclusions: These data show strong links between trauma history, SUDs, and their joint associations with PTSD outcome. They suggest that enhanced awareness of PTSD and substance abuse comorbidity in high-risk, impoverished populations is critical to understanding the mechanisms of substance addiction as well as in improving prevention and treatment. Our findings also add to a nascent body of literature suggesting that resilience characteristics mitigate risks not only for PTSD, major depression, and suicidality, but also for substance use problems in adults exposed to childhood abuse or other traumatic experiences. The fMRI data suggest that the same circuits involved in behavioral inhibition appear to be involved in fear inhibition processes during differential fear conditioning and extinction. Finally, the combined data suggest that shared neural circuitry may mediate risk for both SUDs and PTSD, and that the concept of habitual behavior may be equally applied to both addiction and fear-related behavior.
Disclosure: Nothing to Disclose.

Panel
27. Nicotinic Receptor Signaling in Neurodevelopmental Disorders and Adult Neuropsychiatric Conditions

27.1 Epigenetic Mechanisms Underlying Long-term Developmental Effects of nACHRs on Dendritic Structure in Cortical Neurons

Marina Picciotto

Yale University, New Haven, Connecticut

Background: Developmental nicotine exposure through tobacco smoke causes long-lasting effects in exposed

children and is correlated with later psychiatric symptoms. Nicotine is known to alter neuronal morphology and function through modulation of synaptic transmission and gene expression, likely leading to later behavioral consequences; however, the mechanisms underlying long-lasting maintenance of these changes are currently not known.
Methods: We used diolistic labeling of cortical neurons with DiI to identify changes in dendritic branching and spine number in cortical neurons following developmental nicotine exposure. We then performed microarray screening on cortical tissue from mice exposed to nicotine during development to identify differentially expressed mRNAs in adulthood that might be responsible for maintenance of structural changes. Next generation sequencing was performed to determine whether histone complexes associated with the promoters of genes throughout the genome were altered by developmental nicotine exposure. Changes in dendritic structure, gene expression and histone methylation were validated following nicotine exposure in primary cortical neurons, and shRNA delivery was used to identify causal relationships between gene expression changes and structural effects of nicotine.
Results: Nicotine exposure caused persistent increases in dendritic branching and spine number across cortical regions. We found using microarray screening that developmental nicotine exposure in mice induces expression of a component of a histone methyl transferase complex. We then used ChIP sequencing to evaluate genome-wide changes in histone 3 lysine 4 tri-methylation (H3K4me3) after developmental nicotine treatment to identify long-lasting epigenetic changes in vivo. We identified a number of loci with significant changes, including a second critical member of the same histone methylase complex. Gene ontology and pathway analysis also identified H3K4me3 alterations at several genomic sites involved in regulation of the postsynaptic density, all of which were verified by ChIP-PCR. This histone methylase complex appears to be involved in nicotine-dependent regulation of neuronal structure as evaluated by analysis of dendritic structure in cultured primary neurons.
Conclusions: Taken together, these studies identify a novel molecular target induced by nicotinic stimulation that couples early drug exposure to long-lasting epigenetic and structural changes in the brain. These mechanisms may contribute to the increased vulnerability to smoking and later psychiatric illness that results from developmental tobacco exposure.
Disclosure: Nothing to Disclose.

27.2 Habenular Influences on Anxiety and Compulsive Behavior

Mariella De Biasi

University of Pennsylvania, Philadelphia, Pennsylvania

Background: As of 2010 approximately 4.5% of the global population had an anxiety disorder. In the United States, the lifetime prevalence of anxiety disorders is about 29% and between 11 and 18% of adults have the condition in a given year. Anxiety also develops during the acute withdrawal phase of drugs of abuse, including opiates, methamphetamine, alcohol and nicotine.
Methods: The presentation will include evidence obtained using a combination of pharmacological, optogenetic and designer receptor (DREADDs) approaches. Those techniques were used in vivo and in brain slice preparations to identify brain circuits and cholinergic receptor mechanisms involved in anxiety-related behavior.
Results: We show that the medial habenula/interpeduncular nucleus (MHb-IPN) pathway regulates anxiety levels and compulsive-like behavior in both basal conditions and during drug withdrawal. Activation of the MHb leads to an initial phase of anxiolysis followed by increased anxiety.
Conclusions: nAChRs encoded by the CHRNA5-CHRNA3-CHRNB4 gene cluster are required for the regulation of anxiety and compulsive behavior within the MHb-IPN circuit.
Disclosure: Nothing to Disclose.

27.3 Nicotinic Receptors and Cocaine Reward

Paul Kenny

Mount Sinai School of Medicine, New York, New York

Background: Allelic variation in CHRNA5, the gene encoding the α5 nicotinic acetylcholine receptor (nAChR) subunit, is associated with increased vulnerability tobacco addiction. This action is related to diminished sensitivity of the habenula-interpeduncular aversion system to nicotine. Unexpectedly, the same allelic variation in CHRNA5 appears to protect against cocaine addiction, but underlying mechanisms are unknown. Here, we employed a multidisciplinary approach to investigate the role for the α5* nAChRs in regulating the motivational properties of cocaine.
Methods: To investigate the role for α5* nAChRs in the rewarding effects of cocaine, we trained mice with null mutation in Chrna5 and their wildtype littermates in a discrete-trials current-threshold intracranial self-stimulation (ICSS) procedure and the lowering effects of cocaine on ICSS thresholds was assessed. To investigate the α5* nAChRs in the reinforcing properties of cocaine, α5 subunit wildtype and knockout mice were trained to respond for intravenous cocaine infusions across a broad dose range (0.03-3.0 mg/kg/infusion) and their responding for the drug assessed. To investigate the role for α5* nAChRs in regulating the activity of cholinergic interneurons, we use whole cell patch recordings in α5 wildtype and knockout mice crossed in which channelrhodopsin-2 (ChR2) and eYFP were expressed specifically in cholinergic neurons. To control the activity of corticostriatal and thalamostriatal projection neurons expressing α5* nAChRs, we used Cre-inducible DREADD receptors in mice in which Cre was expressed under the control of the α5 gene promoter.
Results: We found that α5 knockout mice had diminished sensitivity to the lowering effects of experimenter-administered cocaine injections on ICSS thresholds, considered a measure of the reward-enhancing properties of the drug. Consistent with this observation, the knockout mice

self-administered greater quantities of cocaine when a lower unit dose of the drug was available (0.03 mg/kg/infusion) but not a higher (3 mg/kg/infusion) dose of cocaine was available, an effect typically interpreted as a compensatory increase in intake to overcome diminished rewarding effects of the drug. We found that prefrontal cortex and parafascicular nucleus of the thalamus densely expressed α5 subunits. These sites are known to provide excitatory drive onto to cholinergic interneurons in the dorsal striatum, and cholinergic transmission in striatum is thought to negatively regulate drug reward. We found that the firing rate of cholinergic interneurons was dramatically increased, and the inhibitory effects of cocaine on these neurons was decreased, in the α5 knockout mice compared with wildtype controls. Moreover, DREADD-mediated inhibition of neurons in the parafascicular thalamus but not prefrontal cortex that express α5* nAChRs increased responding for cocaine when a lower unit dose was available for self-administration similar to the behavior of the α5 knockout mice.
Conclusions: These data reveal a fundamental role for α5* nAChR signaling in regulating the motivational properties of cocaine. The data suggest α5* nAChRs regulate cocaine intake by controlling the actions of thalamostriatal inputs to cholinergic interneurons. These findings may explain the observed protective effects of allelic variation in CHRNA5 against cocaine dependence in humans.
Disclosure: Nothing to Disclose.

27.4 Nicotinic Receptor Signaling in the Developmental Modulation of Fear-learning Circuits

Lorna Role

Stony Brook University, Stony Brook, New York

Background: The appropriate formation, retention and extinction of cue-associated fear memories are essential behaviors for survival. Dysregulation of the circuits underlying fearful memories can manifest in neuropsychiatric phenotypes crippling to everyday life. Both cortical and subcortical inputs to the basal lateral region of the amygdala (BLA) and the extra-amygdala outputs of the BLA plays a central role in fear memory behaviors. The BLA receives prominent cholinergic input from the Nucleus Basalis of Meynert (NBM) and there is a strong literature on the potential role of cholinergic signaling in certain aspects of BLA based "emotional learning".
Methods: The presentation will include data obtained using a combination of optogenetic stimulation (or inhibition) of endogenous cholinergic inputs from the nucleus Basalis (NBM) to the basolateral amygdala (BLA) and electrophysiological assessment of ACh and nicotine induced effects on cortical vs subcortical input plasticity. Effect measures include both in vivo and slice electrophysiological recording as well as behavioral assessment of the formation and extinction of fear memories.
Results: The presentation will include our findings from studies in which we have examined the efficacy of exogenous nicotine and endogenous cholinergic inputs in modulating cortical vs subcortical excitatory plasticity in slice preparations of BLA from a variety of conditions including (a) WT " adolescent " and adult mice. (b) WT adolescent and adult mice exposed to nicotine for 4 weeks of pre and peri-natal (c) mice heterozygous for Type III Nrg 1 (one of several "high ranking" schizophrenia susceptibility genes). The effects of brief stimulation or inhibition of endogenous cholinergic inputs is also tested in a fear learning behavior paradigm in adult mice.
Conclusions: Overall these studies support an important role for the activation of nicotinic AChRs in fear circuits and learning and reveal dramatic differences in the efficacy of cholinergic modulation of BLA circuits in perinatal nicotine exposed and in Nrg heterozygous animals.
Disclosure: Nothing to Disclose.

Panel
28. Human Stem Cell-based Models of Psychiatric Disease: Studying Schizophrenia and Bipolar Disorder Using Stem Cells

28.1 Alterations in Interneuron Differentiation in an iPSC Model of Bipolar Disorder

K Sue O'Shea

University of Michigan Medical School, Ann Arbor, Michigan

Background: Bipolar disorder (BP) affects millions of individuals worldwide, yet progress in understanding its pathogenesis and improving treatments has been hampered by the lack of viable neuronal models. Patient-derived induced pluripotent stem cells (iPSC) now offer the opportunity to study the development of neural tissues and the prospect of identifying novel disease mechanisms in BP.
Methods: We have derived iPSC from three individuals with BP and three healthy controls and differentiated them into telencephalic neurons. RNAs were extracted from undifferentiated iPSC and neurons derived from them and microarray analysis carried out. To determine if the neuronal phenotype could be altered, iPSC have been exposed to ventralizing agents (purmorphamine) or dorsalizing agents (lithium) and are being evaluated using PCR, western blot and immunohistochemistry.
Results: Expression of transcription factors that convey regional neuronal cell fate was significantly different between the two groups. Neurons derived from control iPSC expressed transcripts that confer dorsal telencephalic fate (EMX2, FEZF2, PAX6, TBR2, TCF3, VGLIT1, ZNF536), while neurons derived from BP iPSC expressed genes involved in the differentiation of ventral (MGE) brain regions (NKX2-2, FOXP2, ASCL1, LHX6). iPSC from both BP and controls are responsive to patterning cues, increasing expression of NKX2-1 (ventral identity) or EMX2 (dorsal). Consistent with the increase in transcripts involved in interneuron cell fate, GABA expression by BP vs C neurons was elevated throughout the culture period, and mature neurons derived from control iPSC expressed higher levels of VGLUT1, those from BP iPSC expressed higher levels of SST.
Conclusions: During development, the majority of cortical interneurons originate in the MGE, undergo tangential

migrations to their adult cortical locations where they form inhibitory GABAergic interneurons. Although only 20% of the total number of cortical neurons, interneurons play a critical role in maintaining the normal balance in cortical activity by making local synapses on long-projecting excitatory (glutamatergic) pyramidal neurons. Since interneuron dysfunction has been suggested to underlie a number of neurodevelopmental and neuropsychiatric conditions, alterations in the specification or function of GABAergic interneurons would be expected to disrupt the excitatory-inhibitory balance in the cortex, contributing to BP.
Disclosure: Nothing to Disclose.

28.2 Mitochondria Improve Impaired Neuronal Differentiation of Hair Follicle-derived Induced Pluripotent Stem Cells of Schizophrenia Patients

Dorit Ben-Shachar

Rambam Medical Center, Technion, Haifa, Israel

Background: Schizophrenia is conceptualized as a neurodevelopmental disorder, involving dysfunction of dopaminergic and glutamatergic systems as well as of mitochondria. Among the major obstacles in studying the pathological processes in this disease are the inaccessibility of the brain and the inability to study brain processes prior to the onset of this neurodevelopmental disorder. Differentiation of induced pluripotent stem cells (iPSC) into neurons is an attractive model to study neurodevelopmental impairments and mitochondrial dysfunction in schizophrenia.
Methods: iPSC from schizophrenia patients and healthy controls were reprogrammed from hair follicle keratinocytes and differentiated into Pax6 + /Nestin + neural precursors and then into ß3-Tubulin + /TH + /DAT + dopaminergic neurons. iPSC were also differentiated through embryonic bodies into β3-Tubulin + /TBR1 + glutamatergic neurons. Mitochondria were assessed by analyzing mitochondrial membrane potential ($\Delta\psi_m$), network dynamics and apoptosis markers.
Results: Schizophrenia-derived dopaminergic cells showed severely impaired ability to differentiate, whereas glutamatergic cells were unable to maturate. Mitochondrial respiration and its sensitivity to dopamine-induced inhibition were impaired in schizophrenia-derived keratinocytes and iPSC. Moreover, we observed dissipation of mitochondrial $\Delta\psi m$ and perturbations in mitochondrial network structure and connectivity in schizophrenia-derived neurons. Our recent data show that amending mitochondrial function improves differentiation of glutamatergic neurons derived from schizophrenia iPSC, expressed by enhanced expression of differentiation markers and increased synaptic contacts.
Conclusions: Our data suggest a direct role for mitochondria in neuronal differentiation. In addition this study unravels perturbations in neural differentiation and mitochondrial function, which are interconnected and of relevance to early neurodevelopmental processes in schizophrenia.
Disclosure: Nothing to Disclose.

28.3 Human Stem Cell-based Models of Psychiatric Disease: Studying Schizophrenia and Bipolar Disorder Using Stem Cells

Kristen Brennand

Mount Sinai School of Medicine, New York, New York

Background: Schizophrenia (SZ) is a debilitating neurological disorder. Though postmortem studies have revealed reduced neuron size and spine density in SZ brain tissue, the molecular mechanisms underlying the disease state remain unclear.
Methods: We directly reprogrammed fibroblasts from SZ patients into human induced pluripotent stem cells (hiPSCs) and subsequently differentiated these disorder-specific hiPSCs into neural progenitor cells (NPCs) and neurons. Gene expression comparisons of our hiPSC-derived neural progenitor cells (NPCs) and 6-week-old neurons to the Allen BrainSpan Atlas indicate that our hiPSC neural cells, from controls and patients with SZ, most resemble fetal rather than adult brain tissue, indicating that hiPSC-based models may not yet be suited for the study of the late features of this disorder. Our analysis confirms that a significant fraction of the gene signature of SZ hiPSC-derived neurons is conserved in SZ hiPSC neural progenitor cells (NPCs), suggesting that at least some of the molecular events contributing to SZ are established prior to neuronal maturation. An unbiased genomic and proteomic analysis observed altered cellular adhesion and oxidative stress proteins in SZ hiPSC NPCs.
Results: We observed aberrant migration and increased oxidative stress in SZ hiPSC NPCs, while SZ hiPSC neurons showed diminished neuronal connectivity in conjunction with decreased neurite number, PSD95-protein levels and glutamate receptor expression. Key cellular and molecular elements of the SZ phenotype were ameliorated following treatment of SZ hiPSC neurons with the antipsychotic loxapine. A genome-wide analysis identified six miRNAs misregulated in SZ NPCs; we now report that rescuing decreased expression of aberrantly expressed microRNA levels in SZ hiPSC NPCs partially rescues some of the phenotypic differences observed in SZ hiSPC derived neural cells.
Conclusions: To confirm these findings across a larger cohort of patients, we have now generated hiPSCs from thirteen patients with childhood-onset SZ (COS) and twelve additional controls. COS is a rare and particularly severe form of the disorder, with an onset of psychosis prior to age twelve. We anticipate that neural cells derived from patients with COS will have accelerated and/or more severe cellular phenotypes relative to those we have already reported for adult-onset SZ, and so might be better suited for stem-cell based models of SZ predisposition.
Disclosure: Nothing to Disclose.

28.4 DISC1 serine-713 Phosphorylation-dependent Neurodevelopmental Switch: Impact on Anatomy and Cognition in Major Mental Disorders

Akira Sawa

John Hopkins Schizophrenia Center, Baltimore, Maryland

Background: Development in cell engineering technology now allow us to establish live neuronal cell lines from

peripheral tissues of human subjects, overcoming a major obstacle that has previously hindered research of major mental conditions, such as schizophrenia and bipolar disorder. We previously identified a key role of specific phosphorylation of the DISC1 protein during neurodevelopment (Ishizuka et al. 2011, Nature). In this study, we have used such human cell engineering technology to study the clinical significance of this specific protein signature.
Methods: We established two neuronal cell models from patients with schizophrenia and bipolar disorder with psychotic features, and from control subjects: olfactory immature neuronal cells (obtained via nasal biopsy) and neurons derived from human induced pluripotent stem (iPS) cells. Using these cells, we compared the specific phosphorylation of DISC1 at the serine-713 residue (pS713-DISC1) between patients and controls, and determined how pS713-DISC1 affected neuronal maturation in vitro and in vivo (where human neuroprogenitors were transplanted into developing rodent brains). Finally, we assessed how pS713-DISC1 levels were associated with clinical features, brain imaging and neuropsychological characteristics from the same subjects.
Results: Levels of pS713-DISC1 were markedly reduced in patient olfactory immature neuronal cells, and in iPS cell-derived neurons from patients with schizophrenia, relative to controls. Importantly, this decrease in pS713-DISC1 in iPS cell-derived neurons delayed neuronal maturation both in vitro and in vivo. Furthermore, reduced pS713-DISC1 was correlated with smaller volume of middle frontal gyrus and impaired working memory.
Conclusions: By using a multi-faceted approach, including human stem cell biology, brain imaging and neuropsychological assessment, we report that decreased phosphorylation of DISC1 at S713 in patients with schizophrenia and bipolar disorder with psychotic features causes delayed neuronal differentiation, which may contribute to subtle anatomical changes in the brain and cognitive deficits.
Disclosure: Nothing to Disclose.

Wednesday, December 10, 2014

Study Group
29. The NIMH Research Domain Criteria (RDoC) Initiative: High Road to Rational Psychiatry or Barrier to Current Progress?

Robert Bilder*, Bruce Cuthbert, William Carpenter, Judith Ford, Stephen Marder, Ralph Hoffman, Daniel Weinberger, Daniel Pine

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

The NIMH RDoC initiative has become a focal point in debates about the pros and cons of overhauling the current diagnostic taxonomy of mental disorders, itself a long-standing target of criticism. Prior to the launch of DSM-5, NIMH Director Insel declared: "patients with mental disorders deserve better" and cited RDoC as part of the solution. The RDoC initiative has convened workshops to create a matrix of dimensions with multiple levels from molecular to symptomatic, and some NIMH program announcements and clinical trials guidance follow the RDoC framework. This study group features diverse perspectives and active discussion of opportunities and challenges posed by RDoC. Some believe the RDoC emphasis is overdue, and that the conventional diagnostic taxonomy has impeded progress. Others see the new emphasis as premature, with proposed dimensions lacking validity and reliability, and believe RDoC may be disruptive. Initial presentations (30 min total) will seed discussion. Bruce Cuthbert (NIMH) provides background about RDoC as a framework to facilitate research on neural and behavioral systems, to explicate heterogeneity within and across current disorder categories, and to help develop more precise treatment targets. Will Carpenter (MPRC) focuses on the extent to which distinctively human attributes of syndromes like schizophrenia map onto RDoC domains. Judy Ford (UCSF) addresses questions about whether specific symptoms (auditory hallucinations) are similar across clinical/non-clinical and diagnostic boundaries. Stephen Marder (UCLA) questions the associations between RDoC domains and symptoms, and asks if RDoC is premature with respect to intervention development. Daniel Pine (NIMH) suggests that RDoC and symptom-based classification are complementary, and explicates how RDoC goes beyond symptom-based classification, specifically in a developmental context, by attempting to predict outcome and treatment response. Ralph Hoffman (Yale) focuses on social salience as a dimension to help map paths to psychosis, and considers if rodent models may elucidate circuit-level mechanisms for hallucinations. Daniel Weinberger (Lieber Institute) challenges the premise that dimensions spanning phenomenology, cognition, neuroimaging, and genetic variation can transcend traditional categories to help predict outcome, disability or response to therapy. Robert Bilder (UCLA) considers the challenges of defining links across levels, focusing on models needed to validate new dimensions with higher credibility than conventional diagnoses.
Disclosure: R. Bilder, **Part 1:** EnVivo/Forum, Johnson & Johnson, Novartis, Takeda-Lundbeck, ThinkNow Inc., **Part 2:** Johnson & Johnson, **Part 4:** Johnson & Johnson; B. Cuthbert, Nothing to Disclose; W. Carpenter, Nothing to Disclose; J. Ford, **Part 1:** Husband consults to BMS.; S. Marder, **Part 1:** Abbvie, Roche, Otsuka, Pfizer, Boehringer-Ingelheim, Synchroneuron, Lundbeck, Takeda, MedAvante, **Part 4:** Sunovion, Amgen, Genentech; R. Hoffman, Nothing to Disclose; D. Weinberger, Nothing to Disclose; D. Pine, Nothing to Disclose.

Study Group
30. Neuroscience Training for Psychiatric Residents

Thomas Insel*, Joyce Chung, David Ross, Amit Etkin, Maria Oquendo

National Institute of Mental Health, Bethesda, Maryland

Neuroscience has emerged as the basic science of psychiatry yet psychiatry training has not evolved to include neuroscience training in its residency programs. Several

experiments are underway to develop neuroscience training experiences and courses for residents. This study group will bring together leaders who are addressing this problem in different ways.
Disclosure: T. Insel, Nothing to Disclose; J. Chung, Nothing to Disclose; D. Ross, Nothing to Disclose; A. Etkin, Nothing to Disclose; M. Oquendo, **Part 1:** My spouse works for Bristol Myers Squibb., **Part 2:** as above, **Part 3:** I receive royalties for the commercial use of the C-SSRS.

Panel
31. Neurodevelopmental Trajectories of Brain Function and Connectivity as Risk Factors for Internalizing and Externalizing Psychopathology

31.1 Using Graph Theory to Inform Heterogeneity in Typical Development and in ADHD

Damien Fair

Oregon Health and Sciences University, Portland, Oregon

Background: Research in psychiatry often relies on the assumption that the diagnostic categories identified in the DSM represent homogeneous syndromes. However, the mechanistic heterogeneity that potentially underlies the existing classification scheme might limit discovery of etiology.
Methods: In our current work we expand on previous brain imaging methods and use graph theory, specifically community detection, to clarifying behavioral and functional heterogeneity in children with and without ADHD.
Results: Using behavioral assays we have been able to identify several unique subgroups of children with ADHD, and importantly, in some cases, in control populations as well. Just as notably, characterizing these unique data driven sub-populations has revealed unique patterns of dysfunction in the children with ADHD. We also show in this longitudinal ADHD sample that this refined nosology is capable of improving our predictive capacity of long-term outcomes relative to current DSM-based nosology. Last, we demonstrate similar phenomena in the form of distinct sub-classifications based on patterns of functional connectivity MRI. As with the behavioral indices, the subgroups yield unique atypical connectivity patterns in the clinical population and shed light on the underlying functional patterns that may contribute to heterogeneity in ADHD.
Conclusions: These findings suggest several principles that have the potential to advance our understanding of typical and atypical developmental trajectories. The first tenet suggests that both children with and without ADHD can be classified into distinct subgroups based on psychometrics or neuroimaging. The second tenet proposes that the information in these data driven neurotypes can assist in predicting future outcomes. We argue that illumination of such phenomena will have significant practical importance for understanding typical development and to identifying the etiologic underpinnings of atypical developmental trajectories.
Disclosure: Nothing to Disclose.

31.2 Development of Brain Connectivity Through Adolescence

Beatriz Luna

University of Pittsburgh School of Medicine, Western Psychiatric Institute & Clinic, Pittsburgh, Pennsylvania

Background: Adolescence is a time of unique behavioral changes and increased vulnerability to the emergence of psychopathology including schizophrenia, mood disorders, and substance abuse. Understanding the neural basis of brain systems specialization during adolescence to adulthood can inform the neurobiological basis of vulnerability to psychopathology and effectiveness of treatment. Until recently, the investigation of the neural basis of development and psychopathology has focused on the integrity of individual brain regions such as prefrontal cortex. However, recent advances in neuroimaging (diffusion tensor imaging (DTI)) and analysis (graph theory) have enabled the assessment of network level processes underlying normal and abnormal development. Studies characterizing normative resting state and structural connectivity through adolescence including rich longitudinal data will be presented.
Methods: Studies using Diffusion Tensor Imaging (DTI) (128 8–28 year olds in a fast longitudinal design) and resting state functional Magnetic Resonance imaging (fMRI) (192 10–26 year olds) were performed. DTI data was analyzed using mixed models to characterize flexible nonlinear growth curves identifying periods of significant white matter development. In an additional study, we collected 5 minutes of resting state data controlling for head motion artifact. Graph analyzes was implemented to characterize network topology including hub network organization as well as community organization and participation coefficients reflecting inter-community integration. Specifically, connectivity to ventral striatum was investigated and its association with DTI results.
Results: Results provide evidence for both mature and maturing brain systems. DTI studies show that white matter integrity supporting prefrontal executive systems and resting state hub architecture and community structure appear on line by adolescence providing a foundation for further specialization. Hierarchical maturation of white matter connectivity is evident reflecting dynamic growth from childhood to adolescence of tracts supporting executive and sensorimotor processes with more protracted maturation through the second decade of life of tracts supporting integration with limbic systems. Resting state connectivity shows mature organization of hub architecture and network community organization. In addition, results showed evidence for dynamic growth of frontal hub interconnectivity through childhood and continued increases in inter-community communication through adolscence.
Conclusions: Together, these results suggest that adolescence is a time of unique executive and limbic system integration that may reflect particular vulnerabilities to impairment that could lead to the emergence of psychopathology but could also indicate a period of particular responsiveness to treatment.
Disclosure: Nothing to Disclose.

31.3 An Emerging Model for Big Data Biomarker Identification

Michael Milham

Child Mind Institute, New York, New York

Background: Central to the development of clinical tools for developmental neuropsychiatry is the discovery and validation of biomarkers. Resting state fMRI (R-fMRI) is emerging as a mainstream approach for imaging-based biomarker identification, detecting variations in the human connectome that can be attributed to developmental and clinical variables (e.g., diagnostic status). As the field moves works to bring together the emerging Big Data and Research Domain Criteria Project models, the challenge of meaningfully relating psychiatric phenotyping with the underlying neurobiology remains central. In this regard, the present work attempts to provide a model by data-driven analysis that can be used to identify psychiatrically-relevant behavioral constructs and map them to variations in brain function across individuals.
Methods: We first applied exploratory factor analysis (EFA) to a battery of dimensional questionnaires designed to probe various psychiatric domains, and then related these factors to an array of commonly measured resting state fMRI (R-fMRI) indices of human brain function. Data for this effort was collected as part of the ongoing Nathan Kline Institute-Rockland Sample initiative, which is acquiring phenotypically rich, multimodal imaging datasets from community-ascertained individuals ages 6.0–85.0 years old (approximately half of which have a lifetime history of one or more DSM-based psychiatric illnesses). The present work limited its focus to 288 participants aged 18–59. Phenotypic data included 16 self-report questionnaires assessing a wide range of behavioral functioning including but not limited to: impulsivity and risk-taking behavior; posttraumatic symptomatology; attention-deficit and hyperactivity symptoms; temperament and personality; depression and anxiety; empathic, callous, and unemotional traits; cognitive failures; adaptive functioning. Participants also completed an abbreviated intelligence testing, as well as an MRI session that included a R-fMRI scan (10 min, TR = 1400ms, voxel size = 2mm isotropic).
Results: EFA (principal axis factoring, oblimin rotation) revealed 5 factors, which were interpreted as: emotional dysregulation, impulse control, sociability, lifestyle factors, and mindfulness. R-fMRI-indices examined included: fractional ALFF (fALFF), Regional Homogeneity (ReHo), and Voxel-mirrored Homotopic Connectivity (VMHC). For each factor, we found a distinct R-fMRI profile across the various measures included. Of particular note was mindfulness factor, which was associated with the dorsal caudate, nucleus accumbens and brainstem. These findings are consistent with existing literature relating striatal function to attentional control.
Conclusions: The present study emphasizes both the value of obtaining broad phenotyping, sampling the wider range of psychiatric variables, and the need to reduce such phenotyping to a core set of latent variables for the purpose of relating behavior with brain function. Next steps for the presented work will focus on the delineation of age-related changes in the brain-behavior relationships, and follow-up in longitudinal designs.
Disclosure: Nothing to Disclose.

31.4 Human Amygdala-Prefrontal Cortex Development and the Role of Early Parental Care

Nim Tottenham

Columbia University, New York, New York

Background: The amygdala and mPFC play a central role in mediating affective behaviors in both typical and atypical populations. Strong evidence indicates that reciprocal connections between the amygdala and mPFC support fundamental aspects of emotional behavior in adults (e.g., fear learning and internalizing problems). However, this circuitry is slow to develop and in species that depend on caregivers for survival, its development is intimately associated with caregiver presence. Rodent and monkey models have demonstrated that this circuitry is heavily influenced by early caregiving environments, and have identified important developmental shifts in neural function associated with maternal care. The current talk will present a series of studies that characterize the development of the human amygdala-prefrontal cortex circuitry and how maternal presence modulates its development.
Methods: We present findings from functional magnetic resonance imaging and behavioral studies collected in children (4-10 years old), adolescents (11-17 years old), and young adults (18–25 years old). In the first set of studies, we examine age-related changes in amygdala-prefrontal function and connectivity during emotional learning and how it relates to developmental changes in normative fear and anxiety. In a second set of studies, we examine the effect of maternal presence when it is phasically manipulated and under conditions of chronic maternal deprivation.
Results: There were large changes across childhood, adolescence, and adulthood in amygdala-prefrontal cortex circuitry. In particular, our findings showed that childhood was marked by developmentally-unique phenotypes that changed upon the transition to adolescence, whereafter phenotypes gradually mature towards adult phenotypes. The developmental switch in amygdala-prefrontal circuitry from an immature to a mature state mediated age-relate changes in anxiety and fear behaviors. Maternal stimuli were effective in modulating the circuitry and associated fear behaviors during this period in childhood, and this effect of the mother ceased to be effective thereafter. In the case of maternal deprivation, the nature of the circuitry was age-atypical such that children showed a mature-like phenotype at an early age, and these group differences in neural phenotype were associated with higher levels of anxiety.
Conclusions: These findings suggest that human amygdala-prefrontal cortex circuitry develops slowly during childhood and adolescence, and this maturation is associated with normative changes in developmental fears and anxiety. Early caregiving environments play a significant role in establishing the neural architecture that supports emotional behaviors in adolescence. Prolonged absence of the mother

is associated with atypical phenotypes of amygdala-prefrontal cortex, with a suggestion of accelerated development. These findings are consistent with previous reports of animal models that show accelerated development of amygdala-PFC circuitry and heightened fear and anxiety. These findings show that stress-related changes in limbic-cortical circuitry may be the mediating factor between early adversity and residual emotional problems experienced by youth with a history of deprivation.
Disclosure: Nothing to Disclose.

Panel
32. When Psychiatry and Neurology Inform Each Other: Astrocyte Dysfunction and Behavioral Disease

32.1 Glial Glutamate and Metabolic Transporters as a Target for Neurodegenerative Therapy and Biomarkers

Rita Sattler

Johns Hopkins University School of Medicine, Baltimore, Maryland

Background: Glial cells play a significant functional role in neuronal function and synaptic transmission and glial dysfunction contributes greatly to the development and progression of a large number of chronic and acute neurodegenerative disorders. Here, we report the importance of two glial plasma membrane transporter proteins, the astroglial glutamate/excitatory amino acid transporters (EAATs) and the oligodendroglial lactate/monocarboxylate transporters (MCTs), both of which have been shown to be dysregulated in disease. Any imbalance of the glutamate and/or lactate homeostasis can lead to neuronal degeneration, as shown in a number of neurodegenerative disorders. The loss of function of these transporters makes them a suitable target for therapeutic intervention as well as potential biomarkers to monitor disease progression as well as therapeutic efficacy during clinical trials.
Methods: Dissociated primary mouse astrocytes as well as organotypic rat spinal cord slice cultures were screened for its ability to activate glutamate transporter EAAT2 and monocarboxylate transporter MCT1. A small molecule library of 1040 FDA approved compounds was tested in these in vitro models. Primary screening of compounds was done at 10uM concentrations, followed by full dose response curves. Select lead compounds were moved into whole animal model studies (SOD1mut mouse model) to test for in vivo efficacy. To biomark EAAT2, a F-18 radiolabeled pro-drug PET ligand was developed and tested in whole animals (rat and monkey) for dynamic measurements of EAAT2 levels in brain and spinal cord.
Results: We were able to selectively activate glutamate or lactate transport with small molecule compounds. The activation of these transporters resulted in neuroprotection of motor neurons both in vitro and in vivo, confirming the possibilities of these transporters as therapeutic targets for clinical drug development. Biomarking EAAT2 using a F-18 radiolabeled pro-drug PET ligand showed the well described loss of EAAT2 expression levels in the spinal cord of SOD1mut rats, suggesting clinical relevance of this PET tracer in patients exhibiting loss of EAAT2 during drug development. The EAAT2 PET tracer was then tested in non-human primates with similarly satisfactory detection levels and imaging signal to noise ratios. Studies are ongoing for preclinical toxicology testing necessary for IND submission to test PET tracer in human subjects.
Conclusions: In conclusion, our studies confirm the value of both glial transporters, EAAT2 and MCT1, as therapeutic targets in a variety of neurodegenerative diseases characterized by a loss of these transporters. In addition, we were able to develop and validate a potential CNS biomarker to monitor expression of EAAT2 for diagnostic purposes as well as a marker to monitor therapeutic intervention of EAAT2 expression. These studies emphasize the role of glial cells in contributing to disease progression in neurodegenerative disorders and most likely also psychiatric disorders.
Disclosure: Nothing to Disclose.

32.2 Comorbidities in Psychiatry and Neurology: Focus on Astrocytes and Adenosine Dysregulation

Detlev Bosion

Legacy Research Institute, Portland, Oregon

Background: Comorbidities between schizophrenia (SZ) and temporal lobe epilepsy (TLE) have been discussed controversially over the past 100 years and remain a highly contentious and unresolved scientific challenge. Clinically, comorbid psychiatric and cognitive impairments are among the most debilitating and persistent concerns of chronic epilepsy. Conversely, complex seizures are common in SZ patients who also suffer from characteristic cognitive impairments. Focal temporal lobe anomalies are particularly frequent in patients with SZ and TLE, and altered astrocyte function has been documented in both conditions. These clinical observations challenge the century-old conception that epilepsy and SZ are mutually antagonistic and suggest that SZ and TLE might share common pathologies. Cognitive impairment is also a characteristic hallmark of Alzheimer's disease (AD) and a common comorbidity of Parkinson's disease (PD) and Amyotrophic Lateral Sclerosis (ALS). Like patients with SZ, patients with AD have a higher likelihood for epileptic seizures compared to the general population, whereas all conditions mentioned above may share certain aspects of psychosis, depression, and sleep dysfunction. This remarkable overlap of symptoms across seemingly unrelated psychiatric and neurological conditions suggests the existence of common pathological mechanisms. The purine ribonucleoside adenosine (ADO) is a homeostatic network regulator of the brain, which is subject to metabolic clearance through astrocytes via adenosine kinase (ADK). ADO affects multiple neurotransmitter systems including dopaminergic, glutamatergic, and GABAergic neurotransmission through interaction with G protein coupled adenosine receptors. In addition, ADO has adenosine receptor independent epigenetic and bioenergetic functions. This presentation will address the 'adenosine hypothesis of comorbidities'.
Methods: Human tissue specimen from patients with TLE, AD, PD, and ALS were analyzed by immunohistochemistry

and Western blot to assess astrogliosis and ADK expression. We generated and analyzed a transgenic mouse 'co-morbidity model' by brain-wide overexpression of ADK (Adk-tg), which decreases the ambient concentration of ADO. Systemic adenosine augmentation was achieved by pharmacological inhibition of ADK, whereas local adenosine augmentation (hippocampus versus striatum) was achieved by the transplantation of engineered adenosine releasing cells.
Results: The following findings will be presented: (1) Astrogliosis is a common pathological hallmark of TLE, AD, PD, and ALS. In human specimen from all four conditions we found marked overexpression of ADK, implicating common ADO deficiency. (2) Adk-tg mice were characterized by attentional impairment, deficits in working memory, deficits in the expression of conditioned freezing, deficits in sleep regulation, altered dopaminergic function, and recurrent electrographic seizures. These findings suggest that ADO deficiency per se can cause a wide range of comorbid symptoms. (3) Using a mouse model of prepulse inhibition we demonstrated that augmenting ADO by pharmacological inhibition of ADK facilitated sensory gating, i.e., yielded an antipsychotic-like effect. In addition, focal ADO augmentation by cellular implants to the hippocampus improved cognitive performance of Adk-tg mice, whereas the same cells grafted into the striatum restored dopaminergic function. Furthermore, focal cell based ADO augmentation therapies were highly effective in the prevention of epileptic seizures.
Conclusions: Our data suggest that dysfunction of ADO signaling is common in psychiatric and neurological conditions, that ADO dysfunction can explain co-morbid phenotypes shared among seemingly unrelated psychiatric and neurological conditions, and that therapeutic ADO augmentation might be a highly effective approach for the treatment of a comorbid spectrum of symptoms in multiple psychiatric and neurological conditions.
Disclosure: Nothing to Disclose.

32.3 Effects of Cocaine Self-administration on Neuron-Astrocyte Communication in the Nucleus Accumbens

Kathryn Reissner

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

Background: In recent years, considerable evidence has indicated that maladaptive changes in synaptic strength and plasticity within the brain's reward circuitry may represent an important cellular mechanism of addiction to drugs of abuse. In parallel, evidence has also indicated an important role for astroglial modulation of adaptive synaptic strength and plasticity. However, it is unclear to what degree drug-induced adaptations in astrocyte physiology within the reward circuitry may contribute to changes in synaptic processing. Studies described herein are designed to test the hypothesis that cocaine self-administration leads to maladaptive neuron-astrocyte communication which may contribute to cellular mechanisms of chronic drug craving.
Methods: Prior to behavioral training, astrocytes in the nucleus accumbens were transduced with an AAV2/5 expressing membrane-tagged Lck-GFP under the control of an astrocyte-specific truncated GFAP promoter. Rats then were trained in chronic cocaine self-administration and extinction, versus yoked saline controls. Following two weeks of extinction training, properties of fluorescent accumbens core astrocytes were measured, including size and branching. Slices were also immunstained for synapsin I, to allow for colocalization of synaptic puncta with GFP-positive pixels containing peripheral astrocyte processes. Z stacks were analyzed using Imaris-Bitplane colocalization, and an intensity based analysis using Mander's overlap coefficient was performed. In a separate series of experiments, Western blots for astrocyte proteins including GFAP, ALDH1L1, vimentin, GLT-1 and NDRG2 were performed on whole cell lysates from nucleus accumbens following cocaine self-administration and extinction.
Results: Measures of astrocyte branching, complexity and overall size revealed no significant differences between the saline and cocaine-administering groups; however, colocalization analysis with immunoreactive puncta for synapsin I revealed a significant increase in synaptic colocalization of astrocytes following a cocaine versus saline history. No difference in overall content of synapsin-positive puncta were observed between cocaine and saline groups, indicating that increased synaptic colocalization is not a consequence of increased synapsin I expression. Moreover, Western blot analysis of astrocyte-specific proteins revealed no evidence for reactive astrocytes, consistent with findings from the fluorescent morphological analysis, but confirmed previous findings that GLT-1 is decreased.
Conclusions: These findings indicate that cocaine self-administration and extinction leads to increased synaptic coverage by astrocytes in the nucleus accumbens, without evidence of reactive astrocytes. The increased astrocyte coverage of synapses supports numerous previous reports that baseline synaptic strength is increased in the nucleus accumbens core of rats withdrawn from cocaine, and suggests astroglial contribution to this phenomenon. To further address how neuron-astrocyte communication is affected by cocaine self-administration, we are employing whole cell patch clamp electrophysiology to measure properties of astrocyte-derived glutamatergic slow inward currents (SICs) in medium spiny neurons within the accumbens core. These findings will further inform existing evidence that astroglial modulation by drug self-administration impacts synaptic and behavioral plasticity. Moreover, findings will aid continued development of glial-modulating compounds with potential as pharmacotherapies for addiction, including N-acetylcysteine, ceftriaxone and propentofylline.
Disclosure: Nothing to Disclose.

32.4 Antidepressive-like Effects of Sleep Deprivation Require Astrocyte-Neuron Communication at the Tripartite Synapse

Philip Haydon

Tufts University, Boston, Massachusetts

Background: Current pharmacological treatments for major depressive disorder take weeks for clinical efficacy, limiting

the ability to bring instant relief to suicidal patients. In contrast, a non-pharmacological intervention that rapidly alleviates symptoms of depression is a night of total sleep deprivation which is effective in ~60% of depressed patients. As the effects of sleep deprivation on depression are not long lasting, sleep deprivation is not always used clinically. However, if the mechanism mediating this action were identified, it might be possible to develop therapeutics that target this pathway as a new treatment for certain forms of depression.
Methods: Depressive-like behaviors were assessed using forced swim, tail suspension and sucrose consumption. Mice were sleep deprived for 12 hours and consequences on depressive like behaviors were assessed. Using molecular genetics and pharmacological approaches we determined the contribution of the astrocyte and adenosine signaling to the antidepressive-like effects of sleep deprivation.
Results: We demonstrate an essential role for the astrocyte in mediating the antidepressive-like effects of sleep deprivation and that these glia exert this effect by controlling extracellular adenosine, which acts through adenosine 1 receptor. Current studies are identifying glial targets that regulate extracellular adenosine in the brain with the long term view of developing pharmacological interventions that will lead to elevated adenosine and rapid antidepressive consequences.
Conclusions: Sleep deprivation modulates depressive-like behaviors in mice and these effects require the astrocyte adenosine signaling pathway that contributes to the control of sleep homeostasis.
Disclosure: Part 1: I am co-founder and President of GliaCure Inc., **Part 2:** GliaCure, Inc, **Part 4:** Sponsored Research Agreement from GliaCure to my laboratory to study Alzheimer's disease.

Panel
33. Loving Food! Peripheral and Metabolic Influences on Mesolimbic and Prefrontal Brain Circuits Controlling Food Intake

33.1 Peripheral and Metabolic Signals Influencing Mesolimbic Circuits, Food Intake, and Drug Addiction

Ralph DiLeone

Yale University School of Medicine, New Haven, Connecticut

Background: Rates of obesity have increased over the past two decades and there is intense interest in the potential causes. By analogy to addiction, studies have focused on the role of mesolimbic circuits and dopamine play in eating behavior, though the environmental inputs to dopamine (DA) circuit function remain ill defined. Interestingly, vitamin D3 deficiency rates have also increased within the last two decades. While there exists an inverse relationship between circulating vitamin D3 levels and body mass index (BMI), a causative role for this deficiency in the development of obesity has not been explored.
Methods: Mice were exposed to a high-fat diet modified to have reduced dietary vitamin D3 (HF-D; containing 15% of control HF vitamin D3). Non-deficient mice were made leptin-resistant by ad libitum consumption of the HF diet, and then given an acute treatment with fully active exogenous D3 (calcitriol, 1–10 μg/kg). Antibodies against vitamin D3 receptor (VDR) protein were used to detect VDR in the brain. Quantitative RT-PCR was used to detect the effects of calcitriol treatment on gene expression in the midbrain and striatum. Fast scan cyclic voltammetry, microdialysis, and amphetamine treatment (2.5 mg/kg) were used to measure dopamine levels after calcitriol treatment. Locomotor activity and consumption of amphetamine solutions were measured in HF-D animals as well as in calcitriol-treated animals.
Results: At day 50 of ad libitum feeding, HF-D mice had low circulating levels of vitamin D3. Moreover, after 50 days of HF-D exposure, the HF-D mice began to display a persistent gain in body weight and food intake. HF diet animals who were leptin resistance showed reduced food intake and body weight after an acute treatment with calcitriol. The VDR protein was detected in DA neurons of the midbrain and striatum of mice. In the midbrain, upregulation of tyrosine hydroxylase and dopamine transporter were observed, while in the ventral striatum, dopamine receptor type 2 was upregulated after calcitriol treatment. Acute calcitriol enhanced amphetamine-induced dopamine release as assessed by microdialysis and fast scan cyclic voltammetry. In the deficient state, HF-D mice showed attenuated locomotor responses to acute amphetamine, but compensatory increases of oral amphetamine intake. Likewise, naïve mice treated with calcitriol showed enhanced locomotor responses, but decreased consumption of oral amphetamine.
Conclusions: Dietary and exogenous vitamin D3 levels have an impact on diet-induced obesity. The expression of VDR, effects of exogenous calcitriol on DA levels, and the changes in gene expression are consistent with direct action of vitamin D3 on DA circuits. The behavioral changes observed in HF-D animals are consistent with a model of reward deficiency. Conversely, the administration of calcitriol, with its potent effects of increasing responding but reduced intake, would represent a state of "reward sufficiency". Overall, these data suggest a causative role of dietary vitamin D3 deficiency in the development of obesity and drug consumption, and that DA centers may be therapeutic targets for exogenous calcitriol.
Disclosure: Nothing to Disclose.

33.2 Interactions of the Orexigenic and Antidepressant Hormone Ghrelin with the Mesolimbic and Limbic Systems

Jeffrey Zigman

University of Texas Southwestern Medical Center, Dallas, Texas

Background: The peptide hormone ghrelin is unique in that it has both orexigenic and antidepressant actions, making it capable of linking eating and affect. Thus, ghrelin and the neuronal circuitry with which it interacts seem well positioned to mediate the strongly interconnected eating and mood-related manifestations of conditions such as anorexia nervosa and obesity. Here, I will review some of

our recent discoveries on mechanisms underlying the coordinated eating and mood-related behavioral responses to caloric restriction and chronic psychosocial stress.
Methods: My group has adapted a series of behavioral tasks with which to study complex eating behaviors in mice, including cue-potentiated feeding and conditioned place preference for high fat diet. When the latter is coupled to the chronic social defeat stress model of prolonged psychosocial stress, we also can study stress-based comfort food eating. These behavioral models are used in conjunction with standardized measures of depressive-like behavior in mice, including social interaction and the forced swim test. Mice which are tested using those behavioral models include many with which we can manipulate the ghrelin system either genetically (e.g. using the cre-lox system to selectively express GHSRs in only certain neuronal subtypes) or pharmacologically. We also use the recently-described P7C3 class of neuroprotective compounds with which to enhance ghrelin-induced effects on neurogenesis.
Results: We have demonstrated that an intact ghrelin signaling system is essential for some key, usual behavioral responses to chronic psychosocial stress and to caloric restriction, both of which increase ghrelin secretion. For example, ghrelin receptor (GHSR) deletion in mice exaggerates depressive-like behavior following chronic stress, blocks the usual food reward behavior induced by chronic stress and prevents the usual antidepressant-like behavioral response to caloric restriction. We have also demonstrated key roles for catecholaminergic neurons in particular in medicating ghrelin's antidepressant actions and its actions on food reward. Furthermore, we demonstrated that ghrelin's antidepressant effect during stress also involves protecting against exaggerated death of hippocampal neuronal precursors undergoing proliferation, leading us to reveal antidepressant efficacy for a recently-described class of neuroprotective drugs.
Conclusions: In the setting of chronic psychosocial stress, ghrelin levels elevate to protect against what would otherwise be worsened depression. Direct engagement of GHSRs on catecholaminergic neurons, including midbrain dopaminergic neurons is sufficient for ghrelin's antidepressant actions in that setting, and also is sufficient for induction by ghrelin of some complex eating behaviors. Of interest, following stress, ghrelin mediates comfort food eating behavior via engagement of those same dopaminergic neurons, suggesting the existence of at least one neurocircuit wherein eating and affect are innately linked. Furthermore, whether it is via indirect or direct effects, ghrelin's strong protection of hippocampal neuroal precursors undergoing adult neurogenesis underlies its antidepressant-like effects.
Disclosure: Nothing to Disclose.

33.3 Insulin and Glucose Manipulations Affecting Mesolimbic and Prefrontal Circuits Underlying Wanting of High-reward Foods: Implications for Obesity

Kathleen Page

University of Southern California School of Medicine, Los Angeles, California

Background: Obesity is a worldwide epidemic resulting in part from the ubiquity of high-calorie foods and food images. In a series of studies, we have shown how metabolic signals such as glucose and insulin influence brain pathways that regulate the motivation to consume high-calorie foods.
Methods: Using functional magnetic resonance imaging combined with blood sampling we examined brain, hormonal and behavioral measures of appetite and food motivation in obese and lean volunteers: 1) under euglycemia vs mild hypoglycemia using a stepped hyperinsulinemic, euglycemic-hypoglycemic clamp; 2) after an acute consumption of glucose or fructose alone; and 3) after glucose or fructose consumption in combination with exposure to high-calorie food cues.
Results: Mild hypoglycemia preferentially activated limbic-striatal brain regions in response to food cues to produce a greater desire for high-calorie foods. In contrast, euglycemia preferentially activated the medial prefrontal cortex (mPFC), an executive control region, and resulted in less interest in food stimuli. Higher circulating glucose levels predicted greater mPFC activation. The prefrontal cortex response was absent in obese individuals. Consumption of glucose, but not fructose, deactivated hypothalamic and striatal regions and increased satiety. In contrast, consumption of fructose compared to glucose resulted in greater food-cue reactivity in the nucleus accumbens and greater desire for food. Circulating levels of glucose and insulin were significantly higher after glucose vs. fructose consumption, and higher insulin levels predicted greater mPFC activation to food cues after glucose consumption.
Conclusions: These findings demonstrate a role for circulating glucose and insulin in modulating neural control over food motivation and suggest a loss of the glucose-linked restraining influence in obesity. Differential brain, hormonal and appetitive responses to fructose compared to glucose consumption may promote overeating behavior.
Disclosure: Nothing to Disclose.

33.4 Caloric Influences on Mesolimbic and Prefrontal Alterations in Obesity

Gene-Jack Wang

National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland

Background: Dopamine (DA) mediates the rewarding effects of food that can lead to overeating and obesity, which then trigger metabolic neuro-adaptations that further perpetuate excessive food consumption. Obese subjects might have impairments in dopaminergic pathways that regulate neuronal systems associated with reward sensitivity, conditioning and control.
Methods: We used positron emission tomography (PET) to assess the involvement of brain DA in adults with obesity.
Results: DA modulates circuits in pathological eating behaviours. We measured DA changes triggered by calorie intake by contrasting the effects of an artificial sweetener (sucralose) devoid of calories to that of glucose. DA changes in ventral striatum triggered by calorie intake were negatively correlated with body mass index (BMI). Individuals with normal BMI the caloric value of food increase dopamine in the ventral striatum independently of palatability. Obese individuals have reduced DA release in

ventral striatum with calorie consumption. The reduced DA release upon calories intake with increased BMI in ventral striatum might contribute to excessive food intake to compensate for a deficit between the expected and the actual response to food consumption. PET studies with food cues showed food cues increase striatal DA, indicating the involvement of DA in the motivational properties of food. Food cues also increase metabolism in the orbitofrontal cortex indicating its association with the motivation for food consumption. Striatal DA D2/D3 receptor availability (D2R) is reduced in obese subjects, which predisposes obese subjects to seek food to temporarily compensate for understimulated reward circuits. Decreased D2R in the obese subjects is also associated with decreased metabolism in prefrontal regions involved in inhibitory control that may underlie their inability to control food intake.
Conclusions: These results suggest that obese individuals suffer from impairments in dopaminergic pathways that regulate neuronal systems associated not only with reward sensitivity and incentive motivation, but also with conditioning and impulse control.
Disclosure: Nothing to Disclose.

Panel
34. Integrative Analyses of Gene Expression in Development and Disease: Focus on Autism and Schizophrenia

34.1 Transcriptional Networks in Post Mortem Autism Brain

Daniel Geschwind

University of California at Los Angeles School of Medicine, Los Angeles, California

Background: Autism, like schizophrenia is an etiologically heterogeneous disorder with over a hundred implicated susceptibility genes. A major question in the field is how these factors might converge in the brain at a molecular or cellular level. Previously we performed gene expression profiling with microarrays in a small cohort of post mortem brains and identified molecular changes that were shared across 2/3 of the cases. These changes involved down-regulation of genes related to synaptic function and up-regulation of neuro-immune genes, enriched in microglia.
Methods: We extracted RNA from 97 unique cases and controls (BA9, BA21/41/42, cerebellum) and performed 50 bp paired end sequencing using the RiboZero Gold kit), so as to analyze mRNA, miRNA and lncRNA in ASD for the first time. Differential expression (DE) analysis was performed with a linear mixed effects model, with age, sex, and brain region as biological covariates and RNA quality and batch effect variables as technical covariates. WGCNA was used to perform network analysis. We analyzed cerebellum and cortex separately.
Results: Analysis of DE in temporal and frontal cortex showed significant overlap in the significantly DE genes with previous results ($p = 2.0x10\text{-}83$, odds-ratio $= 2.6$) validating our initial findings in completely independent individuals and brain samples. In the combined analysis, we found 566 genes downregulated and 718 upregulated in ASD cortex at an FDR 5%. We also calculated that this up- and down- regulation signature is shared among about 2/3 of ASD individuals. In addition, we found 143 DE lncRNAs, many of which were co-expressed with genes down-regulated and related to synaptic function, indicating that these lncRNAs likely regulate synaptic function, several of which may be primate specific. We also identified several dozen novel and known miRNA dysregulated in ASD, and confirmed a subset of them with RT-PCR. Many fewer changes (1/10th) were observed in cerebellum than cortex.
Conclusions: This comprehensive NextGen study of ASD brain reveals profound transcriptional changes shared by 2/3 of ASD cases. This study validates the previous findings of down regulation of synaptic function and up-regulation of neuro-immune genes, indicative of microglial and astroglial up-regulation. Further, it extends these analyses to a much larger cohort of samples, and identifies dysregulated networks consisting of lncRNA and miRNA. These analyses provide a framework for understanding the effects of genetic and epigenetic changes in ASD brain. Additionally, this large dataset based on RNAseq permits comprehensive comparisons with other disorders including schizophrenia.
Disclosure: Part 1: I serve as consultant for SynapDx, **Part 2:** I serve as consultant for SynapDx, **Part 3:** I serve as consultant for SynapDx.

34.2 Co-expression Networks in Schizophrenia

Pamela Sklar

Mount Sinai School of Medicine, New York, New York

Background: Schizophrenia is a highly polygenic disorder that perturbs molecular and cellular networks underlying disease pathophysiology. The most recent Psychiatric Genomic Consortium GWAS reported more than a hundred linkage disequilibrium independent loci as risk factors for schizophrenia. As in autism, it is important to begin to understand the underlying molecular pathology. To do this we have developed a consortium, the CommonMind, to generate and analyze molecular data from human post-mortem brain samples including RNAseq and CHIP-Seq. In this study, we combined high dimensional datasets [genomic; expression quantitative trait loci (eQTL), cis-regulatory elements (CREs) annotations] to study the distribution of risk variants in gene co-expression networks.
Methods: RNA sequencing was performed in 554 human post-mortem samples (265 schizophrenia samples and 289 controls) from the DLPFC (BA9) as part of the CommonMind Consortium (CMC, http://commonmind.org) efforts. Ribozero libraries were constructed to enable detection of non-coding RNAs. Genotype data from Illumina human core and exome were available on all samples. Gene coexpression networks were constructed using WGCNA and high-density eQTL analyses were conducted. A variety of publicly available CRE annotations for promoters, enhancers or open chromatin (DNase hypersensitivity regions) were used. Furthermore, we used in-house

generated CRE (promoter) annotations for neuronal cells sorted from the DLPFC of controls and cases with schizophrenia.
Results: Differential expression was detected with 255 upregulated transcripts and 176 downregulated transcripts in the DLPFC at an FDR of 5%. Differentially expressed genes were enriched for rare nonsynonymous DNA mutations ($p = 0.021$) previously reported in a Swedish case-control exome sequencing study. WGCNA gene coexpression analysis identified 37 modules of which 11 are dysregulated in SCZ at FDR 5%. Among those, 3 modules are upregulated (primarily related to neuronal function) and 8 are down regulated (primarily related to neuronal function, synaptic function, glutamate transmission, PSD and mitochondria/energy production).
Conclusions: In this study, we applied a stepwise approach to identify a subset of putative causal SNPs and genes and then examined their distribution in gene coexpression networks. Overall, these results support the existence of convergent genetic abnormalities in schizophrenia that could potentially drive the disease leading to molecular and cellular alterations.
Disclosure: Part 1: Board of Directors, Catalytic Inc, **Part 4:** Research grant to my institution from Eli Lilly Research grant to my institution from Roche as part of a public-private consortium Research grant to my institution from Takeda as part of a public-private consortium.

34.3 Transcriptional Regulation in Normal Human Brain Development and Psychiatric Disorders

Nenad Sestan

Yale University School of Medicine, New Haven, Connecticut

Background: The development of the human brain is an immensely complex process, which is likely reflected in the complexity of the underlying transcriptional events. Gene expression and its precise spatio-temporal regulation, particularly by cis-regulatory elements and non-coding RNAs, are crucial for normal human brain development and are thought to be altered in major psychiatric disorders.
Methods: Recent advances in functional genomics are providing unprecedented opportunities to dissect the transcriptional regulatory mechanisms underlying normal and abnormal brain development.
Results: I will present unpublished data on the mapping of cis-regulatory elements, epigenetic modifications and gene expression across multiple regions of the developing human brain, which have been generated using RNA-seq, ChIP-seq and the Illumina 450K platforms by the BrainSpan consortium (www.brainspan.org). For many disorders, including psychiatric disorders, genome-wide association studies have identified multiple risk-associated, non-coding single nucleotide polymorphisms (SNPs). To shed light on the possible functional role of these SNPs, we surveyed brain active regulatory elements and non-coding RNAs for the presence of potential disease-associated regulatory SNPs. We observed that three times as many SNPs with strong evidence of association with schizophrenia or with bipolar disorder lie in regions marked with histone modification H3K4me3, as compared to all SNPs assayed. The enrichment for H3K27ac-marked regions (not also marked by H3K4me3) among the schizophrenia SNPs was 11-fold, although the proportion of marked SNPs is lower and no enrichment was observed for bipolar disorder. Since epigenetic and transcriptional dysregulation have been implicated in the pathophysiology of autism spectrum disorders (ASD), I will provide an update on the similar integrated analysis in ASD currently being conducted in our laboratory.
Conclusions: Alterations in brain active cis-regulatory elements and gene expression are associated with psychiatric disorders.
Disclosure: Nothing to Disclose.

34.4 A Functional Role for Non-coding Variation in Schizophrenia Genome-wide Significant Loci

Panos Roussos

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Background: A recent GWAS in schizophrenia (SCZ) identified 22 loci that reached genome-wide significance. The majority of identified SNPs reside within non-coding regions. In order to be able to understand these associations mechanistically, it is important to develop strategies for honing in on regions and SNPs more likely to have functional effects.
Methods: Brain eQTLs were generated in 8 datasets. Brain cis-regulatory elements (CRE) (active promoter, active enhancer, poised promoter, repressed enhancer and open chromatin regions) were generated based on ChIP-seq of histone modifications. GWAS SCZ SNPs were classified into categories: eQTL, CRE, eQTL in a cis regulatory element (creQTL) or functionally unannotated variants. Relative enrichment for the categories was calculated using an empirical cumulative distribution of the GWAS P values after controlling for genomic inflation. We mapped the physical interaction of enhancers in two genes (CACNA1C and NGEF) with the transcription start site of each gene in human prefrontal cortex ($n = 6$) and hiPSC derived-neurons by chromosome conformation capture (3C) assay.
Results: The largest enrichment of GWAS SNPs occurs in eQTLs, active promoters and enhancers. Enrichment is greater when the combined creQTL functional category is analyzed for all types of CREs (CRE range: 1.58–7.08 fold; creQTL range: 4.06–29.51 fold). We detected overlapping eQTL and GWAS signals using the regulatory trait concordance score for 10 of 22 intervals, four times the number expected by chance ($P = 2x10\text{-}5$). The SCZ-related eQTLs are associated with expression of 17 genes, 5 of which are associated with loci within enhancers. For CACNA1C and NGEF genes, we identified enhancer regions that demonstrate increased interaction with the promoter and affect transcriptional activity of each gene.
Conclusions: Our findings point to a functional link between SCZ-associated non-coding SNPs and 3-dimensional genome architecture associated with chromosomal loopings and transcriptional regulation in the brain.
Disclosure: Nothing to Disclose.

Panel
35. State and Trait Findings in Bipolar Disorder: A Series of Imaging Studies

35.1 Functional Prefrontal Differences in Youth with and At Risk for Developing Mania

Melissa DelBello

University of Cincinnati, Cincinnati, Ohio

Background: Offspring of parents with bipolar disorder have a greater risk for developing mood disorders. The risk of developing mania is up to 30% for depressed youth with a bipolar parent. Neuroimaging studies of bipolar youth have found alterations in prefrontal cortical and amygdala activation while performing tasks involving emotional stimuli. During emotional stimuli presentation, healthy youth typically exhibit decreased ventral prefrontal inhibition of amygdala activation and during tasks of attention exhibit increased prefrontal inhibition of amygdala activation. Although structural amygdala abnormalities are present in bipolar youth, they are not present prior to the onset of bipolar disorder. However, few studies have assessed the progression of functional abnormalities in youth at risk for and those with mania. Such studies, might lead to the identification of biomarkers of risk and resilience for bipolar disorder. We examined prefrontal-amygdala dysfunction among four groups of youth with varying risk for bipolar disorder. We hypothesized that there would be a progression of prefrontal cortical and amygdala dysfunction during presentation of emotional stimuli among youth at difference levels of risk for developing mania.
Methods: Youth with new onset mania ($n = 32$), youth with depression and a parent with bipolar I disorder ($n = 32$), youth with no DSM-IV Axis I mood disorder and a parent with bipolar I disorder ($n = 32$), and healthy youth ($n = 32$) without any Axis I disorder in themselves or any first-degree relative underwent an fMRI scan while performing a Continuous Performance Task with Emotional & Neutral Distractors. Region of interest analyses using percent change in activation from baseline stimulus were calculated for emotional (vs. neutral) stimuli. The difference between activation associated with emotional vs. neutral stimuli was also calculated.
Results: There are no statistically significant group differences in demographic variables. There were no statistically significant group differences in either left or right amygdala activation during emotional or neutral stimuli presentation. Within each group there was increased amygdala activation during emotional (vs. neutral) stimuli presentation ($p < 0.001$). The BP group experienced significantly less activation compared to the ARD or HC groups in the anterior cingulate ($p = 0.04$, $p = 0.01$, respectively). No other group differences in anterior cingulate activation were found. Right BA 10 activation was greater in the ARH group than in the ARD group ($p < 0.02$) and in the ARH compared to the BP group ($p < 0.03$) during emotional (vs. neutral) stimuli presentation. Additionally, the BP group exhibited greater activation than HC ($p < 0.04$) in left BA 11-12 during emotional stimuli presentation. Left BA 44 activation was greater in the ARD compared with the ARH group during emotional stimuli presentation, as well as in the ARD and ARH groups compared with HC during emotional (vs. neutral) stimuli presentation ($p < 0.004$ and $p < 0.01$, respectively). Finally, right BA 44 activation was greater in the HC compared with ARH group during neutral stimuli presentation and in the ARD compared with HC during emotional (vs. neutral) stimuli presentation ($p < 0.05$ and $p < 0.03$, respectively).
Conclusions: As expected, there was a consistent increase in amygdala activation during emotional (vs. neutral) stimuli, suggesting similar amygdala functioning across the four groups. Activation differences in ventral prefrontal and anterior cingulate regions during emotional stimuli suggest that these regions may be risk or disease markers involved in bipolar disorder. Specifically, abnormalities in left BA 44 may indicate a risk marker. Furthermore, right BA 10 dysfunction in the ARH group suggests a possible marker for risk or resilience. In summary, amygdala functioning appears to be intact in youth at risk for developing bipolar disorder and at illness onset. However, ventral lateral prefrontal dysfunction may be a useful marker for bipolar disorder. Longitudinal analyses to better determine the progression of ventral lateral prefrontal dysfunction in youth with and at risk for developing bipolar disorder are needed.
Disclosure: Part 1: Research: AstraZeneca, Amylin, Eli Lilly, Pfizer, Otsuka, GlaxoSmithKline, Merck, Martek, Novartis, Lundbeck, Purdue, Sunovion, and Shire Consultant: Bracket, Guilford, Merck, Pfizer, Dey, Lundbeck, Springer, Sunovian, Supernus, and Otsuka Speaker's Bureau: Otsuka, Merck, and Bristol-Myers Squibb. Royalties: Guilford, **Part 2:** Otsuka, Bristol-Myers Squibb, **Part 4:** AstraZeneca, Amylin, Eli Lilly, Pfizer, Otsuka, GlaxoSmithKline, Merck, Martek, Novartis, Lundbeck, Purdue, Sunovion, and Shire.

35.2 NAA Normalization Associated with Lamotrigine Treatment for Bipolar Depression

Mark Frye

Mayo Clinic, Rochester, Minnesota

Background: In the present study we examined N-acetylaspartate (NAA), a general marker of neuronal viability, and total NAA (tNAA), the combined signal of NAA and N-acetylaspartylglutamate (NAAG) in bipolar depression before and after lamotrigine treatment. Given that NAA is synthesized through the direct acetylation of aspartate by acetyl-CoA-L-aspartate-N-acetyltransferase, our hypothesis for this investigation was that treatment with lamotrigine would be associated with an increase in NAA.
Methods: Subjects with bipolar depression underwent two-dimensional (2D) proton magnetic resonance spectroscopy of anterior cingulate ($3\,cm^3$ voxel at 1.5 T 1HMRS) at baseline ($n = 15$) and after 12 weeks of lamotrigine treatment ($n = 10$). A group of age-matched healthy control subjects ($n = 9$) were scanned at baseline for comparison.
Results: At baseline, subjects with bipolar depression had significantly lower NAA and tNAA compared to controls. There were significant increases in NAA levels ($1.39 + 0.21$;

$p = 0.006$) and tNAA levels ($1.62 + 0.25$; $p = 0.014$) after twelve weeks of lamotrigine treatment.
Conclusions: These data suggest a NAA deficit in bipolar depression that is normalized after treatment with lamotrigine. Future research is encouraged to evaluate whether baseline NAA could be a potential biomarker to identify lamotrigine response patterns and whether this functional brain change has an associated clinical response.
Disclosure: Part 1: Grant Support Assurex, Myriad, Pfizer, National Institute of Mental Health (NIMH), National Institute of Alcohol Abuse and Alcoholism (NIAAA), Mayo Foundation, GlaxkoSmith Kline Consultant (Mayo) Janssen Global Services, LLC, Mitsubishi Tanabe Pharma Corporation, Myriad, Sunovion, Supernus Pharmaceuticals, Teva Pharmaceuticals CME/Travel Support CME Outfitters Inc. Speakers' Bureau NONE Financial Interest / Stock ownership / Royalties NONE, **Part 4:** Grant Support Assurex, Myriad, Pfizer, National Institute of Mental Health (NIMH), National Institute of Alcohol Abuse and Alcoholism (NIAAA), Mayo Foundation Consultant (Mayo) Janssen Global Services, LLC, Mitsubishi Tanabe Pharma Corporation, Myriad, Sunovion, Supernus Pharmaceuticals, Teva Pharmaceuticals CME/Travel Support CME Outfitters Inc. Speakers' Bureau NONE Financial Interest / Stock ownership / Royalties NONE.

35.3 fMRI Changes Following Successful and Unsuccessful Treatment in First-episode Bipolar Mania

Stephen Strakowski

University of Cincinnati/UC Health, Cincinnati, Ohio

Background: Functional neuroanatomic abnormalities, compared with healthy subjects, have been commonly reported in bipolar disorder. However, interpreting these findings is complicated by chronic illness and uncertain effects of treatment (and symptom improvement). To address these issues, we used fMRI to identify changes in brain areas associated with bipolar disorder during the course of treatment response in patients during their first episode of mania. The primary focus was in ventral prefrontal networks and affiliated structures (e.g., amygdala).
Methods: Bipolar individuals experiencing a first manic episode were recruited at the University of Cincinnati then treated with either lithium or quetiapine for 8 weeks. The received fMRI scans at baseline (time = 0) then one- and eight- weeks after initiating treatment while performing a mixed affective cognitive task (CPT-END). Forty-two subjects completed all three visits and are the subjects of this report. Healthy subjects ($N = 41$) were also similarly scanned to aid interpretation.
Results: Half of the subjects ($N = 21$) achieved remission by week 8. Region of interest (ROI) analyses demonstrated significant visit by remission effects ($p < .05$) in left BA 10 and right amygdala for emotional stimuli and subgenual cingulate for target and neutral (nonemotional) stimuli. A number of other regions exhibited overall remission and visit effects throughout these emotional networks. Voxel-wise fMRI analyses supported and extended these ROI results.
Conclusions: fMRI data identify regional brain responses in the treatment of first-episode mania that are associated and perhaps may predict treatment response.
Disclosure: Part 1: DSMB chair for Sunovion (pediatric schizophrenia, ADHD and bipolar studies) and Novartis (schizophrenia study). Procter & Gamble, EAP Consultant, **Part 2:** DSMB Chairmanships (cumulatively), **Part 4:** none as PI.

35.4 MRS Measures of Prefrontal Neuronal Activity: A Comparison Between Bipolar Mania and Depression

Caleb Adler

University of Cincinnati College of Medicine, Cincinnati, Ohio

Background: Bipolar disorder is a longitudinal disorder characterized by periods of euthymic mood punctuated by mood extremes including manic and depressive episodes. Controversy remains however, around the nature of those mood episodes. While classically regarded at representing two extremes of a "bipolar" spectrum, some researchers have suggested that bipolar disorder represents a disorder of mood dysregulation that may at times present with predominantly manic and other times depressed symptomatology. Though limited, at least some neurofunctional studies suggest the latter; bipolar patients show a similar pattern of prefrontal changes consistent with a loss of prefrontal regulatory capacity during both manic and depressive periods. In this study we used [1H]MRS to measure neurochemical markers of the excitatory neurotransmitter, glutamate, and prefrontal metabolism during episodes of mania and depression. We hypothesized that these neurochemicals would not differ between bipolar manic and depressed subjects.
Methods: 110 bipolar individuals experiencing a first manic ($n = 59$) episode or early episode of depression ($n = 51$) were recruited at the University of Cincinnati. All subjects participated [1H]MRS scans examining 2x2x2 voxels centered in the left and right ventrolateral prefrontal cortex, and the anterior cingulate. Glx; a combination of glutamate, glutamine, and GABA; N-acetylaspartate (NAA), and Cr-PCr (Cr) were compared between groups. The latter two neurochemicals are associated with neuronal metabolism. A secondary analysis examined the relationship between concentrations of these neurochemicals and mania ratings, depression ratings, and a well-validated mania subscale for aggression.
Results: Manic and depressed bipolar subjects did not differ in glx, NAA, or Cr. differed from healthy controls or each other in any of the MRS measures obtained. Glx, NAA, and Cr in bipolar subjects did not significantly correlate with YMRS or HAM-D scores. Glx inversely correlated with the YMRS aggression subscale across subjects but correlations were significantly greater in manic patients.
Conclusions: MRS data examining correlates of neuronal activity in the prefrontal cortex of bipolar patients suggest that mania and depression are associated with similar neurochemical changes in these brain regions. These findings support suggestions that these affective states may

reflect a singular underlying physiological phenomenon that yields a range of mood symptoms.
Disclosure: Nothing to Disclose.

Panel
36. Drug Development of the Vasopressin and Oxytocin System in ASD

36.1 Melanocortin Receptor Agonists Facilitate Oxytocin-dependent Social Behaviors and Rescue Social Impairments in Prairie Voles: Implications for Novel Therapies for Treating Social Impairments in Autism

Larry Young

Emory University School of Medicine, Atlanta, Georgia

Background: Studies in animals and humans suggest that oxytocin (OT) enhances certain aspects of social cognition, in part by enhancing the salience and reinforcing value of social stimuli. In monogamous male and female prairie voles, OT acts within the striatum and prefrontal cortex to promote enduring pair bonds as assessed by partner preference formation, an index of social cognition. In mice, OT facilitates olfactory based social recognition. Polymorphisms in the OT receptor gene (OXTR) are associated with autism and predict face recognition memory in humans, suggesting that the role of oxytocin in social information processing is conserved from rodent to man. I will discuss a novel pharmacological approach to enhance OT-dependent social cognition and rescue social impairments due to early-life social deprivation by stimulating endogenous OT release in prairie voles. I will also discuss gene by environment interactions in relation to social impairments.
Methods: Neonatal prairie voles were subjected to daily 3 hr per day social isolations for the first week of life. As adults, subjects were tested for the ability to form partner preferences and brain OXTR was quantified. In a separate experiment, neonatally isolated pups were treated s.c. with the melanocortin receptor agonist, melanotan II (MT II), during the first week of isolation and tested for partner preference formation as adults. The effects of acute melanotan II treatment on partner preferences were also examined. Finally, we examined the role of a polymorphism in the vole Oxtr gene on creating susceptibility to early-life social neglects.
Results: As a group, female prairie voles experiencing neonatal social isolation displayed impairments in partner preference formation. This impairment was rescued by neonatal MT II treatment. Interestingly, OXTR expression in the striatum robustly predicted susceptibility to early-life isolation, with animals with low receptor density being severely impacted, while those with high receptor density were resilient. A polymorphism in the Oxtr 3' untranslated region robustly predicted OXTR binding in the striatum. Finally, MT II treatment in adults activated OT neurons, stimulated central OT release, and facilitated partner preference formation in the absence of mating. The effect was blocked by an OT antagonist.
Conclusions: The melanocortin system represents a novel pharmacological target for stimulating endogenous OT release and may be useful for enhancing social cognition in disorders with social impairments, including autism. Furthermore, polymorphisms in the OXTR effect expression in the striatum and susceptibility to early-life social adversity.
Disclosure: Part 1 The author has applied for a Patent for the use of melanocortin agonists to treat psychiatric disorders with social impairments.

36.2 A New Vasopressin V1a Antagonist Restores Normal Social Behavior and Reveals a Specific Brain Network in the Rat Valproate Model of Autism

Christophe Grundschober

F. Hoffmann-La Roche Ltd, Basel, Switzerland

Background: The neuropeptide vasopressin plays an important role in regulating social behavior. We therefore wanted to investigate the role of central vasopressin 1a (V1a) receptor signaling in the phenotype of the rat valproate (VPA) model of autism. In humans, prenatal exposure to the anticonvulsant drug valproate has been associated with an increased risk of autism in the newborn. In rats, a single injection of valproate to pregnant dams at day 12.5 of gestation, the time of the neural tube closure, induces a range of behavioral abnormalities in the offspring, such as deficits in social behavior, working and spatial memory and increased locomotor activity. Based on synaptic and phenotypic similarities, the rat VPA model can be considered a valid model of human autism.
Methods: From postnatal day 60 on rats prenatally exposed to VPA were treated daily during 3 weeks with a new brain penetrant V1a receptor-specific small molecule antagonist. Their behavior was assessed in the Morris water-maze and in the 3-chamber social interaction test. Serotonin, norepinephrine and dopamine levels in prefrontal cortex and nucleus accumbens were measured by microdialysis. Finally, VPA rats and wild-type controls were scanned by functional magnetic resonance imaging at postnatal day 60 and after 1-week chronic V1a antagonist treatment, to reveal changes in brain perfusion due to prenatal exposure to VPA and potential normalization by V1a antagonism.
Results: Chronic treatment for 3 weeks with our V1a receptor-specific small molecule antagonist completely reversed the impairments in social behavior, spatial memory and learning typically seen in VPA rats. Prefrontal serotonin and dopamine levels as well as nucleus accumbens norepinephrine levels were normalized by the compound. In functional magnetic resonance imaging VPA rats were found to be characterized by reduced brain perfusion in cortex, inferior colliculus, hippocampus and hypothalamus and increased brain perfusion in VTA, striatum and superior colliculus compared to control rats. Chronic V1a antagonism specifically normalized brain perfusion in striatum, VTA and colliculus.
Conclusions: Our data show that chronic inhibition of vasopressin V1a receptors restores normal behavior in VPA rats by normalizing perfusion in a brain network important for salience detection, sensory processing and reward.

These results suggest that V1a antagonists have the potential to improve social interaction in autism, a core symptom for which there is currently no drug treatment.
Disclosure: Part 1: Equity Ownership of F. Hoffmann-La Roche Ltd, **Part 5:** F. Hoffmann-La Roche Ltd.

36.3 V1a Antagonist (RG7713) Proof of Mechanism Study in High Functioning Autism Spectrum Disorder: Clinical, Biomarker and Social Learning Effects

Eric Hollander

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Background: Vasopressin plays a critical role in salience of social signals, social reward and social threat, which contribute to the social communication domain in ASD. Experimental therapeutic interventions to block V1a receptor signaling may modulate this domain in ASD and address a large unmet need. Support for a role of the vasopressin system in ASD is provided by studies of the arginine vasopressin receptor 1A (AVPR1A) gene that encodes the V1a receptor and is located on chromosome 12q. Both over- and under- transmission of specific, but different alleles of this gene has been reported in autistic individuals. Consistent with behavioral studies in animals, these risk alleles have been found to modulate activation of the amygdala during emotional face processing and to be associated with specific personality traits in healthy volunteers. Similarly, intranasal administration of vasopressin was shown to modulate the activity of a network involved in processing of emotional information with a specific effect in subgenual cingulate regions. Further evidence for a role of vasopressin in modulating behaviors of relevance to ASD is provided by studies showing altered cerebrospinal levels of vasopressin in aggression, restricted and repetitive behaviors, mood and stress reactivity. Olfaction is important for social interactions in animals. In autism, altered olfaction may contribute to abnormal processing of socially salient information and provide a biomarker indexing disruptions of the embryogenic development within critical time-frames. In summary, evidence both from human and animal studies strongly implicate the V1a receptor in mediating and modulating key social deficits expressed in ASD. The potent and highly selective V1a receptor antagonist RG7713 provides a novel and first approach to address core deficits of ASD.
Methods: To explore the impact of a novel V1a antagonist RG7713 vs. placebo on core social cognition measures, exploratory biomarkers, and safety/tolerability measures in adult high functioning ASD. High-functioning young adults (M = 23.4 years, range = 18 to 40 years) with autism (n = 19) participated in a multi-center (3-site), randomized, double-blind, placebo-controlled, cross-over study of the effects of novel vasopressin 1a receptor antagonist RG7713. Each participant was seen on two separate days (1 week apart) for dosing a 2 hour infusion of RG7713 or placebo. Safety/tolerability, PK, PD, core social cognition (Affective Speech Recognition (ASR), Reading the Eyes in the Mind (RMET)), olfaction (Sniffin Sticks), eye tracking (biological motion) and AVPR1A polymorphisms measures were collected. On ASR, patients listen to recorded sentences of neutral content read with different emotions (angry, disgusted, fearful, happy, lust, neutral, sad, surprised) and circle the correct emotion.
Results: At baseline, better adaptive functioning (Vineland and ADOS) and higher IQ positively correlated with better scores on social cognition measures (Affective Speech Recognition (ASR), Reading the Mind in the Eyes (RMET)). Of note, ASD subjects with better olfaction (N = 10) (vs poorer olfaction (N = 9)) had better scores on social cognition measures (ASR and RMET) at baseline. The Va1 antagonist showed some evidence of anxiolysis. There were large negative effect sizes (ES = -.8) of the Va1a antagonist vs. placebo on the social cognition ASR subscales measures of Lust and Fear. There were selected delayed effects on ASR from infusion 1 to infusion 2 consistent with an effect of social learning. This delayed effect of V1a vs placebo was also seen on an eye tracking measure (biological motion).
Conclusions: This study provides preliminary evidence of the ability of a novel V1a antagonist RG7713 to affect core symptoms of social cognition. Level of adaptive functioning and intelligence at baseline influenced social cognition measures. Patients with impaired olfaction at baseline performed worse on social cognition measures, suggesting that impaired olfaction may contribute to more deficient development of social communication in autism and/or index more severe pathology in brain circuits subserving social communication. Olfactory capabilities may serve as a biomarker easily assessed clinically and useful for stratification in clinical trials. Effects on social learning may persist well past the pharmacokinetic effects of the compound. These results should be taken as preliminary but may help to guide the development of new oral vasopressin antagonist interventions in ASD.
Disclosure: Part 1: scientific advisory board and consulting: Roche, Coronado, **Part 2:** IP licensing agreement with Retrophin, **Part 4:** research grants: Simons Foundation, Prader Willi Research Foundation, Roche, Forest, Sunovion, Coronado.

36.4 Oxytocin Engages Target Neural Systems for Social Motivation and Social Cognition

Kevin Pelphrey

Yale University, New Haven, Connecticut

Background: The hormone oxytocin (OT) plays an important role in regulating social affiliative and perceptual processes known to be impacted in autism spectrum disorder (ASD). Genetic variability in the oxytocinergic system has been linked to ASD susceptibility. Administration of OT to typically developing adults alters key social behaviors, increasing gaze to the eyes, detection of biological motion in noise, accuracy of emotion recognition, and encoding of positive social stimuli. Burgeoning interest in the use of intranasal OT administration as a pharmacological intervention for ASD makes it critical to understand the functional impacts of OT on neural systems supporting social perception. In the present work, we used psychophysiological interaction (PPI) analysis of functional magnetic resonance imaging (fMRI) data to test the hypothesis that

OT administration would increase connectivity between nucleus accumbens (NAcc), a sub-cortical structure innervated by oxytocinergic fi bers, and other brain regions important for basic social perceptual processing and/or for reward and motivational processes.
Methods: Participants were 21 children ages 8.0-16.9 years, with a diagnosis of ASD via expert clinical evaluation guided by the Autism Diagnostic Observation Schedule and the Autism Diagnostic Interview-Revised. We used a randomized, double-blind, placebo-controlled crossover design. The study consisted of two visits (one OT, one placebo (PLC) separated by a minimum of 72 hrs (average: 21 d, range: 3-78 d). Dosing of OT was age-dependent with participants age 16+ receiving 24 IU, ages 12–15 receiving 18 IU, and ages 8-11 receiving 12 IU. Exclusion criteria were 4 mm of movement in any dimension and/or 25% of volumes classified as motion outliers according to the DVARS metric for either visit. In the analyzed samples, scans from OT and PLC visits did not differ in absolute or relative RMS movement or % motion outliers. Standard motion parameters and motion outlier time points were included as covariates in first-level PPI models. FMRI data processing was carried out using FSL's FEAT v. 6.00. NAcc seeds for use in PPI analyses were anatomically segmented from participants' T1-weighted images using FSL's FIRST. At first level, the psychological (PSY) regressor of interest was convolved with a double-gamma hemodynamic response function. The physiological (PHYS) regressor was the mean time series from left or right NAcc. The PPI regressor was the interaction term between the PSY and PHYS regressors. A regressor of no interest was included to account for shared variance between trial types. At second level, PPI estimates for OT versus PLC activity were assessed within individuals, with the OT > PLC contrast being of interest. Group-level analysis used FLAME 1 + 2 with automatic outlier detection and deweighting. Age, full scale IQ, and sex were included as covariates, with the group mean estimate of the PPI effect being the regressor of interest. Participants were scanned at 3 Tesla during two fMRI tasks. In one task, participants viewed blocks (6 per condition, 24 s each) of coherent point-light displays of biological motion interleaved with scrambled versions of these displays. In another task, Participants listened, eyes shut, to angry and happy non-word vocalizations (3 blocks per condition, 15 s per block), separated by 15 s silences.
Results: OT induces an increase in connectivity between NAcc and ventromedial prefrontal cortex (vmPFC) that is selective to the perception of coherent biological motion. OT induces increases in connectivity between NAcc and regions of precuneus and auditory cortex that are selective to the perception of positively valenced vocalizations.
Conclusions: Previously, increases in functional connectivity between vmPFC and NAcc have been linked to increases in the individual specific reward value of a stimulus. Thus, increased NAcc—vmPFC connectivity during biological motion perception may indicate increased valuation of the social stimulus under OT versus PLC. Individuals processing the reward value of music show increases in connectivity between NAcc and auditory cortices related to increases in perceived value. Here, increased connectivity between NAcc and auditory cortices may indicate that happy voices are more rewarding to individuals with ASD under OT versus PLC.
Disclosure: Nothing to Disclose.

Panel
37. Cross-species Research on Social Development: Implications for Neurodevelopmental Disorders

37.1 The Effects of Early, Profound Deprivation on Brain and Behavioral Development

Charles Nelson

Children's Hospital, Boston, Massachusetts

Background: Many aspects of postnatal brain development depend critically on experience for development to proceed normally. Much of the work in neuroscience examining experience-dependent changes on brain development make use of animal models, in which experience can be manipulated and the resulting effects on behavior observed. In the human, however, it is much more difficult to intentionally manipulate behavior; rather, most investigators take advantage of so-called "accidents of nature," such as children born deaf or visually impaired and are then treated for their auditory or visual impairment. I will focus on an extreme version of early deprivation – specifically, infants abandoned and then reared in institutions. I will focus on the effects of early vs. continued institutional rearing on brain development (EEG, MRI), and on the risk for developing an autism spectrum disorder.
Methods: The Bucharest Early Intervention Project (BEIP) was a randomized controlled trial of foster care as an intervention for early institutionalization. A total of 136 children who had been abandoned at birth and placed in various institutions in Bucharest, Romania were targeted for study, along with a sample of 72 children who lived with their biological parents in the greater Bucharest community (and who had no history of institutional rearing). Following an extensive baseline assessment, half of the institutionalized children were placed in high quality foster care group (FCG) created by the research team and the other half to care as usual (CAUG) (continued institutional care; average age at placement = 22 months). This sample has been carefully studied through the first 12 years of life.
Results: I will report that having ever spent time in an institution leads to profound disruptions in EEG; specifically, independent samples t-tests were used to examine differences between the CAUG and FCG groups. Children placed before 18 months and between 18 and 24 months had significantly greater alpha power than children placed after 24 months of age $t(37) = 2.949$, $p = .005$ and $t(40) = 2.134$, $p = .039$, respectively. There were no differences between the groups of children placed before 18 months and those placed between 18 and 24 months ($t(23) = .503$, $p = .620$). Our MRI findings reveal a more nuanced view of development. When EIG children were separated into FCG and CAUG, both groups had significantly smaller total cortical gray matter volume compared with the NIG (CAUG $B = -34.71$, $P = 0.02$; FCG $B = -35.05$, $P = 0.02$). After adjustment for age and sex, children in the CAUG and FCG

continued to have smaller total cortical gray matter volume than children in the NIG. Finally, we report an overall prevalence of autism of about 5 percent of the total sample of ever institutionalized children (there were no children in the never institutionalized group who met criteria for an ASD). Scores on our autism screen (Social Communication Questionnaire; SCQ) were highest among children who were living in institutions at age 8, and lowest among children who had spent all their time post randomization in our high quality foster care families ($p = .022$).
Conclusions: Exposure to early profound deprivation dramatically alters the trajectory of brain development, leading to a smaller cortex, a reduction in both grey and white matter, reductions in EEG activity, and an elevated risk of developing autism. Spending less time in institutional are and more time in a family, however, are associated with increases in white matter and EEG power and a lower risk of developing autism.
Disclosure: Nothing to Disclose.

37.2 Early Life Trauma with Attachment Produces Later Life Neurobehavioral Deficits but are Paradoxically Rescued by the Odors Paired with the Early Life Trauma

Regina Sullivan

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Background: Early life trauma disrupts neurobiological development, including the amygdala, a brain area involved in emotional learning and expression, as well as social behavior. Clinical studies suggest that the caregiver can modulate the infant's response to trauma and influence outcome. We use an animal rodent model and infant trauma induced by either fear conditioning with or without the caregiver or being reared with an abusive mother. The goal of this research is to better understand how the caregiver can modulate the pups' neural response to trauma and identify mechanisms that might initiate pathways to pathology. Our early life trauma manipulations do not have short-term consequences: indeed they produces a new maternal odor. However, amygdala related social behavior deficits emerge as pups reach weaning age and depressive-like behaviors soon follow.
Methods: Infant rat pups were odor-shock conditioned with or without the mother present or reared with an abusive mother. The conditioning groups involved odor paired with a mild 0.5 mA shock (Paired) and controls involving unpaired presentations of the odor and shock (Unpaired) or just the odor (Odor-only). Testing involved assessment of learning but also social behavior. The amygdala was assessed during the early life experience and testing using microarray, electrophysiology and c-Fos. The maternal odor (either natural or one learned through the odor-shock infant conditioning) was presented during some of the testing.
Results: Pups given Paired odor-shock experience or rearing with an abusive mother learned a greatly preferred maternal odor from these experiences. However, compared to controls, trauma experienced (Paired odor-shock) animals showed later life deficits in social behavior and forced swim test (FST) that was rescued by the infant odor. Social behavior ($F(1,24) = 4.64$, $p = 0.0415$) and FST ($F(1,26) = 61.89$, $p < 0.0001$). Unpaired odor-shock did not produce social behavior deficits or depressive-like behavior indicating engagement of the attachment system, not trauma alone is important. A significant difference was also found in the adult amygdala (paired pulse inhibition) that as rescued by the infant odor ($F(1,11) = 6.90$, $p = 0.0236$). Affymatrix gene expression (DAVID analysis) showed the odor was associated with altered serotonin and glucocorticoid activity. A causal relationship was established by blocking serotonin during the FST during infant odor presentation, while increasing amygdala serotonin/blocking systemic corticosterone was sufficient to mimic the infant odor's ability to rescue the FST ($F(1,20) = 14.54$, $p = 0.0011$; drug infusion $F(1,20) = 5.67$, $p = 0.0273$; infant condition $F(1,20) = 5.01$, $p = 0.0368$).
Conclusions: Early life trauma associated with attachment produces long-lasting and persistent changes in social behavior and depressive-like behavior and implicates the amygdala as partially responsible for the behavioral deficits. This effect was not seen following infant experience with control shock presentations. Thus, engagement of the attachment circuit during the trauma provides a major modulatory role in the neural response to trauma. Importantly, while this effect appears to produce no altered neural or behavioral response in early life, engaging the attachment system during trauma may initiate the pathway to pathology. However, interestingly, the maternal odor (as learned by pairing a novel odor with shock in infancy) appears to acquire the ability to rescue adult neurobehavioral deficits produced by early life trauma and implicates the serotonin and glucocorticoids systems.
Disclosure: Nothing to Disclose.

37.3 Individual Differences in Infant Temperament Place Some Children at Risk for Anxiety Disorders

Nathan Fox

University of Maryland, College Park, College Park, Maryland

Background: Infants and young children vary in the manner and intensity of their behavioral responses to novel and unfamiliar stimuli. These differences are associated with the temperament of behavioral inhibition (BI) and with the development of social reticence during childhood. BI increases a child's risk of developing anxiety disorders though not all young children displaying this temperament go on to exhibit anxiety. The goal of our research program has been to identify factors that moderate the link between BI and anxiety disorders.
Methods: Two independent cohorts of subjects were recruited in infancy based upon their pattern of behavioral reactivity to novelty (cohort 1: $N = 165$; cohort 2: $N = 225$). These infants have been followed and assessed during early childhood, elementary school age, and in the case of cohort 1, in adolescence and early adulthood. At the various assessment points, measures of attention to novelty and error monitoring were acquired. Attention bias in both cohorts was assessed using the dot probe paradigm. Error

monitoring and cognitive control was assessed using the Flanker paradigm. For cohort 1, assessment of psychiatric status using the DISC was completed at the young adult age point (mean age 20). For cohort 2, assessment of psychiatric status was most recently completed when children were age 9 using the KSADS.

Results: Results in cohort 1 found that stable BI from infancy through childhood was negatively associated over a fifteen year period with adult mental health ($p = 0.005$). Inhibited temperament was specifically associated with increased risk for anxiety disorders in adulthood ($p < 0.05$). Within cohort 2 there was a significant association between stable BI and anxiety disorders at age 9 ($p < .001$). In addition, these children were more likely to show an enhanced attention bias to threat ($F(1,122) = 5.92$, $p = .02$, $f = 0.22$) and for behaviorally inhibited children the magnitude of this bias was correlated with anxious symptoms ($r = 39) = .498$, $p = .002$). Neuroimaging data of the dot probe identified lower connectivity between amygdala and vlPFC for behaviorally inhibited subjects ($t = -3.88$, $p < .001$). In cohort 1 the magnitude of the ERN during the Flanker task moderated the link between temperament and anxiety (odds ration = 1.3, Wald X2 = 4.4, $p < .05$). Behaviorally inhibited children with heightened ERNs were more likely to display social anxiety compared to children with the same temperament but lower ERN magnitude. A similar significant association was found for cohort 2.

Conclusions: Children with the temperament of BI are at-risk for anxiety disorders. Two biomarkers, attention to threat and error monitoring moderate that risk. The underlying neural mechanisms associated with each suggest that prefrontal and amygdala connectivity is associated with increased risk.

Disclosure: Nothing to Disclose.

37.4 Understanding Heterogeneity in Social Behavior Using QTL Mapping in BXD Mouse Strains

Allison Knoll

Children's Hospital Los Angeles and University of Southern California, Los Angeles, California

Background: Humans exhibit broad heterogeneity in social behavior. Twin and family studies demonstrate that individual differences in core dimensions of social behavior are heritable, yet there are knowledge gaps in understanding the genetic and neurobiological mechanisms that underlie this heterogeneity. In neurodevelopmental disorders, there is also remarkable heterogeneity in social dysfunction, even in individuals with the same causal mutation, which often negatively impacts clinical treatment efficacy. We hypothesize that the genes that cause variation in typical social behavior act in concert with disorder-related genes to influence clinical heterogeneity.

Methods: We began to address these knowledge gaps in understanding genetic influences on social behavioral heterogeneity using the BXD genetic reference panel of mice, which is comprised of >150 recombinant inbred mouse strains derived from the C57BL/6 and DBA/2 parental lines. Mice exhibit rich repertoires of social behavior and provide a powerful experimental approach for identifying genetic mechanisms of heterogeneity. The parental and BXD lines have been fully sequenced, providing highly controlled, replicable, and catalogued genetic variation ideal for determining heritable causes of phenotypic heterogeneity. Moreover, there is open access data on >3000 phenotypic traits, 11 brain region transcriptomes, and 100,000 polymorphic SNPs for the BXD panel. We examined four domains of affiliative social behavior—social approach motivation, social recognition, direct social interaction motivation, and communication—using the 3-chamber social interaction task and a direct social interaction task with synchronous ultrasonic vocalization (USV) recording, across a subset of BXD lines.

Results: We found a remarkable several hundred-fold range in quantitative trait heterogeneity of social behaviors, with moderate to high heritability (h2) of several measures, including USV count (range: 0–704, $h2 = 0.33$) and the percentage of time spent sniffing a social partner (range: 13.4–59.4%, $h2 = 0.48$) in the direct social interaction task. Continuous measures collected for each task were used for quantitative trait locus (QTL) and quantitative trait gene (QTG) mapping to identify genetic causes of variation, with several novel QTLs identified for social approach, direct social interaction, and USV communication. Bioinformatics tools were used to integrate analyses of genome, transcriptome and phenome datasets to identify functional polymorphisms within candidate QTGs and brain regions and biological networks in which they might function to impact different aspects of sociability, resulting in a prioritized list of 10 candidate QTGs.

Conclusions: The identification of candidate QTGs and biological networks will guide next step opportunities for determining gene by environment factors that influence social behavior heterogeneity, which is essential for understanding typical social development and addressing disorder risk in children and families.

Disclosure: Nothing to Disclose.

Panel
38. Translating Clinical Neuroscience into Clinical Practice: Promises and Peril

38.1 Working Memory-related Neural Activity Predicts Future Smoking Relapse

Caryn Lerman

University of Pennsylvania, Philadelphia, Pennsylvania

Background: Brief abstinence from smoking impairs cognition, particularly executive function, and this plays a role in relapse to smoking. This study examined whether working memory-related brain activity predicts subsequent smoking relapse, above and beyond standard clinical and behavioral measures.

Methods: Seventy-three treatment-seeking smokers completed two fMRI sessions (smoking satiety vs. 24hr abstinent) during performance of a visual N-back task. This was followed by brief counseling and a short-term quit attempt. Relapse during the first 7 days was biochemically

confirmed by the presence of the nicotine metabolite cotinine. Mean percent signal change was extracted from a priori regions of interest: bilateral dorsolateral prefrontal cortex (DLPFC), medial frontal cingulate gyrus (MF/CG), posterior cingulate cortex (PCC), and ventromedial prefrontal cortex (vmPFC). Signal from these brain regions and additional clinical measures were used to model outcome status, which was then tested with resampling techniques for validation.
Results: Relapse to smoking was predicted by increased withdrawal symptoms, decreased left DLPFC and increased PCC activation (abstinence minus smoking). ROC analysis demonstrated 81% correct classification of relapses for the full model, a significant improvement over the model with clinical variables only.
Conclusions: The combination of abstinence-induced decreases in left DLPFC activation and reduced suppression of left PCC is a prognostic marker for poor outcome, specifically early smoking relapse. The challenges and the potential for translation of these findings to guide behavioral and pharmacologic interventions for tobacco dependent smokers will be discussed.
Disclosure: Nothing to Disclose.

38.2 Using PET Imaging to Predict Individual Treatment Response in Cocaine Dependence

Diana Martinez

Columbia University, New York, New York

Background: Positron Emission Tomography (PET) imaging studies have shown that cocaine dependence is associated with decreased dopamine signaling in the striatum, which is associated with cocaine seeking behavior. This finding also predicts response to treatment. In a previous study, cocaine dependent subjects underwent two PET scans using [11C]raclopride, before and administration of a stimulant, to measure striatal D2/3 receptor binding (BPND) and dopamine release in response to a stimulant (delta BPND). The cocaine dependent subjects were then enrolled in behavioral treatment using Contingency Management, where subjects earned voucher points for each urine sample that tested negative for cocaine. Subjects who responded to treatment were found to have higher measures of dopamine transmission at the D2/3 receptor (both BPND and delta BPND) compared to subjects who relapsed.
Methods: We have now investigated the ability to predict individual response to treatment on this dataset using a subject's PET scan, the early cumulative clinic attendance, or both. Using logistic regression, we implemented a model with two predictors by plotting the line that divided the two groups, i.e. the decision boundary. We calculated three quantities to measure the performances of the models: the Bayesian Information Criterion (BIC) (Schwarz, 1978), a commonly used, non-cross-validated measure for balancing residual error with model complexity, and two cross-validated performance metrics, which measured the generalization error—the error in prediction during cross-validation. Logistic regression yielded a probability measure, and we thresholded (at 0.5) the probability to make a binary prediction of response vs. non-response.
Results: The results showed that a single neuroimaging predictor (ΔBPND in the ventral striatum) predicted individual treatment outcome with a substantial degree of accuracy (cross-validated correct rate 82%). Adding other clinical predictor variables, including baseline severity measures, does not improve predictive performance. On the other hand, an equally good predictive model can be developed with a behavioral predictor, namely, cumulative clinic attendance over the first 3 weeks of treatment (83% correct rate). Combining the neuroimaging and behavioral predictors into a single multimodal model may significantly improve predictive performance (peaking at 96% correct rate).
Conclusions: These results suggest that a multimodal model can predict treatment success in cocaine dependence at an individual level, and pose hypotheses for the underlying neural circuitry mechanisms responsible for individual variations in treatment outcome. Clinically, our results have a number of implications. Our results represent a proof-of-principle and point to a potentially promising future of combining neuroimaging and clinical biomarkers.
Disclosure: Nothing to Disclose.

38.3 Individual Relapse Prediction: Making Biology Relevant

Martin Paulus

University of California at San Diego, La Jolla, California

Background: A diagnostic tool to help clinicians make decisions regarding treatment does not exist for psychiatric conditions. Identifying individuals with high risk for relapse to substance use following abstinence has profound clinical consequences. This study was aimed to develop neuroimaging as a robust diagnostic tool to predict relapse following substance use treatment programs.
Methods: Patients were followed 1 year after 28-day inpatient treatment to assess abstinence in this longitudinal study. We examined group differences between relapsing and abstaining individuals using a linear mixed-effects model. We also employed a random forest model to generate predictions for each participant regarding their relapse likelihood.
Results: 63 individuals were reached for follow up, and 18 (29%) reported relapse. There were significant group-by-condition interactions for neural activation in the left insula and right putamen and caudate for rewards. Abstaining individuals showed increased activation for larger relative to small rewards, whereas relapsing individuals failed to show differential activation between large and small rewards. A random forest model using brain imaging predictors yielded good test characteristics such that a positive test for relapse indicated a 2.63 increase in risk, whereas a negative test indicated decreased risk by 1.96.
Conclusions: These results suggest that neuroimaging can be developed as a robust diagnostic tool to predict relapse, providing an essential advance in the way treatment providers make decisions about individualized treatment of substance use disorders.
Disclosure: Nothing to Disclose.

38.4 Imaging Biomarkers of Addiction: From Predicting Use Status to Treatment Outcome

Elliot Stein

National Institute on Drug Abuse, Baltimore, Maryland

Background: No behavioral, cognitive or personality characteristic has proven clinically useful in predicting addiction treatment outcome. This may be in part due to the heterogeneous nature of the addiction phenotype. Since neuroimaging provides data that are more proximal to, and putatively more responsible for such phenotype heterogeneities, advanced, multimodal imaging measures, potentially coupled with genetics, offers promise for greater predictive accuracy and specificity than available with the use of any single or combined behavioral characterization. Nevertheless, numerous methodological and scientific uncertainties have limited current clinical utility of neuroimaging. This presentation will discuss several recently published and unpublished imaging studies that have shown promise to predict current drug use and future treatment outcome.
Methods: Drug status predictors were applied using either non-treatment seeking cocaine or nicotine cigarette smokers. Treatment-seeking cocaine dependent individuals were studied for outcome predictions following a two-week inpatient treatment intervention. Since preclinical studies have implicated dysregulation within specific neural circuit in drug addiction, we employed resting state functional connectivity along with gray matter density as our main independent variables. We employed both data driven methods (group ICA and graph theory) and hypotheses based seed analysis methods to analyze resting data. Support vector machine learning (SVM) algorithms were applied to both resting and anatomical data to train classifiers to predict current drug use status. Independent data sets were used as training and testing sets.
Results: SVM models using within network based resting connectivity predicted smoking status at about 80% accuracy. Significant features included higher order executive network components. Gray matter density predicted smoking status at about 70% accuracy. Network analysis predicted cocaine use status based on intermodule connectivity between default-mode and salience module, and between default-mode and medial temporal module. Using a nodal metric, the rostral anterior cingulate connected less with salience and medial temporal modules; the posterior cingulate cortex had reduced connections with executive control module; and the insula demonstrated decreased connections with default-mode, medial temporal and striatum modules. Cocaine treatment outcome, based on recidivism vs. early remission at 30 days, was predicted by the strength of amygdala-cortical and hippocampal-posterior cingulate (default mode) connectivity strength, along with the relationship between impulsivity and posterior insula-putamen circuit strength.
Conclusions: Using multiple analysis strategies in two populations of drug dependent individuals, neuroimaging studies identified specific limbic-cortical circuits and large scale networks that consistently predict drug use status. While these studies were cross sectional, prospective cocaine treatment outcome was predicted with accuracy approaching clinical utility ($>70\%$). However, the clinical utility of these small sample studies and the theoretical limitations to predictively intervene and individualize treatments to improve outcomes will be discussed.
Disclosure: Nothing to Disclose.

Panel
39. The Impact of Anomalies in the Emotional Regulatory Mechanism of Habituation in Psychotic, Anxiety, Personality and Developmental Disorders

39.1 Deficits in Hippocampal Habituation Predict Social Deficits in Schizophrenia

Stephan Heckers

Vanderbilt University, Nashville, Tennessee

Background: Considerable research implicates hippocampal alterations in the pathophysiology of schizophrenia. For example, patients with schizophrenia show hippocampal hyperactivity and memory deficits. In this study we tested the hypothesis that deficits in hippocampal habituation to repeated stimuli underlie both increased hippocampal activation and memory deficits.
Methods: Hippocampal habituation was examined in patients with schizophrenia ($n=21$) and healthy controls ($n=20$). Functional magnetic resonance imaging (fMRI) was used to measure decreases in BOLD signal to a repeated neutral face and a repeated neutral object. Effects of gender (subject gender x stimulus gender) were examined and correlations between habituation, memory, and negative symptoms were performed.
Results: Patients with schizophrenia failed to show the hippocampal habituation to neutral faces that was present in healthy controls (significant clusters in bilateral posterior hippocampi, FWE corrected $p<.05$). Interestingly, this deficit was specific to social stimuli and was moderated by the match between stimulus x subject gender (significant clusters in bilateral anterior and posterior hippocampi, FWE corrected $p<.05$). When viewing same-gender faces, patients showed consistent activation across repeated presentations (habituation failure). However, when viewing opposite-gender faces, patients showed increased activation over time, or sensitization. Across all participants, faster hippocampal habituation correlated with better memory. Within patients, faster hippocampal habituation correlated with lower social withdrawal. ($p<.05$).
Conclusions: These results provide evidence that a failure of hippocampal habituation underlies schizophrenia. Importantly, habituation deficits were specific to social stimuli, moderated by stimulus gender, and associated with social functioning. Thus, habituation deficits do not reflect alterations in basic neuronal processes, but instead are dependent on complex social information. These findings further our understanding of the pathophysiology of schizophrenia and suggest novel targets for treatment.
Disclosure: Nothing to Disclose.

39.2 Amygdala-Ventromedial Prefrontal Functional and Structural Connectivity in Children and Adolescents with Autism Spectrum Disorder

Christopher Monk

University of Michigan, Ann Arbor, Michigan

Background: Amygdala habituation, the initial response and subsequent decrease in responsiveness to the repeated presentation of stimuli, is vital for maintaining adaptive levels of arousal and reduced habituation is associated with heightened anxiety. Input from the ventromedial prefrontal cortex (vmPFC) regulates amygdala activity. The goal of the present set of studies was to examine the following: (1) amygdala habituation to faces in ASD; (2) amygdala-vmPFC connectivity to faces; (3) amygdala-vmPFC connectivity during rest; and (4) structural connectivity of the uncinate fasciculus, the major white matter tract that connects the amygdala with the vmPFC and other structures of the prefrontal cortex.
Methods: Functional MRI (fMRI) data were acquired on 32 children and adolescents with ASD and 56 typically developing controls during a faces task. Amygdala-vmPFC connectivity was examined with psychophysiological interaction analyses. Resting data were acquired on an overlapping sample of 40 youths with ASD and 65 controls as they viewed a fixation cross for 10 min. Diffusion tensor imaging (DTI) was acquired from an overlapping sample of 38 youths with ASD and 58 controls to measure structural connectivity of the uncinate fasciculus with fractional anisotropy (FA).
Results: For habituation, there was an overall interaction of group x emotion x time, $F(3, 688) = 9.90$, $p < .001$ (this and subsequent p values are reported using a correction of the bilateral amygdala region of interest). Relative to controls, youth with ASD evidenced reduced amygdala habituation to sad faces in the left amygdala, $t(86) = 3.78$, $p < .01$, right amygdala, $t(86) = 3.73$, $p < .01$, and to neutral faces in the right amygdala, $t(86) = 4.41$, $p < .01$. Moreover, reduced amygdala habituation correlated with autism severity as measured by the Social Responsiveness Scale, $t(30) = 3.31$, $p < .05$ in the ASD sample. Relative to controls, the ASD group showed weaker amygdala-vmPFC connectivity, $t(86) = 3.34$, $p < .05$ corrected for Brodmann's Area (BA) 25 in the vmPFC. For resting connectivity, both groups showed robust connectivity between the left amygdala and left BA 25, $t(104) = 23.84$, $p < .001$ and connectivity between the right amygdala and right BA 25 $t(104) = 30.46$, $p < .001$ (corrected for BA 25). However, there were no group differences between the amygdala and BA 25 during rest, $p > .2$ uncorrected. Similarly, for DTI, both groups showed strong structural connectivity in the uncinate fasciculus as measured with FA, $t(95) = 209.32$, $p < .001$. Again though, there were no group differences in the uncinate fasciculus, $p > .39$.
Conclusions: Sustained amygdala activation to faces suggests that repeated social stimuli may be overly arousing for individuals with ASD, which could contribute to social impairments. Abnormal modulation of the amygdala by the vmPFC may play a role in reduced habituation. Given the lack of group differences in the resting and structural connectivity, the findings suggest that the impairments seen in amygdala and amygdala-subgenual connectivity are not due to a fundamental physiological or structural disturbance in the circuit. Rather, the reduced habituation and altered functional connectivity selectively emerges in the context of social stimuli (i.e., faces). Currently, we are further evaluating amygdala-vmPFC function in ASD by incorporating more sensitive DTI procedures. In addition, we are acquiring eye-tracking data during the faces task in order to understand the relationship between amygdala habituation and eye gaze in ASD.
Disclosure: Nothing to Disclose.

39.3 Affective Instability Correlates with Borderline Personality Disorder Patients' Rebound Sensitization and Anomalous Habituation in Behavioral and Amygdala Response to Longitudinally Repeated Negative Emotional Cues

Harold Koenigsberg

Mount Sinai School of Medicine, 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 an inversion of the adaptive emotion regulatory mechanism of habituation resulting in sensitization, rather than habituation, when emotionally salient stimuli are reencountered. We hypothesized that when reencountering emotional stimuli, borderline patients, in contrast to healthy volunteers, would show an intensified rather than reduced behavioral reaction and increased rather than reduced amygdala activity.
Methods: 16 BPD patients and 24 healthy volunteers (HC) were shown emotional pictures as fMRI images were obtained on two occasions separated by 2 to 4 days. On each day, the same 30 negative and 30 neutral images were each presented five times, in a pseudorandom sequence over a 35-minute picture viewing period, with affect self-reports being made after each. BOLD signal intensities (beta-weights) in a structurally-defined right amygdala region-of-interest were obtained for each repetition on each day. Change in functional connectivity to this same amygdala seed between first and fifth presentation of each image was assessed by means of psychophysiological interaction (PPI) analyses.
Results: HCs significantly decrease amygdala activation from presentation 1 (P1) to presentation 5 (P5) for negative pictures ($p < .001$, two-tailed) on day 1, while BPDs do not. Further, on day 1 HCs increase functional connectivity from P1 to P5 between amygdala and ventrolateral prefrontal cortex, a region previously implicated in cognitive control, and the extent of this connectivity increase predicts lower affective instability among HCs. BPDs, however, show significantly less functional connectivity change between these regions compared to HCs. Upon re-exposure to the same pictures on day 2, BPDs rate the pictures more negatively than on day 1 ($p < .04$, one-tailed) and show significantly increased amygdala reactivity compared to day 1 ($p < .03$, two-tailed), while HCs do not. Importantly, the

degree to which BPDs sensitize in both task-based affect ratings and amygdala activity from day 1 to day 2 is positively correlated with self-reported affective instability ($r = 0.81$, $p < .01$, two-tailed and $r = 0.52$, $p < .05$, one-tailed, respectively).
Conclusions: BPD patients, a group characterized by intense affective instability, do not show habituation in amygdala activation, as HCs do, to repeated presentations of negative pictures. Moreover when they are rechallenged with these same pictures a few days later, they show sensitization, both in terms of affect self-reports and amygdala activity, and in both cases the degree of sensitization predicts increased affective instability. In addition BPD patients do not show the increases in connectivity between amygdala and prefrontal regions implicated in emotion regulation that HCs do when habituating to negative pictures. These finding suggest that anomalies in amygdala activity and connectivity during habituation and amygdala sensitization to delayed rechallenge underlie the emotional instability in BPD and have implications for psychotherapeutic work with these patients.
Disclosure: Nothing to Disclose.

39.4 Anxiety Type Modulates Immediate Versus Delayed Engagement of Attention-related Brain Regions

Jeffrey Spielberg

VA Boston Healthcare System, Boston, Massachusetts

Background: Habituation of the fear response, critical to exposure treatment for anxiety, is inconsistently observed. This may be due to differential attentional engagement as a function of trans-diagnostic anxiety dimensions, anxious arousal and anxious apprehension. Specifically, anxious arousal is linked to immediate attentional engagement with threat, whereas apprehension (worry) is thought to function as a cognitive avoidance strategy whereby the repetitive verbal processing dampens threat-related imagery (experienced as more arousing).
Methods: We tested this hypothesis by examining patterns of neural habituation associated with anxious arousal vs. anxious apprehension. fMRI was collected while participants ($n = 75$) performed the emotion-word Stroop. The first set of analyses examined habituation in task activation. To index within-participant habituation, activation to negatively valenced stimuli (vs. neutral) during the second half of the task was contrasted against activation during the first half. At the group level, habituation was regressed on measures of the anxiety dimensions. A second set of analyses examined habituation in topological network properties using the Graph Theoretic GLM toolbox. Assortativity matrices were computed separately for each task condition and half, and graph theoretic properties indexing functional integration, segregation, and centrality were computed for each matrix. Task condition and half were then entered as repeated factors into a GLM with anxiety dimensions as between participant predictors.
Results: As predicted, anxious arousal was linked to immediate attention-related activation that habituated over time, whereas apprehension predicted delayed attention-related activation that occurred only after habituation in a region related to verbal rehearsal. Overall, network analyses supported a model in which apprehension-related verbal rehearsal suppressed attentional engagement.
Conclusions: Results further elucidate mechanisms involved in attention to negatively valenced stimuli, indicate that anxiety is a heterogeneous construct with regard to attention to such stimuli, and support the hypothesis that apprehension functions as a cognitive avoidance strategy.
Disclosure: Nothing to Disclose.

Panel
40. Next Generation Phenotyping in Search of Genes for Psychiatric Disorders

40.1 New Data to Investigate an Old Epidemiological Puzzle: The Negative Association Between Schizophrenia and Rheumatoid Arthritis

Enda Byrne

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Background: A long-standing epidemiological puzzle is the reduced rate of rheumatoid arthritis (RA) in those with schizophrenia (SCZ) and vice versa, made even more puzzling because smoking is a major risk factor for RA and smoking rates are high in those with SCZ. Traditional epidemiological approaches to determine if this negative association is underpinned by genetic factors would test for reduced rates of one disorder in relatives of the other. However, since both disorders affect only $\sim 1\%$ of the population very large samples of families with multiple family members measured for both disorders are needed, which are difficult to achieve. The genomics era presents an alternative paradigm for investigating the genetic relationship between two uncommon disorders using samples of cases and controls that are unrelated in the classical sense.
Methods: We use data from genome-wide association studies comprising 8479 cases and 26775 controls for RA and 12,920 cases and 15,954 controls for SCZ. We used the single nucleotide polymorphism (SNP) genotypes to estimate the genetic similarity between all pairs of individuals, both across the whole genome and for regions annotated by function. We tested the hypothesis that SCZ cases are genetically different to RA cases.
Results: We estimated a small but significant negative genetic correlation between RA and SCZ of -0.07 (s.e. 0.03, $p = 0.01$) across the whole genome. The negative correlation increased to -0.23 (s.e.0.078, $p = 0.004$) when only coding and regulatory regions were considered. Previous analyses of RA have highlighted the importance of genes expressed in CD4+ effector memory T cells. As expected we found that the variance attributable to this pathway from coding and regulatory regions was enriched for RA (19 fold, $p = 8.4E\text{-}38$). The variance attributable to this pathway was also enriched in SCZ (3 fold, $p = 0.001$) highlighting the potential importance in this pathway with SCZ.
Conclusions: We provide evidence that some genetic risk factors for RA are protective for SCZ and vice versa and that

these risk factors are clustered according to functional annotation. We hypothesise that these pathways are of particular relevance in the context of environmental immunological challenges. The existence of antagonistically pleiotropic alleles may explain why common risk variants are maintained in the population.
Disclosure: Nothing to Disclose.

40.2 Pharmacogenomic Endophenotypes: What Can the Subjective Response to D-Amphetamine Tell Us about Risk for Psychiatric Disorders?

Abraham Palmer

University of Chicago, Chicago, Illinois

Background: The subjective response to d-amphetamine is heritable and may serve as an endophenotype for a variety of psychiatric disorders, especially those related to dopaminergic signaling.
Methods: We performed a Genome Wide Association Study (GWAS) for the subjective responses to amphetamine using data from 398 non-drug abusing healthy volunteers. Response to amphetamine were measured using a double-blind, placebo-controlled, within-subjects design. We used sparse factor analysis to reduce the dimensionality of the data and then performed GWAS using genotypes from Affy 6.0 imputed to 1000 Genomes.
Results: We identified several putative associations; the strongest was between a factor reflecting the positive subjective effects of amphetamine and a SNP (rs3784943) in the 8th intron of cadherin 13 (CDH13; $P = 4.58 \times 10\text{-}8$), a gene previously associated with a number of psychiatric traits, including methamphetamine dependence. We have examined both CDH13 knockout rats and adiponectin knock out mice and observed differences in conditioned place preference, which offers additional support for the role of CDH13 in modulating the sensitivity to the subjectively positive effects of amphetamine. Additionally, we observed a putative association between a factor representing the degree of positive affect at baseline and a SNP (rs472402) in the 1st intron of steroid-5-alpha-reductase-α-polypeptide-1 (SRD5A1; $P = 2.53 \times 10\text{-}7$), a gene whose protein product catalyzes the rate-limiting step in synthesis of the neurosteroid allopregnanolone. This SNP belongs to an LD-block that has been previously associated with the expression of SRD5A1 and differences in SRD5A1 enzymatic activity. However, due to the lack of replication datasets we view all of these results as preliminary. We extended these studies by exploring co-enrichment of SNPs associated with response to d-amphetamine and SNPs associated with psychiatric disorders. We found that SNPs nominally associated ($P \leq 0.05$ and $P \leq 0.01$) with schizophrenia and attention deficit hyperactivity disorder (ADHD) were also nominally associated with d-amphetamine response. Furthermore, we found that the source of this enrichment was an excess of alleles that increased sensitivity to the euphoric effects of d-amphetamine and decreased susceptibility to schizophrenia and ADHD. In contrast, three negative control phenotypes (height, inflammatory bowel disease, and Parkinson's disease) did not show this enrichment. Ongoing studies to extend this work to include other endophenotype datasets and additional GWAS that use priors based on this approach are currently underway.
Conclusions: Taken together, our results suggest that alleles identified using an acute challenge with a dopaminergic drug in healthy individuals can be used to identify alleles that confer risk for psychiatric disorders commonly treated with dopaminergic agonists and antagonists. More importantly, our results demonstrate a broadly applicable approach to the integration of small endophenotype datasets with the results of well-powered GWAS for psychiatric diseases.
Disclosure: Nothing to Disclose.

40.3 Medical Internship as a Model to Identify Genes in Depression

Srijan Sen

University of Michigan, Ann Arbor, Michigan

Background: While there has been substantial progress in identifying genes in many complex phenotypes, large-scale genomic studies into major depression have been disappointing. Three factors that have likely made the identification of genes in depression particularly difficult: 1) inaccurate assessment of the lifetime depression history 2) heterogeneous triggers of depression and 3) failure to account for life stress. Medical internship, the first year of professional physician training, provides a model to potentially overcome these barriers. Internship is a rare situation where we can prospectively predict the onset of a uniform, chronic stressor and a dramatic increase in depressive symptoms. This model allows us to follow subjects as they experience a common stressor and allow for the assessment of current depressive symptoms.
Methods: In this study, we assessed 1288 subjects for depressive symptoms in a low-stress environment before internship and then quarterly, through the stressful internship year. Subjects were genotyped on a 5000 SNP Illumina array consisting of variants in neurotransmitter and endocrine function genes and GWAS hits from other phenotypes (Solovieff et al Neuropsychopharmacology 2014).
Results: The proportion of subjects who met PHQ-9 criteria for depression increased dramatically, from 4% prior to internship to a mean of 25% during internship. Variants in multiple genes of interest were associated with an increase in depressive symptoms under stress. Of particular interest, variants in SLC6A15, the only gene to date implicated in a depression GWAS study, were significantly associated with depression under stress. In addition, a coding variant in the Fibroblast Growth Factor 2, a gene implicated in depression with strong preclinical and post-mortem human evidence, was also associated in this sample.
Conclusions: While these associations require replication and biological validation, this work demonstrates the value of studies employing alternative designs, specifically tailored to identify genes involved in depression under stress.
Disclosure: Nothing to Disclose.

40.4 Molecular Genetic and Epigenetic Mechanisms of FKBP5 Gene by Environment Interaction

Torsten Klengel

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Background: Psychiatric disorders result from an interaction of the individual's genetic predisposition and environmental factors to a varying degree. A substantial number of independent studies now provide evidence for the interaction of genetic variants in the GR-regulating co-chaperone FKBP5 and childhood adversity to predict long term risk for a number of psychiatric disorders. We previously proposed a molecular mechanism for the interaction of a common SNP in FKBP5 with childhood trauma on the development psychiatric disorders in adulthood which combine a modified 3D architecture of the locus with differences in the molecular and system wide response to stress that lead to long-term epigenetic changes. In this talk we present novel data on gene by environment interaction on DNA methylation in the FKBP5 locus in additional samples and novel gene by environment interactions with rare functional SNPs in this gene as well as copy number variants.
Methods: Genome wide genotyping, Gene by environment interaction, DNA methylation analysis, next generation sequencing, reporter gene assays, Copy Number Variation analysis, GWAS, stimulated mRNA and protein expression.
Results: Using a next generation sequencing approach we identified a 3.3 kb long insertion in intron 1 of the FKBP5 locus that occurred in 21.3 % of individuals ($N = 413$) and consists of LINE and SINE element sequences and is associated with differential local DNA methylation. The insertion only occurs on the previously reported risk haplotype tagged by rs1360780. When testing the insertion for interaction with child abuse on predicting current and lifetime PTSD, as well as depressive symptoms, we observed a significant interaction, with the presence of insertion having protective effects ($pint = 0.0004$ on mPSS, $N = 231$; $pint < 0.00001$ on BDI, $N = 232$). In-vitro experiments suggest, that this CNV influences the stress-elicited transcriptional activation of FKBP5 in interaction with rs1360780. In sum, individuals carrying this insertion on the risk haplotype background were protected from the increase in risk, suggesting a complex interplay of common and structural variants in gene environment interactions of the FKBP5 locus. In addition, sequencing of FKBP5 revealed several rare SNPs that led to in-vitro modulation of the transcriptional response of FKBP5 to GR activation and may also contribute to altered gene by environment interactions with the common variant. We will further present additional data in larger cohorts on how such complex gene by environment interactions effect local DNA methylation and correlation of these changes in DNA methylation with neuroimaging, neuroendocrine and gene expression biomarkers for stress-related disorders.
Conclusions: The presented data delineate the relevance of a complex interplay of environmental risk factors with common and rare polymorphisms as well as structural variants and epigenetic changes to shape risk for psychiatric disorders. Given such complex interaction within a single locus, more complex risk models should be considered for psychiatric disorders also on the genome-wide level.
Disclosure: Nothing to Disclose.

Panel
41. Sex Differences in the Brain: Insights into CNS Therapeutics

41.1 Prenatal Immune Programming of Adult Stress Response Circuitry Deficits Across Disorders (Psychoses and Mood) and Sex

Jill Goldstein

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Background: Maladaptive responses to stress generated by negative valence stimuli are implicated in every psychiatric disorder. Studies have related stress responses, i.e., disruptions of HPA axis functioning, to prenatal abnormalities in immune activation. In this study, we characterized neural circuitry associated with stress response across ill [major depressive disorder (MDD) and psychoses (PSY)] and non-ill populations and tested associations with prenatal (mid-gestation) maternal immune activation 45 years earlier. Stress circuitry included hypothalamus (HYPO), amygdala (AMYG), hippocampus (HIPP), medial and orbital prefrontal and anterior cingulate cortices (mPFC, OFC, ACC), and periaqueductal gray (PAG). Cytokines included those whose associated receptors are located in stress circuitry regions and for which we demonstrated at the population level significant sex differences in risk for MDD and/or PSY (IL-1β, IL-6, TNF-α, IL-10).
Methods: 50-year cohort study following mothers through pregnancy and offspring, 99 adult offspring were assessed with fMRI using a mild visual stress task of negative, neutral and fixation images adapted from International Affective Picture System:58 cases (27 recurrent MDD; 31 PSY half schizophrenia/half bipolar); 41 healthy controls; groups equally divided by sex and comparable within sex by group. Maternal mid-gestation assays (listed above) were assessed using a multiplexed, bead-based immunoassay on a Luminex 3D detection platform. Assay sensitivities were 0.1-0.4 pg/mL. Using SPM8, blood oxygen-level dependent (BOLD) signal changes comparing negative valence/high arousal and neutral valence/low arousal stimuli were estimated. Anatomically defined masks of HIPP, AMYG, HYPO, OFC, mPFC, ACC, and PAG were overlaid on FWE-corrected ($p < .05$) negative minus neutral contrasts, and mean BOLD responses were extracted. General linear models related cytokine exposure to BOLD changes associated with stress, group and sex.
Results: FWE-corrected task-related activations were significant across group and sex in all regions but ACC. However, cases showed significantly higher BOLD response in PAG than controls ($p = .01$, Cohen's $d = .49$); and MDDs were significantly higher than PSY in HYPO ($p = .01$, $d = .71$). Case and sex by cytokine interactions showed higher TNF-α associated with higher BOLD changes in bilateral mPFC ($p = 0.005$) in cases, regardless of sex, but

not in controls. All females, regardless of group, exposed to higher TNF-α showed lower BOLD left HIPP activity ($p = 0.01$), but all males exposed to higher TNF-α showed higher BOLD left HIPP activity ($p = 0.04$). Further, male cases (but not controls) regardless of type, exposed to higher levels of IL-1β showed lower BOLD in left HIPP ($p = 0.02$) and right AMYG ($p = 0.02$), suggesting differential consequences for male offspring dependent on type of immune exposure.
Conclusions: Neural circuitry associated with stress response to negative stimuli is shared across sex and ill and non-ill populations. However, the primary arousal regions (i.e., PAG and HYPO), associated with substantial HPA response, were significantly higher in cases than controls, and for the latter, particularly MDD. Further, prenatal immune programming associated with TNF-α and IL-1β contributed to long-term programming of stress response regions that were shared (mPFC) and sex-dependent (HIPP and AMYG), demonstrating fetal origins of stress response circuitry deficits 50 years later.
Disclosure: Nothing to Disclose.

41.2 Sex Differences in Behavioral and Neuroendocrine Responses to Stress: Roles for Estrogen Receptors

Robert Handa

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Background: Animals respond to real and perceived threats to their welfare by activating neurons that control neuroendocrine (e.g. the Hypothalamo-pituitary-adrenal (HPA) axis) and autonomic responses. For HPA axis, stress drives the release of corticotropin releasing hormone (CRH) into the hypophyseal portal vasculature to stimulate ACTH from the anterior pituitary gland followed by adrenal glucocorticoid (GC) secretion. In rodents, basal and stress-induced GC secretion are greater in females than in males and stimulation of hormonal stress responses and PVN neuron activation are correspondingly higher. This sex difference may be due to estradiol changes occurring across the estrous cycle. Similarly, anxiety and depressive-like behaviors are also hormone regulated. Previous studies show that selective activation of estrogen receptor beta (ERβ) in rodents reduces the hormonal and behavioral indices of stress. Determining the neuroanatomical projections and phenotypes of ER expressing neurons can inform us of mechanisms by which E2 modulates stress responses and behavior. In these studies we have utilized transgenic mouse models targeting ERβ neurons or where select phenotypes of neurons can be manipulated or tagged through their expression of cre-recombinase. These studies will lead to a better understanding of how estrogen signaling in brain might underlie sex differences in stress-related responses.
Methods: Our studies utilize mouse models to explore the functions of ERβ in PVN and their projections to limbic brain areas. The Esr2(ERβ)-EGFP mouse line expresses an EGFP reporter inserted downstream of the ERβ coding sequence to visualize cell specific EGFP expression in ERβ neurons. Transgenic mouse lines expressing cre recombinase driven by the CRH, OT, AVP or ERβ promoters were crossed to a reporter mouse to allow fluorescent protein expression in cre recombinase neurons.
Results: We found a sex difference in the ability of ERβ agonists to inhibit hormone responses and behavior (F > M). The distribution of ERβ was also found to be sexually dimorphic in some brain areas such as the AVPV (F > M) and the BnST (M > F), whereas, there was no sex difference in the PVN. ERβ-EGFP was detected in CRH, AVP and OT neurons in the mouse PVN; a distribution that differs from that of the rat. Transcription factors (e.g. MeCP2 and CBP) were found in most CRH-tdTomato and ERβ-EGFP neurons in the PVN. Glucocorticoid receptors were also localized in CRH and ERβ neurons in the PVN and GC regulation of gene expression in CRH and ERβ neurons has been determined using PCR array. Tract tracing studies are currently being used to define the projections of PVN ERβ neurons.
Conclusions: These studies have defined the anatomy and physiology of ERβ neurons in PVN and will define the mechanisms where ERβ agonists can act in a sexually dimorphic fashion to modulate neuroendocrine responses and behavior.
Disclosure: Nothing to Disclose.

41.3 Fetal Antecedent Mouse Studies Demonstrate Sex Differences in PVN and BBBDevelopment with Physiologic and Behavior Consequences after Puberty

Krystle Frahm

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: Neurons of the paraventricular nucleus of the hypothalamus (PVN) integrate peripheral signals and coordinate responses that are important for maintaining homeostasis, vasomotor tone, energy balance, stress responses and integrative behaviors, including several potentially related to psychiatric-like disorders. In addition to the density of its cytoarchitecture, the PVN also contains 3-fold more blood vessels than surrounding brain regions. This vascularity may also vary in the extent to which there is regional regulation of the blood brain barrier (BBB).
Methods: Mice were either genetically deficient for GABAB receptor signaling, or exposed to either vehicle (veh) or the synthetic glucocorticoid dexamethasone (dex; 0.1 mg/kg) during embryonic days 11–17. On postnatal (P)20 or P50 male and females were perfused with the low molecular weight dye fluorescein isothiocyanate (FITC). Brains were then examined for leakage of FITC out of blood vessels, immunoreactive (ir)-platelet endothelial cell adhesion molecule (PECAM), ir-Glial fibrillary acid protein (GFAP) for astrocytes, and ir-desmin as a marker for pericytes.
Results: Results showed that genetic deficiency for GABAB signaling or fetal exposure to excess glucocorticoids decrease the vascularity of the PVN at P20. Fetal dex exposure led to increased leakage out of PVN blood vessels at P20. There were no longer dex-dependent differences in vascularity or leakage by P50. However, veh-treated females had more total ir-GFAP than dex-treated females in the PVN ($p < 0.05$). There was a similar pattern of decreased ir-GFAP covering blood vessels in dex-treated females compared to veh-treated females ($P < 0.05$). For ir-desmin

pericytes in the PVN, there was a significant increase in ir-desmin normalized to blood vessel density in dex-treated compared to veh-treated males ($p < 0.05$).
Conclusions: Taken together the data suggest that fetal antecedent exposure to excess glucocorticoids (e.g., direct injection or perhaps stress) has different consequences pre and postpubertally. Before puberty there are no sex differences in dex-dependent effects that include vascularity and vascular leakage. After puberty sex differences emerge in BBB characteristics, but they do not lead to alterations in vascular function in basal physiological states. We hypothesize that these alterations in BBB components in combination with environmental or physiological challenges may result in sex-related changes in BBB competency.
Disclosure: Nothing to Disclose.

41.4 Neurosteroids and Sex Differences: Relevance to Biomarkers and Therapeutics

Christine Marx

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Background: There are pronounced sex differences in the prevalence and clinical expression of a number of psychiatric disorders, including anxiety disorders, depression, and schizophrenia. The precise neurobiological underpinnings of these sex differences, however, remain to be comprehensively elucidated. Many neurosteroids exhibit sex differences in humans and rodents. For example, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) levels are generally higher in men compared to women, and allopregnanolone (a GABAergic neurosteroid) exhibits variation across the menstrual cycle. Accruing data suggest that neurosteroids are altered in PTSD (Pinna and Rasmusson 2012), depression (Uzunova et al 2006), and schizophrenia (Marx et al 2006, Ritsner et al 2007), and may have potential as biomarkers in these conditions. In addition, sex differences in therapeutic responses to neurosteroid interventions have been reported in several randomized controlled clinical trials. For example, women with schizophrenia (Strous et al 2003) appear to respond more favorably to treatment with DHEA, and women with bipolar depression (Brown et al 2014) and schizophrenia (Marx et al 2014) appear to have greater therapeutic responses to pregnenolone augmentation. Neurosteroids are also critical modulators of the stress response, and may contribute to resilience in the setting of extreme stress (Morgan et al 2009). We thus quantified neurosteroid levels in serum samples from men and women who served in the U.S. military since September 11th, 2001 (many of whom experienced the stress of multiple deployments to Iraq or Afghanistan) to investigate possible relationships to stress and depression symptoms post-deployment.
Methods: DHEA and DHEAS levels were quantified by radioimmunoassay in serum samples from 662 male and 403 female OEF/OIF veterans by radioimmunoassay. Additional neurosteroids were quantified by gas chromatography/mass spectrometry in a subset of 485 male veterans. Behavioral symptoms were assessed by the Beck Depression Inventory-II (BDI-II), Davidson Trauma Scale (DTS), and other rating scales. Resilience was assessed by the Connor-Davidson Resilience Scale (CD-RISC). ANCOVA analyses were conducted examining the effect of depression and PTSD symptoms on DHEA and DHEAS levels, co-varying for age and smoking. Contrast analyses were also conducted (DTS $\geq$ 40 vs. DTS $<$ 10). Pearson partial correlation coefficients were determined for CD-RISC assessments, controlling for smoking and age.
Results: DHEAS levels were significantly lower in male veterans with DTS total scores $\geq$ 40 (consistent with PTSD; $n = 213$) compared to male veterans with DTS total scores $<$ 10 (consistent with no/minimal PTSD symptoms; $n = 291$); these associations were not found in female veterans. In addition, DHEAS levels were significantly lower in male veterans with depression (BDI $\geq$ 20 vs. BDI $<$ 10), $p = 0.026$, but not in female veterans. DHEAS levels were positively correlated with resilience (as assessed by the CD-RISC), $r = 0.15$, $p = 0.0002$ in male veterans. As anticipated, both DHEA ($p < 0.0001$) and DHEAS ($p < 0.0001$) decreased markedly with age, and were higher in men compared to women. In 485 male veterans, androsterone (a GABAergic neurosteroid and DHEA metabolite) was inversely correlated with both PTSD ($r = -0.151$; $p = 0.001$) and depression symptoms ($r = -0.147$; $p < 0.002$).
Conclusions: Neurosteroids have potential as biomarker candidates for depression symptoms, PTSD symptoms, and resilience in male veterans, and merit additional investigation as therapeutic interventions.
Disclosure: Part 1: No financial disclosures. Applicant/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).

Panel
42. Linking Information Processing Impairment to Local Circuit Dysfunction in Schizophrenia and Related Disorders

42.1 Early Visual and Auditory Perception in Schizophrenia and Bipolar Disorder: What Is Common and What Is Distinctive?

Michael Green

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Background: People with schizophrenia show impairments in early visual and auditory processing within the first few hundred ms of visual and auditory processing. Impairments are seen using simple stimuli (e.g., pure tones, line drawings) and complex stimuli such as faces. Our group previously demonstrated the implications of early perception deficits for daily functioning of patients. Perceptual impairments in schizophrenia show relationships to social cognitive deficits, which affect negative symptoms and daily functioning. We know very little about the diagnostic specificity of perceptual impairments to schizophrenia versus other chronic psychiatric disorders. In this talk, we will review studies that compared patients with schizophrenia (SZ), those with Bipolar Disorder (BD), and healthy controls (HC) on: 1) early visual processing, 2) early auditory processing, and 3) early face processing.

Methods: For early visual perception, 43 BD, 43 SZ, and 51 HC received 3 paradigms: 1) location backward masking (for early stage object formation, 2) 4-dot masking (for middle stage of object substitution), and 3) rapid serial visual processing (RSVP) task for a later stage of the perception-attention interface. For auditory processing, 52 BD, 30 SZ, and 27 HC received a duration-deviant auditory oddball paradigm to assess mismatch negativity (MMN) and P3a. For face processing, participants identified the emotion of a face, the gender of a face, or whether a building was one or two stories tall. The N170 (structural encoding facial features) and N250 (decoding facial features) were assessed in 57 BD, 30 SZ and 30 HC.
Results: For visual processing, SZ performed significantly worse than BD and HC on location and 4-dot masking. BD did not significantly differ from HC on either masking task. Both patient groups performed significantly worse than HC on the RSVP task. For early auditory processing, significant MMN and P3a amplitude reductions were present in BD and SZ relative to HC. The MMN reduction was more prominent in SZ than BD, at a trend level. P3a did not differ significantly between patient groups. For face processing, N170 amplitude was significantly smaller in SZ compared to BD and HC, which did not differ from each other. For N250, both patient groups showed significantly smaller amplitudes compared with controls, but did not differ from each other.
Conclusions: For early visual processing, BD were intact at the early and middle stages of visual processing but intermediate between the SZ and HC groups at a later processing stage involving perceptual and attentional processes (RSVP task). A similar pattern was observed on the face processing waves in which BD only showed impairment on the N250. For auditory processing, both groups showed impairment on both waveforms, but BD was less affected than SZ, at a trend level. These findings suggest that SZ is characterized by diffuse pathophysiology affecting all stages of auditory and visual processing. BD has relatively intact visual processing, showing disruption only at the latest stages. Auditory processing is also dysfunctional in BD, though at attenuated levels compared to SZ. Overall, findings confirm auditory and visual sensory-level deficits in SZ and suggest that investigation of underlying mechanisms may provide important insights into the pathophysiology of psychotic disorders.
Disclosure: Part 1: Consultant to AbbVie, DSP, Forum, and Roche. On the scientific board for Mnemosyne, **Part 4:** Grant from Amgen.

42.2 Time Frequency Analysis of Visual Sensory Dysfunction in Schizophrenia (Sz)

Antigona Martinez

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Background: Deficits in early visual processing have been extensively documented in Sz using behavioral, event-related potential (ERP) and fMRI approaches. In the traditional time domain ERP approach, responses to repeated stimuli are averaged together prior to analysis in order to increase signal to noise. In the time domain, reliable deficits have been observed in the generation of the visual P1 potential, which serves as a trait marker of Sz vulnerability. By contrast, newer, frequency domain analyses permit trial-by-trial analysis of neurophysiological data and translation to underlying local circuit mechanisms within cortex. Such measures have only recently become tractable in large datasets, permitting application to clinical data.
Methods: Visual ERPs were analyzed from Sz, clinical high risk (CHR) and Ctl subjects in two separate paradigms: 1) feature selective attention (19 Sz, 19 Ctl), and 2) multi-feature stimulation (30 Sz, 15 CHR, 21 Ctl). For both paradigms, phase-locked intertrial coherence (ITC) and non-phase locked "induced power" analyses were conducted. Phase-locked responses, corresponding to the time domain (e.g. P1) are considered to reflect the interaction between glutamate cells and parvalbumin (PV) interneurons, whereas non-phased locked responses reflect interaction with other interneuron subtypes.
Results: Across paradigms, significant decrements were observed in ITC within the theta (4-7 Hz) frequency band, corresponding to impaired time-domain P1 generation. In addition, significant deficits were noted in stimulus-induced suppression (i.e. event-related desynchronization, ERD) of ongoing activity within the alpha (8-12 Hz) frequency band. In the feature-attention paradigm, alpha ERD was prolonged to attended vs. unattended stimuli across groups, suggesting a critical role in stimulus evaluation. This prolongation was equivalent in both groups, suggesting intact top-down effects. Deficits in theta ITC and stimulus-induced alpha ERD convergently predicted impairments in MCCB domains of attention/vigilance and visual learning. In the multi-feature passive stimulation paradigm, differential decrements in theta ITC and alpha ERD were observed in Sz vs. CHR, suggesting differential contribution to Sz risk. Differential deficits were observed as well in stimulus motion-induced ERD, suggesting that risk phenotypes in Sz may extend to the early visual system.
Conclusions: Suppression of ongoing alpha activity is a critical process by which the brain brings cortical regions "on line" for continued stimulus processing. Here, we provide the first evidence for deficits in stimulus-induced alpha ERD in Sz, in addition to deficits in the visual P1 component. Furthermore, impairments in alpha ERD contributed along with impaired P1 generation to higher order visual dysfunction. Alpha ERDs are slow and not tightly phase locked to stimulus onset and thus likely reflect interaction of glutamate cells with non-PV types of GABA interneuron. Both ITC- and ERD-type mechanisms therefore may serve to independently link clinical findings to underlying cortical pathology.
Disclosure: Nothing to Disclose.

42.3 Disruption and Repair of Synaptic Plasticity and Excitatory-inhibitory Balance

Robert Froemke

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Background: Impaired NMDA receptor signaling is believed to contribute to schizophrenia and other psychiatric

disorders. NMDA receptors are critical for neural development as well as learning, memory, and cognitive processes in adults. Thus disruption of NMDA receptor activation and synaptic transmission might lead to schizophrenia. Drugs that act as NMDA receptor antagonists (e.g., ketamine) lead to schizophrenic-like symptoms, and human genetic studies have highlighted de novo mutations in NMDA receptors and associated proteins in humans with schizophrenia. However, little is known about how NMDA receptor activation is directly involved in cognitive processes such as perception and memory. Here I will present new work in mouse models showing the connections between a gene implicated in schizophrenia (CNTNAP2), NMDA receptor activation, and control of local circuit dynamics by synaptic plasticity and regulation of excitatory-inhibitory balance in mouse sensory cortex.

Methods: We performed electrophysiological experiments in brain slices and in vivo. Some behavioral experiments to examine perception and seizures were also performed. For in vitro experiments, brain slices of wild-type and Cntnap2 mutant animals were made. Synaptic transmission (strength of inhibition relative to strength of excitation) and long-term synaptic plasticity (pairing pre- and postsynaptic spikes to examine spike-timing-dependent plasticity) were assessed with whole-cell current-clamp and voltage-clamp recordings. Methods are similar to our past studies in brain slices (Froemke et al., Nature 2005; Southwell et al., Science 2010). For in vivo experiments, animals were anesthetized and head-fixed, and whole-cell recordings made in auditory or visual cortex. Sensory stimuli (pure tones/vocalizations or light flashes/sequences, respectively) were presented to the animals; response strength and short- and long-term plasticity were measured. Methods are similar to our past studies in vivo (Froemke et al., Nature 2007; Froemke et al., Nature Neurosci 2013).

Results: In Cntnap2 mutant animals, we found that: 1) NMDA receptor amplitude was decreased relative to AMPA receptor transmission, 2) long-term synaptic plasticity was impaired, 3) GABAergic inhibition was imbalanced and uncorrelated to excitation, and 4) sensory responses were unreliable and more variable than in wild-type animals. Each of these latter results (#2-4) might come as a direct consequence of impaired NMDA receptor activation (#1). However, treatment with D-serine could selectively rescue and boost NMDA receptor currents in Cntnap2 mutant mice, suggesting that glycine site modulation could improve synaptic and local circuit function in these animals.

Conclusions: Many of the disruptions in Cntnap2 mutant mice (serving as a genetic model of schizophrenia) could have as a root cause reductions in current flux through NMDA receptors. However, glycine site agonists may provide a reasonable therapeutic approach for enhancing NMDA receptor function and improving cognitive processes, especially in the context of training-based approaches to recruit mechanisms of long-term synaptic plasticity and rebalancing inhibition with excitation.

Disclosure: Nothing to Disclose.

42.4 Diverse Neocortical Interneuron Subpopulations Contribute Fast and Slow Inhibitory "Blankets" Controlling Distinct Oscillatory Biomarkers of Schizophrenia in Mice

Rafael Yuste

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Background: The neocortex is a complex circuit, composed of dozens of subtypes of neurons with very different morphological and functional properties. Work from our lab and others depict a diversity of GABAergic interneurons including fast acting parvalbumin (PV) containing cells and slower acting somatostatin containing cells (SOM), each contributing a locally dense blanket of inhibition, while other interneuron subtypes such as vasoactive intestinal peptide (VIP) containing cells may provide more locally precise disinhibition of these blankets. Given this heterogeneity, these distinct subtypes could have very different (or even opposite) effects on the generation of spatiotemporally patterned cortical activity underlying cognitive and sensory processing known to be disrupted in schizophrenia (Sz). Indeed, patients with Sz display alterations in specific neocortical GABAergic interneuron markers and, likewise, display well-defined disruptions in intrinsic and sensory-elicited neural oscillatory dynamics ("biomarkers") dependent on local inhibitory feedback mechanisms. Here we demonstrate links between oscillatory visual biomarkers and specific inhibitory interneuron populations and discuss how this relates to patterned activity of the neocortical circuit in healthy and Sz mouse models.

Methods: In a variety of transgenic mice (C57Bl/6 origin) we selectively inhibited or stimulated PV, SOM, and VIP interneurons in V1 in vivo using optogenetics (ArchT, C1V1) while measuring i) spontaneous and sensory-elicited oscillations using linear multi-electrode arrays and ii) the activity of hundreds of cortical neurons simultaneously using two-photon calcium imaging.

Results: In the absence of sensory stimulation, inhibiting PV and SOM interneurons introduced increased synchrony in the cortical network as a whole as reflected in an increase in broadband oscillatory power and larger numbers of coactive neurons. Inhibiting PV interneurons also suppressed early phase locked response to visual stimuli (standard visual gratings and flashes) and decreased inter-cell correlations in the local network. Inhibiting SOM cells augmented early gamma oscillations to the onset of stimuli, but suppressed slower oscillatory dynamics (alpha band). Preliminary data suggest that ketamine exposure in mice (a putative pharmacological model of Sz) enhances intrinsic gamma oscillations but disrupts the structure of patterned cortical activity, overall decreasing inter-cell correlations over slower time courses, suggesting effects in multiple interneuron subtypes.

Conclusions: Distinct functional aspects of the neocortical circuit are controlled by local inhibitory interneuron subpopulations and are reflected in local field oscillations. These findings provide a series of hypotheses to be examined in a number of genetic mouse models of Sz, and suggest links between biological mechanisms and specific biomarkers readily measurable in patients. In the

future, a sophisticated therapeutic approach to Sz and other psychiatric disorders could be to specifically target the neuronal subtypes that play a key role in the controlling cortical activity, and by activating or inactivating them, block abnormal patterns and potentially, reduce or eliminate symptoms.
Disclosure: Nothing to Disclose.

Panel
43. Selective Genetic Targeting Reveals New Insights into Function and Dysfunction of the Noradrenergic Locus Coeruleus Brain System

43.1 Regulation of Cortical Processing and Behavior Through Selective Optogenetic and Pharmacogenetic Manipulation of Locus Coeruleus-Norepinephrine neurons

Elena Vazey

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Background: The nucleus locus coeruleus (LC) projects widely throughout the CNS and is the nearly exclusive source of norepinephrine (NE) to cortex. LC has been implicated in a range of core neural processes including arousal, mood and executive functions. Dysfunction in NE regulation of forebrain activity is also implicated in many disorders including ADHD, depression and dementia. Because of its role in core neural functions and disease, it is important to selectively target and manipulate LC-NE. Until recently, this has not been possible in non-genetically modified animals due to the small size and brainstem location of the LC nucleus.
Methods: We used viral vectors with a synthetic dopamine-β-hydroxylase promoter (PRSx8) to specifically restrict expression to LC-NE neurons in outbred rats. These cell-type specific vectors containing channelrhodopsin (ChR2) or DREADD designer receptors (hM3Dq and hM4Di) were targeted to LC-NE neurons in rats in vivo. We used ChR2 to specifically drive phasic or tonic LC-NE firing patterns. DREADDs were used to tonically manipulate LC-NE unit activity by local or systemic clozapine N-oxide administration (CNO, DREADD ligand). We characterized cortical responses with single neuron and electroencephalographic recordings. Finally, we selectively manipulated LC-NE activity with DREADDs while rats performed cognitive tasks to study the functional outcome of these changes in cortical processing.
Results: Optogenetic stimulation of LC-NE neurons drove phasic busting or tonic entrainment up to 15Hz. DREADD-based manipulations of LC-NE neurons altered tonic activity but not sensory-evoked phasic bursting. Stimulation of LC-NE either optogenetically or with DREADDs drove cortical arousal even in anesthetized animals. Under deep anesthesia, sustained LC-NE activation was sufficient to reduce burst suppression ratio (a measure of anesthetic depth) and drive a rightward shift in EEG, producing a theta dominant electroencephalogram. These LC-NE mediated changes in cortical state reliably modulated behavioral state transitions between consciousness and unconsciousness, favoring the conscious aroused state. In cortical neurons and fields, phasic (but not tonic) LC-NE stimulation produced a distinct late response (~200-400ms) following mild tactile stimuli that is normally only observed after intense stimuli. This late response reveals novel aspects of LC modulation of sensory processing. Given these effects on cortical activity and processing of environmental stimuli, we studied behavioral responses to LC-NE manipulation in cognitive function, specifically, attentional set-shifting. We found that activation of LC-NE neurons, particularly those that project to mPFC, facilitated behavioral flexibility as measured with extradimensional set-shifting.
Conclusions: The LC-NE system plays a critical role in a wide range of functions – from consciousness to behavior. Our ability to control this system with phenotypic and temporal specificity permits dissection of phasic vs. tonic LC-NE function in regulating sensory and cognitive processing. Our results show phasic LC-NE stimulation may encode a saliency signal integrated in late but not early cortical responses to sensory events. We also showed influences of tonic LC-NE manipulation on consciousness and cognitive function. This understanding will be important for developing treatments for psychiatric and neural disorders in which these functions are compromised.
Disclosure: Nothing to Disclose.

43.2 Is Norepinephrine Reinforcing?

David Weinshenker

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Background: The brain reward system controls motivated behavior by linking pleasurable effects to stimuli and mediating reinforcement, defined as an increase in the probability of a behavior occurring. Because this system mediates the positive subjective effects of natural rewards, as well as drugs of abuse, and its dysregulation contributes to addiction, identifying its functional neuroanatomy and neurochemistry is crucial. Beginning in the 1950s, intracranial self-stimulation (ICSS) paradigms were used to map the neuroanatomical substrates of reward and reinforcement in the brain. The correlation between the location of sites that supported ICSS and the location of catecholamine projections led to the "catecholamine hypothesis of reward," which posited that neurons containing norepinephrine (NE) or dopamine (DA) were critical for reward and reinforcement. Subsequent studies revealed that, whereas electrodes placed in the dopaminergic ventral tegmental area (VTA) robustly and consistently supported ICSS, stimulation of the noradrenergic locus coeruleus (LC) produced mixed results, with some studies supporting and others refuting a reinforcing effect of NE. These and other results identified DA neurons as a vital component of the brain reward system, while the LC came to be recognized as a major stress and aversion nucleus; a potential role for NE in reward was then mostly forgotten. However, LC ICSS experiments were hampered by caveats, including suspect electrode placement, activation of non-noradrenergic neurons and fibers of passage in and near the LC, and the use of non-physiological stimulation parameters. Thus, more than

half a century after the initial hypothesis and investigations, we lack a definitive answer to the fundamental question "Is NE reinforcing?". We used optogenetics, which overcomes the limitations of previous studies, to address this issue.
Methods: To achieve channelrhodopsin 2 (ChR2) expression selectively in VTA DA neurons, we used TH-Cre transgenic rats, which express Cre recombinase under control of the catecholamine neuron-specific tyrosine hydroxylase (TH) promoter, and unilateral intra-VTA injection of a Cre-dependent adeno-associated virus (AAV) containing ChR2 and the yellow fluorescent protein marker (DIO-ChR2-YFP). To achieve ChR2 expression selectively in LC NE neurons, we used unilateral intra-LC injection of a lentiviral vector containing ChR2 tagged with the mCherry marker under control of the noradrenergic-specific PRSx8 promoter (PRSx8-ChR2-mCherry). Four weeks later, an optic ferrule was implanted 1 mm dorsal to the viral injection site. One week later, rats were food trained for 1 day on an FR1 schedule to facilitate the learning of lever-pressing behavior (1 active lever press = 1 food pellet + cue light until 100 rewards earned, 6 h max; presses on the inactive lever had no programmed consequences). They were then switched to either LC stimulation (1 active lever press = 1 sec of 10 Hz stimulation + cue light), VTA stimulation (1 active lever press = 1 sec of 10-Hz stimulation + cue light), or control during daily 2-h sessions. Controls consisted of either no stimulation (cue light alone) or stimulation of a rat that received a PRSx8-mCherry virus with no ChR2.
Results: Preliminary results indicate that the control group showed a classic extinction burst on day 1, then subsequently extinguished active lever-pressing behavior over the next several days. As expected and shown before by others, VTA stimulation maintained operant behavior over several days. Importantly, LC stimulation also maintained operant behavior of a magnitude similar to VTA stimulation.
Conclusions: These data indicate that selective optogenetic LC activation can, in fact, be reinforcing. We are currently testing additional stimulation parameters, such as higher frequency stimulation, shorter stimulation bursts, and tonic-like stimulation rather than phasic bursting.
Disclosure: Part 1: I am co-inventor on a patent covering the use of selective dopamine beta-hydroxylase inhibitors for the treatment of cocaine dependence (Patent No. US-2010-0105748-A1 "Methods and Compositions for Treatment of Drug Addiction").

43.3 Locus Coeruleus Optogenetically Stimulated Activity During Sleep Suppresses Sleep Spindles, Increases REM Sleep Density and Impairs Reversal Learning

Gina Poe

University of Michigan, Ann Arbor, Michigan

Background: The locus coeruleus (LC) falls silent during rapid eye movement (REM) sleep and the transition to REM (TR) sleep spindles. Some have hypothesized that such silence is essential to REM sleep generation, although this hypothesis has not been tested. The presence of noradrenaline in the thalamus depolarizes the thalamic network and prevents spindle generation in anesthetized preparations, but its effect on sleep spindles in freely behaving animals has not been tested. Finally, noradrenaline acting at beta receptors increases the likelihood of long term potentiation (LTP) and prevents its opposite, depotentiation. It is our hypothesis that LC silence during TR and REM sleep uniquely allows bidirectional plasticity (LTP and depotentiation) and renders memories pliable to modification during sleep dependent consolidation and reconsolidation. Such bidirectional plasticity would be important to incorporate new information into old schema, such as when associating new safety-signalling contextual cues with old fear-signalling cues during extinction learning. There is evidence of sustained high noradrenergic activity during sleep in those suffering from PTSD. We had previously shown, using the antidepressant noradrenergic reuptake inhibitor desipramine, that sustained noradrenergic presence at synapses was enough to impair novel reversal learning in addition to impairing the reconsolidation of familiar information. Desipramine reduced REM and TR sleep significantly while impairing reversal learning. We sought to determine whether LC silence during REM and TR sleep is necessary for reversal learning by optogentically preventing such noradrenergic silence while leaving sleep amounts intact. Our experimental design also allowed us to test whether LC silence is necessary for REM sleep generation and whether sustained noradrenergic activity would permit sleep spindle generation.
Methods: We did not allow the LC to fall silent during TR and REM sleep states following a reversal learning period. A DBH promoter-linked channel rhodopsin gene with an mCherry red reporter gene was targeted to noradrenergic cells of the locus coeruleus. Fiber optic light guides coupled to bright LED's were stereotaxically targeted to the LC and mounted into a headstage. EEG and nuchal EMG electrodes were also inserted and attached to the headstage to measure sleep state parameters. After 8-10 days recovery, 8 male Long Evans and Sprague Dawley rats were retrained 30 minutes a day on a familiar 8-box track in which 3 of 8 boxes contained accessible food in constant positions relative to the room layout. Errors were counted whenever the rat checked unbaited box positions or omitted checking baited boxes. On reversal training day, the familiar maze session was immediately followed by a session on the same maze where the positions of the baited boxes were moved to new permanent locales. Twenty millisecond pulses of blue light were delivered at a sub-arousal frequency of 2-4 Hz to optogenetically stimulate the LC noradrenergic cells during the sleep period after training. Stimulation was begun after 30 seconds of sleep and lasted the duration of each sleep bout (no stimulation during waking) for 4 hours. Outcome measures were percent sleep states, sleep quality characteristics, especially sleep spindle number and REM density, sleep architecture (bout lengths and number of bouts), and performance measured in errors per lap on the familiar and reversal versions of the 8-box maze.
Results: Reversal learning and familiar performance on the 8-box maze were significantly worsened during the next training day when reversal learning was followed by a 4 hour sleep period that included LC low frequency stimulation during sleep. Sleep spindles were reduced by 2/3rds and REM sleep density, measured by myoclonic jerks, was nearly doubled. Sleep state percentages including REM sleep

amounts were unchanged from circadian matched baseline recordings in the same animals. Thus consistent 2-4 Hz LC noradrenergic cell activity did not prevent REM sleep but instead increased REM density, while sleep spindles were diminished. Histological results with the reporter mCherry and experiments that included simultaneous LC single and multiunit recording confirmed proper channel rhodopsin expression and optic fiber placement.
Conclusions: These results are consistent with our hypothesis that reversal and extinction learning require normal LC silence during sleep in order to incorporate new information into older schema. REM density increase under LC stimulation resembles the phasic REM sleep and increased REM sleep intensity of those suffering from PTSD and suggests that spindle quantity and quality should be more closely examined in PTSD patients. Both results are in line with the hypothesis that the hypernoradrenergic system during sleep in patients with PTSD is an underlying cause of PTSD symptoms including disturbed sleep metrics and abnormal fear memory extinction consolidation. We propose a mechanism whereby hippocampal firing patterns that are consistent with depotentiation during REM sleep combine with a lack of noradrenaline to allow resetting of memory synapses to prevent saturation within the CA1 region such that new contextual cues can be learned during e.g. extinction training. Our results further support our idea that hippocampal firing during TR sleep spindles in the absence of noradrenaline can serve to reformat prefrontal cortical information in light of new information associated in the hippocampus, a process critical to normal recovery after trauma and prevention of PTSD.
Disclosure: Nothing to Disclose.

43.4 An Optogenetic Means to Deconstruct Locus Coerulear Modular Function: Wagging the Tail with the Dog

Anthony Pickering

University of Bristol, Bristol, United Kingdom

Background: The locus coeruleus (LC) extends wide-spread projections across the neuroaxis and is linked to the regulation of diverse functions like sleep-wake cycles, signaling salience, autonomic control, pain and affect. We are interested in the role of noradrenergic neurons in the production of endogenous analgesia. Previous studies have shown that direct electrical or chemical stimulation of the LC can produce anti-nociception - mediated by spinal release of noradrenaline. Consistent with these findings we used a vector strategy to inhibit the pontospinal noradrenergic projection and demonstrated a pro-nociceptive action on acute pain (Howorth et al. 2009, J Neurosci). However, direct optogenetic activation of LC neurons showed both pro- and anti-nociceptive effects – evoked apparently from different subgroups within the LC (Hickey et al. 2014, J Neurosci). We hypothesized that this may be a consequence of the recruitment of distinct LC modules with opposing functions. Therefore we have developed a generalizable retrograde targeting strategy for noradrenergic neurons to specifically manipulate the pontospinal LC module.
Methods: All experiments conformed strictly to the UK animals (scientific procedures) 1986 Act and complied with Home Office guidance. We generated a canine adenoviral type 2 vector (CAV2) to express the light sensitive channelrhodopsin2 driven by a catecholaminergic specific promoter (CAV2-PRS-ChR2-mCherry). Under recovery anaesthetic Wistar rats received stereotaxic injections of vector either direct to the LC or into the spinal dorsal horn at L3-4. After 2-3 weeks analysis of expression patterns was enabled by immunocytochemistry for Dopamine β-Hydroxylase. Whole cell recordings were made from transduced LC neurons in pontine slices to confirm functional expression of ChR2. Extracellular recordings were made from opto-identified pontospinal LC neurons in anaesthetized animals to characterise their naturalistic response properties. The effects of engaging the pontospinal module on nociception were studied in behaving animals using the Hargreaves thermal withdrawal assay.
Results: Stereotaxic LC injection of CAV2-PRS-ChR2-mCherry to adult Wistar rats produced dense, selective transduction of noradrenergic neuronal somata throughout the nucleus (seen from one week to 5 months after injection). Recordings from transduced neurons in vitro showed robust light-evoked inward currents consistent with functional expression of ChR2 (1-2 weeks after transduction, p28-35 animals). Comparison of the electrophysiological properties of transduced and non-transduced LC neurons showed no differences in their intrinsic properties arguing indicating that transfection with CAV was well tolerated. These experiments validate the use of CAV2-PRS-ChR2-mCherry to express produce robust transduction of LC neurons. Injection of CAV2-PRS-ChR2-mcherry into spinal dorsal horn of Wistar rats resulted in retrograde, selective transduction of pontine NA neurons predominantly in LC ($78 \pm 9\%$, $n = 3$) but also in A5 ($7 \pm 2\%$) and A7 ($15 \pm 10\%$) after 2-3 weeks. Compared to our previous experience with human adenoviral vectors the CAV2 vector showed ~ 5 fold increased retrograde efficacy but this still only labelled a ventral subset of the LC neurons ($\sim 10\%$). Whole cell recordings from retrogradely transduced pontospinal neurones (7-14 days after CAV-PRS-ChR2-mCherry injection) showed reliable light-evoked spike discharge and evidence of characteristic ChR2 inward currents. The pontospinal LC neurones showed distinctive electrophysiological properties with shorter action potentials and smaller AHPs compared to non-transduced LC neurones. Spinally projecting LC neurones identified in vivo were shown to exhibit biphasic response to thermal and mechanical nociceptive stimuli consistent with a role in the processing of nociceptive information. In conscious behaving rats, opto-activation of the spinally projecting LC neurons prolonged withdrawal latency in Hargreaves test (from 9.7 ± 0.7 s vs 15.0 ± 2.5 s, $n = 8$, $p < 0.001$) and also produced immediate increase of alertness and arousal accompanied by self-grooming behaviour.
Conclusions: These data indicate that the CAV2-PRS-ChR2 viral vector is of utility for both retrograde neuronal tracing and functional circuit dissection of the noradrenergic systems and further that selective engagement of the pontospinal module of the LC is capable of exerting a potent anti-nociceptive action.
Disclosure: Nothing to Disclose.

Thursday, December 11, 2014

Panel
44. Neural Circuitry of Decision Making and Value-related Signals and Suicidal Behavior

44.1 Cortical and Subcortical Encoding of Prospective Reward Value

Joseph McGuire

University of Pennsylvania, Philadelphia, Pennsylvania

Background: Everyday rational decision-making requires estimating the subjective value, or utility, of available alternatives. Subjective value is a single quantity integrated across the attributes of each alternative (e.g., reward magnitude, delay, and risk). Subjective value estimates, when computed prospectively, serve to facilitate value-maximizing choice. This talk will present a general overview of neural mechanisms involved in subjectively evaluating future prospects. The relevant neural structures overlap with those involved in registering the arrival of rewards, including the mesolimbic dopamine system and its projection targets in striatum and prefrontal cortex. Electrophysiological evidence has implicated these structures in encoding a signal of reward prediction error. Human neuroimaging research has examined effects of subjective value in a wide array of task contexts. This talk will focus on a quantitative meta-analysis seeking to identify neural responses consistently associated with subjective value across approximately 200 fMRI experiments.
Methods: To be included in the meta-analysis, a study had to report whole-brain results for a comparison between different levels of subjective value. Peak activation foci were tested for significant spatial clustering across studies, relative to the null hypothesis that activation foci were spatially random within gray matter. Finer-grained tests were conducted using subsets of the meta-analytic corpus. Separate analyses examined positive effects (greater fMRI BOLD signal associated with higher subjective value) and negative effects (greater signal associated with lower subjective value). Positive effects were further subdivided based on whether they focused on the time of decision making vs. outcome receipt, and whether primary rewards vs. monetary incentives were at stake.
Results: One set of brain regions showed a mixture of positive and negative subjective value responses across studies. This might be explained by an underlying U-shaped effect, reflecting a quantity such as salience or motivational relevance rather than subjective value per se. A second set of regions showed positive effects exclusively. These regions, including ventromedial prefrontal cortex (VMPFC), ventral striatum (VS), and posterior cingulate cortex (PCC), may constitute a core system for subjective evaluation. VMPFC and VS each responded during both decision making and outcome receipt, and to both monetary and primary-reward outcomes. VMFPC has been implicated in a variety of effects other than subjective evaluation (e.g., episodic cognition, self-referential cognition, and task-related deactivation). Meta-analytic comparisons suggest considerable anatomical overlap between these disparate domains. Higher-resolution methods will be needed to determine definitively whether these functions engage the same or merely adjacent cortical territory.
Conclusions: A core neural system consisting of VMPFC, VS, and PCC, consistently reflects subjective value in human neuroimaging studies across a variety of task contexts and reward modalities. These cortical and subcortical structures are thought to interact with the mesolimbic dopamine system to support the prospective appraisal of individual alternatives during value-based decision making.
Disclosure: Nothing to Disclose.

44.2 Neurotransmitters and Decision Making in Suicidal Behavior

J. Mann

Columbia University, New York, New York

Background: Postmortem studies of suicide have found abnormalities in serotonin transporter and 5-HT1A receptor binding in ventral prefrontal cortex, brainstem raphe nuclei and in anterior cingulate, brain regions known to be involved in willed action and decision-making. A number of studies using structural and functional MRI have found structural and functional deficits in suicide attempters and cognitive tests of decision-making performance. What has been lacking are neurotransmitter imaging studies related to these brain regions involved in decision-making and their status in suicidal behavior.
Methods: We have conducted PET studies of the serotonin system in three medication-free groups: DSM-IV major depression suicide attempters, major depression nonattempters and healthy volunteers. We have quantified binding to the serotonin transporter and to the 5-HT1A receptor. Our goal was to determine if there was a parallel to the findings in the same serotonin indices as observed in suicides.
Results: We found a deficit in serotonin transporter binding in major depression associated with suicidal behavior independent of major depression. This deficit was most pronounced in the midbrain raphe nuclei. Findings in other brain regions were a trend in the hypothesized direction. We also found great 5-HT1A binding in the midbrain raphe nuclei that correlated with lethality of suicidal behavior. Binding in prefrontal cortex correlated with severity of suicidal ideation.
Conclusions: Serotonin abnormalities associated with more lethal suicidal behavior map approximately on to the similar brain regions as observed in suicides. These findings are important for two major reasons. The biological abnormalities of nonfatal suicidal behavior resemble suicide only when the attempts are more lethal and demonstrate the need to consider type of nonfatal suicidal behavior in efforts at understanding cause and prediction of risk. Second, the biological stigmata of suicide are present in living patients and may be a tool for detection of high risk patients and a biomarker for interventions that ameliorate risk.
Disclosure: Part 1: Stock options from Qualitas Health a startup company developing a PUFA product, **Part 2:** Royalties for commercial use of C-SSRS from Research Foundation for Mental Hygiene.

44.3 Paralimbic Value Signals, Impulsivity, and Suicidal Behavior

Alexandre Dombrovski

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: Suicide can be viewed as an escape from unendurable punishment at the cost of any future rewards. Could faulty estimation of these outcomes predispose to suicidal behavior? We investigated the neural circuit abnormalities that underlie disadvantageous choices in people at risk for suicide. We also examined the relationship between these neural circuit abnormalities putative components of vulnerability to suicide: impulsivity, poor cognitive control, and depression.
Methods: We conducted a series of behavioral decision-making experiments and a functional magnetic resonance imaging (fMRI) study augmented with a computational model in older (60+) suicide attempters. Fifty-three participants in the fMRI study included 15 depressed patients who had attempted suicide, 18 depressed patients who had never attempted suicide (depressed control subjects), and 20 psychiatrically healthy controls.
Results: On gambling and reward learning tasks, suicide attempters tended to make disadvantageous decisions that neglected useful information. In the imaging study, impulsivity and a history of suicide attempts (particularly poorly planned ones) were associated with a weakened expected reward signal in the paralimbic cortex (particularly the ventromedial prefrontal cortex, BA32), which in turn predicted the behavioral insensitivity to contingency change. This blunting of paralimbic expected value signals was distinct and doubly dissociated from alterations in the corticostriatothalamic responses to unexpected rewards (positive prediction errors) associated with depression and poor cognitive control. The findings were robust to effects of possible confounders, including severity of depression, medication exposure, possible brain injury from suicide attempts, and premorbid IQ.
Conclusions: Faulty integration of value in the paralimbic cortex may facilitate suicidal acts through suboptimal decisions under uncertainty. In a crisis, affected individuals may ignore deterrents to suicide and alternative solutions. This behavioral pattern, also seen in gambling and cocaine use, may reflect a primary deficit in the paralimbic cortex or in its mesolimbic input. This pattern is distinct from a general disruption in integrating reinforcement seen in individuals with poor cognitive control.
Disclosure: Nothing to Disclose.

44.4 Social Decision Making in Suicidal Behavior

Katalin Szanto

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: One can conceptualize suicide as an extreme reaction to stressors that involves a distorted cost-benefit analysis. Suicide is often a solution to mounting conflicts, albeit at a catastrophic personal cost. Social motivations range from a need to escape and relieve others of burden, to revenge, or a wish to see that others care. Perceived unfairness is a common theme in suicide notes. Social injustice compels us to punish offenders, often at a cost to ourselves. Economic exchange games provide a controlled environment in which to measure decision biases in a social context.
Methods: Our study focused on older adults because of the high proportion of medically serious (high-lethality (HL) suicide attempts in this age group. We used the Ultimatum Game (UG) which measures responses to unfairness in 26 depressed older adults with a history of HL attempts (HL), 20 low-medical lethality suicide attempts (LL), 35 non-suicidal depressed older adults, and 22 elders with no psychiatric history. In the UG, players decide whether to accept or punish (reject) unfair monetary offers from another player, trading personal gain against social fairness. Despite the fact that rejection is personally costly, people typically punish unfair counterparts by rejecting their offers. Yet, the demand for social fairness is sensitive to price in healthy subjects; with increasing reward size unfair offers are rejected less frequently. In our analyses we took advantage of complete trial-by-trial data, using hierarchical models including within-subject and between-subject levels.
Results: On the UG, HL failed to incorporate the cost of prosocial punishments into their choices. We ascertained that HL attempters' disadvantageous social decision making was not due to poor cognitive control or impulsivity. It was also evident in a condition when no actual rewards were delivered, suggesting that HL's behavior is due to the specific effects of social emotions and not to a general insensitivity to any rewards. Such interference of social emotions with the estimation of expected rewards has been noted in neuroeconomic research with healthy subjects. Delgado and colleagues found that information about the opposing players' social reputation interfered with striatal reward responses during an economic exchange. These striatal responses to unexpected rewards are thought to represent the critical learning signal, which shapes reward-guided behavior. Extending these findings Dr. Szántó will also present new data related to emotional interference in cooperation and trust using a trust game experiment.
Conclusions: Economic bargaining games can model social influences on decision-making and help to identify individuals at risk for suicide. We propose that social decision making deficits are part of the suicidal diathesis, and they underlie interpersonal conflicts that are often described by suicidal individuals. In real life, high-lethality attempters' relative insensitivity to the cost of retaliation and their emotional interference in cooperation and trust may lead to uncompromising, catastrophic responses to conflicts.
Disclosure: Nothing to Disclose.

Panel
45. Measuring, Modulating, and Manipulating alpha7 nicotinic acetylcholine Receptors (α7-nAChR): Biology, Behavior, Biomarkers

45.1 Drug Actions on Nicotinic Receptors. Chronic vs Acute; Outside-in vs Inside-out

Henry Lester

California Institute of Technology, Pasadena, California

Background: The superfamily of nicotinic acetylcholine receptors (nAChRs) comprises various homopentameric

and heteropentameric ligand-gated channels (such as alpha7 and alpha4beta2*, respectively). Continuing studies show how the acute effects of exposure to nicotine (ms to min) arise from an "outside-in" event: activation and desensitization of sodium, potassium, and calcium selective ion channels in the various nAChRs. The "outside-in" events of nicotine resemble the actions of acetylcholine itself. These events are becoming known on the neuroanatomical scale of micrometers, the time scale of μs, the structural distance scale of Å, and the genomic resolution of single bp.
Methods: Evolving methods are also revealing new features of nicotinic drug action during **chronic** exposure (hr to wk). The new methods include fluorescently labeled nAChRs, expressed both in knock-in mice and in cultured model systems; proteomics of molecules that interact with nAChRs; and RNA-Seq of genes activated by chronic nicotine.
Results: An "inside-out" mechanism is becoming a likely mechanism to explain some chronic actions of nicotine and other nicotinic drugs. The "inside-out" pathway occurs at much lower nicotine concentrations than channel activation. The "inside-out" pathway begins with pharmacological chaperoning: nicotine and related drugs permeate into the cytoplasm, then into the endoplasmic reticulum. There, the drugs interact with nAChRs and stabilize some nascent nAChRs. The best-known result of "inside-out" action is the classical posttranslational "upregulation" of some nAChRs. In addition, the "inside-out" pathway also leads, via pharmacological chaperoning, to other events including decreased endoplasmic reticulum stress, decreased unfolded protein response, and possibly perturbed nAChR interactions with other molecules.
Conclusions: The "inside-events" are not straightforward continuations of transduction pathways activated during "outside-in" activation and desensitization, but result when prolonged (hr to wk) nicotine binding to intracellular nAChRs activates an entirely different set of pathways. The "inside-out" pathway is accreting many biophysical, biochemical, thermodynamic, cell biological, neuroanatomical, and electrophysiological details. Yet, "inside-out" mechanisms have arisen, in part, from re-examining classical pharmacokinetic and pharmacodynamic facts: nicotine can interact with intracellular molecules many orders of magnitude more strongly than can the endogenous neurotransmitter, acetylcholine. There is no current evidence that the "inside-out" events have counterparts in the normal cell or organismic biology of nAChRs. The present challenge is pursue the hypothesis that the "inside-out" events have the power and selectivity to underlie two aspects of chronic exposure to nicotine: nicotine dependence and apparent neuroprotection.
Disclosure: Nothing to Disclose.

45.2 Alpha7 Nicotinic Receptor Agonists as Pro-cognitive Drugs for CNS Diseases

William Kem

University of Florida College of Medicine, Gainesville, Florida

Background: Alpha7 nicotinic receptors (α7s) have been implicated in the pathophysiology of schizophrenia, Alzheimer's disease and Parkinson's disease. A considerable body of data now exists that indicates α7 agonists enhance cognitive function in animal models of these diseases and in humans. Besides its involvement in synaptic signaling within the cognitive circuitry of the brain, this calcium-permeable ion channel also indirectly influences gene transcription and long term memory through its stimulation of intracellular signaling cascades that are responsive to elevations in intracellular calcium. Most neuronal α7s are located within the CNS, but macrophages (also microglia) secreting a variety of inflammatory cytokines also express these receptors; α7 agonists have been demonstrated to inhibit cytokine secretion from these cells. Many schizophrenics have subnormal α7expression. Most (~83%) are heavy smokers and may be self-medicating through this nAChR subtype, which displays a relatively low affinity for nicotine. While antipsychotic drugs are generally successful in controlling positive symptoms, they have not been very successful in treating negative symptoms, including cognitive deficits, that are now a major target for improving treatment of this mental disease. Evidence for α7 involvement in other CNS diseases is less extensive. Brains of AD and PD patients show significant loss of α4β2 and α7 receptors at death in comparison to age-matched controls. Aβ1-42 binds to brain α7s and inhibits α7 activation in neurons and microglia. α7 knock-out mice over-expressing human Aβ1-42 develop a more extensive AD histology and cognitive deficits than control mice. DA secreting neurons in the striatum also express α7 receptors and recent studies indicate that α7 agonists can improve locomotor and cognitive function in a primate PD animal model, as well as being neuroprotective in rodent PD models.
Methods: A variety of behavioral tests were used to assess α7 effects on sensory gating and cognition in animals and in the clinical trials.
Results: Several α7 agonists have been shown to improve cognitive dysfunction in various animal models and several are now in clinical trials. DMXB-A (GTS-21), a derivative of the marine toxin anabaseine, entered clinical tests in the mid-1990s. This compound has been made available to a large number of laboratories and consequently >130 publications have appeared in which it was used either as a research tool or tested as a drug candidate. DMXB-A has been shown to have procognitive and sensory gating effects in schizophrenics, and results from a recent phase II test that for the first time included smokers will be presented. Recently several quinuclidine derivatives including EVP-6124, ABT-107, R-3487 and TC-5619 have also been subjected to phase II trials for schizophrenia or AD. DMXB-A and EV-6124 are partial agonists and the others are full agonists. The cognitive effects of these drug candidates, which occur at plasma concentrations that are much lower than would be predicted from in vitro functional experiments on human α7s will be presented.
Conclusions: Although α7 receptors are readily desensitized by high agonist concentrations, low agonist concentrations can improve sensory gating and cognition in schizophrenic test subjects as well as in experimental animal models. A "receptor priming" hypothesis rationalizes this situation and in vivo PET imaging, by assessing the extent of α7 occupancy generated at clinical test doses, can provide a test of this hypothesis.

Disclosure: Part 1: WRK is a coinventor on several Univ Florida patents regarding DMXB-A (GTS-21).

45.3 Development of [18F]ASEM, the First Highly Specific α7-nAChR Radioligand for PET Imaging

Andrew Horti

Johns Hopkins University, Baltimore, Maryland

Background: The nicotinic acetylcholine receptor subtype α7-nAChR and α7 drugs constitute a vibrant field in modern medicine. Positron-emission tomography (PET) is the most advanced imaging technique for non-invasive research of receptors. PET imaging would provide a significant advance in the understanding of α7-related CNS disorders. We will review the α7 PET radioligands that have been synthesized previously globally, but most of them manifested low specific binding in the brain. Here we describe development of the first highly specific and selective radioligand [18F]ASEM for PET imaging of α7-nAChRs.
Methods: New series of α7 dibenzothiophene ligands was synthesized and their inhibition binding affinities (Ki, nM) at various nAChR subtypes (α7, α2β2, α2β4, α3β2, α3β4, α4β2-nAChR, α4β4-nAChR) and 5-HT3 were determined. The compound with the best α7 binding affinity and selectivity, ASEM (4-(6-fluorodibenzo[b,d]thiophen-3-yl)-1,4-diazabicyclo[3.2.2]nonane 5,5-dioxide), was radiolabeled with 18F. Regional brain distribution of [18F]ASEM was studied in control mice and after blockade with the selective α7 ligand SSR180711, clinical α7 drugs GTS-21, EVP-6124, varenicline and non-α7 ligands (α4β2-nAChR, 5-HT3, 5-HT2, D1, D2, D3 and opioid). The effect of GTS-21 on the brain distribution of D2/3 PET tracer [11C]raclopride in the mouse brain was also assessed. The radiometabolites of [18F]ASEM were determined in the mouse plasma and brain by HPLC.
Results: ASEM exhibits higher α7 binding affinity (Ki = 0.4 nM) and selectivity than at heteromeric nAChR subtypes and 5-HT3 (selectivity > 1000). [18F]ASEM was prepared with a radiochemical yield of 50% and specific radioactivity of 296 to 2180 GBq/μmol. In mice, [18F]ASEM readily entered the mouse brain and specifically labeled α7-nAChRs with BPND = 5 – 8. The α7-nAChR selective ligand SSR180711 blocked the binding of [18F]ASEM in the mouse brain in a dose-dependent manner. None of the non-α7-nAChRs central drugs reduced accumulation of radioactivity when compared with controls. GTS-21, EVP-6124 or Varenicline significantly blocked the [18F]ASEM binding in the brain. The GTS-21 challenge significantly reduced the uptake of [11C]raclopride at a low dose (3 mg/kg) of GTS-21 (− 50%). At a higher dose of GTS-21 (25 mg/kg) the [11C]raclopride uptake was increased (+55%) ($P < 0.001$, ANOVA). [18F]ASEM undergoes rapid metabolism in mouse blood (∼1% parent at 30 min), but the radiometabolites do not enter the brain (>95% parent).
Conclusions: [18F]ASEM, a new selective α7-nAChR radioligand with high binding affinity, has been synthesized and its pre-clinical evaluation in mice was performed. [18F]ASEM readily entered the mouse brain and labeled cerebral α7-nAChR receptors with high specificity and selectivity. The binding potential of [18F]ASEM (BPND = 5.3 – 8.0) in control mice is superior to previous α7-nAChRs PET radioligands. Another advantage of [18F]ASEM is the low amount of radiometabolites that accumulate in the brain tissue. [18F]ASEM is suitable for studying the α7-nAChR occupancy by various nicotinic drugs including the current α7 drugs in clinical trials (GTS-21 and EVP-6124). A study of the effect of administration of GTS-21 on the D2/3 radiotracer [11C]raclopride demonstrates that low doses of GTS-21 activate α7-nAChR and stimulate a release of dopamine that blocks [11C]raclopride, whereas high doses of GTS-21 desensitize the α7-nAChR, inhibit dopamine release, and, thus, reduce the competition with [11C]raclopride. A combination of [18F]ASEM and [11C]raclopride imaging is a powerful tool for studying effect of α7 drugs. [18F]ASEM is the first highly specific PET radioligand for quantification of α7-nAChRs.
Disclosure: Nothing to Disclose.

45.4 First Successful PET-[18F]ASEM Imaging of the α7-nAChR in Human Subjects: Global Efforts and JHU Novel Studies

Dean Wong

Johns Hopkins Medical Institutions, Baltimore, Maryland

Background: Until recently existing PET radioligands for α7-nAChR were not suitable for quantitative imaging largely due to suboptimal in vivo kinetic properties, especially insufficient specific binding in preclinical studies. Recently we developed [18F]ASEM, a radioligand that showed high specific α7-nAChR binding in the mouse1 and baboon brain2 and humans as of the 2014 spring. The α7-nAChRs have been implicated in the pathophysiology and treatment of psychiatric and neurodegenerative disorders including schizophrenia (SCZ) and dementia. A goal of this presentation is to demonstrate our novel PET human studies of α7 with [18F]ASEM. We will also review existing PET non-human imaging attempts globally in comparison with α7-nAChR PET imaging in healthy human subjects with [18F]ASEM. Another goal is to determine suitability of [18F]ASEM for occupancy studies with DMXB-A, an α7-nAChR drug that demonstrated cognitive enhancement in SCZ using a non-human PET study.
Methods: For our unpublished data , following preclinical toxicology, radiation dosimetry and a successful eIND with the FDA, four healthy male subjects were imaged for 90 min after bolus IV injection of [18F]ASEM (12-15 mCi, specific radioactivity > 10 Ci/□mol). After MRI co-registration, time-activity curves from 23 brain regions were generated. Plasma input graphical analysis (PRGA), using HPLC metabolite corrected arterial plasma, was employed. Baboon studies were carried out in the absence and presence of DMXB-A given (oral 10 mg/kg) baseline binding potential as previously described in Horti, et al Nuclear Medicine 2013.
Results: [18F]ASEM readily entered the human brain after an IV bolus injection and showed reversible kinetics with a peak (%SUV = 300 - 400) at 10-15 min. In human subjects the regional brain distribution of [18F]ASEM matched our previous in vitro data and was comparable with prior baboon [18F]ASEM-PET results. Distribution volumes (VT) generated by PRGA proved to be more stable within

individual regions than those generated by compartmental models. Precuneus, parietal, occipital, and cingulate cortices, and putamen showed relatively high values of VT (>20 mL/mL) and binding potential (>1; BPND = VT/VT(corpus callosum) - 1) while entorhinal cortex, cerebellum, caudate and corpus callosum showed lower values of VT (<15 mL/mL). The baboon PET imaging studies show baseline VT and a significant blockade was demonstrated after the administration of DMXB-A.
Conclusions: [18F]ASEM can quantify α7-nAChRs in human brain with regionally specific imaging characteristics and high values of VT and BPND. Binding specificity and occupancy studies in human subjects with DMXB-A are being vigorously pursued. Research support: NIH grants MH079017 and AG037298 References 1. Gao et al., J Med Chem 2013, 56, 7574-9. 2. Horti et al., J Nucl Med 2014, 55, 672-7.
Disclosure: Part 1: Dartneuroscience, **Part 4:** Avid, Biotie, GE, Intracellular, J&J, Lilly, Lundbeck, Merck, Pfizer, Roche.

Panel
46. Modifiable Risk Factors for Cognitive Decline and Neurodegeneration

46.1 The Importance of Risk Factor Modification over the Lifecourse

Kristine Yaffe

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Background: Studies of mid and late life exposures have identified a number of modifiable risk factors for the prevention of cognitive decline and neurodegeneration. A small but growing body of data suggests that risk factors earlier in the lifecourse may also contribute to an increased risk of cognitive aging.
Methods: We present data on early adult modifiable risk factors from the Coronary Artery Risk Development in Young Adults Study (CARDIA), a biracial cohort of 3,499 adults (18-30 years old at enrollment). Cumulative exposures estimated as the area under the curve and long-term exposures to cardiovascular risk factors and lifestyle behaviors were evaluated using repeated measures over 25 years. At Year 25, cognitive function was assessed using the Digit Symbol Substitution Test (DSST), Stroop Interference Score, and Rey Auditory Verbal Learning Test (RAVLT). An additional study using meta-analytic data from mid and late life epidemiological studies were used to estimate the population attributable risks and projected impact of seven major modifiable risk factors (diabetes, midlife hypertension, midlife obesity, depression, physical inactivity, smoking, and low education) on Alzheimer's disease (AD) prevalence.
Results: Over 25 years, cumulative exposure to elevated levels of blood pressure and fasting blood glucose was associated with worse cognitive performance on RAVLT, DSST, and Stroop in midlife ($p<0.05$ for all). Similarly, a greater number of the American Heart Association's components for ideal cardiovascular health over the follow up period was associated with better cognitive function (p for trend <0.01 for all) while long-term patterns of low physical activity over 25 years were associated with increased likelihood of poor cognitive performance on DSST and Stroop in midlife ($p<0.05$ for both). Projected data derived from mid and late life epidemiological studies indicate that modifiable risk factors account for up to half of AD cases worldwide (diabetes: 2%, midlife hypertension: 5%, midlife obesity: 2%, depression: 10%, physical inactivity: 13%, smoking: 14%, low education: 19%), and a 25% reduction of these combined risk factors could prevent up to 3 million cases of AD.
Conclusions: Compelling evidence supports an increased risk of cognitive decline associated with exposure to modifiable risk factors over the lifecourse. Prevention strategies which target these risk factors could significantly reduce the public health burden of cognitive impairment.
Disclosure: Part 1: Dr. Yaffe is a consultant for Novartis and Pfizer. She serves on DSMBs for Takeda, Inc.

46.2 Treatment Strategies to Modify Disease Course in Comorbid Depression and Cognitive Impairment

Davangere Devanand

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Background: Depression and cognitive impairment are common disorders in the elderly. Epidemiological and clinical studies show that individuals presenting with both depression (DEP) and cognitive impairment (CI), DEP-CI, often progress to dementia. Whether depression is an independent risk factor, or an early feature comorbid with cognitive decline in individuals with Alzheiimer's or vascular dementia brain pathology, remains a topic of vigorous debate. Evidence from treatment trials in patients with DEP-CI is limited.
Methods: Thirty five depressed, cognitively impaired (DEP-CI) patients 50–90 years old participated in this 48-week treatment trial. Escitalopram alone was prescribed for the first 2 weeks and memantine was added to escitalopram for the remainder of the trial. Comprehensive assessments for depression were conducted every 2–4 weeks, and neuropsychological testing with diagnostic assessment was conducted at baseline, 12, 24, and 48 weeks.
Results: Of the 35 DEP-CI patients, 28 completed week 12 and 26 completed week 48. 24-item Hamilton Depression Rating Scale scores improved markedly during the 48-week treatment period. One of 35 patients (2.9%) converted to dementia, diagnosed as probable Alzheimer's disease (AD), over 48 weeks. This rate is lower than that observed in naturalistic follow-up studies of DEP-CI subjects. There was improvement in Selective Reminding Test (SRT) total recall ($F=6.60$; $p=0.0147$) with significant improvements in category fluency for letters ($p=0.01$) and Boston Naming test ($p=0.001$), but not for any of the other cognitive measures. The presence of amnestic mild cognitive impairment (MCI, $n=22$), non-amnestic MCI ($n=13$), and antidepressant response (responders $n=25$, non-responders $n=10$) were not significantly related to the change in SRT total recall from baseline to end-study.
Conclusions: In a meta-analysis of memantine treatment for AD, the effect size was 0.24 using the ADAS-cog scale. The effect size in our study with SRT total recall was 0.45. The

similarities in time course of response to memantine with published trials in AD indirectly suggest that many DEP-CI patients have incipient AD. The cognitive improvement and low conversion rate to dementia in DEP-CI patients suggests that the combination of escitalopram and memantine may delay both cognitive decline and clinical conversion to a diagnosis of dementia. In the context of our prior work showing that donepezil in patients with DEP-CI may improve cognition to a greater extent than placebo, the strategy of combined antidepressant and cognitive enhancer treatment in patients with DEP-CI appears to be a viable therapeutic option that may delay the clinical diagnosis of dementia over time. The findings indirectly support the view that depression in older patients with cognitive decline is often a comorbid feature rather than an independent risk factor for dementia.
Disclosure: Part 1: Research Support: Eli Lilly Consultant: AbbVie, Lundbeck.

46.3 FDDNP-PET Brain Imaging Patterns in Retired Professional Football Players Differ from Those of Patients with Alzheimer's Dementia

Gary Small

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Background: Mild traumatic brain injury due to contact sports may cause chronic behavioral, mood, and cognitive disturbances associated with tau protein deposition resulting in a condition known as chronic traumatic encephalopathy (CTE). National Football League (NFL) players have a higher rate of behavioral, mood, and cognitive disturbances compared with controls. We used positron emission tomography (PET) scans after intravenous injections of FDDNP, which measures tau and amyloid deposition in living brains, to determine if brain tau deposition patterns in retired players with suspected CTE differ from those of patients with Alzheimer's dementia (AD) and normal controls.
Methods: Retired NFL players ($N = 14$) with mood and cognitive symptoms, patients with AD ($N = 24$), and cognitively intact controls ($N = 28$) received neuropsychiatric evaluations and FDDNP-PET. PET signals in subcortical (caudate, putamen, thalamus, midbrain, cerebellar white matter) and cortical (amygdala, frontal, parietal, posterior cingulate, medial, lateral temporal) regions were compared.
Results: Compared with controls, players had significantly higher subcortical and amygdala FDDNP binding. This FDDNP PET imaging pattern for the retired players was distinctively different from the pattern of high temporal, parietal, posterior cingulate, frontal and parietal binding observed in AD. This is consistent with the progressive neuropathology observed in AD, which typically begins in the medial temporal lobe along the cortical default mode network, with no or minimal involvement of subcortical structures.
Conclusions: This particular FDDNP-PET imaging pattern in cases of suspected CTE is consistent with the tau distribution observed at autopsy in subjects with a history of traumatic brain injury, cognitive and behavioral symptoms, and an autopsy-confirmed diagnosis of CTE. These results point to different tau and amyloid deposition patterns in retired football players compared with AD patients, and suggest that FDDNP-PET may offer a means to identify neurodegeneration in contact-sports athletes.
Disclosure: Part 1: Adviser/Speaker: Lilly, Novartis, Pfizer, Janssen, **Part 2:** Novartis.

46.4 The Dopamine System in Frontotemporal Dementia and Related Illnesses

Edward Huey

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Background: The frontal lobes receive extensive dopaminergic projections. Early evidence suggests that the phenotype and course of neurodegenerative disorders that primarily affect the frontal lobes and connected subcortical structures, such as Frontotemporal dementia (FTD), can be affected by functional polymorphisms in genes that code for enzymes that breakdown dopamine in the frontal lobes. We examined the effect of such a polymorphism, the Catechol O-methyltransferase (COMT) val158met polymorphism, on the clinical and MRI findings in 174 patients with FTD-spectrum illness and the cognitive, behavioral, and fMRI effects of inhibiting COMT with a COMT inhibitor (tolcapone) in 28 patients with FTD.
Methods: COMT genotyping, MRI scans and measures of executive function, motor function, memory, and neuropsychiatric symptoms in 110 patients with FTD and 64 patients with Corticobasal syndrome (CBS) were obtained. A single ANOVA was performed on the effect of val dosage in the COMT val158met polymorphism on the domains listed above. A second ANOVA was performed in SPM8 comparing the effects of val dosage in the COMT val158met polymorphism on brain volume in a whole-brain analysis. Clusters surviving an uncorrected threshold of $p < 0.001$ and a cluster size of 30 voxels were considered significant. A 4-week placebo-controlled crossover study of the effects of 200 mg po tid of tolcapone on the behavioral, cognitive, and language symptoms of FTD was performed on 28 patients with FTD. Resting fMRI data and fMRI data during N-back and other task performance was collected at baseline and during placebo and active treatment phases.
Results: The COMT val allele at position 158 was associated with selective impairment of executive function, as measured by the Delis-Kaplan Executive Function System (D-KEFS), and decreased volume of the bilateral caudate heads on MRI. Within the caudate heads, grey matter density was negatively correlated with verbal fluency and sorting on the D-KEFS. Results are pending on the trial of tolcapone for FTD, but will be available by the meeting.
Conclusions: The frontal lobes receive extensive dopaminergic projections from the basal ganglia. These data indicate that the COMT system, which affects brain dopamine, influences neurodegeneration in the basal ganglia, which provide dopaminergic projections to the frontal lobes. Tolcapone is an inhibitor of COMT that is currently

FDA-approved and may provide benefit for the symptoms of FTD-spectrum disorders.
Disclosure: Nothing to Disclose.

Panel
47. Sleep, Schizophrenia and Spindles

47.1 Sleep Spindle Deficits in Schizophrenia: A Treatable Mechanism of Impaired Cognition?

Dara Manoach

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Background: Chronic medicated patients with schizophrenia have marked reductions in sleep spindle activity and a correlated deficit in sleep-dependent memory consolidation. In a randomized placebo-controlled trial we found that eszopiclone – a non-benzodiazepine hypnotic agent that acts on γ-aminobutyric acid (GABA) neurons in the thalamic reticular nucleus where spindles are generated – significantly increased spindle activity in chronic, medicated schizophrenia. In the present study, we used archival data to investigate whether antipsychotic-naïve early course patients with schizophrenia and young non-psychotic first-degree relatives of patients with schizophrenia also show reduced sleep spindle activity and whether spindle activity correlates with cognitive function and symptoms.
Methods: Sleep spindle activity during Stage 2 sleep was compared in antipsychotic-naïve adults newly diagnosed with psychosis (n = 26: 15 with schizophrenia; 11 with other psychotic disorders), young non-psychotic first-degree relatives of schizophrenia patients (n = 19) and two samples of healthy controls: one matched to the patients (n = 25) and the other matched to the relatives (n = 12). Spindle parameters were correlated with cognitive measures and symptom ratings.
Results: Early course schizophrenia patients showed significantly reduced spindle activity relative to both healthy controls and to early course patients with other psychotic disorders. Relatives of schizophrenia patients also showed reduced spindle activity compared with controls. Reduced spindle activity correlated with worse performance on measures of executive function and IQ and with reduced severity of positive symptoms.
Conclusions: This study presents the first demonstration that, like chronic medicated schizophrenia patients, antipsychotic-naïve early course schizophrenia patients and young non-psychotic relatives of individuals with schizophrenia show reduced sleep spindle activity. In contrast, early course psychotic patients with other diagnoses showed normal spindle activity. These findings indicate that the spindle deficit is not due to antipsychotic medications, is not a product of chronic illness nor is it a general feature of psychosis. Moreover, consistent with a growing basic literature that links sleep spindles to a range of cognitive functions, the present study found that sleep spindle activity correlated with cognition. Our data are consistent with the hypothesis that the spindle deficit is an endophenotype of schizophrenia that predates its onset, persists throughout its course and contributes to cognitive dysfunction. The present data, together with our prior findings that sleep spindles deficits correlate with impaired memory consolidation and can be effectively treated, suggest sleep spindles as a novel target for treatment development. Collaborators: Charmaine Demanuele, Erin Wamsley, Mark Vangel, Debra Montrose, Jean Miewald, David Kupfer, Daniel Buysse, Robert Stickgold, Matcheri Keshavan Support: K24 MH099421; R01 MH092638.
Disclosure: Nothing to Disclose.

47.2 Anatomical Volume of Interest Analysis and Sleep Spindle Source Modeling Point to a TRN-MD Thalamus-Prefrontal Cortex Circuit Deficit in Schizophrenia

Fabio Ferrarelli

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Background: Marked deficits in sleep spindles have recently been reported in patients with schizophrenia compared to both healthy individuals and psychiatric non-schizophrenia patients. Sleep spindles are waxing and waning NREM sleep oscillations that are generated within the thalamus by the interplay of the Thalamic Reticular Nucleus (TRN) with dorsal thalamic nuclei and then amplified and sustained in the cortex and have been inked to memory consolidation and plasticity. After reviewing our previous findings on sleep spindle deficits in schizophrenia and their relation to cognitive impairment, we will present new results combining anatomical and functional indices and their association with impairment in an abstraction/working memory task.
Methods: Anatomical Volume of Interest analysis (i.e., Whole Thalami (WT), Mediodorsal nuclei (MD), and Lateral Geniculate Nuclei (LGN)), whole night sleep high density (hd)-EEG recordings, as well as EEG source modeling (sleep spindle-related cortical currents) were performed in patients with schizophrenia and healthy comparison subjects. A subset of schizophrenia patients also completed the Abstraction, Inhibition, and Working Memory (AIM), task, a computerized test from the PENN battery that assesses cognitive executive functions.
Results: Schizophrenia patients had reduced mediodorsal (MD) thalamic volumes, especially on the left side, compared to healthy controls, whereas WT and LGN did not differ between the two groups. Furthermore, left MD volumes were strongly correlated with the number of scalp-recorded spindles in an anterior frontal region. Cortical currents underlying these anterior frontal spindles were localized to the rostral prefrontal cortex. Finally, prefrontal currents at the peak of spindle activity were significantly reduced in schizophrenia patients in relation to healthy comparison subjects, and spindle prefrontal activity was correlated with the performance in the AIM task in patients with schizophrenia.
Conclusions: Altogether, these findings point to deficits in a specific TRN-MD thalamus-prefrontal cortical circuitry in patients with schizophrenia, associated to cognitive deficits commonly reported in those patients.
Disclosure: Nothing to Disclose.

47.3 Optogenetic Study of the Role of Parvalbumin-containing Thalamic Reticular Nucleus Neurons in Spindle Generation: Implications for Schizophrenia

Robert McCarley

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Background: Sleep spindles have been found to be consistently reduced in schizophrenia (Sz). Human sleep spindles are 12–15 Hz EEG oscillations during nonREM sleep. They are thought to originate in the thalamic reticular nucleus (TRN), a structure composed primarily of inhibitory GABAergic neurons, many of which contain the calcium binding protein parvalbumin (PV). Spindles are postulated to be generated by the interaction of rhythmically bursting PV TRN neurons and thalamocortical (TC) relay neurons. Of relevance to Sz, PV neurons have been shown to be abnormal in cortex in post-mortem Sz studies; there is some evidence they may be abnormal in TRN leading to speculation of abnormalities in PV neurons causing spindle abnormalities. However, at the cellular level there have been no studies of the role of identified TRN PV neurons in spindle generation. Thus, we investigated for the first time alteration of sleep spindle oscillatory activity in mice expressing Cre recombinase in PV neurons (PV Cre mice) in response to optogenetic bilateral channelrhodopsin2 (ChR2) excitation or archaeorhodopsin (ArchT) inhibition. We hypothesized that direct, selective stimulation of PV TRN neurons would promote spindle activity, and inhibition would produce attenuated spindle activity, consistent with evidence of reduced PV expression and abnormal spindle activity in both animal models and clinical studies of Sz.
Methods: In our first experiment to test the role of PV neurons in spindle control, adeno-associated virus(AAV)-ChR2 with EYFP marker was first bilaterally injected into TRN in the PV Cre mouse ($N = 5$) to specifically transduce PV neurons of TRN, and blue 473 nM laser light was then applied three weeks later for optogenetic excitation. Histology confirmed transduction of TRN PV neurons. In a second experiment, we tested the effect of bilateral ArchT inhibition on spindle activity. Tests with 532 nM laser light illumination began 3 weeks after injection of AAV ArchT and GFP marker in the PV Cre mouse into the TRN ($N = 2$).
Results: Optogenetic ChR2 excitation was tested in 5 mice at varying frequencies (2-60 Hz), producing the largest frontal cortical EEG response at $\sim$10 Hz. This suggested activation of an intrinsic oscillator tuned to the mouse spindle frequency (8-12 Hz). 10 Hz excitation elicited both a significant increase of cortical EEG power at the stimulation frequency, and regularly produced a cortical EEG spindle when the mouse was in NREM sleep (298/300 trials). To test ArchT inhibition our strategy was to halt an ongoing series of NREM spindles, thus assuring that inhibition was occurring in a state with ongoing production of spindles. Upon detecting 2 spindles we began ArchT illumination for 10 sec. In all trials (28/28) in one mouse there was an immediate cessation of spindling for 4 sec, whereas with no ArchT stimulation 19/30 trials showed a 3rd spindle in this epoch). Similarly, a second mouse showed no third spindle in 25/25 trials of ArchT stimulation vs. 21/31 trials with no ArchT. (For each mouse, Fisher's exact Chi Square $P < 0.0001$). Interestingly when spindles occurred during prolonged ArchT application, there was a striking reduction in their amplitudes. A post inhibition rebound of 1 or several spindles occurred in 11/28 trials after the end of ArchT simulation. Of note, Chr2 PV TRN excitation increased NREM sleep by 30% (10 Hz, 1sec/min for 5 hr, $N = 2$ mice).
Conclusions: Our experiments show that PV-positive TRN neurons are essential for cortical spindles and influence NREM sleep. Moreover, since TRN PV inhibition results in spindle abnormalities of reduction in number and amplitude replicates clinical findings, it supports a model of spindle abnormalities in Sz that is based on TRN PV neuronal abnormalities. Collaborators from our lab in this work were Drs. Stephen Thankachan, James McNally, James McKenna, Robert Strecker, & Ritchie Brown. Support: VA Merit and MH039683 to RWM.
Disclosure: Nothing to Disclose.

47.4 Decoding Sleep-dependent Signatures of Thalamic-Limbic-Cortical Dysfunction in Neurodevelopmental and Genetic Models of Schizophrenia

Matthew Jones

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Background: Sleep spindles occur in concert with other the network signatures of non-REM sleep, namely slow-waves (0.1–4 Hz) reflecting synchronized windows of cortical activity and fast (140-200 Hz), brief ($<$200 ms) hippocampal ripple oscillations associated with CA1 pyramidal neuron population bursts. Quantifying the interrelationships between spindles, slow-waves and ripples provides direct insights into the mechanisms and functions of limbic-cortical interactions during sleep. Our previous work (Phillips, Bartsch et al. 2012, Neuron 76: 526-33) characterised decoupling of limbic-cortical activity during non-REM sleep in the MAM-E17 rat neurodevelopmental model of Sz. We have now assessed whether the source of spindle abnormalities in the MAM-E17 model might lie in the thalamic reticular nucleus (TRN). We have also extended analyses of sleep neurophysiology into mice harbouring mutations in a Sz risk allele, with a view to linking genetic and neurophysiological features of the disease.
Methods: All procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986. Timed pregnant Sprague–Dawley rat dams (Charles River UK) were injected intraperitoneally with saline (SHAM) or methylazoxymethanol acetate (MAM, 22 mg/kg, in a volume of 1 mg/ml) on Embryonic Day 17. TRN slices were taken from 2-6 month old male offspring and in vitro multi-electrode array recordings of extracellular multi-unit activity used to quantify spontaneous spindle-frequency bursts. 'Ext2mut' mice with a nonsense mutation leading to a premature stop codon in Exon 2 of zfp804a (the orthologue of ZNF804a, a common risk allele for Sz and bipolar disorder) were identified from an ENU mutagenesis program (MRC Harwell UK). Adult ($>$2 months) male mice backcrossed onto a C57BL6 background were chronically implanted with either arrays of tetrode recording

electrodes targeting CA1 of the dorsal hippocampus (5 mutants, 5 wildtype littermates) or with dual-site silicon probes targeting CA1 and medial prefrontal cortex (mPFC). Post-mortem immunohistochemistry was used to assess the density of parvalbumin-expressing (PV) interneurons in CA1 and mPFC.
Results: The densities, durations and spectral characteristics of in vitro TRN spindles were similar in slices taken from SHAM and MAM rats. Ext2mut mice showed normal PV densities in CA1 and mPFC. Consistent with this, the spatially-modulated firing properties of CA1 pyramidal cells (place cells) during maze running appeared grossly normal in Ext2mut mice, as did the overall pattern of hippocampal local field potential oscillations during active exploration. However, during rest, Ext2mut place cells ($n = 45$) were significantly over-active during ripples when compared to controls ($n = 44$; $p < 0.05$ ranksum). Silicon probe recordings are currently being analysed to quantify ripple and spindle-associated CA1-mPFC interactions in these mice.
Conclusions: Intact spindle activity in TRN slices from MAM-E17 rats implies that the in vivo reduction in spindle density and mis-timed spindle activity relative to CA1 ripples in this model do not stem from core TRN dysfunction. Rather, we suggest that dysfunction of cortical PV interneurons (which are reduced in density in MAM-E17 rats) leads to aberrant cortico-thalamic feedback and/or cortico-cortical propagation of slow-wave and spindle activity. Spindle abnormalities in Sz may therefore derive from cortical, as well as thalamic, pathologies. The aberrant ripple-associated firing in CA1 of zfp804a mutant mice is similar to that reported in a calcineurin knockout mouse model of Sz (Suh et al. 2013, Neuron 80: 484–93) and may be a common signature of sleep-dependent hippocampal dysfunction in Sz models. This may reflect impaired local interneuronal networks and, alongside spindle abnormalities, is likely to contribute to impaired sleep-dependent memory consolidation in Sz and related diseases. Collaborators: TRN recordings performed by Richard Gardner (University of Bristol) and Stuart Hughes (then at Eli Lilly & Co.); zfp804a mouse work performed by Lynsey Forsyth (University of Bristol) with Lawrence Wilkinson (Cardiff University, UK).
Disclosure: Nothing to Disclose.

Panel
48. From Animal Models and Brain Circuits to Functional Outcomes: Testing Models, Target Engagement, Mechanisms, and Modulators of Social Cognition across Psychiatric Disorders

48.1 Genomic Variation of the Oxytocin Receptor and Its Impact on Social Cognition Across Neurodevelopmental Disorders; Early Evidence for Feasibility of Oxytocin Manipulation in Neurodevelopmental Disorders

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Background: Social deficits are a core/defining symptom domain of ASD. The nature of such deficits is complex but primary social perception/social cognition deficits, such as affect recognition deficits and face identity deficits have been repeatedly demonstrated (e.g. Kuusikko et al., 2009). Although social deficits are not a core feature of ADHD, children with ADHD experience considerable social isolation, and have difficulty integrating into groups. Some early data suggest that affect recognition deficits exist in at least a subgroup of individuals with ADHD, and that such deficits may be more prominent in the inattentive subtype (Miller, Hanford, Fassbender, Duke, Schweitzer, 2011). In the case of OCD, social deficits have been repeatedly demonstrated. Very preliminary data suggest that there are affect recognition deficits in at least a subgroup of individuals with OCD, although such deficits may be specific to certain affective expressions, such as disgust (Grisham, Henry, Williams & Bailey, 2010) or sadness (Aigner et al, 2007). We have chosen to study empathic accuracy across these neurodevelopmental disorders and examine the effect of genomic variation associated with the oxytocin receptor on this domain across groups. We also have begun evaluating the effect of administering oxytocin to children with ASD on social cognition.
Methods: Recruitment is taking place through the Province of Ontario Neurodevelopmental Disorders Network funded by the Ontario Brain Institute. More than 800 children with neurodevelopmental disorders have already been recruited. Empathic accuracy is measured by the Reading the Mind in the Eyes (RMET) task across disorders. Social function is also measured using the Social Communication Questionnaire. All participants have contributed biological samples for genomics. Several snps of the oxytocin receptor have been genotyped across this group. We are evaluating the effects of several oxytocin snps on overall empathic accuracy performance as well as on accuracy based on emotional valence. In addition, we will present the data for a subgroup of children with ASD who have been exposed to oxytocin manipulation over 12 weeks.
Results: We are presenting on a sample of 200 children and youth. Empathic accuracy differences across groups are evident both in terms of difficulty of items ($p = 0.0001$) as well as emotional valence of items ($p = 0.005$ for positive emotions). The effect of oxytocin receptor genomic variation on such differences will be presented. Lastly, a maximum tolerated dose (MTD) study of intranasal oxytocin run under Health Canada jurisdiction suggests feasibility for this population, good tolerability and highlights areas of potential efficacy. Specifically, of the 4 doses tested for 12 weeks duration (0.2, 0.26, 0.33 and 0.4 IU/kg/dose), the 0.4 IU/kg/dose was the maximum tolerated dose, as no serious adverse events (SAEs) or severe adverse events were noted at any dose level. Adverse events were mild to moderate, and either expected or typically associated with the disorder. Ten of 15 participants were noted to be global responders based on Clinical global Impression CGI-I-Global at week 12 and 6/14 participants remained responders 3 months after the last dose was administered. Statistical within group differences after treatment were noted in the ABC-SW ($p = 0.05$), SRS ($p = 0.002$) and the BASC relevant subscales of social skills and functional communication ($p = 0.03$ and $p = 0.0006$). Additional effects were seen in measures social cognition, in particular the Lets Face it battery and Irony and Empathy tasks.

Conclusions: Oxytocin is neuropeptide with effects on social cognition and function. Manipulation of this peptide seems to be feasible and shows good tolerability in children and youth with ASD. Genomic variation of the oxytocin receptor has effects on aspects of social cognition across neurodevelopmental disorders and may have therapeutic implications.
Disclosure: Part 1: consultation fees from NOVARTIS, Roche, **Part 4:** Sanofi Aventis Canada unrestricted grant Research funding from SynapDx.

48.2 Effects of Intranasal Oxytocin on Social Cognitive Processes in Schizophrenia

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Background: Individuals with schizophrenia often have significant deficits in social cognitive abilities, and these deficits are predictive of poor functioning. The oxytocin (OT) system, given its role in human social cognition and behaviors, has emerged as a potential therapeutic target. We have previously reported that intranasal OT may acutely improve high-level social cognitive performance as well as learning of social cognitive skills when administered prior to training sessions. We will present the findings from 2 studies – one completed and one in progress.
Methods: Twenty-seven male outpatients (mean age = 40) who met DSM-IV-TR criteria for schizophrenia and were taking antipsychotic medications participated in a 6-week (12-session) course of Social Cognitive Skills Training (SCST). Participants were randomized (double-blind) to receive intranasal OT (40 IU) or placebo 30 minutes prior to each session. Baseline (1-week before treatment), post-treatment (1-week after treatment), and one-month follow-up assessments included measures of low-level (1. facial affect recognition, 2. social perception, 3. detection of lies) and high-level (4. detection of sarcasm, 5. empathy, 6. management of emotions) social cognition. We also assessed event-related potentials (ERPs) during a face processing paradigm (N170 and N250). The individuals who completed this study are currently participating in a follow-up single-dose cross-over study to investigate the mechanism of OT's effects. Participants are being randomized to receive intranasal OT (40 IU) or placebo 30 minutes prior to EEG assessments of mu-suppression and measurements of pupil dilation in response to social vs. non-social stimuli. One week later, participants receive the other nasal spray treatment and repeat the same assessment battery. Electrophysiological and pupillometric response to social vs. non-social stimuli in the context of OT vs. placebo treatment are being compared to assess the acute effects of intranasal OT on engagement with social information.
Results: In our completed study, 13 patients were randomized to receive OT and 14 to placebo, and there were no significant demographic differences between the groups. On the social cognitive tests, subjects receiving OT demonstrated significantly greater improvements on empathic accuracy than those receiving placebo ($p = .03$, $d = .92$ post-treatment and $p = .03$, $d = .98$ at 1 month follow-up). There were no OT-related effects for the other individual tests. On the social cognition composite scores, there was a trend for larger improvements on high-level social cognition in the OT vs. placebo group ($p = .07$, $d = .50$ post-treatment). We also found a significant increase in left-hemisphere N170 amplitude for emotion identification in participants who received OT ($p < .05$ at 1 month follow-up), but not in those receiving placebo. In our ongoing follow-up study, seven participants have completed both visits and the study will be complete by August, 2014.
Conclusions: Evidence is accumulating in support of targeting the OT system for improving social cognitive abilities in patients with schizophrenia. OT may have a potential use as a treatment prior to sessions of psychosocial interventions targeting social cognitive skills. Additional results will help clarify the mechanism of OT's effects on social cognition in schizophrenia.
Disclosure: Part 1: Co-inventor on a University of California patent application entitled 'Methods of using (S)-Hydroxyzine and (R)-Hydroxyzine,' **Part 4:** Research support (materials): Theravalues, Inc.

48.3 Converging Multimodal Evidence of Social Cognitive Abnormalities in Borderline and Schizotypal Personality Disorders: Circuits, Modulators And Mechanisms

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Background: Despite the severe interpersonal dysfunction that characterizes personality disorders, social cognitive abnormalities have only recently been recognized as core features of personality disorders. Borderline (BPD) and schizotypal (SPD) personality disorders are characterized by social cognitive impairments, including abnormalities in activity and emotional modulation of social cognitive networks including the amygdala and prefrontal cortex; emotion recognition; understanding of mental states (mentalizing); salience/reward processing of social information; and attention to social cues.
Methods: We examined social cognition and emotion processing abnormalities in BPD and SPD using multimodal methodologies, including functional (fMRI) and structural (DTI) neuroimaging, imaging-genetics, genetics, and behavioral paradigms. We examined the effect of dialectical behavioral psychotherapy (DBT) on neuroimaging biomarkers of social information processing in BPD, and we tested two untested models of the effect of intranasal oxytocin on social cognitive abnormalities in BPD and SPD ("interactionist model": effects of intranasal oxytocin depend on individual factors including baseline social cognitive abilities; and "optimizing model": intranasal oxytocin optimizes social cognition regardless of baseline).
Results: Genetics data in a cohort 179 patients with BPD, 139 with SPD and 155 healthy controls support the involvement of opioidergic, oxytocinergic, and vasopressinergic systems (neurobiological systems that are critical for normative social functioning) in modulating different aspects of social cognition including attachment and empathy in BPD and SPD. In a cohort of 78 male and

female veterans, understanding of mental states, a key mediator of social cognition, is impaired among BPD patients at high risk for suicide ($F = 4.7$; $df = 1$; $p = 0.03$). Multimodal neuroimaging data suggest abnormalities in brain networks subserving emotion processing and social cognition in BPD and SPD. Adolescents with BPD have decreased FA in white matter tracts connecting the amygdala with regions involved in recognition of facial affect (Wilks $F(3,57) = 3.55$, $p < 0.02$). BPD patients have abnormal amygdala reactivity and a habituation deficit to social stimuli during an event-related fMRI task, which are modulated by BDNF genotypes ($F[1,51] = 4.48$, $p < 0.04$, Wilks). These abnormalities improved after DBT ($F(1,20) = 4.89$; $p < 0.04$), and correlated with symptomatic improvement ($r = 0.70$, $p < 0.02$). Data on the effect of intranasal oxytocin in BPD and SPD support an "interactionist" model.
Conclusions: Converging evidence from multimodal neuroimaging, genetics, imaging-genetics, behavioral and treatment data suggest that social cognitive impairment is a core feature of BPD and SPD, and that opioids, oxytocin, vasopressin and BDNF modulators are potential molecular targets for development of novel treatments. Data also suggest that evidence-based psychotherapies and drugs such as intranasal oxytocin can improve emotion-processing deficits in disorders characterized by social cognitive abnormalities.
Disclosure: Nothing to Disclose.

48.4 The Biology of Social Impairments: Findings from a Novel Monkey Model and Children with Autism

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Background: Autism spectrum disorder (ASD) is characterized by core social impairments, affects 1 in 68 US children, but remains poorly understood. No robust biomarkers of ASD have been identified, hindering the understanding of its basic biology; nor are there any pharmacotherapies that treat the social features of ASD. Elucidating the underlying biology of these social deficits, testing therapeutics that improve social functioning, and identifying novel biomarkers of treatment response are important challenges that require urgent attention. Neurobiological systems that are critical for normative social functioning are arguably the most promising signaling pathways for ASD biomarker discovery. Two such candidates are the neuropeptides oxytocin (OXT) and arginine-vasopressin (AVP). Preclinical research has shown that brain OXT and AVP pathways are critically involved in social behavior (e.g., affiliation, bonding, social learning and memory), whereas OXT and AVP peptide and receptor impairments induced by pharmacologic or genetic manipulations produce social deficits in rodents.
Methods: We investigated OXT and AVP biology in a large child ASD cohort and in a novel rhesus monkey model of naturally occurring social impairments.
Results: In the largest ASD cohort tested to date ($N = 193$), we found no evidence to support the "OXT-deficit hypothesis" of ASD. Rather, carriers of the "A" risk allele of OXTR rs2254298 exhibited greater global social impairments ($p = 0.0234$), and plasma OXT concentrations strongly and positively predicted theory of mind ($p = 0.0327$) and social communication ($p = 0.0060$) performance, regardless of disease status. Furthermore, plasma OXT concentrations showed significant heritability between ASD discordant siblings ($h2 = 85.5\%$; $p = 0.0005$); a heritability estimate on par with that of height in humans. In contrast, we found strong evidence that plasma AVP concentrations strongly and positively predicted theory of mind performance in children with ASD, but not in unaffected children ($p = 0.0203$). Post-hoc inspection provided evidence that ASD children with AVP concentrations in the first quintile had significantly lower theory of mind scores compared to all other participants ($p = 0.0125$). We recently "reverse translated" these clinical findings to a monkey model of naturally occurring social impairments. Preliminary data indicate that cerebrospinal fluid (CSF) AVP concentrations predicted group classification ($p = 0.0253$), such that monkeys with social deficits ($N = 15$) have diminished CSF AVP concentrations compared to those without social deficits ($N = 15$). CSF AVP concentrations also positively predicted initiation ($p = 0.0108$) but not receipt ($p =$ ns) of social grooming for all monkeys.
Conclusions: There are several implications of these findings. First, OXT biology is not uniquely associated with ASD, as is commonly believed, but instead, exerts independent, additive, and highly heritable influences on individual differences in human social functioning, including the severe social impairments which characterize ASD. Second, our preclinical and clinical AVP findings suggest that AVP is an important biological target for ASD diagnostics and therapeutics. There is a critical need for treatment trials to include biological markers of disease symptomatology in order to enhance the therapeutic potential of novel drugs. We are currently pursuing two follow up studies: (1) intranasal AVP administration to socially impaired monkeys; and (2) a Phase II clinical trial of intranasal AVP administration to children with ASD.
Disclosure: Nothing to Disclose.

Panel
49. Molecular and Cellular Neurobiology of Bipolar Disorder

49.1 Ankyrin-G: Forebrain Specific Conditional Mouse Model and Potential Pathway

Christopher Ross

Johns Hopkins University School of Medicine, Baltimore, Maryland

Background: The ANK3 (Ankyrin-G) locus is a risk factor for bipolar disorder and schizophrenia. Ankyrin-G localizes to the axon initial segment and node of Ranvier of CNS neurons, and tethers sodium and potassium channels. In local cortical circuits that are believed to be altered in psychiatric disorders, chandelier cells are fast spiking interneurons that form axo-axonic inhibitory synapses on pyramidal cells, forming GAT-1 and GAD67 positive cartridge-like structures wrapping around their axon initial segments. Previous studies from David Lewis's lab have found decreased Ankyrin-G label in postmortem schizophrenia cerebral cortex, suggesting loss-of-function effects.

Ankyrin-G may also have interactions with other risk factors for psychiatric disorders.
Methods: We established a forebrain-specific Ankyrin-G conditional knockout mouse model (CamKII-Cre (JAX) X Flox-AnkG exon 23 from Vann Bennett's lab), which deletes all major forms of Ankyrin-G (vs a previous model which deleted one isoform, highly expressed in cerebellum). In our current model, CaMKII-Cre expression, and thus Ankyrin-G knockdown, begins in adulthood. We characterized behavior, examined the effects of psychiatric medications, and explored the cellular and molecular mechanisms, using electrophysiology and double label immunofluorescence. We also examined interactions between Cav1.2 calcium channels, Ankyrrin-G and voltage gated channels in cells in culture. The work was done by Shanshan Zhu, a postdoc in the lab.
Results: Ankyrin-G knockout mice displayed increased motor activity in the open field apparatus, increased exploratory activity and less anxiety-like behavior in the elevated plus maze, and only mild cognitive deficit in the Y-maze, reminiscent of affective disorder. Treatment with several anti-mania agents, such as Clozapine, Lithium and Valproic Acid, strikingly ameliorated the hyperactivity. Pyramidal neuron excitability from injected current was altered in slices from the knockouts (collaboration with Solange Brwon's lab). Chandelier GABA interneuron cartridge structures on pyramidal neuron axon initial segments were strikingly diminished in the Ankyrin-G KO mice.
Conclusions: Our forebrain-specific Ankyrin-G conditional knockout mice may be useful as a model of aspects of the brain changes due to alterations at the ANK3 locus, associated with bipolar disorder and schizophrenia. The striking alterations of GABA-positive cartridges on the pyramidal axon initial segments suggest that, in this potential model of psychiatric disease, there can be rewiring of cortical microcircuitry, even in adulthood. The cell model studies suggest a pathway involving CACNA1C, Ankyrin-G, and voltage gated sodium and potassium channels. These mouse and cell models may help elucidate psychiatric disease pathophysiology, facilitate development of tools to explore the mechanisms of psychiatric drugs, and make possible the testing of new therapeutic strategies for bipolar and schizophrenia.
Disclosure: Nothing to Disclose.

49.2 Function of the Ankryin 3 Bipolar Disorder Risk Gene in Brain and Behavior

Tracey Petryshen

Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts

Background: Genome-wide association studies (GWAS) have identified the ankyrin 3 gene (ANK3) among the strongest and most replicated genetic risk factors for bipolar disorder (BD). ANK3 encodes the ankyrin G protein that tethers integral membrane proteins to the cytoskeleton. Ankyrin G has critical functions in neuronal activity and other essential cellular processes, although its role in BD is unknown.
Methods: We are conducting a series of studies using mouse models to investigate the role of ANK3 in brain and BD pathophysiology. Using transgenic, RNA interference, and genome editing methods, we are examining the behavioral, neurobiological, and physiological effects of modulating ankyrin 3 expression in mouse brain.
Results: Ankryin 3 reduction in mouse brain robustly increases impulsivity, exploration, and motivation for reward, key features of BD mania. Ank3 + / − mice also exhibit heightened response to stress and elevated corticosterone levels, indicative of hypothalamic-pituitary adrenal (HPA) axis dysfunction, compared to wild-type Ank3 + / + mice. Altered synaptic protein levels and dendritic spine density in mice with reduced ankyrin 3 suggest a role in synaptic function. Reversal of the behavioral and synaptic changes by chronic lithium treatment support the disease relevance of these findings. Altered adult neurogenesis and neuronal activity in dentate gyrus of Ank3 + /- mice suggest that hippocampal dysfunction may underlie the behavioral abnormalities.
Conclusions: Behavioral, neurobiological, and physiological abnormalities in mice with reduced ankyrin 3 expression suggest ANK3 functions in neural circuits regulating mania-related behaviors and stress response, as well as synaptic function and neuronal activity. This study supports the investigation of genetic risk factors using mouse models to elucidate the neural mechanisms underlying BD.
Disclosure: Nothing to Disclose.

49.3 Modulating CACNA1C Leads to Altered Mesolimbic Dopamine System Function

Todd Gould

University of Maryland School of Medicine, Baltimore, Maryland

Background: CACNA1C codes for the L-type calcium channel Cav1.2, and genetic variation in this gene has been associated with clinical diagnoses of bipolar disorder, schizophrenia, and depression. L-type calcium channels are associated with normal function of the mesolimbic dopamine (ML-DA) system, dysregulation of which is linked to these disorders. We hypothesized that decreased levels of Cav1.2 leads to decreased ML-DA system function, resulting in attenuation of a subset of DA mediated behaviors.
Methods: Cacna1c heterozygous (HET) and wild-type (WT) mice were tested in several behaviors following stimulant challenge, including acute locomotor response, sensitization, conditioned place preference (CPP), and stereotypic behavior. Using fast-scan cyclic voltammetry (FSCV), subsecond DA release and reuptake in the nucleus accumbens of HET and WT mice was measured following stimulation of the ventral tegmental area. Recordings were taken at a series of stimulation amplitudes and after GBR12909 administration. Western blot was used to determine levels of dopamine transporter (DAT) protein.
Results: HET mice manifested significantly reduced hyperlocomotion following acute administration of psychostimulants specific to DAT (amphetamine, cocaine, and GBR12909) but not to glutamate (MK-801), as well as delayed sensitization. There was no effect of genotype on stereotypic behavior or CPP. FSCV revealed that HET mice had significantly more rapid DA reuptake following

GBR12909 administration compared to WT mice. There was no effect of genotype on DAT protein levels.
Conclusions: Cacna1c haploinsufficiency was associated with attenuation of selective DA dependent behaviors. FSCV revealed that Cav1.2 has a role in presynaptic ML-DA system function, including a likely role in regulating DAT function. However, this is not due to total levels of DAT protein suggesting that DAT activity is regulated through an alternative mechanism. Current experiments include using conditional Cacna1c knockout and transgenic lines to examine the specific role of Cav1.2 in the nucleus accumbens and ventral tegmental area.
Disclosure: Nothing to Disclose.

49.4 Impaired Striatal Neural Synchrony in Genetic Model of Mania

Kafui Dzirasa

Duke University Medical Center, Durham, North Carolina

Background: Alterations in affect-related processing are observed across many neuropsychiatric disorders including bipolar disorder. Though polymorphisms in a number of circadian genes confer risk for this disorder, little remains known about how changes in circadian gene function disrupt brain circuits critical for anxiety-related processing.
Methods: Here we characterize neural network function simultaneously across five limbic brain areas as wild-type (WT) mice and mice with a mutation in the circadian gene, CLOCK (Clock-D19 mice), perform a battery of task related used to model affective behaviors in mice.
Results: Our findings demonstrate the Clock-D19 mice demonstrate dysfunctional oscillatory phase timing within striatum. This timing dysfunction results in altered functional connectivity across striatal-dependent neural networks that modulate multiple aspects of affect-related behavior including reward processing, anxiety, and mood function.
Conclusions: These results demonstrate that disruptions of Clock gene function are sufficient to promote alterations in NAC microcircuits, and raise the hypothesis that dysfunctional NAC phase signaling may contribute to the mania-like behavioral manifestations that result from diminished circadian gene function.
Disclosure: Nothing to Disclose.

Panel
50. Keeping the Periphery in Mind: Programming Behavior Beyond the Brain

50.1 Maternal Stress and the Vaginal Microbiome: Impacts on Neurodevelopment

Tracy Bale

University of Pennsylvania, Philadelphia, Pennsylvania

Background: Stress pathway dysregulation is the most pervasive symptom in neuropsychiatric disease, yet we understand little as to the developmental programming and maturation of this system and the sensitive periods during which perturbations may be disruptive. Stress during pregnancy has been strongly associated with an increased incidence of neurodevelopmental disorders, including depression, anxiety, schizophrenia, and autism. The mechanism through which fetal antecedents contribute to disease development involves complex interactions between the maternal and fetal environments. One such interaction that has not been explored is the effect of prenatal stress on the vaginal microbiome. As the neonate's gut is initially populated by the maternal vaginal microbiome, changes produced by maternal stress can alter this initial microbe population. Substantial evidence now points to the neonatal gut ecology as having important influence on brain development and behavior, including programming of the HPA stress axis and serotonin pathway development, and has been shown to impact the developing immune system as well. Importantly, studies have pointed to a critical window of early postnatal development in which the microbiota have an influence on these outcomes. Further, as most neurodevelopmental disorders exhibit a sex bias (e.g., autism affects 4X as many boys as girls), mechanisms contributing to sex-specific neurodevelopmental programming such as changes in the host microbiome by stress will likely yield significant mechanistic insight into disease susceptibility factors.
Methods: Using targeted approaches in our EPS mouse model, we have examined the maternal and offspring microbiome by MiSeq analyses for both alpha and beta diversity as well as overall levels of Lactobacillus, the most common species between vaginal and gut microbiota. In addition, we have used metabolomics analyses to examine the link between neonate gut microbiota and nutrient absorption by measuring serum extractions for both free fatty acids and water-soluble metabolites. At these same neonatal time points, we have also micropunched the hypothalamus and submitted the isolated RNA to RNA-Seq analyses for changes in programmatic gene sets related to an altered gut microbiome. To confirm the importance of the neonate gut microbiota during this early postnatal developmental window, we have also utilized antibiotic treatment and c-section delivery to examine the role of the microbiota in programming aspects of the EPS phenotype and hypothalamic reprogramming.
Results: We found that EPS affects both the maternal vaginal and offspring gut microbiome, and further that there is a significant positive relationship between the maternal vaginal microbiome and her offspring's gut microbiome. We also have found changes in the beta diversity with EPS in postpartum vaginal samples, supporting broad effects of maternal stress on microbial populations here. Postnatal pup serum samples showed interesting results in our analysis of the water-soluble extraction, where EPS significantly reduced plasma histidine levels in male pups only. Additionally, we have identified early-postnatal, EPS-specific hypothalamic gene expression patterns with monotonic relationships to neonate gut levels of Lactobacillus, a genus of bacteria known to have neuromodulating properties.
Conclusions: These studies demonstrate the important link between the maternal vaginal microbiome and her offspring's gut at birth, and the profound effect of maternal

stress experience on this microbial population and in early brain development. As an important model in this relatively new research area, our model of EPS allows us to provide concrete evidence through rescue and recapitulation studies that the neonate gut plays a very important role in setting up the gut immune niches, activating the immune system, and determining the early nutrients available in shaping the brain. These studies have enormous translational potential, as many countries are already administering oral gavage of vaginal lavages to c-section delivered babies to ensure appropriate microbial exposure occurs. Knowledge of how maternal experiences such as stress during pregnancy can alter the vaginal microbiome are critical in determination of at-risk populations.
Disclosure: Nothing to Disclose.

50.2 Microbiome-Gut Brain Axis: A Key Regulator of Brain & Behavior

John Cryan

University College Cork, Cork City, Ireland

Background: Bacterial colonisation of the gut plays a major role in postnatal development and maturation of key systems that have the capacity to influence central nervous system (CNS) programming and signaling, including the immune and endocrine systems. Individually, these systems have been implicated in the neuropathology of many CNS disorders and collectively they form an important bidirectional pathway of communication between the microbiota and the brain in health and disease. Regulation of the microbiome-brain-gut axis is essential for maintaining homeostasis, including that of the CNS. Moreover, there is now expanding evidence for the view that commensal organisms within the gut play a role in early programming and later responsivity of the stress system. Research has focused on how the microbiota communicates with the CNS and thereby influences brain function. The routes of this communication are not fully elucidated but include neural, humoral, immune and metabolic pathways. The concept of a microbiome-brain-gut axis is emerging which suggests that modulation of the gut microflora may be a tractable strategy for developing novel therapeutics for complex stress-related CNS disorders where there is a huge unmet medical need. Indeed, the initiation of large-scale metagenomic projects such as the Human Microbiome Project has allowed the role of the microbiota in health and disease to take center stage. Emerging data from ours and other laboratories have shown a critical role for the microbiota in brain development and function. This talk highlights recent evidence that point to an essential role for the gut microbiota in brain development and the regulation of behaviors relevant to anxiety, cognition and autism.
Methods: Germ-free animals are used to assess the effects of microbiota on anxiety, stress and social-related behaviours as well as on stress-related responses. Probiotic bacteria (Bifidobacteria and Lactobacillus) are administered chronically to mice and assessed in animal tests of anxiety depression and cognition. Early life exposure to antibiotic drugs is assessed to investigate the ontogeny of brain-gut-microbe disregulation on behabiour. Finally, a model of Caesarean delivery or exposure to stress in early life is investigated to assess a naturalistic perturbation of the gut microbiota on behaviour.
Results: Studies in germ free animals suggest a role for the gut microbiota in the regulation of anxiety, mood, pain cognition and social behaviors. Studies with specific bifidobacteria and lactobacilli show that some but by no means all bacterial strains have psychobiotic potential. finally, exposure to early life stress and to Caesarean delivery can significantly alter behaviors in adulthood which may be due to microbial dysbiosis.
Conclusions: The emerging concept of a microbiota-gut-brain axis suggests that modulation of the gut microbiota may be a tractable strategy for developing novel therapeutics, 'psychobiotics', for complex CNS disorders.
Disclosure: Part 1: The authors are supported in part by Science Foundation Ireland in the form of a centre grant (Alimentary Pharmabiotic Centre Grant Number SFI/12/RC/2273); by the Health Research Board of Ireland (Grant Numbers HRA_POR/2011/23 and HRA_POR/2012/32) and received funding from the European Community's Seventh Framework Programme Grant MyNewGut under Grant Agreement No. FP7/2007-2013. The Centre has conducted studies in collaboration with several companies including GSK, Pfizer, Cremo, Suntory, Wyeth and Mead Johnson. The author has spoken at meetings sponsored by food and pharmaceutical companies.

50.3 The Rewarding Life of a Gut Peptide: Pathways and Mechanisms Supporting the Central Contributions of GLP-1 Signaling to Psychostimulant Action

Gregg Stanwood

Vanderbilt University, Nashville, Tennessee

Background: Glucagon-like peptide-1 (GLP-1) is both a peripherally expressed incretin and a centrally active neuropeptide that is released in response to food intake. GLP-1 regulates energy homeostasis and metabolism by interacting with its receptors expressed on neurons in the gut and in the brain, and several GLP-1 analogs are approved for clinical use in type 2 diabetes to improve glycemic control and support weight loss. Recent studies have begun to elucidate the central pathways that mediate GLP-1 effects on food intake and reward, including the mesolimbic dopamine system. This relationship between central dopamine and GLP-1 is reminiscent of previous work demonstrating that insulin also signals together with brain dopamine to coordinate and calibrate brain reward signals and modify consummatory behaviors. This presentation will discuss how gut signaling mechanisms, as exemplified by GLP-1, can coordinate inter-dependent food and drug reward circuits, at least in part through effects on the brain dopamine transporter (DAT).
Methods: Animals: Mice were either purchased from commercial vendors or bred internally at Vanderbilt University. All experiments were in accordance with the directives of the "Principles of Laboratory Animal Care" (NIH publication No. 85-23) and local institutional animal care and use committees. BAC transgenic Conditioned Place Preference: Testing commenced within the first 3 h of the

light phase. Mice were randomly assigned a drug treatment (pretreatment/treatment): 10 μg/kg Ex-4/SAL, 30 μg/kg Ex-4/SAL, 100 μg/kg Ex-4/SAL, SAL/Cocaine, 10 μg/kg Ex-4/Cocaine, 30 μg/kg Ex-4/Cocaine, or 100 μg/kg Ex-4/Cocaine. Ex-4 (10, 30, and 100 μg/kg body weight), cocaine HCl (20 mg/kg; NIH/NIDA), and 0.9% saline (SAL) were administered i.p. Mice were re-assessed for preference on day 10, and their side preference was compared to their pretreatment values. Self-Administration: The self-administration procedure was conducted in the laboratory of our collaborator Dr. AndersFink-Jensen and has been described previously (Sorenson et al, 2012, Psychopharmacology 222:565-577). The paradigm uses nose-poke responses that result in tail vein injection of cocaine on an FR1 schedule. Biochemistry and Neuroanatomy: DA uptake assays, high-speed chronoamperometry, slice biotinylation and immunoblotting were all conducted using standard protocols. Glp1r transcript was measured using fluorescent in situ hybridization. GLP-1 receptor expressing cells were mapped in BAC transgenic mice using antibody-mediated amplification of signal.

Results: We have demonstrated that systemic administration of a GLP-1 analog, Ex-4, reduces cocaine reward, as measured both by conditioned place preference and self-administration. GLP-1 receptor activation also alters central DA homeostasis, at least in part through increases in DAT function and membrane expression. Our new transgenic reporter mouse is allowing us to delineate the sites of GLP-1 receptors and GLP-1 responsive circuits. New GLP-1 mimetics that cannot enter the brain are allowing us to dissect the pertinent peripheral mechanisms through which GLP-1 receptor activation ultimately alters the brain.

Conclusions: We have demonstrated new roles for the peptide hormone and satiety signal GLP-1 in cocaine reward and the regulation of DA signaling and psychostimulant action. These studies expose new avenues to examine in understanding the complex mechanisms that underlie gut-brain interactions. They also point to GLP-1 receptors as a potential target for the treatment of drug abuse, and point to mechanisms in which the autonomic and behavioral processes that regulate energy balance may also be recruited in drug addiction.

Disclosure: Nothing to Disclose.

50.4 Brain and Blood, Guts and Drugs: Might the Study of Disrupted Serotonin Signaling Offer Insights into Both the Behavioral and Peripheral Features of Autism Spectrum Disorder and Support the Identification of Novel Therapeutics?

Randy Blakely

Vanderbilt University School of Medicine, Nashville, Tennessee

Background: In addition to patterns of restricted, repetitive behavior and deficits in communication and social interactions, subjects with autism spectrum disorder (ASD) often exhibit elevated whole blood serotonin (5-HT) levels (hyperserotonemia), as well as alterations in immune signaling and disrupted gastrointestinal (GI) function. In a search for genetic determinants of ASD risk, we (Sutcliffe et al, ASHG 2005) identified five rare coding variants in the SLC6A4 gene that encodes the antidepressant 5-HT transporter (SERT). Subsequent studies with SERT variant-transfected cells and human lymphoblastoid cells revealed that all variants lead to constitutively elevated 5-HT transport function. Further studies with the most common of these variants, SERT Ala56, yielded evidence consistent with transporter hyperfunction as arising from a p38 MAPK-dependent shift of SERT to higher affinity recognition of 5-HT. Consistent with these studies, SERT Ala56 knock-in mice (Veenstra-VanderWeele, PNAS 2012) demonstrate constitutively elevated 5-HT clearance in vivo, accompanied by p38 MAPK-dependent SERT hyperphosphorylation. Moreover, SERT Ala56 mice demonstrate hyperserotonemia and 5-HT receptor sensitivity, as well as multiple behavioral changes, including increased repetitive behavior, diminished pup vocalizations, and disrupted social behaviors. Given significant expression of SERT in the immune system and GI tract, we hypothesized that peripheral alterations linked to these tissues might also be present in the SERT Ala56 model, and thereby support a broader relevance of altered 5-HT homeostasis to the features of ASD. Additionally, we wondered whether any or all of these alterations might be remedial through pharmacological interventions that target p38MAPK-dependent, SERT activation pathways.

Methods: SERT Ala56 mice and their wild type (WT) littermates on a 129S6/S4 background were examined under an approved protocol of the Columbia University Institutional Animal Care and Use Committee (IACUC) for evidence of functional GI disturbances, using measures of gastric and intestinal transit, colonic rhythmicity, and lower GI elimination. Neuronal populations in the myenteric and submucosal plexi were evaluated by immunofluorescence approaches. Activation of immune signaling was assessed by qPCR and Luminex-based cytokine arrays, as well as through western blots of activated cytokine signaling enzymes. Transcriptome profiles of human lymphoblastoid cells were obtained using Affymetrix-based microarray procedures. Pharmacological suppression of p38 MAPK-dependent SERT hyperactivation in vitro and in vivo was achieved using MW108, a recently developed p38 MAPKalpha-specific antagonist developed by Martin Watterson, Northwestern University. In vitro studies were performed with transiently transfected CHO cells. For in vivo studies, SERT Ala56 mice and WT littermates were given i.p. qid 10 mg/kg MW108 for 1 week prior to assessing social interactions in the Tube Test, a procedure that scores the frequency that animals withdraw from a forced encounter with a conspecific.

Results: SERT Ala56 mice were found to display slower GI transit time, though normal gastric emptying, relative to WT littermates. Additionally, disruptions in the rhythmic propagation of contractions in the gut of SERT Ala56 were observed. Sensitivity of GI peristalsis to 5-HT receptor activation was found to be elevated, similar to that seen for CNS 5-HT receptors. These functional changes were supported by a significant loss in the number of late arriving myenteric and submucosal neurons, consistent with a critical role of 5-HT availability in GI neural crest cell division and differentiation during development. SERT Ala56 expressing cells and mice displayed transcriptional and peptide signatures of elevated native immune system activation with specific relevance to ectopic activation of

p38 MAPK signaling pathways. Finally, treatment of cells and adult mice with MW108 blocked SERT activation and reversed social behavior deficits, respectively.
Conclusions: Our findings demonstrate that genetically-imposed SERT hyperactivity produces not only multiple behavioral disturbances that align with the core diagnostic deficits of ASD, but also leads to peripheral perturbations seen in many ASD subjects, including altered immune signaling and bowel dysfunction. These efforts also reveal mechanisms by which central and peripheral deficits seen in ASD may be linked via disrupted 5-HT homeostasis. Additionally, our studies illustrate how the pursuit of a basic understanding of transporter regulation has provided novel, mechanistic insights into the functional perturbations that arise in the SERT Ala56 model. and has led to new possibilities for treatment of adults with ASD. Finally, they provide a cogent example as to how the study and modeling of rare, penetrant gene variation can provide insights that may generalize to idiopathic forms of neuropsychiatric disorders.
Disclosure: Part 1: Lundbeck, Prexa Pharmaceutics, NeuroDetective International, **Part 2:** Prexa, **Part 4:** Lundbeck, NeuroDetective International, Psychiatric Neuroscience Institute.

Panel
51. Pyramidal Cell Heterogeneity and Schizophrenia: On the Nosology of Psychiatric Disease

51.1 Pyramidal Neurons in Layers 3 and 5 of the Human Prefrontal Cortex: Cell Type-specific Transcriptomes and their Alterations in Schizophrenia

David Lewis

Western Psychiatric Institute & Clinic, Pittsburgh, Pennsylvania

Background: Authors: David A. Lewis (1,3) ,Dominique Arion (1), John Corradi (4), Shaowu Tang (2), Dibyadeep Datta (3), Franklyn Boothe (3), Aiqing He (4), Angela Cacace (4), Robert Zaczek (4), Charles Albright (4), and George Tseng (2) Departments of Psychiatry (1), Biostatistics (2), and Neuroscience (3) University of Pittsburgh and Bristol-Meyers Squibb (4) Schizophrenia is associated with alterations in working memory that reflect dysfunction of dorsolateral prefrontal cortex (DLPFC) circuitry. Working memory depends on the activity of excitatory pyramidal cells in DLPFC layer 3, and to a lesser extent in layer 5. Although a number of studies have profiled gene expression in DLPFC gray matter in schizophrenia, little is known about cell type-specific transcript expression in these two populations of pyramidal cells.
Methods: Individual pyramidal cells in DLPFC layers 3 or 5 were captured by laser microdissection from 36 matched pairs of subjects with schizophrenia or schizoaffective disorder and normal comparison subjects (Study 1), and from 19 matched tetrads of subjects with schizophrenia, bipolar disorder, major depression and normal comparison subjects (Study 2). The mRNA from cell collections was subjected to transcriptome profiling by microarray followed by qPCR validation. Expression levels of selected transcripts were also assessed in pyramidal neurons from the DLPFC of monkeys exposed to antipsychotic medications.
Results: In Study 1, expression of genes involved in mitochondrial (MT) or ubiquitin-proteasome system (UPS) functions were markedly altered in the patient group. MT-related gene alterations were more prominent in layer 3 pyramidal cells, whereas UPS-related gene alterations were more prominent in layer 5 pyramidal cells. Many of these alterations were not present, or found to a lesser degree, in samples of DLPFC gray matter from the same subjects, suggesting that they are pyramidal cell-specific. Furthermore, these findings principally reflected alterations in the schizophrenia subjects, were not present or present only to a lesser degree in the schizoaffective disorder subjects, were not attributable to factors frequently comorbid with schizophrenia, and were not present in pyramidal neurons from monkeys exposed chronically to haloperidol or olanzapine. In Study 2, the pyramidal cell transcriptomes from each patient group differed from that of the normal comparison subjects. In addition, preliminary analyses suggest that gene expression patterns in layer 3 pyramidal neurons, but not in layer 5 pyramidal neurons, differed among each of the three patient groups.
Conclusions: These findings suggest that the disease process of schizophrenia involves pyramidal cell-specific alterations in gene expression regulating MT and UPS functions that are 1) both common to and distinctive between pyramidal cells in DLPFC layers 3 and 5, and 2) not shared by subjects with schizoaffective disorder. Importantly, these findings suggest that layer 3 pyramidal cells are hypometabolic, and by inference hypoactive, in individuals with schizophrenia. Furthermore, preliminary findings suggest that the pattern of transcriptome alterations specifically in layer 3 pyramidal cells might differ across subjects with schizophrenia, schizoaffective disorder, bipolar disorder and mood disorder, perhaps providing a cellular/circuitry basis for differences in the clinical phenotypes of these syndromes. In concert, these findings support the idea of heterogeneity of pyramidal neurons across cortical layers and their differential alterations in disease states.
Disclosure: Part 1: David A. Lewis currently receives investigator-initiated research support from Bristol-Myers Squibb and Pfizer and in 2012–2014 served as a consultant in the areas of target identification and validation and new compound development to Autifony, Bristol-Myers Squibb, Concert Pharmaceuticals, and Sunovion, **Part 4:** Bristol-Myers Squibb and Pfizer.

51.2 A Vulnerable Set of Pyramidal Cells in Prefrontal Cortex: Relevance for Attention Deficits in Schizophrenia

Evelyn Lambe

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Background: The cognitive symptoms of schizophrenia are disabling and not well addressed by current treatments. These symptoms, which include attention deficits, arise early in the illness and suggest aberrant maturation and adult functioning of the prefrontal cortex. Recent work in animal models shows that layer VI corticothalamic neurons

of the prefrontal cortex and their cholinergic inputs are essential for normal attention under demanding conditions. Here, we show new data investigating how disruptions during development affect the ability of these neurons to integrate inputs across the cortical mantle as well as the type of potassium channels required for the control of instantaneous spike frequency by acetylcholine. Given the necessary signals and precise timing required for optimal performance on attention tasks, the vulnerability of corticothalamic feedback neurons to aberrant development points to a cellular mechanism relevant to the cognitive deficits of schizophrenia.

Methods: In order to assess the adult consequences of developmental manipulations, pyramidal neurons in the major corticothalamic output layer of mouse prefrontal cortex were examined through a combination of electrophysiological recording, multiphoton imaging, and detailed morphometry. In particular, we examined dependence of neuronal changes on developmental perturbation of the nicotinic acetylcholine receptor subunit encoded by chrna5, which has been linked to increased risk for schizophrenia.

Results: Prefrontal layer VI of adult cerebral cortex is unusual compared to other cortical regions because more than half its pyramidal neurons have apical dendrites that stretch across the cortical mantle to the pial surface. Here, we show for the first time that this subpopulation of layer VI neurons has significantly greater dendritic spine density ($P < 0.0001$) compared to the population typical of layer VI neurons in other cortical regions, suggesting a prominent role in input integration across both superficial and deep layers of the prefrontal cortex. Most strikingly, we find this high spine density is selectively vulnerable to developmental disruption of the nicotinic receptor subunit encoded by chrna5 ($P < 0.001$), whose gene variants increase risk for schizophrenia, nicotine dependence, and attention deficits. In this particular subset of vulnerable layer VI neurons, moreover, developmental disruptions of chrna5 lead selectively to cholinergic responses with aberrant timing ($P < 0.01$), resulting from recruitment of a distinct set of potassium channel effectors.

Conclusions: Here, we demonstrate the heterogeneity of prefrontal layer VI neurons in their vulnerability to developmental insult. The vulnerable neurons are an unusual subpopulation positioned to integrate incoming, excitatory information across all the layers of the prefrontal cortex. The observed changes to the integration of information and the timing of excitation are highly relevant to the type of attention deficits observed in schizophrenia.

Disclosure: Nothing to Disclose.

51.3 Excessive Dopamine D2 Receptor Activation May Contribute to Prefrontal Dysfunction by Driving Hyperactivity within a Specific Subpopulation of Prefrontal Pyramidal Neurons: Optogenetic and Pharmacologic Studies in Behaving Mice

Vikaas Sohal

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Background: Prefrontal dysfunction drives cognitive deficits that are the major source of disability in schizophrenia. One prominent hypothesis is that aberrant dopaminergic modulation contributes to prefrontal dysfunction, but details about such a mechanism, as well as its possible relationship to other hypothesized mechanisms, e.g. parvalbumin interneuron dysfunction, remain sparse. Our laboratory that has described subtypes of layer 5 (L5) pyramidal neurons within the mouse medial prefrontal cortex (mPFC) that differentially express dopamine receptors and receive defined distinct patterns of input. Specifically, L5 pyramidal neurons in the mPFC comprise two subtypes: (1) thick-tufted neurons that project to subcortical targets, e.g. MD thalamus, and express D2Rs (and possibly D1Rs as well); (2) thin-tufted neurons that project callosally and express D1Rs but not D2Rs. We have shown that D2R activation can drive hyperexcitability in the D2R-expressing subtype, and hypothesized that under certain conditions, D2R-induced hyperexcitability in these neurons may contribute to prefrontal dysfunction.

Methods: We tested our hypothesis using a combination of optogenetic and pharmacologic manipulations during a mPFC-dependent social exploration task. Specifically, we used D1-Cre or D2-Cre mice to drive selective expression of ChR2 or eNpHR within D1R or D2R-expressing neurons within the PFC, and delivered either optogenetic excitation using 5-10 Hz trains of 5 msec, 470 nm flashes, or optogenetic inhibition using continuous 530nm light to the mPFC. For pharmacologic manipulations, we injected various quantities (1-3 mg) of either SKF38393 or quinpirole into the mPFC to activate D1Rs or D2Rs, respectively.

Results: Consistent with our hypothesis, both optogenetic excitation of D2R-expressing mPFC neurons and infusion of the D2R agonist quinpirole into the mPFC reduced social exploration. Notably both manipulations also increased exploration of a novel object during a similar task. Furthermore, optogenetic inhibition of D2R-expressing mPFC neurons rescued quinpirole-induced deficits in social exploration. In contrast, neither optogenetic activation of D1R-expressing mPFC neurons, nor infusion of the D1 agonist SKF38393 impaired social exploration. These results suggest that (1) excessive D2R activation can disrupt aspects of mPFC function, (2) these effects reflect excessive activity within D2R-expressing neurons, and (3) at least some of these effects are specific to D2Rs and D2R-expressing neurons and do not generalize to activation of D1Rs and/or D1R-expressing neurons.

Conclusions: These findings support the specific hypothesis that excessive D2R activation in the mPFC induces hyperactivity within D2R-expressing L5 pyramidal neurons that can contribute to prefrontal dysfunction in conditions such as schizophrenia. In addition, we have previously reported that the D2R-expressing subtype of L5 pyramidal neurons is preferentially inhibited by parvalbumin interneurons within the mPFC, which are hypothesized to be dysfunctional in schizophrenia. Thus, hyperactivity of D2R-expressing L5 pyramidal neurons within the mPFC represents a point of convergence for dopaminergic and GABAergic hypotheses about schizophrenia, and may drive aberrant signals to subcortical targets, e.g. MD thalamus, that disrupt corollary discharge and related forms of long-range communication that are implicated in schizophrenia.

Disclosure: Nothing to Disclose.

51.4 Layer V Prefrontal Cortical Pyramidal Cells Innervating Different Targets Differ in Dendritic Spine Response to Dopamine Loss: Structural, Proteomic, and Genomic Analyses

Ariel Deutch

Vanderbilt University Medical Center, Nashville, Tennessee

Background: Postmortem studies of schizophrenia have reported a loss of dendritic spines on pyramidal cells (PCs) in the prefrontal cortex (PFC). Structural and functional data also point to a decrease in the dopamine (DA) innervation of the PFC. We have previously reported that partial DA denervation of the PFC results in a decrease in dendritic spine density on PCs. However, not all cells are affected. We determined if distinct subpopulations of PCs, categorized by either laminar position or projection target, lose spines after PFC DA denervation.
Methods: Adult rats with partial DA loss in the PFC were injected with a fluorescent latex microspheres into one of five different sites to which the prelimbic cortex (area 32) projects. Three weeks later animals were sacrificed and the retrogradely-labeled neurons intracellularly filled to permit determination of spine density and morphology. In other animals a similar protocol was followed, but retrogradely-labeled PCs projecting to the different target sites were harvested by laser capture microdissection. These different populations of PCs were analyzed by qPCR to identify catecholamine receptors expressed, or were subjected to shotgun proteomics to interrogate the proteome of PCs.
Results: Dopamine depletion of the PFC resulted in spine loss only in PCs located in layer V (L5), but not PCs of L2/3 or L6. Even within L5, however, the dendritic spine changes were seen only in certain PCs. L5 PCs that project to the mediodorsal thalamus (MD) and nuc. accumbens (NAS) lost spines after DA depletion; L5 PCs that innervate the basolateral amygdala (BLA), ventral tegmental area (VTA), or contralateral PFC did not. In L5 cortico-thalamic and cortico-accumbens PCs the spines that remained had a greater spine head diameter:spine length ratio than seen in PCs that did not undergo spine loss. qPCR analyses of PCs separated on the basis of projection target do not indicate a simple explanation for dopamine denervation-induced loss of spines, with both D1 and D2 mRNAs being expressed in susceptible PCs; however, the alpha 2c adrenoceptor mRNA was differentially distributed. Proteomic analyses revealed that PCs were highly enriched in isoforms of Na + -K + ATPases (excitable cells) and CaMKIIapha (PCs), but had very little to no GAD65 (interneurons) or GFAP (astrocytes). Analysis of the proteome of different populations of PCs based on projection target is ongoing.
Conclusions: Dopamine loss in the PFC induces dendritic spine loss that is both lamina-specific and restricted to distinct groups of PCs defined on the basis of projection target. The mechanisms that underlie the vulnerability of certain types of PCs to structural remodeling are beginning to be understood by applying contemporary approaches to biochemical and genetic analyses of different sets of PFC PCs. This opens the door to relating specific symptom domains to different populations of PCs that define specific circuits, a goal of psychiatric nosology dating back to the late 19th century.
Disclosure: Part 1: Eli Lilly & Co.

Panel
52. It's all in the Sperm! Paternal Epigenetic Mechanisms Underlying Transgenerational Programming of Neuropsychiatric Disease Risk and Resilience

52.1 Paternal Cocaine Exposure Elicits Transgenerational Learning Deficits

Chris Pierce

University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

Background: Cocaine addiction is associated with profound cognitive impairments, including deficiencies in memory function. In some cases, these memory deficits predict poor treatment retention and outcome. Additionally, growing evidence indicates that drug abuse can influence behavior and neurophysiology not only in adults, but also in offspring. We previously showed that paternal cocaine self-administration reduces the reinforcing efficacy of cocaine in male offspring.
Methods: Behavioral and electrophysiological techniques were combined to examine the influence of paternal cocaine self-administration on memory formation and synaptic plasticity in offspring. Object-based paradigms are ideally suited to evaluate memory traces because they circum vent potentially confounding alterations in motor function or stress response, and the underlying neural circuits have been well defined. Male rats self-administered cocaine daily for 60 days and controls received yoked saline infusions. Cocaine-experienced and saline-experienced sires were then bred to drug-naïve females and memory formation was assessed in the resulting adult (60 days and older) offspring.
Results: Paternal cocaine self-administration elicited short-term (30 minutes) spatial memory deficits in male, but not female, offspring (F1 generation) and grand-offspring (F2 generation). F1 and F2 animals showed normal novel object recognition in a hippocampus-independent version of this task. These findings indicate that paternal cocaine self-administration produces transgenerational spatial learning deficits and disrupts hippocampal function. Theta bursts-induced LTP, a cellular model of memory formation, was evaluated to directly test hippocampal plasticity in the descendants of cocaine-exposed sires. LTP induction was impaired in adult male F1 offspring, suggesting that NMDA receptor signaling was reduced. Consistent with this hypothesis, bath application of the endogenous NMDA receptor co-agonist D-serine restored LTP induction in hippocampal slices from F1 rats.
Conclusions: Taken together, these findings indicate that the offspring and grand-offspring of cocaine-experienced rats show spatial learning deficits, which may be caused by reduced NMDA receptor signaling in the hippocampus.
Disclosure: Nothing to Disclose.

52.2 Transgenerational Transmission of Stress

Eric Nestler

Mount Sinai School of Medicine, New York, New York

Background: There have been numerous reports that behavioral experience of an adult animal can be passed on to subsequent generations. However, the mechanisms underlying this transmission have remained obscure.
Methods: We have bred male mice, previously subjected to chronic social defeat stress, to normal female mice and studied baseline behaviors as well as stress susceptibility in the adult male and female offspring of these mice. We are now comparing the offspring of normal sexual reproduction to those of in vitro fertilization (IVG) and artificial insemination to distinguish between behavioral and true epigenetic modes of inheritance. We are also carrying out genome-wide maps of DNA methylation and microRNA expression in sperm of stressed and control animals.
Results: Published work to date has demonstrated robust transmission of stress susceptibility to male and female offspring of male mice previously subjected to chronic social defeat stress. Offspring of IVF, in contrast, display only minor behavioral abnormalities. Studies are underway with artificial insemination to test the mode of inheritance of stress susceptibility. Studies are also underway to look for epigenetic modifications (changes in DNA methylation or microRNA expression) in the sperm cells of stressed males that might provide a mechanism for epigenetic inheritance.
Conclusions: Together, this work will provide definitive evidence for or against a true epigenetic mechanism of transgenerational transmission of stress susceptibility in mice.
Disclosure: Nothing to Disclose.

52.3 Paternal Stress Reprograms Offspring Stress Neurocircuitry via Sperm miRNAs

Alison Rodgers

University of Pennsylvania, Philadelphia, Pennsylvania

Background: Neurodevelopmental disorders including autism and schizophrenia have been highly associated with parental factors, including maternal stress. However, much less is understood as to how paternal lifetime experiences are able to contribute to offspring disease risk. Epidemiological data supports unique windows over the lifespan in which male germ cells may be re-programmable and thus more susceptible to external environmental perturbations. Such plasticity in the germ cell epigenome would likely influence offspring neurodevelopmental disease risk.
Methods: To investigate the potential mechanisms by which paternal stress may contribute to offspring hypothalamic-pituitary-adrenal (HPA) axis dysregulation, we exposed mice to six weeks of chronic stress prior to breeding. As epidemiological studies support a heightened susceptibility of paternal germ cells to reprogramming during limited developmental windows, male stress exposure occurred either throughout puberty or in adulthood. Male and female offspring were then examined as adults for changes in stress responsive behaviors and physiology. Brains from these groups were then micropunched in stress-relevant brain regions, and analyzed by microarray and gene set enrichment for programs of functional gene sets predictive of stress pathway dysregulation. Paternal sperm was examined for changed in miRNA content. Identified miRs were then synthesized and injected into single cell zygotes, transferred to surrogate dams, and offspring examined for stress phenotype as adults. Single cell amplification was also conducted from cultured zygotes following miR injection using Fluidigm technology. Stored maternal mRNA targets of sperm miRs were then determined.
Results: Remarkably, offspring of sires from both paternal stress groups displayed significantly reduced HPA axis stress responsivity. Gene set enrichment analyses in offspring stress regulating brain regions, the paraventricular nucleus (PVN) and the bed nucleus of stria terminalis (BNST), revealed global pattern changes in transcription suggestive of epigenetic reprogramming and consistent with altered offspring stress responsivity, including increased expression of glucocorticoid-responsive genes in the PVN. In examining potential epigenetic mechanisms of germ cell transmission, we identified robust changes in sperm miRNA (miR) content, where nine specific miRs were significantly increased in both paternal stress groups. In order to determine potential epigenetic mechanisms by which these parental stress experiences can be transmitted to and affect offspring brain development, we identified significant changes in miRNAs from paternal germ cells. To test the relevance and potential mRNA targets of these miRNAs, we synthesized and injected the nine miRNAS into single cell zygotes and found that the adults recapitulated the stress phenotype. In addition, we have now completed single cell amplification from injected zygotes and ascertained the stored maternal mRNAs that are the targets of these sperm miRs and thus affecting post-fertilization development that results in a reprogrammed brain that is stress hypo-responsive.
Conclusions: These studies provide valuable insight into novel paternal contributions to sex-biased disease vulnerability following paternal stress exposure impacting the developing brain. Overall, these results demonstrate that paternal experience across the lifespan can induce germ cell epigenetic reprogramming and impact offspring HPA stress axis regulation, and may therefore offer novel insight into factors influencing neuropsychiatric disease risk. Identification of the specific miRNAs in germ cells that are altered long-term following stress experience may point to unique biomarkers that could identify at-risk populations.
Disclosure: Nothing to Disclose.

52.4 Dynamic Epigenetic Patterning in Germ Cells: Role in Normal Development

Jacquetta Trasler

McGill University, Montreal, Canada

Background: Perturbing the epigenetic marking of the genome during germ cell development can lead to decreased developmental potential and long term health consequences for the offspring. The methylation of DNA at 20-30 million sites across the mammalian genome is the best characterized epigenetic modification associated with the modulation of gene activity. Regulated activity of the DNA (cytosine-5) methyltransferases (DNMTs) and the availability of methyl

donors are required for the establishment of normal DNA methylation patterns in sperm. Abnormalities in genomic methylation patterns can be transmitted to the offspring and have been associated with perturbations in growth, placental function, neurobehavioral processes and cancer.
Methods: Mouse models were used to determine the normal timing of DNA methylation patterning and the types of insults that result in altered DNA methylation patterns in sperm including, decreased DNMT activity, methyl donor deficiency and supplementation, folate pathway enzyme (e.g. methylenetetrahydrofolate reductase or MTHFR) deficiencies and exposure to anticancer agents. Locus-specific techniques such as pyrosequencing and genome-wide approaches, such as reduced representation bisulfite sequencing (RRBS), were used to examine DNA methylation in germ cells and tissues of the offspring, including examination of transmission of epigenetic defects across generations.
Results: DNA methylation is initially erased in primordial germ cells and then re-acquired in males during both prenatal and postnatal phases of germ cell development. DNA methylation at most of the 20 million sites in the mouse genome is acquired as male germ cells develop in the prenatal period (i.e. in utero); in contrast oocytes only acquire their epigenetic patterns postnatally. In adult life, male germ line stem cells stably maintain the DNA methylation patterns acquired during in utero germ cell development; further remodelling of DNA methylation patterns occurs as male germ cells develop into mature sperm in the testis. DNMT deficiency, exposure to anticancer drugs, and both folate deficiency and supplementation can induce DNA methylation defects in sperm; physiological effects of the initial treatment can be transmitted between generations. RRBS is being used to determine the types of epigenetic defects that are transmitted and the numbers of generations affected. A specific hypothesis is that sequences associated with neurobehavioral processes may be more susceptible to perturbation.
Conclusions: Our data indicate that male germ cells are susceptible to both prenatal (in utero) and postnatal conditions that interfere with acquisition of DNA methylation patterns that are essential for normal germ cell development and subsequent fertility. Altered germ cell DNA methylation patterns are subject to reprogramming but can be passed across generations. Controversies in the field of epigenetic inheritance and interactions with other epigenetic modulators such as histone methylation and small RNAs will also be discussed.
Disclosure: Nothing to Disclose.

Panel
53. Developmental Stress and Development of Schizophrenia: Dysregulation in Whole Body and Brain Coordinating Systems

53.1 Glucocorticoid Resistance and a Schizophrenia-like Phenotype in an Animal Model

Ron de Kloet

Leiden University, Leiden, Netherlands

Background: An overarching question in the neurobiology of mental disorders is how cortisol the endproduct of the hypothalamic-pituitary-adrenal axis can change from protective to harmful. To address this question it is of interest that cortisol acts as a two edged sword via mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). During the initial phase of the stress response, cortisol affects appraisal of novel information and regulation of emotional reactivity through MR localized in the limbic salience network. Next while cortisol reaches the peak of the stress response, GR activation leads to reallocation of energy resources to limbic-frontocortical regions engaged in executive functions underlying behavioral adaptation. Prolonged stress exposure or treatment with synthetic glucocorticoids, both known to induce psychotic episodes, cause an imbalance in these MR:GR dependent cortisol actions. From this MR:GR balance perspective, I will address the outcome of cumulative stress exposures during development in a rat strain genetically selected for a schizophrenia-like endophenotype.
Methods: We have used apomorphine-susceptible (APO-SUS) rats, which were selected from Wistar rats on the basis of extreme stereotypic gnawing response to administration of the dopamine agonist, apomorphine. The parental Wistar strain was used for comparison. Adult rats exposed as pups to either poor or high maternal care and to post-weaning social or isolation rearing were examined for pre-pulse inhibition of acoustic startle (PPI), T-maze spontaneous alternation, contextual fear-conditioning and hormonal stress-responses to a conditioned emotional stressor. Hippocampal tissue was dissected and subjected to RNA-sequencing.
Results: Adult APO-SUS rats that had experienced poor maternal care, as judged from low maternal licking and grooming (LG) scores, showed dramatically enhanced stress-induced ACTH levels in the face of modest increases in circulating corticosterone and prolactin levels. These Low LG offspring also developed a basal PPI-deficit, reduced acoustic startle and impaired contextual fear-conditioning, but showed enhanced T-maze short-term memory. Additional isolation rearing abolished entirely basal PPI and impaired short-term memory. The low LG animals were also resistant to corticosterone. High LG offspring, on the contrary, displayed enhanced PPI in both rearing conditions that was reduced after corticosterone challenge.
Conclusions: We conclude that a schizophrenia-like phenotype characterized by severe deficits in sensorimotor gating and brain glucocorticoid resistance precipitates, if individuals genetically predisposed for enhanced dopamine responsiveness are exposed to cumulative stress during development.
Disclosure: Nothing to Disclose.

53.2 Childhood Trauma and Psychosis Resilience: Role of the Mineralocorticoid Receptor

Christiaan Vinkers

University Medical Center Utrecht, Utrecht, Netherlands

Background: Childhood maltreatment is a major risk factor for the development of psychosis and is associated with long-lasting changes in the brain including gray matter volume reductions and dysregulation of the

hypothalamic-pituitary-adrenal (HPA) axis. However, considerable inter-individual differences exist in outcomes after childhood trauma exposure and many individuals do not develop psychosis. The mineralocorticoid receptor (MR), an essential link in the processing of stress, has received surprisingly little attention, yet there is increasing evidence that increased expression of the MR in the brain confers resilience to traumatic stress and may therefore protect against psychosis. The MR may therefore be an important moderator of the effects of childhood maltreatment on psychosis risk.
Methods: To examine the role of the MR in encountering and overcoming childhood maltreatment in the context of psychosis, we used three indepdendent angles:. 1. The effects of a functional and common MR CA haplotype (based on rs5522 and rs2070951) on the impact of childhood maltreatment on psychotic symptoms in the general population (N = 1262). 2. The functional effects of this MR haplotype on experimental cortisol stress reactivity (using the Trier Social Stress Test, TSST) in healthy participants (N = 66) with differentiating levels of childhood maltreatment, including the moderating role of schizotypy using the Schizotypal Personality Questionnnaire (SPQ). 3. The effects of childhood maltreatment on methylation of 22 CpGs in the MR of healthy individuals (N = 89) and schizophrenia patients (N = 15), including their effects on experimental stress reactivity.
Results: The MR CA haplotype was associated with an increased resilience to the detrimental effects of childhood maltreatment on psychotic symptoms in the general population (p = 4.4xe-5). Moreover, we found significant increased stress reactivity in carriers of this MR haplotype and increased resilience to the HPA-axis-blunting effects of childhood maltreatment. Moreover, childhood maltreatment was associated with changes in peripheral MR methylation levels, even though schizophrenia patients displayed a significantly blunted cortisol response.
Conclusions: Our data indicate that genetic variation in the MR is associated with resilience to psychosis after childhood maltreatment and results in an adaptive cortisol stress response. Both genetic and epigenetic factors play a role in these pro-resilience effects. Together, these data underscore the potential role of the MR in determining long-lasting resilience after exposure to childhood maltreatment.
Disclosure: Nothing to Disclose.

53.3 Hypothalamus-Pituitary-Adrenal (HPA) Axis and Inflammation as Mediators of the Association between Childhood Trauma and Onset of Psychosis

Valeria Mondelli

King's College London, Institute of Psychiatry, London, United Kingdom

Background: Previous studies have reported an association between childhood trauma and the onset of psychosis. However, the mechanisms underlying this association are still unclear. We have previously shown that patients at the onset of psychosis have an abnormal biological stress response, including high diurnal cortisol levels, a blunted cortisol awakening response, increased levels of pro-inflammatory cytokines (interleukin-6, IL-6; tumor-necrosis-factor alpha, TNF-alpha) and reduced levels of brain-derived neurotrophic factor (BDNF). In this presentation I will show our recent findings on the effect of childhood trauma on the biological response to stress in subjects at their first episode of psychosis and in healthy controls.
Methods: BDNF and pro-inflammatory cytokines messenger RNA levels were measured in the leukocytes of 49 first episode psychosis patients and 30 healthy controls (age mean ± SEM 28.2 ± 0.9 and 27.0 ± 0.8 years respectively). In a different sample of 47 first episode psychosis patients and 35 healthy controls (age mean ± SEM 31.0 ± 1.5 and 32.5 ± 2.2 years respectively), we measured salivary cortisol levels at 6 time points during the day. We calculated area under the curve for diurnal cortisol (using awakening, noon and 8pm time points) and for the cortisol awakening response (using 0, 15, 30, 60 minutes after awakening time points). In all the subjects we collected information about childhood trauma using the Childhood Experience of Care and Abuse questionnaire.
Results: Patients had reduced BDNF levels and increased levels of IL-6 and TNF-alpha when compared with controls (respectively effect size, d = 1.3, p < 0.001; d = 1.1, p < 0.001; d = 1.7, p < 0.001). Number of childhood trauma were negatively correlated with levels of BDNF (p = 0.006) and TNF-alpha (p = 0.02) at the onset of psychosis. Patients and controls with childhood sexual abuse had significantly higher diurnal cortisol levels when compared with patients and controls without sexual abuse (p = 0.02). We found a significant interaction between status (patients/controls) and presence of childhood sexual abuse on the cortisol awakening response (p = 0.007), with healthy controls with sexual abuse having higher cortisol awakening response than controls without abuse (727.1 ± 112.4 vs 477.2 ± 50.7 nmol min/l) and patients with sexual abuse having lower cortisol awakening response than patients without abuse (356.3 ± 57.1 vs 486.2 ± 48.0 nmol min/l).
Conclusions: Childhood traumas contribute lower BDNF levels and higher TNF-alpha levels found at the onset of psychosis. First episode psychosis patients exposed to sexual childhood abuse show different HPA axis abnormalities when compared with healthy controls exposed to sexual childhood abuse.
Disclosure: Nothing to Disclose.

53.4 Developmental Vulnerability from Disrupted Developmental Modularity: A Longitudinal MRI Study of Synchronized Cortical Maturation in Typical Development and Childhood-onset Schizophrenia

Aaron Alexander-Bloch

University of California at Los Angeles, New York, New York

Background: The brain's functional and anatomical modularity may confer resilience to environmental stressors. Modular systems are built from semi-autonomous subsystems (modules), with greater within-module connectivity compared to between-module connectivity. This property is central to systems from gene expression networks to computers. In whole brain networks, fMRI studies have

demonstrated modules of increased inter-regional functional connectivity within the brain, while longitudinal structural imaging studies have demonstrated developmental modules of synchronized growth between regions during typical development. Both theoretical and empirical studies indicate that more modular systems are better able to adapt and to survive to changing developmental environments with multiple developmental stressors, suggesting the hypothesis that decreased brain modularity could represent a risk factor for developmental diseases including schizophrenia. It has previously been suggested that schizophrenia is a disease of dysmodularity, both in a functional and in an anatomical sense. We have demonstrated decreased modularity and altered modular community structure in childhood-onset schizophrenia (COS), using resting-state fMRI to show disproportionately decreased short-distance, within-module functional connectivity. Here, we use longitudinal measures of cortical thickness acquired in COS to demonstrate developmental dysmodularity. The cingulo-fronto-temporal module, composed of cortical regions whose thickness develops synchronously in typical development, disproportionately contains regions of cortex that demonstrate altered trajectories of thickness maturation in COS. To assess their clinical relevance, we relate these alterations to correlations between positive and negative symptoms and cortical thickness within the COS population.

Methods: We acquired 525 longitudinal structural MRI scans from 208 subjects (100 COS), in the age range of 10-30, using a single 1.5T GE scanner in Bethesda, MD. MNI's CIVET pipeline estimated thickness at ~80,000 cortical regions. Statistical analysis was performed in R using a nonparametric local smoothing technique, penalized splines, to fit maturational trajectories at every region. Essentially, thickness was estimated as a non-linear function of age across the cortex. In both COS and typical development, regions were clustered into developmental modules using a k-means algorithm, with modules defined as regions with highly similar maturational trajectories of cortical thickness. Statistically, we tested the hypotheses that the maturational trajectory of cortical thickness is altered in COS, in each brain region; in addition, we tested for group differences in the inter-regional modular structure of synchronized growth. Finally, using the Scale for the Assessment of Positive Symptoms (SAPS) and Negative Symptoms (SANS) acquired in the patient sample, we tested for correlations between positive and negative symptomatology and cortical thickness in COS, relating these correlations with alterations in modular structure described above.

Results: As has previously documented using different methodologies, most cortical brain regions showed monotonically decreasing cortical thickness in this age range, although there was significant variability between regions in the rate, acceleration and deceleration of cortical thickness changes. Five developmental modules of synchronized cortical growth were isolated in typical development, composed of brain regions with similar maturational trajectories. A distributed group of brain regions showed altered maturational trajectories in COS compared to typical development, specifically a relatively steep decline in thickness in the age range 10-20 ($P < .05$, FDR-corrected for 80,000 multiple comparisons). These regions included inferior frontal, cingulate and medial temporal cortex. Moreover, these regions were found disproportionately within a single module of synchronized cortical trajectories in typical development. Finally, while controlling for the non-linear affect of age, a similarly-distributed group of brain regions showed significant correlations with SAPS scores within the COS population ($P < .05$, FDR-corrected). Surprisingly, 95% of these correlations were positive, with increases in cortical thickness observed in direct proportion to increases in positive symptoms, possibly indicative of a compensatory mechanism in the context of overall decreased cortical thickness in the patient population. While correlations with SANS scores did not meet stringent criteria for statistical significance, regions defined with an exploratory threshold ($P < .05$, uncorrected) showed 90% negative correlations with cortical thickness, suggesting specific relationships with cortical thickness for positive and negative symptoms respectively.

Conclusions: In COS, alterations in cortical thickness maturation appear to target or disrupt growth within a particular developmental module, the cingulo-frontal-temporal module. These findings support previous work demonstrating disrupted modularity in fMRI functional connectivity networks in schizophrenia. There is ample theoretical and empirical support for the notion that modularity facilitates the construction, development and evolution of complex systems under environmental pressure. Because of its developmental ramifications, modularity is a property of brain networks of particular interest in the study of schizophrenia and its relationship with developmental stress. In ongoing studies, we test for altered developmental modularity in the healthy siblings of patients with COS and between high- and low-stress groups of healthy subjects.

Disclosure: Nothing to Disclose.

Panel
54. Disentangling the Medial and Lateral Habenula in Emotion and Reward Mechanisms

54.1 Roles of the Dorsal Medial Habenula in Motivated Behavior

Eric Turner

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Background: It has long been known that the medial habenula has two distinct components, a dorsal subnucleus (dMHb) characterized by the expression of the neuropeptide substance P, and a ventral subnucleus (vMHb) which expresses cholinergic markers. However, few specific functions have been assigned to these nuclei.

Methods: To understand the specific function of the dMHb we have created two kinds of models in transgenic mice. In the first model we have used the Cre-mediated excision of a developmental transcription factor, Pou4f1, to specifically ablate the dMHb neurons in early postnatal development (dMHb CKO mice). In the second model system, we have used Cre-mediated conditional expression of channelrhodopsin-2 and halorhodopsin to allow the in vivo stimulation

and silencing of dMHb neurons (dMHb ChR2, dMHb Halo mice). dMHb CKO mice were examined in a variety of behavioral tests, including voluntary wheel running activity (WRA), measurement of circadian parameters, the sucrose preference test, and a battery of tests of locomotion and motor function. dMHb ChR2 mice were examined in an intracranial self-stimulation (ICSS) protocol, and dMHb Halo mice were examined in an acute place-preference protocol.
Results: dMHb CKO mice show normal basal locomotion and minor defects in a battery of motor tests. However, they show a profound deficit in voluntary WRA. Wheel running is linked to other measures of hedonic state in rodents, and dMHb CKO mice also show deficits in another hedonic measure, sucrose preference. Stimulation of the dMHb in dMHb ChR2 mice is highly reinforcing in a wheel-preference ICSS protocol. Conversely, silencing of dMHb fibers at the target of dMHb innervation, the interpeduncular nucleus, is aversive in an acute place preference assay.
Conclusions: We conclude that the dMHb may has a role in exercise reinforcement and the maintenance of hedonic state in mice.
Disclosure: Nothing to Disclose.

54.2 Cocaine-evoked Synaptic Plasticity in the Lateral Habenula: Encoding Good or Bad States?

Manuel Mameli

Institut du Fer a Moulin, Paris, France

Background: The lateral habenula (LHb) stands in the epithalamus and functionally bridges the forebrain with midbrain structures. Recent advances indicated a crucial role of the LHb in encoding negative and aversive states, and its dysfunction has been suggested to participate in the etiology of mood disorders. We and others have previously shown that cocaine exposure alters the efficacy of glutamatergic synaptic transmission onto LHb neurons, however the underlying mechanisms and the behavioral relevance remain elusive.
Methods: We combined electrophysiology in acute brain slices containing the LHb together with viral-based strategies to map specific anatomical connections and to overexpress specific proteins to understand the role of AMPAR in cocaine-evoked synaptic plasticity in the LHb. We used behvioral paradigms modeling drug-driven reward and depressive-like states to assess the behavioral relevance of cocaine-evoked synaptic adaptations in the LHb.
Results: Using patch clamp recordings in acute slices, we report that cocaine experience enhances excitatory synaptic transmission onto LHb neurons projecting to the rostromedial tegmental nucleus (RMTg). We mapped RMTg-projecting neurons by employing a modified herpes simplex virus, stereotactically injected in the RMTg two weeks prior treating the animals. When recording from fluorescent neurons, we find that the amplitude of mEPSCs, recorded in voltage-clamp mode and in presence of tetrodotoxin, increases 24 hours after a two-days exposure protocol. Cocaine-evoked plasticity in LHb is transient as it is not present one week after the last exposure, and it depends on D2 receptors activation as it is prevented by pre-treatment with eticlopride. We further investigated the mechanisms of expression and identified that AMPA receptors trafficking is a requirement for cocaine-evoked plasticity. These results are in line with a projection-specific cocaine-evoked synaptic plasticity, which requires activation of dopamine D2 receptors and trafficking of AMPARs as induction and expression mechanisms. We further find that blocking the activity-dependent delivery of AMPAR by means of a viral strategy overexpressing a dominant negative for the c-tail of the GluA1 subunit prevents the synaptic plasticity and also impairs specific cocaine-evoked behavrioral adaptations.
Conclusions: In conclusion we find that cocaine exposure alters the efficacy of excitatory transmission onto LHb neurons specifically projecting to the RMTg. This requires dopamine receptors activation for its induction and exocytosis of AMPARs for its expression. Altogether, our results suggest that a neural circuit mediating negative emotional responses plays a primary role in encoding certain aspects of cocaine-mediated responses.
Disclosure: Nothing to Disclose.

54.3 DREADD'ed Addiction: Investigating the Role of the Lateral Habenula and Its Neuronal Circuitry in Cocaine-reinforced Operant Responding and Reinstatement

Sunila Nair

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Background: Cocaine addiction has become a worldwide epidemic with major social and economic burdens on society. In addition to on-going drug use, an important problem in the treatment of addiction is the vulnerability of individuals to relapse long after cessation of drug use. The lateral habenula (LHb), an epithalamic brain region, is uniquely positioned anatomically to participate in reward-related brain circuits, and is an important regulator of the midbrain dopaminergic system that is known to be involved in the neuronal circuitry underlying operant cocaine self-administration and cocaine-seeking behaviors. However, very little is known about the precise role of this nucleus in regulating these complex behaviors. Here, we utilized the DREADD (Designer Receptors Exclusively Activated by Designer Drugs) technology to parse out the role of the LHb and its circuitry in operant cocaine self-administration and reinstatement.
Methods: Firstly, we examined the role of LHb in operant cocaine self-administration and reinstatement by selectively and transiently inhibiting or activating LHb neurons, using viral vectors that express Gi/o-coupled DREADDs (hM4Di) or Gq-coupled DREADDs (hM3Dq). These receptors have lost their affinity for their native ligand, acetylcholine, while gaining high affinity for the otherwise inert synthetic ligand clozapine-N-oxide (CNO). DREADD receptors are activated by CNO with nanomolar potency, but are insensitive to endogenous ligands, allowing specific activation of Gi or Gq-coupled signaling depending on which DREADDs are expressed. Secondly, we examined the role of the LHb neurons projecting to the ventral tegmental area (VTA) in cocaine reinforced operant responding. A Cre-recombinase dependent viral vector based flip-excision method was

employed that involved injecting a combination of floxed, inverted hM4Di into LHb neurons and a canine adenovirus 2 (CAV-2) engineered to express Cre recombinase into the VTA. CAV-2 efficiently infected VTA axon terminals and was retrogradely transported to the neuronal cell bodies in the LHb, resulting in the expression of hM4Di receptors exclusively in LHb neurons that project to the VTA. For all experiments, male, Long-Evans rats were implanted with jugular venous catheters and infused intracranially with viral vectors. Approximately 10-14 days after viral infusions, rats were trained to self-administer cocaine (0.75 mg/kg/infusion) and effects of activation of specific G-protein coupled signaling pathways in the LHb was examined on cocaine self-administration on a FR1 reinforcement schedule, progressive ratio schedule or reinstatement of cocaine seeking.

Results: Our results indicate that activation of LHb Gi/o-coupled signaling by CNO, increases cocaine reinforced operant responding on a FR1 reinforcement schedule. In contrast, activation of LHb Gi/o-coupled signaling fails to alter food reinforced operant responding. Activation of Gq – coupled signaling in these neurons decreases operant responding on a progressive ratio schedule. Interestingly, activation of Gi/o-coupled signaling in LHb neurons projecting to the VTA has no influence on on-going cocaine self-administration. In contrast to the effect of activation of LHb Gi/o-coupled signaling on self-administration, CNO-mediated activation of LHb hM4Di decreases reinstatement of cocaine-seeking induced by a cocaine prime. Preliminary results indicate that this manipulation also modestly decreases footshock stress induced reinstatement of cocaine seeking.

Conclusions: Our cocaine self-administration results are consistent with a neuronal mechanism wherein inhibition of LHb neuronal activity enhances cocaine taking behaviors due to the powerful stimulatory effects that inhibiting LHb neuronal activity is anticipated to have on VTA dopaminergic neurons. Interestingly, our results indicate that LHb neurons projecting to the VTA do not appear to be involved in this effect; we are currently exploring the role of LHb projections to the rostromedial tegmental nucleus (a GABAergic nucleus and target for LHb glutamatergic afferents) in cocaine-taking behaviors. In contrast to the effect of activation of Gi/o-coupled signaling on operant cocaine self-administration, this manipulation decreases cocaine-priming, and presumably stress-induced reinstatement of cocaine seeking, suggesting opposite effects of modulating LHb neuronal activity on drug self-administration versus reinstatement.

Disclosure: Nothing to Disclose.

54.4 The Habenula as a Biomarker of Tobacco Addiction and Suicidal Ideation

Ramiro Salas

Baylor College of Medicine, Houston, Texas

Background: The habenula has been shown to play a role in major depression and in substance abuse, two medical conditions with enormous public health impact in the world. However, little is known about the role of the habenula in these conditions on humans. We studied habenular activity and connectivity in the human using different MRI techniques.

Methods: We performed two MRI experiments, using 3T Siemens Trio scanners. In the first, healthy non-smokers and smokers were scanned using a passive learning paradigm using sweet juice as reward, and delay of expected juice as "disappointment". Smokers were scanned twice, once smoking as usual, and once abstinent since previous midnight. Data was analyzed by studying putamen and habenular activity at reward (juice received) events, and at "disappointing" events (juice expected but not delivered). In a second experiment, 159 psychiatric in-patients at The Menninger Clinic in Houston, TX were scanned using resting state functional connectivity (RSFC) and diffusion tensor imaging (DTI), to study habenular connectivity. These patients were a heterogeneous group with several diagnoses including major depression, substance abuse, anxiety disorders, personality disorders and more. Most of the patients in this sample showed co-occurring disorders. RSFC data was analyzed by studying resting state functional connectivity between the habenula and 9 other brain areas (Striatum, Nucleus Accumbens, Insula, Amygdala, Prefrontal cortex (Sup, med, inf), Suppl motor cortex, Anterior cingulate cortex). DTI was studied by tractography using a small, manually-placed habenular region of interest.

Results: In the passive learning fMRI task, we observed reduced putamen activity following reward in smokers (both smoking as usual and abstinent) and increased habenula activity following reward delay, but only in abstinent smokers. No difference in habenula activation was observed when the same group of smokers were scanned after having smoked normally. In the habenular connectivity experiment in the psychiatric population, both RSFC between the habenula and insula and the number of habenular DTI streamlines negatively correlated with suicidal ideation in these patients. These measures were also significant predictors of suicidal ideation at discharge.

Conclusions: First, we showed that the activation of reward areas (putamen) upon natural reward is decreased in both abstinent and "smoking as usual" smokers. In addition, we showed that in abstinent smokers, the habenula is hyperactive when a disappointing event happens. This may have important implications for the tobacco field, since a hyperactive habenula may translate in a continuous state of negative reward. We hypothesize that tobacco withdrawal symptoms are likely to arise from habenular hyperactivation during tobacco abstinence. In addition, we studied habenular connectivity in a sample of psychiatric in-patients and found that habenulo-insular connectivity and habenula-associated white matter are predictors of the presence of suicidal ideation in those patients.

Disclosure: Nothing to Disclose.

Panel
55. The Role of Neuroinflammatory Pathways in Opioid, Stimulant, and Alcohol Abuse: Preclinical and Clinical Studies

55.1 Proinflammatory Activity Mediates Escalation of Alcohol Drinking Induced by Stress

Markus Heilig

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Background: Liver inflammation in alcoholism may trigger inflammatory activity in the brain, potentially influencing motivational and emotional processes underlying alcohol seeking. MCP1 elevations have been found in post mortem alcoholic brain and may play a role in this process. We first studied CSF levels of MCP1 in healthy volunteers and alcoholics 1 and 4 weeks following detoxification. We then examined the mechanistic role of proinflammatory signaling for alcohol consumption, alcohol reward and stress-induced drinking using genetically modified mice.
Methods: Treatment seeking alcohol dependent inpatients were assessed 1 and 4 weeks into abstinence. CSF was samples and analyzed for a cytokine panel. Serum liver enzymes were obtained as markers of alcohol-induced liver inflammation. We then used mice with deletions of IL-1 and TNF- receptors, and evaluated the role of these signaling pathways for alcohol consumption (two bottle free choice home cage drinking), alcohol reward (conditioned place preference, CPP) and stress-induced drinking (chronic social defeat). Central NFkB activation in response to stress and alcohol was evaluated by measuring p65 phosphorylation.
Results: In patients, MCP-1 levels were elevated in alcoholics both on day 4 and day 25 ($p < 0.0001$). Using multiple regression analysis, we found that MCP-1 concentrations were positively associated with the liver enzymes gamma glutamyltransferase (GGT; $p = 0.03$) and aspartate aminotransferase/glutamic oxaloacetic transaminase (AST/GOT; $p = 0.004$). In mice, deletion of the IL-1RI gene resulted in modestly decreased alcohol consumption, but affected neither the rewarding properties of alcohol, nor escalation of drinking induced by social defeat stress. Double KOs for TNF-1R and IL-1RI consumed significantly less alcohol than control mice. The combined deletion of TNF-1R and IL-1RI did not influence alcohol reward as measured by CPP, but did prevent escalation of alcohol consumption resulting from exposure to repeated bouts of social defeat stress.
Conclusions: These data indicate that proinflammatory activity i present in the CNS of alcohol dependent patients. The animal model data indicate that pro-inflammatory activity contributes to regulation of stress-induced, negatively reinforced drinking, while leaving rewarding properties of alcohol largely unaffected.
Disclosure: Nothing to Disclose.

55.2 Toll-like Receptor 4 Involvement in Cocaine Seeking

Ryan Bachtell

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Background: Drugs of abuse are thought to produce their rewarding and addictive properties by enhancing the activity of the mesolimbic dopamine system through their neuronal mechanism of action. Recent evidence suggests that drugs of abuse, such as cocaine, also activate innate immune signaling within the brain. It remains unresolved how cocaine engages the brain's innate immune system, and whether cocaine's effects on the immune system contribute to the development of abuse and/or relapse. The current studies explore (1) the role of the immunosurveillance receptor complex, Toll-Like Receptor 4 (TLR4), on microglial cells to initiate central innate immune signaling upon cocaine administration and (2) identify how TLR4 signaling contributes to cocaine self-administration and reinstatement in rats.
Methods: Male Sprague-Dawley rats were implanted with jugular catheters and trained to self-administer cocaine or saline. Rats were then administered a single cocaine challenge (15 mg/kg, ip) and tissue punches were collected from regions comprising the mesocorticolimbic dopamine pathway. Quantitative RT-PCR was used to identify changes in proinflammatory markers (interleukin 1β (IL1β), tumor necrosis factors, etc). Behavioral studies assessed both cocaine reinforcement and the reinstatement of cocaine seeking in animals that were trained to self-administer cocaine. The effect of a systemic administration of the TLR4 antagonist, (+)naltrexone, was tested on a fixed ratio dose response and cocaine-primed cocaine seeking following extinction training. We next identified whether TLR4 signaling in the nucleus accumbens or ventral tegmental area is necessary for cocaine seeking. This was tested by microinfusing lipopolysaccharide from the photosynthetic bacterium Rhodobacter sphaeroides (LPS-RS), a TLR4 antagonist, into the nucleus accumbens or ventral tegmental area prior to a cocaine prime.
Results: Administration of cocaine produced a robust increase in the proinflammatory cytokine, IL1β, in the ventral tegmental area. This increase was impaired by a co-administration of the TLR4 antagonist, (+)naltrexone. Systemic administration of (+)naltrexone also dose-dependently inhibited cocaine seeking, but not on the reinstatement of sucrose seeking. Microinfusion of LPS-RS into either the nucleus accumbens or ventral tegmental area also inhibited cocaine seeking suggesting these areas as primary sites of action for cocaine-TLR4 interactions.
Conclusions: The data presented here indicate that cocaine-induced activation of TLR4 triggers a proinflammatory response that is necessary for cocaine self-administration and reinstatement. Based on these and other findings, it is becoming more evident that drugs of abuse are proinflammatory and drug addiction may be better conceptualized as an issue of neuroimmunopharmacology. Thus, it may be that both neuronal and glial cell functioning are necessary for the development of drug addiction and identifying strategies to offset these proinflammatory

responses may guide the development of pharmacotherapies to treat addictive disorders.
Disclosure: Nothing to Disclose.

55.3 Safety and Early Efficacy of Ibudilast as a Pharmacotherapy for Methamphetamine Addiction

Steven Shoptaw

University of California at Los Angeles, Los Angeles, California

Background: Amphetamine type stimulants, including methamphetamine, represent the second most abused class of illicit drugs worldwide, following cannabis. Yet no medications are available to treat addiction to methamphetamine. Ibudilast is a 4, 10 phosphodiesterase inhibitor approved in Asia for asthma, ocular allergies and post stroke dizziness. Ibudilast has multiple mechanisms of action, including modulation of microglia and astroglia, suppression of inflammatory immune responses (TNF-α, IL-1β, IL-6, NO), and promotion of anti-inflammatory immune responses (BDNF, GDNF, neurotropin-4 and IL-10). In preclinical experiments, ibudilast reliably prevents stress induced relapse to methamphetamine and blunts methamphetamine-induced locomotor activity in rats, presumably via action on microglia and BDNF. This study evaluated the safety (cardiovascular) and early efficacy (subjective and neurocognitive measures) of ibudilast (0 mg, 50 mg, 100 mg) in 11 methamphetamine dependent, non-treatment seeking adults.
Methods: A randomized, double-blind phase 1b design involved 27 days of inpatient hospitalization. On days 1 and 2, subjects completed I.V. methamphetamine challenges (15 mg, 30 mg) to document they could safely tolerate the challenges. Order of ibudilast condition was randomized to ensure completion of ascending doses (0 mg, 50 mg, 100 mg or 50 mg, 100 mg, 0 mg). Following safety challenges, subjects began a study week that involved being brought to steady state at each dose level for 4 days. At steady state, subjects completed a 15mg methamphetamine challenge (day 5) and a 30 mg methamphetamine challenge (day 7). The entire 7-day procedure then repeated for the next two dose levels in the design. Cardiovascular and subjective effects data were collected during to document safety and early efficacy. Neurocognitive testing, including the Connors Continuous Performance Test-II (CPT-II), a measure of sustained attention was conducted upon admission (at confirmed methamphetamine negative status) and 48 hours after exposure to the last I.V. 30 mg challenge before discharge at either 100 mg ibudilast or placebo.
Results: At steady state for 50 mg or 100 mg ibudilast, there were no statistically significant or meaningful effects on heart rate, systolic or diastolic blood pressure in the presence of 15 or 30 mg I.V. methamphetamine. Similarly, no statistically significant subjective effects were detected along any measures during the methamphetamine challenges. Along neurocognitive functioning, subjects were similar on their baseline CPT-II performances. At follow-up high-dose ibudilast subjects showed reduced variability in response times ($U = 0.00$, $p = .02$, $r = -.82$) and perseverative responses ($U = 1.50$, $p = .04$, $r = -.71$) in contrast to placebo subjects.
Conclusions: Ibudilast has an acceptable safety profile in the presence of relevant doses of I.V. methamphetamine, which makes this medication an appropriate candidate for testing in an outpatient RCT. Findings of significant improvement along neurocognitive measures of attention suggest the medication may preserve cognitive resources. Importantly, the FDA "fast-tracked" ibudilast for methamphetamine dependence, which builds enthusiasm for evaluation of this promising compound.
Disclosure: Part 4: Medicinova, Inc (clinical supplies); Pfizer Inc (clinical supplies).

55.4 Effects of Minocycline and Ibudilast on Opioid-mediated Responses in Human Research Volunteers

Sandra Comer

Columbia University, New York, New York

Background: Preclinical studies have reliably demonstrated that opioids induce a neuroimmune response by increasing glial cell activity, which results in production of a variety of immune factors including cytokines and chemokines. This proinflammatory response has been shown to contribute to the development of opioid tolerance and dependence. In rodents, inhibiting opioid agonist effects on glial activity with glial cell modulators, such as ibudilast, attenuates behavioral signs of withdrawal (Hutchinson et al., 2009), verifying that the opioid-induced proinflammatory response contributes to the development of dependence. In addition to their effects on opioid dependence, inhibitors of glial activation, such as minocycline, reduce opioid-induced rewarding effects as measured by conditioned place preference in rats (Hutchinson et al., 2008).
Methods: We therefore examined the ability of ibudilast to alter withdrawal symptoms using a between-groups, inpatient study design. Participants were maintained on morphine (30 mg QID, PO) for 2 weeks. During the 3rd week, they received placebo morphine (0 mg QID, PO). Ibudilast (0, 20 and 40 mg BID) was also administered throughout the study. All participants received 0 mg ibudilast during the 1st study week, and then they were randomized to receive 0, 20 or 40 mg BID ibudilast during the 2nd and 3rd weeks ($N = 10$ per group). In a separate study, we examined the ability of minocycline to reduce the abuse liability of oxycodone in non-dependent prescription opioid abusers using a within-subjects, outpatient study design. Participants ($N = 12$) received a dose of minocycline and a dose of oxycodone simultaneously and subjective, physiological, and analgesic effects were assessed.
Results: Although overall peak SOWS (Subjective Opiate Withdrawal Scale) scores did not significantly differ across the 3 groups (0, 20 and 40 mg BID ibudilast), a subset of symptoms (anxiety, perspiration, restlessness, and stomach cramps) were significantly lower in the active dose groups compared to the placebo group ($p < 0.05$). In general, ibudilast was safe and well tolerated under these conditions. In the second study, minocycline produced dose-related reductions in ratings of drug "Liking" and "Good Effects." It did not increase oxycodone-induced ratings of "Bad

Effects," nor did it alter oxycodone-induced physiological or analgesic effects. Minocycline was safe and well tolerated under these conditions.

Conclusions: The preclinical findings, as well as our own clinical laboratory results, provide evidence to support the hypothesis that glial-cell inhibitors, such as ibudilast and minocycline, may have potential to treat opioid-related substance use disorders.

Disclosure: Part 1: AstraZeneca, Salix, Camarus, Pfizer, Janssen, Mallinckrodt, Reckitt-Benckiser, **Part 2:** AstraZeneca, Salix, Reckitt-Benckiser, **Part 4:** Reckitt-Benckiser.

Panel
56. Fear and Loathing in the Amygdala: Novel Insight into the Mechanisms of Amygdala-mediated Regulation of Fear and Anxiety

56.1 Neurons Are Recruited to an Amygdala Fear Memory Trace Based on Relative Neuronal Excitability Immediately Before Training

Sheena Josselyn

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Background: Different brain regions may specialize in storing different types of memories. For instance, the amygdala is known to be important in storing fearful memories. However, computational and observational findings indicate that only a tiny portion of neurons within a region encodes any one memory. The critical question of "why is a particular amygdala neuron (and not another) involved in a particular fear memory?" was virtually unexplored until my lab discovered that neurons actively compete against one another for recruitment into the memory trace (Han et al., Science, 2007). Competition is common in countless biological (and non-biological) systems and creates selective pressure between individual elements. Using a combination of viral-mediated gene transfer, transgenic mice, cellular imaging and behavioral approaches, my lab made what we believe are the first steps in answering the fundamental question of how a small subpopulation of amygdala neurons is recruited to a given fear memory trace. Specifically, we manipulated levels of the transcription factor CREB in individual neurons within the lateral amygdala (LA). We found that LA neurons with relatively increased CREB function were more likely to be recruited (or allocated) into a memory trace whereas neurons with decreased CREB function were excluded from the memory trace (Han et al., Science, 2007, 2009, Josselyn, J Psychiatry Neurosci 2010, Sargin et al., Frontiers in Behavioral Neuroscience, 2013). Moreover, we showed that increasing CREB function in as few as 10% of all LA neurons was sufficient to enhance memory formation. Together, these findings show that eligible neurons compete for inclusion in a memory trace and that winners of this neuronal competition are determined by relative CREB function. CREB is a ubiquitous transcription factor that is implicated in an ever-growing list of cellular processes. Which of these is important to neuronal allocation and fear memory formation? We examined one potential mechanism: neuronal excitability. Previous research indicates that increasing CREB function increases, while decreasing CREB decreases, intrinsic neuronal excitability. Hebbian plasticity requires co-incident firing of the pre-synaptic, and depolarization of the post-synaptic, neuron. A post-synaptic neuron that is more excitable than its neighbor would be more likely to be depolarized and subsequently "fire together" and "wire together" with a pre-synaptic partner. Therefore, neurons with relatively higher intrinsic excitability may be "primed" for recruitment into a given memory trace. To test this, we used three different techniques to manipulate intrinsic excitability in a small portion of LA neurons and showed that the relative intrinsic excitability of a given neuron at the time of training helps determine which neurons win the competition for allocation to a fear memory trace.

Methods: First, we increased excitability by manipulating the function of K+ channels (using a dominant negative KCNQ2 channel) in a random small portion of neurons using viral vectors. Next, we blocked the memory-enhancing effects of CREB overexpression by co-expressing a K+ channel (Kir2.1) that decreased excitability in the same neurons. Second, we used chemicogenetic approaches developed by Dr. Bryan Roth's lab to show that transiently increasing excitability just before (and during) training in a similarly small portion of neurons was sufficient to influence memory allocation and formation. Finally, we used optogenetics developed by Dr. Karl Deisseroth's lab to show that increasing excitability in the seconds before training similarly enhanced memory formation.

Results: First, we found that increasing excitability in a small portion (roughly 10%) of LA neurons using a vector that expresses the dnKCNQ2 construct increased fear memory by roughly 40% ($n = 12$) over mice microinjected with a control vector ($n = 9$). Importantly, subsequent deletion of these neurons "erased" the fear memory. Next we observed that co-expressing CREB and the K+ channel that decreased excitability resulted in no fear memory enhancement ($n = 13$). These results indicate that although CREB has many functions, its memory enhancing effects are mediated by excitability. Second, we showed that transiently increasing neuronal excitability in roughly 10% of LA neurons using chemicogenetics increased fear memory formation ($n = 12, 12$). Subsequent artificial reactivation of this "memory trace" was sufficient to induce a fear response. Importantly, the time of the increase in excitability was key in this memory enhancement as using chemicogenetics to increase excitability in 10% of LA neurons after training failed to enhance memory. Finally, we used ChR2 to increase excitability in a small subset of random LA neurons immediately before training and found that as with CREB overexpression, increasing excitability using optogenetics also enhanced memory formation ($n = 10, 10$). Together, these novel results indicate that neuronal memory allocation is based on relative neuronal excitability immediately before training.

Conclusions: Our results show that amygdala neurons are recruited to a fear memory trace based on relative excitability at the time of training. In these experiments, we artificially modified intrinsic excitability. However, endogenous changes in intrinsic excitability are linked to

learning in a variety of species (Hermissenda to rodents), suggesting that the current manipulations tap into an underlying fundamental memory process. Moreover, this process of neuronal allocation based on relative excitability may also play a role in "preplay", an intriguing phenomenon in which the emergence of hippocampal place cell firing is predicted by neuronal activity patterns that occur in the minutes before actual exposure to a novel spatial context (Dragoi & Tonegawa, Nature, 2011, PNAS, 2013). Although our work was performed in mice, functional neuroimaging findings from Dr. Ray Dolans' lab (Bach et al., J Neurosci, 2011) suggest that a similar mechanism may underlie fear memory in humans. Our finding that neurons compete for allocation to a fear memory trace has been successfully replicated in computational modeling studies (Kim et al., J Neuroscience, 2013).
Disclosure: Nothing to Disclose.

56.2 Pathway-specific Corticoamygdala Mediation of Fear Extinction

Andrew Holmes

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Background: Anxiety disorders such as PTSD are characterized by deficits in fear extinction. Growing evidence firmly establishes the importance of intact corticoamygdala function for successful fear extinction. However, there remain outstanding questions regarding the specific corticoamygdala pathways that are critical to extinction. One key issue for the field relates to the centrality of the reciprocal pathway connecting the infralimbic cortex (IL) and amygdala (intercalated cell masses and basolateral amygdala) to fear extinction. Prior studies have shown that IL inputs to the BLA are recruited and exhibit synaptic plasticity with extinction training, while optogenetically inhibiting inputs from the BLA to the IL during extinction training impairs retrieval. However, though IL inputs to the amygdala are commonly theorized to mediate fear extinction, this remains to be definitively demonstrated.
Methods: We first examined whether recruitment of the IL-amygdala pathway was predictive of successful fear extinction by quantifying immediate-early gene (c-Fos) expression of neurons in the IL, intercalated cell masses and basolateral amygdala, in good and poor extinguishing mice. Next, we tested the necessity of IL-amygdala inputs for fear extinction, using optogenetics to control IL inputs to the amygdala. C57BL/6J mice were conditioned to associate a novel tone with foot-shock and then given extinction training, consisting of fifty non-reinforced tone presentations, followed by an extinction retrieval test of five non-reinforced tones. We bilaterally infused an AAV-CaMKIIa-eArchT3.0-eYFP viral vector into the IL, and bilaterally shone green light into the amygdala during extinction training in order to silence IL inputs to the amygdala. We then tested for extinction retrieval the following day (without shining light into the amygdala).
Results: We found that low levels of c-Fos expression in the IL, intercalated cell masses and basolateral amygdala following fear extinction predicted poor extinction retrieval ($P < .01$ via t-tests for each region, $n = > 9$ per subgroup and region). Next, we found that silencing IL inputs to the amygdala during extinction training did not alter freezing during extinction training trials. Importantly, however, silencing IL inputs to the amygdala during extinction training impaired extinction retrieval the following day. This was evidenced by significantly higher freezing in mice infected with AAV-CaMKIIa-eArchT3.0-eYFP, as compared to mice infected with the control eYFP virus ($P < .01$ via t-test, $n = 10$-14 per virus group).
Conclusions: These studies provide a critical advance in current understanding of the specific neural pathways underpinning corticoamygdala mediation of fear extinction. In addition, they provide a foundation for investigating how manipulations of these pathways can be used to rescue extinction deficits in models of impaired extinction, with potential implications for therapeutically enhancing extinction.
Disclosure: Nothing to Disclose.

56.3 Corticotropin Releasing Hormone Regulates Endocannabinoid Hydrolysis within Principal Neurons of the Amygdala to Modulate Anxiety Behavior

Matthew Hill

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Background: Endocannabinoids (eCB) have gained significant attention in the regulation of emotional behavior in recent years. The work of our group, and others, has clearly demonstrated the importance of the amygdala, particularly the basolateral nucleus (BLA), as representing a hub mediating the ability of eCB signalling to influence anxiety. For example, disruption of eCB signalling within the BLA exclusively has been shown to increase anxiety, activation of the HPA axis and impair fear extinction. Interestingly, stress exposure causes a loss of eCB signalling which contributes to the generation of an anxious state; however, the mechanisms by which stress exposure alters eCB function is poorly understood. Using a combination of genetic, biochemical and electrophysiological approaches, we have identified several pathways by which stress can regulate both the synthesis and hydrolysis of eCB molecules within the amydgala to modulate anxious states.
Methods: To determine the role of CRH and CRHR1 receptor in mediating the effects of stress on eCB function within the amygdala, we examined the effects of icv administration of CRH or specific agonists to the CRHR1 and CRHR2 receptors, and determined their effect on the eCB system within the amygdala. To support these findings, we also examined the effects of CRH over expression within forebrain neurons on eCB function within the amygdala. Next, using either a pharmacological antagonist to CRHR1 or genetic deletion of CRHR1 exclusively from glutamatergic neurons within the BLA, we determined the necessity of CRHR1 activity in mediating the effects of stress on changes in eCB function. Using a reporter mouse to visualize the expression of CRHR1 receptors within the BLA, as well as colormetric in-situs, we examined colocalization between CRHR1 and the enzyme fatty acid amide hydrolase (FAAH), which metabolizes the eCB anandamide, within principal neurons of the BLA. Additionally, we examined the effects of inhibition of FAAH within the BLA following

administration of CRH on the generation of anxiety-like behaviour. Similarly, we employed a line of selectively bred rats which exhibit elevated emotionality and examined eCB function within the amygdala. Finally, we employed electrophysiology to examine the effects of FAAH inhibition on stress-induced excitability within the BLA to develop a mechanistic understanding of how eCB signalling within this structure regulates anxiety.

Results: First, we found that administration of CRH, or the CRHR1 agonist cortagine (but not the CRHR2 agonist Urocortin II) triggered an increase in the hydrolytic activity of FAAH and resulted in a rapid decline in anandamide levels within the amygdala (n = 6-9). These effects were independent of changes in corticosterone indicating that they were directly related to the activation of CRHR1. Consistent with this, we also found that CRH over expression in forebrain neurons resulted in a sustained increase in FAAH activity and reduction in anandamide content within the amygdala. Next, we determined that pharmacological blockade of CRHR1, or genetic deletion of CRHR1 exclusively from glutamatergic neurons within the BLA, prevented the ability of acute stress to increase FAAH activity and reduce anandamide levels within the amygdala (n = 7-9). Consistent with the finding that CRHR1 in glutamatergic neurons is an important mediator in the effects of stress on eCB metabolism, we found that principal neurons within the BLA which expressed CRHR1 exhibited over a 90% co-expression with FAAH indicating that the ability of CRHR1 to increase FAAH activity is mediated through crosstalk between these systems within glutamatergic principal neurons within the BLA. Next, we found that administration of a FAAH inhibitor prior to stress-exposure reduced stress-induced increases in glutamatergic currents within BLA principal neurons (n = 14-21 cells / condition), but had no effect of GABAergic currents within the BLA. This would suggest that in response to stress, CRH release triggers FAAH activity which results in a decline in the signalling pool of anandamide within the BLA. This loss of anandamide within the BLA disinhibits excitatory inputs to principal neurons in the BLA, resulting in an increased excitatory drive to these cells. Given that increased activation of BLA principal neurons has been associated with increased anxiety, we next determined that inhibition of FAAH within the BLA proper resulted in a suppression of CRH-mediated anxiety (n = 9-11). Consistent with this finding, we also found that selectively bred rats which exhibit heightened anxiety and elevated CRH within the amygdala possess significantly reduced levels of anandamide within the amygdala (n = 6). More so, we also found that sustained exposure to corticosterone (which up-regulates CRH within the amygdala) results in a CRHR1-mediated induction of FAAH activity and reduced anandamide levels within the amygdala (n = 8-10).

Conclusions: Taken together, these data suggest that there is an eCB "tone" within the amygdala which is mediated by anandamide and functions to limit excitatory inputs to the BLA keeping the amygdala in a quiescent state during conditions of low stress. In response to stress exposure, there is a rapid release of CRH in the amygdala which triggers FAAH activity through the activation of CRHR1 within glutamatergic principal neurons of the BLA. This increase in FAAH activity results in a decline in the signalling pool of anandamide within the BLA, which in turn disinhibits glutamatergic inputs to the BLA and contributes to activation of this nucleus in response to stress. The increase in BLA activation from this loss of anandamide signalling increases anxiety. Under conditions of chronic stress, the up-regulation of CRH from chronic exposure to glucocorticoids results in a sustained increase in FAAH activity and reduction in anandamide signalling. As we have previously demonstrated that this loss of anandamide signalling contributes to structural changes within the BLA and the generation of an anxious state, these data indicate that CRH mediates both the acute and chronic effects of stress on the eCB system. They also illustrate the importance of cross-talk between the CRH and eCB systems in the regulation of emotional behavior.

Disclosure: Part 1: Scientific consultant for Pfizer.

56.4 Using Imaging Genetics to Dissect the Neural Circuits of Fear & Anxiety in Humans

Ahmad Hariri

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Background: As the three companion abstracts in this panel submission illustrate, animal models employing genetic and optogenetic techniques are producing increasingly refined maps of the neural circuitry underlying fear and anxiety. Translating these discoveries to human brain, behavior, and psychopathology represents a significant challenge. Here, I will demonstrate how the integration of human neuroimaging and molecular genetics (i.e., imaging genetics) can begin to address this challenge and allow for similarly incisive studies in humans. Based on the work presented by Josselyn and Holmes, I will first describe how fMRI can be used to measure the differential response of human amygdala subregions to emotional facial expressions associated with threat as well as the habituation of these responses, which reflects changes in the functional coupling between these subregions and the medial prefrontal cortex over time. I will then demonstrate how measurement of human genetic polymorphisms can be leveraged to test the potential impact of molecular signaling pathways on these circuit responses as presented by Hill. Specifically, I will describe the impact of interactions between two common SNPs in the human genes for CRHR1 (rs110402) and FAAH (rs324420), respectively, on individual differences in amygdala habituation. FAAH rs324420 was selected based on prior work demonstrating relatively decreased FAAH enzyme function (and presumably increased AEA) as well as increased amygdala habituation and decreased negative affect in carriers of the A allele in comparison with homozygotes for the C allele. CRHR1 rs110402 was selected based on prior work demonstrating relatively increased cortisol reactivity to an acute stressor in homozygotes for the A allele in comparison with carriers of the G allele.

Methods: Data were derived from the first 873 participants of the ongoing Duke Neurogenetics Study, which seeks to map predictive links between genes, brain, behavior, and experience in the emergence of individual differences in behavior and related risk for psychopathology in 18-22 year old college students. BOLD fMRI was used to measure subregional

amygdala reactivity and habituation during the perceptual processing of emotional facial expressions including fear and anger, which represent conditioned stimuli predicting threat. CRHR1 rs110402 and FAAH rs324420 genotypes were identified from saliva-derived DNA using customized Illumina whole-genome arrays in a subsample of 709 participants. After stringent multilevel quality control procedures applied to both imaging and genetics datasets, BOLD fMRI and genotype data were available for analyses in 661 participants. All analyses controlled for ancestrally informative principal components, gender, as well as past or present DSM-IV Axis I disorder determined through clinical interview using the eMINI. Consistent with recent recommendations, all analyses further controlled for all possible genotype x covariate interactions.

Results: A significant interaction effect between CRHR1 rs110402 and FAAH rs324420 was observed on habituation of the basolateral amygdala in both right ($F(1,636) = 7.36$, delta $R2 = .011$, $p = .007$) and left ($F(1,636) = 7.14$, delta $R2 = .011$, $p = .008$) hemispheres. Post-hoc t-tests revealed that the CRHR1 AA/FAAH A carrier group exhibited significantly less amygdala habituation in comparison with either the CRHR1 AA/FAAH CC (Right: $b = .091$, $t = 2.34$, $p = .019$; Left: $b = .12$, $t = 2.63$, $p = .009$) or CRHR1 G carrier/FAAH A carrier groups (Right: $b = .1279$, $t = 2.96$, $p = .0032$; Left: $b = .14$, $t = 2.77$, $p = .006$). The CRHR1 G carrier/FAAH A carrier group, which has the highest predicted AEA signaling, exhibited the nominally greatest rate of amygdala habituation. There were no significant differences in habituation between CRHR1 AA/FAAH CC and CRHR1 G carrier/FAAH CC genotype groups.

Conclusions: The observed genetic interaction pattern on human amygdala habituation supports the demonstrated importance of FAAH in mediating the effects of CRHR1 agonism in the preclinical model presented by Hill. Moreover, the interaction pattern is consistent with the proposed "eCB tone" model wherein there is a dominant inhibitory effect of AEA signaling in the basolateral amygdala in the presence of relatively decreased cortisol reactivity (i.e., CRHR1 G carrier/FAAH A carrier genotype combination). These data highlight the potential of human imaging genetics to model interactions between specific molecular signaling pathways involved in modulating the neural circuitry of fear and anxiety identified in preclinical animal models. This strategy may be similarly effective in translating other novel discoveries in preclinical models to humans.

Disclosure: Nothing to Disclose.

Panel
57. Blood and Brain Gene Expression Convergence: Implications for Blood-based Biomarkers

57.1 Multi-omic Expression Profiling in a Mouse Model Simulating Aspects of Post-traumatic Stress Disorder

Rasha Hammamieh

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Background: Post-traumatic stress disorder (PTSD) affects about 3.8 % of Americans with lifetime prevalence of 6.8 %, and about 4–17 % of veterans. PTSD is a growing major problem due to lack of prognostic and diagnostic mechanisms and lack of effective biological therapeutic targets. Many patients (40–50%) do not respond to the many extant treatments such as psychological therapies (e.g., cognitive, behavioral) and pharmacological interventions (e.g., serotonin reuptake inhibitors), and develop chronic PTSD. PTSD patients exhibit deficits in inhibition of conditioned fear association, in attention regulation, in cognitive functions, in frontal cortex circuitry involved in executive functions, and in fear extinction with dysfunction of corticolimbic circuits mediating extinction, and impaired memories except the unrelenting (intrusive) memories of the traumatic event. PTSD-like symptoms are also observed in different species of animals, both in the wild and in controlled study settings. Rodents exposed to electric shock, social stress (aggressor-exposed) and predator stress often exhibit behaviors parallel to human PTSD. Animal models that can closely mimic the phenotype of human PTSD are critical in identifying molecular signatures and pathways leading to the pathological state of neural circuits in PTSD or depression. The major behavioral responses of PTSD patients such as avoidance of trauma reminders, increased vigilance (startle response), social withdrawal, anxiety, impaired cognition (impaired object recognition) and arousal symptoms were replicated in our aggressor-exposed "mouse models of social stress. In addition, these mouse models also showed behaviors and biological processes similar to PTSD co-morbid conditions: depression (frozen motion), obesity, diabetes and peripheral inflammation (similar to immune mediators of chronic PTSD in humans). We performed global gene and miRNA expression and DNA methylation profiling of blood and different brain regions collected from socially stressed mice showing PTSD-like behaviors, to elucidate molecular mechanisms underlying stress-induced plasticity in fear circuits.

Methods: The social stress (SS) model in C57/BL6 mice reliably elicits PTSD-like behaviors. SS or control (C) mice were housed in a small box (without food or liquid) within a larger cage for 6 h, for 5 days or 10 days. SS, but not C, was exposed to an aggressor mouse at random intervals (3x/day). Mice were sacrificed and blood and brain samples taken after 1 day, 10 days or 42 days of rest. Blood samples as well as brain regions associated with fear circuitry were collected from aggressor-exposed (5 or 10 days) or control C57BL/6J mice. The samples were subjected to gene expression profiling, after various periods of rest. Changes in gene expression after each of the 3 periods of rest were profiled using microarray and real time PCR.

Results: Transcripts from blood samples demonstrating increased expression following longer rest periods are those known to be associated with T-cell immunity, serotonergic and dopaminergic synaptic processes and axonal guidance. In contrast, transcripts in blood with decreased expression following longer rest periods were those known to be involved in associative learning, opioid signaling and glutamatergic pathways. Changes at the transcriptome level for both blood and brain indicated the pervasive nature of traumatic stress-induced alteration in functions and pathways reported to participate in stress-related phenotypic behavioral disorders and pathologies. After the longer rest period, some of the important functions and pathways

tended to return toward control levels, suggesting recovery with time.
Conclusions: As part of our systems biology approach, we carried out proteomics, genomics and metabolomics analyses on blood, organs and brain regions collected from our mouse models. A battery of hormones and other tissue-specific biomarkers were also assayed. Transcripts involved in PTSD and social stress related behaviors such as startle and fear response, social withdrawal, circadian rhythm of sleep and metabolism, associative learning and long-term fear memory were significantly changed though different member transcripts were changed at different time points and in different tissues. Transcripts of nervous system processes and pathways which are important in stress responses such as synaptic transmissions critical for anticipation of good and feeling of wellness (dopaminergic and serotonergic), axon guidance, nerve growth signaling, post-synaptic membrane potential regulation, long-term synaptic potentiation and depression were also affected.
Disclosure: Nothing to Disclose.

57.2 Expression Profiling Associates Blood-Brain Glucocorticoid Receptor Signaling with Trauma-related Individual Differences

Nikolaos Daskalakis

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Background: Delineating the molecular basis of individual differences in the stress response is critical to understanding the pathophysiology and treatment of posttraumatic stress disorder (PTSD).
Methods: In this study, 7-days after predator-scent-stress (PSS) exposure, male and female rats were classified into vulnerable (i.e., "PTSD-like") and resilient (i.e., minimally affected) phenotypes on the basis of their performance on a variety of behavioral measures. Genome-wide expression profiling in blood and two limbic brain regions (amygdala and hippocampus), followed by quantitative PCR validation, was performed in these two groups of animals as well as in an unexposed control group.
Results: Genes were identified in association with both PSS-exposure and with distinct behavioral profiles post-exposure in blood and brain. There was a small, but significant, across-tissue overlap (4-21%) for the genes associated with exposure-related individual differences, indicating convergent gene expression in both sexes. To uncover convergent signaling pathways across tissue and sex, upstream activated/de-activated transcription-factors were first predicted for each tissue and then the respective pathways were identified. Glucocorticoid receptor (GR) signaling was the only convergent pathway associated with individual differences when using the most stringent statistical threshold. A corticosterone treatment after PSS-exposure prevented anxiety and hyperarousal 7-days later, confirming the GR involvement in the PSS behavioral response.
Conclusions: Genes and pathways associated with extreme differences in the traumatic stress behavioral response can be distinguished from those associated with trauma exposure, in both sexes. Blood-based biomarkers can predict aspects of brain signaling. GR signaling is a convergent signaling pathway, associated with trauma-related individual differences.
Disclosure: Nothing to Disclose.

57.3 Next Generation Blood Biomarkers for Psychiatric Disorders: The Power of Longitudinal Designs

Alexander Niculescu

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Background: Following our ground-breaking work published over the last 6 years, we will present new data on second generation blood biomarkers for mood disorders, psychosis, and suicide.
Methods: Discovery gene expression studies in psychiatric patients using a within-subject longitudinal design, coupled with Convergent Functional Genomics (CFG) integration with animal model data and human genetic data, yielded a series of biomarkers that were then validated in independent cohorts.
Results: We demonstrate functional prediction of future clinical events and hospitalizations based on these biomarkers, alone or by integration with quantitative phenomic (clinical) data, for which we have developed easy to use apps for clinicians and patients.
Conclusions: Combined blood biomarker and clinical data approaches may enable may enable precision medicine in psychiatry.
Disclosure: Part 1: Consultant: Otsuka, Sunovion, **Part 2:** Sunovion, **Part 3:** Co-founder, Mindscape Diagnostics.

57.4 On the Outside Looking in: Comparison of Blood and Brain Gene Expression in Schizophrenia and Other Neuropsychiatric Disorders

Stephen Glatt

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Background: For nearly a decade, we and others have been pursuing the prospect of using blood-based gene-expression measurements to detect, classify, and understand schizophrenia and other neuropsychiatric disorders. Much of this work has progressed without a fundamental understanding of the comparability of the blood and brain transcriptomes.
Methods: In our recent work and that of others using gene-expression microarrays and RNA-sequencing technologies, we have examined the degree to which the expression of coding and non-coding RNAs is preserved across blood and brain tissue samples from healthy control populations and samples of populations with schizophrenia, bipolar disorder, autism spectrum disorder, and other neuropsychiatric disorders, as well as animal models.
Results: As a surrogate of brain transcriptional activity, blood gene expression is moderately well preserved relative to other sampled tissues. Across the transcriptome, there are numerous genes that are very well correlated between blood and brain, while many genes are entirely uncorrelated in their expression across the two tissues. Extensive results regarding one gene (SELENBP1) and one pathway

(ubiquitin proteasome pathway) found to be dysregulated in both brain and blood in schizophrenia will be presented and discussed.

Conclusions: We do not view absolute comparability of the biomarker tissue (blood) and the target disorder tissue (brain) as necessary for the construction of useful blood-based transcriptome signatures, but understanding the degree and manner in which blood and brain transcriptomes correlate enables other inferences; e.g., regarding putative etiologic mechanisms and the source of dysregulation (genetic vs. environmental).

Disclosure: Nothing to Disclose.