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Panels, Mini-Panels and Study Groups
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Panel
1. Gut Feelings: How the Microbiome May Affect Mental Illness and Interact With Treatment

1.1 Gut Microbial Community and Behavioral Changes in a Chronic Mild Stress Model of Depression in Rats

Emily Jutkiewicz

University of Michigan, Ann Arbor, Michigan, United States

Background: Recent evidence demonstrates that the gut microbiome affects brain function and emotional behavior, suggesting that the microbiome-gut-brain axis may play a pathophysiological role in psychiatric diseases such as depression. At this time, there are limited studies investigating whether gut microbiome and brain interactions have directional causality. To this end, the current study evaluated time-dependent changes in gut microbial communities in male Sprague-Dawley rats during exposure to 7 weeks of chronic, variable mild stress and evaluated the effects of microbial exposures on mood-related behaviors.
Methods: Behavioral measures and fresh fecal samples were collected weekly prior to, during, and after stress exposure. Microbial communities were evaluated by DNA sequencing of the V4 region of the 16S gene. Behavioral changes were measured by weekly determinations of sucrose drinking and preference scores, as a measure of anhedonic-like behavior, as well as changes in despair-like behavior in the forced swim test.
Results: Exposure to chronic mild stress leads to a decrease in bacterial species diversity as compared with non-stressed controls and these changes precede decreases in sucrose preference. Further, we identified specific bacterial populations that were associated with the stressed and non-stressed behavioral phenotypes. In addition, exposure to feces from stressed rats rapidly (within 5 days) altered behavioral outcomes in naïve rats. However, exposure to feces from non-stressed rats did not rapidly improve depressive-like phenotypes, but there is a tendency to accelerate recovery.
Conclusions: Overall, these findings suggest that gut microbiome-brain interactions play an important role in modulating brain function and behavioral outcomes and may be more likely to exacerbate stress-induced behavioral disruptions than to serve as an intervention.
Disclosure: Nothing to Disclose.

1.2 The Gut Microbiome Composition Varies in Bipolar Disorder and Associates With Self-Reported Severity of Illness

Simon Evans

University of Michigan, Ann Arbor, Michigan, United States

Background: The gut microbiome is emerging as an important factor in regulating mental health yet it remains unclear what the target should be for psychiatric treatment. We aimed to elucidate the complement of the gut-microbiome community and its relationship with burden of disease measures in a population of individuals with bipolar disorder.
Methods: We compared the stool microbiome from individuals with bipolar disorder ($n=115$) and control subjects ($n=64$) using 16S ribosomal RNA (rRNA) gene sequence analysis to assess case-control differences. We further tested for relationships between expression of Operational Taxonomic Units (OTUs) and self-reported burden of disease measures.
Results: Analysis of Molecular Variance (AMOVA) revealed global community case-control differences (AMOVA $p=0.047$). OTU level analysis revealed lower levels ($p<0.001$) of Firmicutes Faecalibacterium after adjustment for age, sex, BMI and False Discovery Rate (FDR) correction at the $p<0.05$ level. Within individuals with bipolar disorder, Faecalibacterium levels positively associated with better self-reported health outcomes based on the Short Form Health Survey (SF12); the Patient Health Questionnaire (PHQ9); the Pittsburg Sleep Quality Index (PSQI); the Generalized Anxiety Disorder Scale (GAD7); and the Altman Mania Rating Scale (ASRM).

Conclusions: This study provides the first analysis of associations between the gut microbiome and multiple psychiatric domains from a bipolar population. The data supports the hypothesis that targeting the microbiome may be an effective treatment paradigm for bipolar disorder.
Disclosure: Nothing to Disclose.

1.3 Effects of Major Depression and SSRIs on the Gut Microbiota

Chadi Calarge

Baylor College of Medicine, Houston, Texas, United States

Background: Human studies suggest that manipulation of the gut microbiome can alter emotions. In rodents, such manipulations are associated with a variety of changes in emotion-relevant brain areas. Alterations in the gut microbiota (i.e., dysbiosis) in mood disorders have not been well investigated, neither have mechanisms that mediate such associations.
Methods: Stool samples ($n = 200$) were collected from participants ($n = 162$, 57% females, age: 19.8 ± 1.8 years) enrolled in a longitudinal study examining the skeletal effects of selective serotonin reuptake inhibitors (SSRIs). As a result, repeated samples were available when participants were in a depressive episode ($n = 79$), in remission ($n = 66$), or never depressed ($n = 55$), while medicated with SSRIs ($n = 62$) and not ($n = 138$). Microbiome composition is analyzed by culture-independent methods using Illumina sequencing. Further, the production of indole/oxindoles was measured. Analyses include alpha and beta-diversity measures and multivariate statistics are used to identify differences in microbiome profiles and indole/oxindoles production across different states.
Results: The extant literature relating changes in the gut microbiota to emotion regulation in rodents and humans will be briefly reviewed. Findings from our study, 31% during SSRI use, Between and within individual analyses will show differences in gut bacterial composition in depressive disorders, particularly in relation to the presence of neurovegetative symptoms, and comorbid generalized anxiety disorder ($n = 58$). Further, the prevalence of the tryptophanase gene will be shown as it relates to indole/oxindoles production, diverting tryptophan from the serotonin pathway. Finally, relevance of the findings to potential innovative treatments will be discussed.
Conclusions: Emerging evidence suggests the microbiota-gut-brain axis may play a role in mood disorders but further studies, particularly in humans, are needed to validate the mechanisms involved and develop potential treatments modulating the gut microbiome.
Disclosure: Nothing to Disclose.

1.4 Interaction Between Atypical Antipsychotics and the Microbiome in a Bipolar Disease Cohort

Stephanie Flowers

University of Michigan, Ann Arbor, Michigan, United States

Background: The atypical antipsychotic (AAP) class is often associated with metabolic disease but the mechanistic underpinnings of this medication-related risk are not understood. A recent study in adolescent males determined that new-onset risperidone treatment resulted in a gradual change in gut microbiota in association with body-mass index (BMI) gain. Our primary hypothesis is that AAP-treatment in adults results in gut dysbiosis that potentiates metabolic disease criteria such as increased BMI.
Methods: In a cross sectional design, 117 subjects (49 Bipolar Disorder (BD)-diagnosis and 68 Controls) from the Prechter Longitudinal Study of Bipolar Disorder were analyzed to assess the effects of AAP-treatment on the gut microbiome. Fifty-two of the BD subjects were treated with an AAP. Age, gender and BMI metadata were also collected for each subject. The V4 sequences of 16S rRNA were analyzed using mothur v1.36. Analysis of molecular variance was used to detect significant clustering of treatment groups and the inverse Simpson index was used to calculate alpha diversity. Detection of deferentially abundant OTUs between treatment groups was performed using metastats.
Results: Regression analysis of AAP-treated BP-subjects revealed higher BMI values that remained significant after correcting for age ($p = 0.016$). There were statistically significant differences in overall gut microbial profiles between AAP-treated and non-AAP treated BD subjects ($p = 0.04$). There were three abundant organisms that were important in this separation. OTU #2, Bacteroides (Phylum : Bacteroidetes; $p =$ 2E-05) and OTU #7, Roseburia (Phylum: Firmicutes; $p =$ 8E-06) and OTU #11, Alstipes (Phylum: Bacteroidetes; $p = 0.0002$). Species diversity between medication groups did not differ until stratified by gender. AAP-treated females showed a significant decrease in species diversity when compared to non-AAP treated females ($p = 0.02$). Males did not show a difference in diversity between groups ($p = 0.38$).
Conclusions: These data suggest that AAP-treatment is associated with specific representation of gut bacterial families and decreased species richness in females, which is a measure of gut health.
Disclosure: Nothing to Disclose.

Panel
2. Synaptic Plasticity and its Dysregulation in Neuropsychiatric Disorders

2.1 Experience-Dependent Expression of Neurogranin, a Schizophrenia-Associated Gene, Gates Memory Formation and Synaptic Plasticity via Adrenergic Signaling

Weifeng Xu

Massachusetts Institute of Technology, Cambridge, Massachusetts, United States

Background: Schizophrenia is a complex neurological disorder caused by a combination of genetic and environmental factors. Recent genetic evidence has revealed several genes in the NMDA receptor (NMDAR) complex and its downstream Ca2+-dependent signaling cascade that are associated with schizophrenia. Among these is neurogranin, a small neuron-specific protein, expressed primarily in the

postsynaptic compartments of forebrain projection neurons. Neurogranin binds to calmodulin, a major Ca2+-binding protein which activates Ca2+/calmodulin-dependent enzymatic pathways. Neurogranin prevents calmodulin from binding calcium, thereby influencing the dynamics of Ca2+/calmodulin in Ca2+-dependent signaling events in the postsynaptic compartment, including NMDAR-dependent synaptic plasticity. The gene encoding neurogranin has been associated with schizophrenia and a rare genetic disorder with symptoms of intellectual disability, Jacoben syndrome. Neurogranin levels are profoundly decreased in the cortex of post-mortem brain from human subjects with schizophrenia. Recent studies indicate that the schizophrenia risk allele associated with the neurogranin gene correlates with changes in the brain structure, brain activity during behavioral tasks and expression levels of neurogranin. These findings implicate the involvement of neurogranin in the pathophysiology of schizophrenia. Our preliminary data indicate that neurogranin levels are dynamically regulated by environmental, hormonal and behavioral factors, suggesting state-dependent dynamics in neurogranin levels can influence Ca2+ homeostasis and Ca2+-dependent signaling events including NMDAR-dependent synaptic plasticity important for cognition.

Methods: We used a combination of pharmacological and genetic manipulations of neurogranin and contextual memory formation, a hippocampal-based behavior, to examine how changes in neurogranin expression can influence memory formation and synaptic plasticity.

Results: We found that novel context exposure induces upregulation of neurogranin in hippocampus. Blocking adrenergic signaling blocked novel context induced upregulation of neurogranin in the hippocampus also context memory formation. In addition, activation of adrenergic pathway induced activity-dependent translation of neurogranin and facilitated context memory formation. Constitutive hippocampal expression of the 3'UTR of neurogranin, which blocks activity-dependent upregulation of neurogranin *in vitro*, blocked adrenergic-dependent facilitation of contextual memory formation and LTP.

Conclusions: Taken together, these results document that novel context exposure acts via adrenergic signaling to promote neurogranin translation in the hippocampus. The newly synthesized neurogranin is required for facilitation of LTP and contextual memory formation. Therefore, potential defects in neurogranin expression and its regulation in schizophrenic patients may disrupt synaptic plasticity and cause deficits in cognition.

Disclosure: Nothing to Disclose.

2.2 Regulation of Glutamate Receptors and Cognitive Disorders

Richard Huganir

Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Background: Neurotransmitter receptors mediate signal transduction at synaptic connections between neurons in the brain. We have been studying the regulation of glutamate receptors, the major excitatory receptors in the central nervous system. Studies in our laboratory have found that glutamate receptors are regulated by a variety of protein kinases and we have identified a variety of proteins that directly or indirectly interact with the receptors and are necessary for their proper subcellular trafficking and downstream signaling. Receptor modulation and these interacting proteins are crucial for expression of synaptic plasticity and are important for several forms of learning and memory. One of these proteins called SynGAP is a Ras-GTPase activating protein that is highly enriched at excitatory synapses in the brain and is critical for downstream signaling from the NMDA receptor. However, the molecular mechanisms coupling NMDA receptor to SynGAP activity are not known. In addition, recent human genetic studies have identified SynGAP mutations in intellectual disability (ID), autism spectrum disorder (ASD) and schizophrenia (SCZ); however, the effects of these mutations on SynGAP function are not known.

Methods: Here we use molecular biological, biochemical, imaging and electrophysiological techniques to study the regulation of SynGAP function and synaptic targeting in neurons. In addition, we study the role of SynGAP in the regulation of AMPA receptor trafficking during chemically induced long-term potentiation (LTP) in neuronal cultures and organotypic hippocampal slice cultures. We also use SynGAP knockout mice to examine the role of SynGAP in synaptic plasticity and learning and memory. Finally, using mutational analysis, we study the effect of SynGAP mutations found in ID on synaptic signaling and plasticity.

Results: We found that SynGAP is rapidly dispersed from spines upon LTP induction in hippocampal neurons and this dispersion depends on phosphorylation of SynGAP by CaMKII. Moreover, the degree of acute dispersion predicts the maintenance of spine enlargement. Thus, the synaptic dispersion of SynGAP by CaMKII phosphorylation during LTP represents a key-signaling component that transduces CaMKII activity to small G protein mediated-spine enlargement, AMPA receptor synaptic incorporation and synaptic potentiation. Recently de novo deleterious SynGAP mutations have been associated with ID, ASD and SCZ. Interestingly, many of the non-sense mutations of SynGAP found in ID lack the domain critical for SynGAP dispersion. Furthermore, SynGAP heterozygote knockout mice recapitulate some of the pathophysiology of ID. In preliminary results we have found that small molecules that regulate RAS activity can ameliorate the synaptic plasticity and behavioral deficits in these model mice.

Conclusions: These studies indicate that the modulation of receptor function and proper signaling at excitatory synapses is a major mechanism for the regulation of synaptic transmission and is a critical determinant of animal behavior. Importantly, recent genetic studies evidence has implicated glutamate receptors and interacting proteins are disrupted in several neuropsychiatric disorders including ID, ASD and SCZ. This study demonstrates that SynGAP is a critical synaptic protein that controls synaptic plasticity and learning and that disruption of SynGAP function is a significant factor in cognitive disorders.

Disclosure: Part 1: Pfizer Inc., Advisory Board, **Part 2:** Pfizer Inc., Advisory Board, **Part 3:** Pfizer Inc., Advisory Board.

2.3 Experience-Dependent Regulation of NMDA Receptors in Brain Development and its Dysregulation by Maternal Deprivation, an Environmental Risk Factor in Schizophrenia

R. Suzanne Zukin

Albert Einstein College of Medicine, New York, New York, United States

Background: Research in our laboratory focuses on epigenetic remodeling of NMDARs in synaptic plasticity and psychiatric disorders. A hallmark feature of hippocampal NMDARs is a developmental switch in receptor subunit composition and function that coincides with closure of 'critical period', a window of heightened plasticity in development. Whereas NMDARs at immature synapses are primarily GluN2B-containing, NMDARs at mature synapses are primarily GluN2A-containing. This is significant in that GluN2B-containing NMDARs exhibit slower kinetics, carry more Ca2+ and preferentially tether to the plasticity protein CaMKIIα. Whereas the switch in NMDAR phenotype has been of intense interest for nearly two decades, mechanisms that regulate the switch are, as yet, unclear. REST is a gene silencing factor that is widely expressed in pluripotent stem cells and neural progenitors, where it actively represses a wide array of genes important to synaptic plasticity and spine structure including the NMDAR subunit GluN2B. Polycomb group proteins are gene-silencing factors that are widely expressed during embryogenesis and are essential to epigenetic memory, pluripotency, and stem cell self-renewal. Polycomb proteins are recruited to target genes by REST via HOTAIR9 and together orchestrate epigenetic modifications. Our recent discovery that maternal deprivation prevents REST activation and aberrantly elevates GluN2B documents the ability of adverse experience to dysregulate the synaptic NMDAR phenotype. This is significant in that early childhood adversity or neglect is an environmental risk factor in schizophrenia. Findings by Carol Tamminga that GluN2B is aberrantly elevated in hippocampal CA3 tissue from drug-free subjects with schizophrenia underscore the clinical relevance of these findings.
Methods: We use molecular genetics and electrophysiology to examine the synaptic NMDAR phenotype. We examine how maternal deprivation can influence NMDAR properties and memory formation, and how paradigms such as communal nesting can rescue acquisition of the mature NMDAR phenotype and cognition.
Results: We have examined a role for polycomb proteins in acquisition of the mature NMDAR phenotype. The polycomb protein EZH2 is a histone methyltransferase that confers a trimethylation mark on histone 3 at lysine 27, a mark of enduring repression. EZH2 is expressed in the hippocampus during the first two weeks postnatal, peaks at P15, and is recruited to the grin2b promoter just prior to the NMDAR switch. Whereas REST is transiently expressed in hippocampal neurons at P15 followed by a decline, EZH2 is sharply upregulated at P15 and remains high as late as P60. EZH2 acts in a REST-independent manner to suppress GluN2B expression as late as P60. Adverse experience in the form of maternal deprivation prevents epigenetic remodeling of NMDARs by EZH2. Studies to rescue the impact of maternal deprivation on synaptic NMDARs are ongoing.
Conclusions: REST and EZH2 together orchestrate remodeling of the synaptic NMDAR phenotype in development. Maternal deprivation blocks activation of REST and EZH2 and acquisition of the mature NMDAR phenotype, a finding relevant to schizophrenia. REST and EZH2 are critical to the fine-tuning of proteins involved in synaptic plasticity.
Disclosure: Nothing to Disclose.

2.4 Altered Glutamatergic Plasticity in Hippocampal Subfields in Schizophrenic Psychosis

Carol Tamminga

University of Texas Southwestern Medical Center, Dallas, Texas, United States

Background: Our laboratory has analyzed molecular markers in individual hippocampal subfields from human subjects with schizophrenia to develop a testable mechanistic model for the hippocampal hyperactivity associated with psychosis. Hippocampal tissue from humans with schizophrenia shows evidence of dysfunction in excitatory synaptic transmission that originates in the dentate gyrus and projects to the CA3. This, in turn, is associated with increased glutamatergic excitability in CA3 and in downstream nuclei such as CA1, where hyperactivity can be detected by means of *in vivo* brain imaging of individuals with schizophrenic psychosis. We have invoked altered metaplasticity processes in hippocampus to explain this pathology. Recently, we have developed a reverse translation mouse model of this complex phenomenon, a dentate gyrus-selective GluN1 KO mouse.
Methods: We have used a combination of methodologies to comprehensively examine molecular, cellular, electrophysiological and behavioral endpoints in these mice. We have analyzed molecular markers of excitation and inhibition, electrophysiological characteristics of inhibitory and excitatory neurons, and behavioral readouts related to psychosis. Currently, we are using DREADD pharmacology to examine whether deficits in DG transmission is causally related to CA3 hyperactivity.
Results: To date we have demonstrated psychosis-like behaviors in the DG-GluN1 KO mice (reduced PPI, altered Morris Water Maze performance and increased fear conditioning), increased excitatory synaptic transmission at synapses onto CA3 pyramidal cells, and increased c-fos activity in pyramidal neurons of the hippocampal CA3 and CA1. We have documented our ability to induce psychosis-relevant behaviors in the mice by expression of an excitatory DREADD in the CA3. We are currently examining the impact of expression of an inhibitory DREADD in DG on activity in CA3.
Conclusions: The full description of tissue pathology in hippocampus associated with the psychosis dimension in schizophrenia requires an animal preparation that is available for dynamic studies of plasticity in hippocampus. The animal preparation we have developed mimics specific correlates of the dysfunction observed in humans with schizophrenia and enables us to examine causality between distinct hippocampal pathologies. We have the potential to identify pharmacological inhibitors that reverse these

pathologies in the mouse hippocampus. The idea that a reduction in dentate gyrus function could produce a paradoxical hyperactivity in CA3, with implications for hippocampal function, is an interesting consequence of these studies. These data argue for altered metaplasticity in the hippocampal subfields of an animal model of schizophrenia, consistent with the concept that psychosis is a disorder of learning and memory.
Disclosure: Part 1: Astellas, Ad Hoc Consultant, Autiphony Therapeutics, Ad Hoc Consultant, Intracellular Therapies (ITI), Chair, Medical Advisory Board, Taisho Pharmaceuticals, Ad Hoc Consultant, **Part 2:** Intracellular Therapies (ITI), Chair, Medical Advisory Board, **Part 4:** Sunovion, Investigator Initiated Scientific Grant.

Panel
3. Large-Scale Network Approaches to Addiction

3.1 Whole-Brain Resting-State Connectivity in Cocaine Addiction: Impact of Inhibitory Control and Recency of Cocaine Use

Rita Goldstein

The Icahn School of Medicine at Mount Sinai, New York, New York, United States

Background: Resting-state functional connectivity has emerged as a reliable marker of abnormal brain functioning in addiction. However, resting-state connectivity has rarely been investigated using a whole-brain approach and whole-brain resting-state studies which integrate neuropsychological data are yet to be performed in individuals with cocaine use disorder (iCUD). By tapping into individual differences in brain connectivity at rest and in baseline cognitive functioning we aim to advance the study of the neurocognitive deficits underlying drug addiction, to ultimately develop targeted training for preventing relapse.
Methods: We assessed neuropsychological functioning with the Cambridge Neuropsychological Test Automated Battery (CANTAB) and acquired resting-state functional scans in iCUD ($N=44$) and gender-/race-matched healthy controls ($N=31$), covarying for age. Factor analysis on ten CANTAB variables [including measures from Delayed Matching to Sample, Spatial Span, Verbal Recognition Memory, Intra-Extra Dimensional Set Shift, Stop Signal Task (SST)] revealed a group difference (controls > iCUD) in an Inhibitory Control factor (three SST variables, loadings >0.70). To evaluate the overlap with the expected group differences in resting-state (a 10 min scan, with eyes open, using the 3 T Siemens Skyra), factor scores for Inhibitory Control were used as regressors in a whole-brain resting-state connectivity analysis employing complex network analysis (graph theory). The measure of interest in the graph theory analysis was functional integration, both locally (within-region: local efficiency) and globally (across large-scale brain networks: global efficiency). Within iCUD, we also compared individuals with non-recent (iCUD-, urine negative) and recent drug use (iCUD+, urine positive).
Results: The iCUD demonstrated linearly decreased global functional integration of the executive control network (e.g., cingulate/supplementary motor area), memory circuit (hippocampal region) and subcortical brain regions involved in reward processing (e.g., putamen) as a function of recency of use (iCUD+ < iCUD- < Controls). The local functional integration in the visual stream (basic and higher visual processing: e.g., cuneus, ventral temporal cortex, inferior/superior parietal cortex) and in frontal regions involved in value representation (e.g., orbitofrontal cortex) was linearly increased (iCUD+ > iCUD- > Controls). Global disintegration of the executive control network and increased local connectivity within the visual stream were correlated ($r=-0.61$), indicating a shift in balance from frontal (and global) to sensory (and local) brain networks. Impaired Inhibitory Control was linked to abnormalities in the same networks, being associated both with decreased global functional integration of the executive functioning/memory circuit and increased local connectivity within the visual stream. This association between worse Inhibitory Control and abnormalities in functional integration was strongest in iCUD-.
Conclusions: These novel results extend previous reports to show that whole-brain connectivity states are altered both as a function of Inhibitory Control and recency of cocaine use in iCUD. They demonstrate an impairment of executive control extending beyond immediate task demands, suggesting that altered baseline functioning underlies compromised control in cocaine addiction. It remains to be studied if altered baseline functioning is a predispositional factor or a consequence of drug use. Targeting these whole-brain states by cognitive training (e.g., to improve self-control) and other interventions (e.g., pharmacologically enhanced neurofeedback) may lead to the development of novel individually-tailored treatment approaches grounded in neuroscience.
Disclosure: Nothing to Disclose.

3.2 Whole-Brain Static and Dynamic Connectivity as a Window Into the Brains of Substance Users

Vince Calhoun

The Mind Research Network, Albuquerque, New Mexico, United States

Background: Recent years have witnessed a rapid growth in moving functional magnetic resonance imaging (fMRI) connectivity beyond simple scan-length averages into approaches that capture time-varying properties of connectivity. In this perspective we use the term "chronnectome" to describe such metrics that allow a dynamic view of coupling. We discuss the potential of these to improve characterization and understanding of brain function, which is inherently dynamic, not-well understood, and thus poorly suited to conventional scan-averaged connectivity measurements. It is well known that users of alcohol, nicotine and marijuana show altered brain function. However little work has been done studying the transient connectivity dynamics related to substance use. In this work we discuss an analysis of resting fMRI data from a large number of substance users showing substantial changes in both static and dynamic connectivity.
Methods: Data were obtained from a total of 426 subjects grouped as: 121 controls (CTR), 62 drinkers (DRN), 65

smokers (SMK), 50 smoking and drinking (SAD), 35 marijuana smoking and drinking (MAD), 31 smoking nicotine and marijuana (SAM), and 62 consumers of all three substances (ALL). Five minute eyes open resting functional MRI data were collected on a 3T Siemens TIM Trio scanner. Data were pre-processed using the SPM software including slice-timing correction, realignment, co-registration and spatial normalization to the Montreal Neurological Institute (MNI) standard space. Data were subject to a group independent component analysis resulting in a total of 75 non-artifact components. Components were clustered in the following groups: cerebellum (CER), sub-cortical (SBC), auditory, sensorimotor (SEN), visual (VIS), cerebellum/visual (CVS), default mode network (DMN), salience network (SAL), executive control network (ECN) and attentional (ATT). Static functional network connectivity was computed and tested for group differences. In addition, dynamic connectivity revealed a set of 5 states. Mean dwell time for each state was evaluated as a percentage of the scanning time. Group differences of dwelling time were assessed using ANOVA and post hoc analysis.
Results: Both static and dynamic connectivity showed significant differences, however the static approach showed substantially more effects, suggesting that substance use primarily impacts the transient aspects of functional connectivity. ANOVA results revealed group differences in state dwell time for centroids A and B. The SAM group spent significantly less time in state A and tended to spend more time in state B compared to the CTR and DRN groups. The SMK group spent significantly less time in state B than the SAD and SAM groups.
Conclusions: Results suggest that the combined effect of smoking nicotine and marijuana reduces the ability to maintain connectivity among DMN, SAL and ECN. This effect also tends to increase time dwelling in an rsFNC state where these 3 networks are hypo-connected. In contrast, smoking alone may tend to keep the brain away from this hypo-connected state.
Disclosure: Nothing to Disclose.

3.3 Beyond Reward Learning: A Network-Based View of Fronto-Striatal Interactions in Pleasure and Pain

Tor Wager

University of Colorado Boulder, Boulder, Colorado, United States

Background: Brain circuits involved in emotion and motivational drives are thought to be central in addiction and related mental health disorders. A consensus view is that a circuit connecting ventromedial prefrontal cortex (vmPFC) and nucleus accumbens—both targets of the mesolimbic dopamine pathway— plays a critical role in reward learning. Aberrant reward-related processes in this circuit are thought to play a central role in addiction. I present recent evidence suggesting that this view must be broadened in several ways.
Methods: This work uses a combination of several emerging techniques. The first is multivariate pattern recognition or "machine learning" approaches to developing brain signatures. These signatures are designed to make accurate predictions about new, individual participants. Developing signatures allows us to validate them across multiple studies and research groups. Secondly, our work relies on two kinds of effective connectivity models. The first is multi-level mediation, which allows us to test path models of how experimental manipulations affect outcomes via mediating brain mechanisms. This technique allows us to search for brain mediators. The second technique is Dynamic Causal Modeling (DCM), an effective connectivity technique that models the bidirectional relationships in dynamic changes among a small set of regions.
Results: Findings from our studies suggest that 'value-related' portions of vmPFC plays a prominent role in shaping pain avoidance and responses to distress as well. These circuits may play an important role in addiction by governing distress-avoidance. DCM findings outline a circuit connecting the vMPFC and midbrain PAG, which jointly govern pain avoidance learning. Secondly, this circuit is not merely a passive learner of value driven by reinforcement. It is responsive to conceptual thought, including expectations, beliefs, and self-regulation. Multi-level mediation results demonstrate that cognitive self-regulation of pain works by shaping functional responses in a vmPFC-nucleus accumbens circuit. This circuit also mediates placebo effects on pain.
Conclusions: Together, these findings implicate processes beyond reward learning in substance use, and indicate a role for midline fronto-striatal circuits in avoiding anticipating negative outcomes.
Disclosure: Part 1: Janssen, Consulting fees, **Part 4:** NIH, grants.

3.4 Pleasures of the Brain: Insights From Whole-Brain Computational Modelling

Morten Kringelbach

University of Oxford Warneford Hospital, Oxford, United Kingdom

Background: The pleasure system serves adaptive evolutionary functions; relying on wanting, liking and learning neural mechanisms mediated by mesocorticolimbic networks driving pleasure cycles with appetitive, consummatory and satiation phases. Liking is generated in a small set of discrete hedonic hotspots and coldspots, while wanting is linked to dopamine and distributed brain networks. Breakdown of the pleasure cycle can lead to anhedonia, a main characteristic of affective disorders and prevalent in addictive disorders.
Methods: Recent advances in whole-brain computational modelling of human neuroimaging data have now opened the possibility of providing probabilistic causal information on the underlying networks and mechanisms. Briefly, such models can capture the global dynamics of the human brain's large-scale network of local neural networks – or regions, linked by long-range connections. The global dynamics of the whole-brain network are determined by the intrinsic dynamics of regions, i.e. the dynamics of a region in absence of all couplings, as well as the extrinsic network couplings, allowing communication between the regions of the network. The local spontaneous dynamics of a single region can be modelled as attractors of a network of spiking neurons coupled through AMPA, NMDA and GABA

receptor synaptic dynamics, where the emergent collective macroscopic behaviour of brain models has been shown to be only weakly dependent on the details of individual neuron behaviour.
Results: We show the results from whole-brain models using the underlying anatomical skeleton of each individual obtained using diffusion tensor imaging to explicitly link regions, shaping the interplay between the local dynamics of each region, to fit with functional neuroimaging data from MEG, EEG and fMRI in health and disease. We demonstrate how whole-brain models can be manipulated off-line to provide probabilistic causal information on hedonic brain networks in health and disease. This in turn has helped launch the field of computational neuropsychiatry.
Conclusions: The pleasure system has started to be better understood in health and disease in other animals but a causal understanding of the human pleasure system is still missing. Whole-brain computational models of human neuroimaging data are offering novel insights on the underlying mechanisms; potentially even linking eudaimonia to optimal metastability in the pleasure system.
Disclosure: Nothing to Disclose.

Panel
4. Oxidative Stress: Linking Cellular and System Pathophysiology Towards Clinical Trials in Schizophrenia

4.1 Novel and Established Therapies in Early Psychosis and Their Transdiagnostic Potential

Patrick McGorry

University of Melbourne, Parkvile, Victoria, Australia

Background: Despite the availability of effective therapies for schizophrenia and psychotic illness, recovery rates remain less than optimal and there have been no dramatic therapeutic advances for some time. There are two avenues for further progress. The first involves a search for new therapeutic targets and agents both biological and psychosocial, and the use of biosignatures and "profiling" using both biological and psychosocial features. The second has more to do with the timing of interventions and is aided by the clinical staging framework which supports early intervention as a way of "bending the curve" of outcome. Both these approaches may be assisted by a transdiagnostic mindset and research framework.
Methods: A systematic review and analysis of the literature on novel mechanisms, therapies and therapeutic outcomes in early psychosis and beyond.
Results: A range of new therapeutic options were identified and encouraging data in support of further research and translation identified. The biological therapies are being linked to specific mechanisms notably oxidative stress, neuroinflammation and lipid metabolism. Psychological profiling is a complementary approach and new technologies are making this more feasible as well. Timing of intervention will also be critical aspect.
Conclusions: The field of therapeutics in schizophrenia offers some exciting possibilities which can be based on neurobiological and psychological profiles and mechanisms. Sophisticated and sequential clinical trials with biomarker linkage are needed and these need to extend transdiagnostically and across stages of illness.
Disclosure: Nothing to Disclose.

4.2 Redox Dysregulation in Schizophrenia Revealed by *In Vivo* NAD+/NADH Measurement

Fei Du

Harvard Medical School, Belmont, Massachusetts, United States

Background: Schizophrenia (SZ) is a severe psychiatric disorder characterized by abnormal cognition and perception. Despite its public health impact and a century of biological research, the pathophysiology of SZ remains poorly understood. A growing body of evidence suggests that an "immuno-oxidative" pathway including oxidative stress, mitochondrial dysfunction, and neuroinflammation may contribute to disruptions in brain activity in SZ. Nicotinamide adenine dinucleotide (oxidized form, NAD+ and reduced form, NADH) has long been implicated in energy metabolism, antioxidant activity as well as other biological activities such as calcium homeostasis, gene expression, and immunological functions. Balance between the redox pair of oxidized NAD+ and reduced NADH, reflecting the oxidative state of cells and the ability of biological systems to carry out energy production, plays an essential role in the pathophysiology of the living system. Despite the crucial roles of NAD+ and NADH, its noninvasive *in vivo* detection is extremely challenging. Here we demonstrated the feasibility of 31P MRS-based NAD quantification at 4 T MRI scanner and applied this novel method to investigate the brain's redox state (i.e., NAD +/NADH ratio) in SZ patients. The major aim of this study is to assess possible redox imbalance in SZ patients.
Methods: First we implemented and validated 31P-MRS based NAD+/NADH detection method at 4 T with phantom experiments as well as Monte-Carlo simulations. Generally, NAD+ and NADH components were determined by nonlinear least-square fitting of the model simulated spectra; these incorporated prior information of chemical shift and coupling constant to *in vivo* resonances obtained from 31P MRS experiments. Then participants included 40 healthy controls, 21 chronic SZ, 13 first-episode SZ, and 18 first-episode bipolar disorder (BD) patients (as a psychiatric control group) were recruited and underwent structural imaging at a 3 T and 31P MRS measurements on a 4 T MR scanner.
Results: By applying this novel method, we found a significant reduction in the NAD+/NADH ratio in chronically ill SZ patients (38%, $p < 0.001$) compared to a matched healthy control group. This finding was driven by a 53% NADH elevation with no significant change in NAD+. To investigate whether this significant abnormality is found in other phases of the illness, we also collected data from first-episode SZ patients ($N = 13$) and age-matched (i.e., young) controls ($N = 20$) as well as first-episode BD patients ($N = 18$). Analysis revealed first-episode SZ patients had a highly significant reduction (48%) in NAD+/NADH ratio

compared with healthy controls ($p < 0.001$) as well as a more modest 23% reduction compared with first-episode BD patients ($p = 0.032$). Again this pattern was driven by the elevated NADH without abnormalities in NAD+. The first-episode SZ group also had 15% lower NAD+/NADH ratio compared with chronic SZ, adjusting for age effects. We found no correlations between NAD+/NADH ratio and demographic (other than age) and clinical variables, which included CPZ equivalents (measure of antipsychotic dose) and tobacco smoking.
Conclusions: Using a novel and noninvasive MR-based *in vivo* NAD assay, we have provided direct evidence for redox imbalance in the brain in all phases of SZ, potentially reflecting oxidative stress. Our work provides new insights into the pathophysiology of SZ, as well as a potential biomarker for tracking the impact of treatment interventions.
Disclosure: Nothing to Disclose.

4.3 Oxidative Stress as one Core Mechanism in Schizophrenia Pathophysiology and Impact of Antioxidant N-Acetyl-Cysteine in a Clinical Trial With Early Psychosis Patients

Kim Do

Lausanne University Hospital, Prilly-Lausanne, Switzerland

Background: A large body of evidence suggests that oxidative stress, tightly coupled with neuroinflammation and NMDA hypofunction, is involved in schizophrenia (SZ) pathophysiology, particularly affecting structural and functional integrity of parvalbumin interneurons (PVI) which are critical for cognitive functions (Hardingham and Do, 2016). Various SZ animal models, ranging from genetic manipulations to environmental insults, present an increase in oxidative stress associated with an impairment of PVI and their perineuronal net in the medial prefrontal cortex. In some models, the antioxidant N-acetyl-cysteine (NAC) prevents the cellular as well as physiological and behavioral alterations (Cabungcal et al, 2014). These preclinical results suggest a convergence of various genetic and environmental risk factors on oxidative stress induced PVI impairment as one core mechanism (Cabungcal &al, SFN 2016). In chronic SZ patients, add-on clinical trials with NAC improved negative symptoms (Berk et al, 2008; Farokhnia et al, 2013), mismatch negativity (Lavoie et al, 2008) et local synchronization (Carmeli et al, 2012). We present here a NAC clinical trial with early psychosis patients.
Methods: Early psychosis patients (EP, SZ spectrum disorders, with less than 5 years of illness, $N = 63$; NAC = 32, placebo = 31) was supplemented with NAC (2.7g/day) over 6 months in a double-blind randomized placebo-controlled trial (RTC). Outcome measures: primary: PANSS negative; secondary: PANSS positive, and neurocognition (MATRICS Consensus Cognitive Battery excluding MSCEIT; $n = 36$); quantification of brain glutathione levels (GSHmPFC) by 1H-magnetic-resonance-spectroscopy and of blood cells glutathione (GSHBC) and glutathione peroxidase activity (GPxBC) at the beginning and end of treatment.
Results: No difference between NAC and placebo was observed on global changes in PANSS negative and positive. NAC led to increases of GSHmPFC (+23%, $p = 0.005$) and GSHBC (+19%, $p = 0.05$). In patients with high-baseline GPxBC activity levels (>22.3U/gHb), subgroup explorations revealed an improvement of PANSS positive compared to the low-baseline GPx group ($p = 0.02$). The change in GPxBC activity correlated negatively with the change of PANSS positive, showing that positive symptoms improved in parallel with an amelioration of the redox status. There was no difference in an overall cognition score, but a significant interaction showing improvement in Processing Speed (NAC > Placebo; $F(1, 30) = 5.849$, $p = .022$), with the interaction favoring NAC on 2 of 3 PS tasks (Trail Making A, $F(1, 30) = 4.279$, $p = .048$ and Verbal Fluency, $F(1, 30) = 5.749$, $p = .023$).
Conclusions: This is the first RCT assessing the impact of NAC treatment in a sample of EP and the potential predictive role of peripheral biomarkers of redox dysregulation. The hypothesis that NAC would be more beneficial on negative symptoms in an earlier phase of illness was not confirmed in this rather small sample. The increase in cerebral GSH levels by NAC demonstrates its capability to cross the blood-brain barrier. Addition of NAC significantly improves Processing Speed showing for the first time its cognitive enhancer ability. Peripheral redox status associated with brain GSH levels allows identifying a subgroup of patients who improve their positive symptoms. Future biomarker guided, antioxidant interventions in larger EP samples are needed to replicate these findings.
Disclosure: Nothing to Disclose.

4.4 Redox Regulation of Cortical Plasticity

Takao Hensch

Harvard University, Cambridge, Massachusetts, United States

Background: Oxidative stress and the specific impairment of perisomatic gamma-aminobutyric acid circuits are hallmarks of the schizophrenic brain and its animal models. Proper maturation of these fast-spiking inhibitory interneurons normally defines critical periods of experience-dependent cortical plasticity.
Methods: Here, we linked these processes by genetically inducing a redox dysregulation restricted to such parvalbumin-positive cells and examined the impact on critical period plasticity using the visual system as a model. Conversely, we measured oxidative stress markers across a panel of mouse models in which the window of cortical plasticity was extended or delayed.
Results: Oxidative stress was accompanied by a significant loss of perineuronal nets, which normally enwrap mature fast-spiking cells to limit adult plasticity. Accordingly, the neocortex remained plastic even beyond the peak of its natural critical period. These effects were not seen when redox dysregulation was targeted in excitatory principal cells. Conversely, in mouse models of extended critical period plasticity, signatures of redox imbalance were observed.
Conclusions: A parvalbumin cell-specific regulation of redox state thus balances plasticity and stability of cortical

networks. In other words, proper critical period closure is neuroprotective. Mistimed developmental trajectories of brain plasticity may contribute, in part, to the pathophysiology of mental illness. Such prolonged developmental plasticity may, in turn, offer a therapeutic opportunity for cognitive interventions targeting brain plasticity in schizophrenia.
Disclosure: Part 4: Pfizer, coi.

Panel
5. Novel Antidepressant Targets

5.1 Molecular Dissection of Cholinergic Contributions to Behaviors Related to Anxiety and Depression

Marina Picciotto

Yale University, New Haven, Connecticut, United States

Background: Progress in developing new medications for depression may be facilitated by understanding the interaction between neurotransmitter systems involved in behaviors related to depression. Imaging studies suggest that nicotinic acetylcholine receptor (nAChR) occupancy by acetylcholine (ACh) is increased in the brains of human subjects during a depressive episode. Conversely, blockade of nicotinic or muscarinic ACh receptors can have antidepressant effects in human subjects and in preclinical models. Stress can increase ACh levels in specific brain areas and alter acetylcholinesterase (AChE) activity. Further, increasing ACh signaling systemically or locally in the hippocampus can induce behaviors related to anxiety and depression. These studies suggest that cholinergic innervation of the hippocampus is involved in behaviors related to anxiety and depression, but the interaction between cholinergic and monoaminergic pathways innervating the hippocampus remains understudied.
Methods: We first investigated the interaction between cholinergic and serotonergic signaling in behavioral models of antidepressant efficacy pharmacologically. We then used AAV-shRNAs to knock down 5-HT1A receptors in either the dorsal raphe (presynaptic autoreceptors) or the hippocampus (a brain area with high expression of 5-HT1A heteroreceptors, also sensitive to cholinergic effects on affective behaviors) or used shRNA's to knock down the α7 nAChR subunit in the hippocampus and determined the effect of molecular manipulations on pharmacological responses to cholinergic compounds in models of antidepressant efficacy. Finally, we silenced or activated cholinergic neurons innervating the hippocampus by infusing AAV-floxed-Gi-DREADD or AAV-floxed-Gq-DREADDs locally into the medial septum or hippocampus of Chat-CRE mice followed by peripheral administration of CNO to modulate the activity of cholinergic inputs or intrinsic hippocampal cholinergic interneurons, respectively and evaluated the effects on depression-like behaviors and response to social stress.
Results: The SSRI fluoxetine and the 5-HT1A agonist 8-OH-DPAT both had synergistic effects with the nicotinic partial agonist cytisine in a mouse model of antidepressant efficacy, whereas serotonin depletion blocked the effects of cytisine. Knockdown of 5-HT1A receptors in hippocampus, but not dorsal raphe, significantly decreased the antidepressant-like effect of cytisine. With respect to the nAChRs that might mediate cholinergic effects in the hippocampus on stress-related behaviors, local knock down of the α7 nAChR subunit prevented the increase in stress-related behaviors observed following systemic administration of the AChE blocker physostigmine but had no effect in the absence of pharmacological challenge. While there was little effect of stimulating or inhibiting the medial septum, which provides about 70% of the cholinergic input to the hippocampus, stimulating the sparse population of ChAT-Cre-positive cells in the hippocampus in mice with AAV-floxed-Gq-DREADD using CNO increased anxiety-like behaviors, immobility in tests of antidepressant efficacy and decreased interaction in the social defeat paradigm.
Conclusions: These results suggest that serotonin signaling through postsynaptic 5-HT1A receptors and ACh signaling through α7 nAChRs in hippocampus are critical for the antidepressant-like effects of cholinergic agents, and begins to elucidate the molecular mechanisms underlying interactions between the serotonergic and cholinergic systems related to mood disorders. Further, activating the ChAT-positive neurons in the hippocampus, but not the inputs from the medial septum/diagonal bands of Broca, can induce behaviors related to anxiety and depression in the mouse. These data identify ACh-5HT interactions in hippocampus as a target for further investigation.
Disclosure: Nothing to Disclose.

5.2 RGS4 Modulates Responses to Chronic Stress and the Actions of Fast Acting Antidepressants

Venetia Zachariou

The Icahn School of Medicine at Mount Sinai, New York, New York, United States

Background: RGS4 is a signal transduction protein which controls the function of monoamine, opiate, matabotropic glutamate, muscarinic, and other G protein-coupled receptors via interactions with Galpha subunits. RGS4 is expressed in several brain regions involved in mood, movement, cognition, and addiction, and is regulated by psychotropic drugs, stress, and corticosteroids. Chronic stress promotes long term changes in the expression of RGS4 in the medial prefrontal cortex, a brain region expressing high amounts of RGS4.
Methods: To study the brain region-specific role of RGS4 in behavioral responses to stress, we have generated mouse models for conditional deletion of RGS4 in specific netowrks or specific brain regions. We have crossed floxed RGS4 mice with Drd1-Cre and Drd2-Cre BAC lines, whereas mPFC specific knockdown of RGS4 is achieved using stereotaxic infection of this area with adenoassociated viral vectors expressing Cre recombinase (AAV-Cre).
The sudy also applies next generation Sequencing (Illumina), qPCR, western blotting, immunoprecipitation analysis, as well as mouse brain imaging (fMRI).
Results: We hypothesized that RGS4 interactions with serotonin receptor 5HT1A and metabotropic glutamate receptors (mGluR) in the mPFC modulate responses to

chronic stress and affect vulnerability to depression-like states. Next generation RNA Sequencing revealed that RGS4 is significantly downregulated in the mouse mPFC following one month of chronic unpredictable stress. Consistent with this finding, chronic stress promotes significant decreases in the mRNA and protein levels of RGS4 in the mPFC of male and female mice. Conditional deletion of RGS4 in the mPFC increased susceptibility to chronic unpredictable stress, as mutant mice developed depression-like behaviors after only two weeks of stress. fMRI imaging revealed that global knockout of RGS4 decreased thalamocortical and striatotcortical connectivity, whereas whole cell patch clamp recordings showed that ablation of RGS4 enhanced the excitability of mPFC neurons. Chronic stress also promoted robust intracellular adaptations in RGS4 knockout brain, including changes in the levels of several components of the mTOR signaling pathway, as well as changes in the levels of mGluR5/Galpha subunits in the mPFC. In addition, prevention of RGS4 action in the mPFC enhances the efficacy of ketamine in models of acute and chronic stress. Next generation RNA Sequencing was applied to identify the genes and pathways affected by RGS4 inactivation. Our data suggest that prevention of RGS4 action prominently affects the Akt/mTOR pathway, and the expression of several monoamine receptors, and this effect is reversed by ketamine treatment.
Conclusions: Our findings point to a critical role of RGS4 in depression vulnerability, and provide new information on intracellular adaptations to chronic stress in male and female mice. Furthermore, our findings suggets that interventions in RGS4 activity or in donstream pathways may alleviate depression-like behaviors and enhance the efficacy of fast acting antidepressants.
Disclosure: Nothing to Disclose.

5.3 Epigenetic Regulation of Sex Differences in Stress Susceptibility

Georgia Hodes

Virginia Polytechnic Institute and State University, Blacksburg, Virginia, United States

Background: Men and women manifest different symptoms of depression and under current diagnosis depression is a predominantly female disease. Yet little is known of the mechanisms contributing to these important sex differences and how they may impact potential new therapeutics. Recent research has indicated that an epigenetic mechanism leading to increased DNA methylation, contributes to sexual differentiation of the brain during development (Nugent et al, 2015). Here we examine how DNA methylation contributes to sex specific stress vulnerability in adult animals.
Methods: Mice of both sexes were exposed to variable stress and given a behavioral test battery to examine stress sensitivity. A separate cohort was used to examine transcriptional patterns in the Nucleus Accumbens (NAc) using RNA sequencing. Markers of pre and post synaptic plasticity and spine morphology were also examined using a combination of immunohistochemistry and cell filling. Viral overexpression in combination with transgenic knockout strategies was used to manipulate DNA methyltransferase (DNMT) 3a levels in NAc and examine behavior. Transcriptional profiles were measured following DNMT3a knockout.
Results: Female mice expressed depression-associated behavior across all tests following 6 days of variable stress, whereas males were behaviorally resilient. Increasing the expression of DNMT3a site specifically in NAc shifted both males and females to a stress susceptible state following exposure to a sub-threshold variable stress. Females demonstrated circuit specific pre-synaptic alterations that may contribute to stress susceptibility in the absence of postsynaptic alterations in spine density or phenotype. Excising DNMT3a site specifically from NAc in adulthood made female mice behaviorally resilient to variable stress. Removal of DNMT3a shifted the female transcriptome to a more male like state.
Conclusions: These studies examine an epigenetic mechanism that regulates transcriptional sex differences in reward circuitry mediating behavioral susceptibility and resilience to variable stress. By altering DNA methylation, we sex specifically shift female transcriptional expression and behavior to a resilient state.
Disclosure: Nothing to Disclose.

5.4 Small Non-Coding RNA Targets of Antidepressant Treatment

Juan Pablo Lopez

McGill University, Montreal, Canada

Background: Antidepressants (ADs) are the most common treatment for major depressive disorder (MDD). However, only about 30% of patients experience adequate response after a single AD trial, and this variability remains poorly understood. Genes can be regulated through the activity of several non-coding RNA (ncRNA) transcripts that act as fine-tuners and on-off switches of gene expression patterns. Among ncRNAs, microRNAs (miRNAs) are particularly interesting because they play a key role in the regulation of essential brain processes and seem to mediate psychiatric treatments. Moreover, they are emerging as excellent candidates for biomarkers because of evidence that they circulate in blood and other fluids in vesicles called exosomes. Here we conducted a series of complementary studies investigating miRNA to gain insight into possible mechanisms mediating antidepressant response.
Methods: We used a series of different methods. We first performed small RNA-sequencing in a randomized placebo-controlled trial of duloxetine ($N = 516$) using blood samples collected before and eight weeks after treatment. We then confirmed our results in an independent clinical trial of antidepressant ($N = 122$), and subsequently, we investigated response to treatment in a well-characterized animal model of depression ($N = 26$). Further, we conducted a series of studies investigating postmortem brain samples and neural progenitor cells.
Results: The small RNA-sequencing in the randomized placebo-controlled trial of duloxetine revealed differential expression of miR-146a-5p, miR-146b-5p, miR-425-3p and miR-24-3p according to response to treatment. These results were replicated in an independent clinical trial of depressed

patients, and were further supported by AD treatment in the animal model of depression, and in postmortem human brain samples. Using bioinformatic analyses, we identified common target genes of the differentially expressed miRNAs, and found a significant dysregulation of genes involved in MAPK/Wnt signaling pathways. We confirmed these results and downstream interactions experimentally through *in vitro* experiments using cellular functional assays.
Conclusions: Together, our results postulate miR-146a-5p, miR-146b-5p, miR-425-3p and miR-24-3p as consistent markers of AD response, as well as regulators of the MAPK and Wnt systems. Furthermore, these results provide important steps in the development of early diagnostic tools, preventive strategies, and effective pharmacological targets for MDD treatment.
Disclosure: Nothing to Disclose.

Panel
6. Advancing Human Biomarker Discovery in Severe and Treatment-Resistant Depressive Disorders

6.1 Ketamine and Family History of an Alcohol Use Disorder: Strange Bedfellows in Treatment Resistant Depression

Mark Niciu

National Institute of Mental Health, Bethesda, Maryland, United States

Background: Ketamine has rapid and robust antidepressant efficacy in treatment-resistant major depression (TRD). Due to ketamine's more blunted dissociative, psychotomimetic and acute dysphoric effects in recently-detoxified alcoholics and healthy volunteers with a family history of alcohol dependence (family history positive, FHP) and the association of alcohol dependence with glutamate receptor genes, we have investigated ketamine's antidepressant efficacy in treatment-resistant depressed subjects with a personal or family history of an alcohol use disorder.
Methods: We probed our database of treatment-resistant major depressive disorder and bipolar I/II depressed subjects who received a single subanesthetic dose (0.5mg/kg over 40 minute) ketamine infusion to investigate potential biomarkers of antidepressant efficacy. Subjects may have had a lifetime history of a substance use disorder but not an active substance use disorder (excluding nicotine and/or caffeine) in the three months prior to inpatient admission. Univariate Pearson correlations and multivariate linear regression (to evaluate potential multicollinearity) were used to examine the association between a family or personal history of an alcohol use disorder and ketamine's antidepressant efficacy at 230 minutes ($n = 108$), day one ($n = 82$) and one week ($n = 71$) after infusion. Factorial linear mixed models with restricted maximum likelihood estimation were used to examine ketamine's extended antidepressant response (up to four weeks) in subjects who participated in a riluzole vs. placebo extension trial ($n = 52$). Kaplan-Meier survival analysis was used to assess relapse (defined as two consecutive days of $\leq 25\%$ improvement from baseline) in pre-randomization responders ($\geq 50\%$ improvement from baseline at ≤ 230 minutes' post-infusion).
Results: In a univariate analysis, lifetime personal history of an alcohol use disorder ($n = 12$, 28.6%) was not correlated with antidepressant efficacy at all three points. FHP ($n = 40$, 39.2%) was associated with ketamine's antidepressant response at all time points (230 minutes: $r = -0.21$, $p = .03$; day one: $r = -0.33$, $p = .004$; one week: $r = -0.47$, $p < .001$). In the multivariate linear regression, a correlation was not observed between FHP and ketamine's antidepressant effects at 230 minutes ($\beta = -0.17$, $p = .08$) but persisted at day one ($\beta = -0.27$, $p = .01$) and one week ($\beta = -0.41$, $p < 0.001$), explaining up to 22% of the variance in ketamine's antidepressant efficacy at this final time point. As the strength of this association increased over time, we next investigated the strength and duration of this response in a four-week, double blind, placebo controlled trial of riluzole extension following open-label ketamine. In the placebo arms, FHP subjects ($n = 9$) displayed a greater antidepressant response to ketamine over the next four weeks [$F(1,50) = 9.69$, $p = .003$]. In the time-to-relapse survival analysis, subjects without a family history of an alcohol use disorder in a first-degree relative randomized to placebo ($n = 9$) relapsed more quickly (3.6 days, SE = 1.0) than the FHP responders randomized to placebo ($n = 8$; 17.0 days, SE = 3.9) ($X2 = 7.38$, $p = .007$).
Conclusions: FHP TRD subjects have a more robust and extended antidepressant response to subanesthetic dose ketamine infusion. As family history of an alcohol use disorder status also correlates with alcohol sensitivity and functional neuroimaging findings, our group is currently investigating alcohol-responsive biomarkers in the following protocol: "The Neurophysiological Effects of Intravenous Alcohol as Potential Biomarkers of Ketamine's Antidepressant Effects in Major Depressive Disorder" (ClinicaTrials.gov identifier: 02122562).
Disclosure: Nothing to Disclose.

6.2 The Impact of Ketamine on Global Brain Connectivity in Treatment Resistant Depression

Chadi Abdallah

Yale University School of Medicine, West Haven, Connecticut, United States

Background: Capitalizing on recent advances in resting state functional connectivity magnetic resonance imaging (rs-fcMRI) and the distinctive paradigm of rapid mood normalization following ketamine treatment, the current study investigated large scale intrinsic brain networks in treatment resistant depression (TRD) during a depressive episode and following treatment with ketamine.
Methods: Eighteen medication-free patients with TRD and 25 healthy control subjects (HC) completed baseline rs-fcMRI. MDD patients received a single infusion of ketamine and underwent repeated rs-fcMRI at 24h after treatment. Global brain connectivity with global signal regression (GBCr) values were computed as the average of correlations of each voxel with all other gray matter voxels in the brain. Whole-brain voxel-wise fully data-driven analyses with appropriate correction were conducted.

Results: We found widespread GBCr alterations in MDD, showing reduced GBCr in the prefrontal cortex, and increased GBCr in the posterior cingulate, precuneus, lingual gyrus, and cerebellum. Ketamine significantly increased GBCr in the prefrontal cortex and reduced GBCr in the cerebellum, showing a pattern of connectivity normalization. At baseline, 2174 voxels of altered GBCr were identified, with 310 voxels significantly different relative to controls following treatment (corrected $p < 0.05$). Treatment response was associated with increased GBCr, such that responders to ketamine showed increased GBCr in the right later prefrontal cortex and left anterior insula.
Conclusions: Ketamine treatment appears to normalize functional connectivity alterations identified in TRD and this pattern of normalization is specifically associated with treatment response. In addition, the extent of the functional dysconnectivity identified in TRD and the swift and robust normalization of intrinsic brain connectivity following treatment, suggest that GBCr may serve as a treatment response biomarker for the development of rapid acting antidepressants.
Disclosure: Nothing to Disclose.

6.3 Electrophysiological Mapping of Immediate and Long-Term Antidepressant Effects of Subcallosal Cingulate DBS

Helen Mayberg

Emory University School of Medicine, Atlanta, Georgia, United States

Background: Antidepressant effects of subcallosal cingulate white matter deep brain stimulation (SCC DBS) occur in two stages: a rapid change in interoceptive and exteroceptive awareness with first stimulation at the optimal target and a slower progressive improvement in symptom ratings over weeks to months. Ongoing, longitudinal electrophysiological monitoring with next-generation DBS systems guided by tractographic identification of the optimal stimulation site now allows characterization of this chronology.
Methods: Electrophysiological changes with acute DBS were measured in 14 patients during implantation surgery. Change in SCC local field potentials (LFP) were quantified before and after two minutes of stimulation at contacts later used for chronic DBS (130Hz, 60us, 6mA). Oscillatory power was extracted from both Left and Right LFP channels. In a subset of patients, multiple daily epochs of SCC LFPs were also measured to characterize the evolution of oscillatory changes mediating long-term antidepressant effects over 6 months.
Results: Left > right SCC LFP changes were seen with first exposure to optimally targeted unilateral DBS consistent with lateralized behavioral self-reports. Ipsilateral decreases in theta and alpha power were seen with left DBS; changes following right DBS were not significant. The magnitude of change in left theta power was significantly correlated with weeks to achieve a sustained antidepressant response with bigger decreases predicting faster recovery. Repeat testing of acute stimulation 1 month after surgery and immediately prior to initiation of chronic DBS showed a more variable response, with bilateral stimulation at optimal contacts inducing broad band increases in power (left > right). Chronic stimulation induced more selective band-specific changes over weeks to months with increased theta and decreased beta power correlated with stable improvement in depression severity scores.
Conclusions: Initial stimulation in the SCC area evokes local oscillatory changes that predict the time course of symptom resolution with chronic DBS. The time course of SCC LFP changes is not linear and suggests a process of ongoing network adaptation to chronic stimulation. Combined SCC LFP with high density EEG will allow more nuanced analysis of these postulated network changes over time.
Disclosure: Part 1: St. Jude Medical, Inc., licensing of IP, **Part 2:** St. Jude Medical, Inc, licensing of IP, **Part 4:** Medtronic, DBS device donation, self, St. Jude Medical, Inc, DBS device donation.

6.4 Multimodal Imaging Predictors of ECT Response in Major Depression

Katherine Narr

University of California, Los Angeles, Los Angeles, California, United States

Background: A third of patients with major depressive disorder (MDD) will not respond to two or more standard antidepressant medications following weeks to months of treatment. Electroconvulsive therapy (ECT) is a highly effective, well-tolerated and rapidly acting intervention for severe MDD. As such, ECT provides a powerful model for determining biological indicators predictive of treatment response over shorter time frames. Here we use multimodal neuroimaging data and machine learning algorithms to determine if pretreatment measures of neural structure and function might determine future clinical response to ECT.
Methods: To identify imaging biomarkers predictive of ECT response, we acquired structural, functional (resting state) and diffusion MRI data in patients with MDD ($N = 27$, 16 females, age = 41.96 ($\pm$13.54)) prior to the start of an index treatment series of ECT. Mood ratings, collected using the Montgomery–Åsberg Depression Rating Scale (MADRS), were obtained before treatment and after patients completed their ECT index (mean: 12 ECT sessions). Informed by recent ECT imaging findings focused on specific imaging modalities [1-4], we extracted measures of brain structure and structural and functional connectivity within and between the dorsal (dACC) and subgenual anterior cingulate cortex (sgACC) and hippocampus. Radial kernel support vector machines (tuned with grid search) with 10-fold cross validation were used to establish if multimodal MRI measures examined for each region separately or combined better predict change in mood ratings. Treatment response was defined as 50% or greater improvement in MADRS score over the course of ECT. Patients were defined as remitters if MADRS scores were < 10 at the end of the ECT index.
Results: Eighteen participants were identified as responders, 13 as remitters and 9 as nonresponders. Using combined structural and functional features of the hippocampus and

dACC and sgACC while excluding non-remitting responders, our classifier achieved an accuracy of 72% and an area under the curve (AUC) of 76% [95% CI: 54-97%]. Hippocampal measures alone yielded a significant AUC of 75% [95% CI: 52-97%]. dACC features afforded a non-significant AUC of 54% [95% CI: 27-81%] while sgACC features were significantly predictive (AUC = 75% [95% CI: 53-97%]). Combining hippocampal and sgACC features provided the best performance (AUC = 80% [95% CI: 61-99%]; accuracy = 77%).

Conclusions: Biomarkers that predict an individual's therapeutic response may allow for more personalized and accelerated treatment strategies. Using a multivariate machine learning approach, our results show that structural and functional features of the hippocampus and sgACC at baseline predispose patients to successful ECT outcomes. These findings complement independent reports showing structural [2] and functional connectivity markers [3] of the sgACC and hippocampal structure [1] correlate with or predict treatment response. Multimodal imaging probes could play an important role in predicting clinical response to ECT and potentially other treatments for major depression.

Disclosure: Nothing to Disclose.

Panel
7. Impact of Sex Differences on Developmental Mechanisms in Mood and Anxiety

7.1 Sex Differences in Estrogen Receptor Expressing Neural Circuits in the Mouse Brain: Implications for Programming Effects on Stress Responses and Stress Related Behaviors

Robert Handa

Colorado State University, Fort Collins, Colorado, United States

Background: Estrogen receptors (ERs) regulate many brain functions in adulthood, including (but not limited to) reproductive behaviors, stress-related behaviors and neuroendocrine responses, and cognition. Sex differences exist in each of these functions and are in part, a consequence of the organizing actions of steroid hormones acting during a critical period in neonatal life, coupled with differential sensitivity to estradiol in adulthood. ERs (alpha and beta) change across development in the rodent brain, in a region specific pattern, and these changes may provide a cellular basis for the concept of 'critical periods' for the actions of steroid hormones on sexual differentiation of brain and behavior. In these studies, we have carefully examined the expression and distribution of estrogen receptors in multiple brain regions throughout perinatal development of the mouse.

Methods: These studies utilized an Estrogen Receptor beta-eGFP transgenic mouse where a BAC transgene containing the ERbeta promoter drives the expression of eGFP. We have validated this mouse model and shown almost complete overlap between eGFP expression and ERbeta-immunoreactivity (-ir). ERalpha-ir distribution can also be visualized using standard dual label IHC procedures. ERbeta-EGFP animals of both sexes were euthanized by intracardiac perfusion across the perinatal period and brains processed for dual- and triple-label immunofluorescence. Tissue was visualized by confocal microscopy and dual and triple labeled cells were identified and counted in 3D using Imaris (v8.2) imaging software.

Results: Examination of ERbeta-EGFP fluorescence demonstrated several brain areas that showed sexually dimorphic development of ERbeta. These include the prefrontal cortex (FC), the anteroventral periventricular n., the bed n. of the stria terminalis (BST) and the dorsal raphe (DR). In the frontal cortex, both males and females expressed ERbeta early in postnatal life, but the distribution differs from adult patterns. The adult pattern of ERbeta-eGFP expression develops later in females than in males. These neurons do not express doublecortin, a marker of immature neurons, thus indicating that they are mature. In the BST, there are greater numbers of ERbeta neurons in the lateral BST of males than females. Many of these neurons express corticotropin releasing factor (CRF) and several other neuropeptides. The CRF neurons of the BnST reportedly project to the PVN (which is not sexually dimorphic). In the dorsal raphe, ERbeta-EGFP is detected in numerous tryptophan hydroxylase (TPH)-ir neurons throughout development. Neonatal male mice have more TPH neurons than do females. The projections of these serotonergic neurons are currently under examination with the possibility that ERbeta may program serotoninergic output from the DR to forebrain regions such as the prefrontal cortex.

Conclusions: Our studies have identified a potential estrogen receptor beta-ergic neural circuit (DR – FC – BST) that is sexually dimorphic in ontogeny. Moreover, there are sex differences in responses to estradiol in adulthood where estrogen receptor beta mediated effects are anxiolytic and anti-depressive and inhibitory to the neuroendocrine responses to stress. These sex differences in the ontogeny of stress-related circuitry may underlie the early developmental programming by hormones of sex differences in stress-related behaviors in adulthood.

Disclosure: Nothing to Disclose.

7.2 Programming Prenatal Female Resilience: Epigenetic Mechanisms of the X

Tracy Bale

University of Pennsylvania, Philadelphia, Pennsylvania, United States

Background: There is a clear sex bias in neurodevelopmental disorders where a greater prevalence exists for boys, especially related to any prenatal insults. Prenatal stress is a risk factor for neurodevelopmental disorders, including early onset schizophrenia and autism, which display marked male-biases in onset, presentation, and treatment. In our mouse model of early prenatal stress (EPS), stress exposure during the first week of gestation imparts long-term neurodevelopmental programming deficits in male offspring resulting in a hypersensitivity to stress, cognitive impairments, and metabolic programming. As the placenta, a fetally-derived organ reflecting fetal sex chromosome complement, is a key

modulator between the maternal and fetal compartment, functional changes occurring here in response to maternal insults can have significant and sex-specific effects on neurodevelopment. We previously identified the X-linked gene and chromatin regulator, O-linked-N-acetylglucosamine (OGT), as a placental biomarker of prenatal stress. OGT escapes X-inactivation in the placenta, providing females with an increased capacity to regulate transcription at the level of the trophoblast cell. Placental-specific reduction of OGT recapitulates the neurodevelopmental and metabolic impairments associated with EPS exposure.

Methods: As OGT is known to modify several epigenetic regulators associated with histone methylation, specifically the transcriptional repressive mark, H3K27me3, we hypothesized that sex differences in levels of OGT mediate sex differences in histone methylation and promote robust sex-specific programs of gene expression. Females have significantly more H3K27me3 in placental trophoblasts than males, thus we hypothesize that female-biased epigenetic repression protects females from prenatal insults such as EPS. EPS or deletion of OGT only in trophoblast cells was also compared between male and female placental tissue for changes in H3K27me3 and transcriptional responses to stress using RiboTag transgenic mice. We have used an array of methods including ChIP-Seq, protein biochemistry, and RNA-Seq in mouse placentas with trophoblast-specific OGT reduction and mapped the corresponding changes in the embryonic brain. Further, we specifically reduced H3K27me3 levels in female placentas to that of male levels using trophoblast-specific genetic manipulations of the H3K27me3 methyltransferase, EZH2, and then compared sex-specific vulnerability to EPS.

Results: We found that OGT profoundly regulates genome-wide sex differences in levels of H3K27me3 and gene expression in placental trophoblast cells. EPS significantly increased transcriptional changes in male trophoblast cells, but not in females. Decreased placental H3K27me3 or EZH2 increased female vulnerability to the effects of EPS including changes in transcriptional repression in the placenta, and in programming of offspring behaviors including increased HPA axis sensitivity, metabolic deficiencies, and cognitive impairments in adulthood.

Conclusions: These studies reveal profound sex-differences in mechanisms of transcriptional regulation in placental trophoblast cells that directly contribute to increased risk for the developing male brain to maternal insults across gestation. Such outcomes may point to useful biomarkers in predicting neurodevelopmental disease risk or resilience.

Disclosure: Nothing to Disclose.

7.3 Fetal Programming of Neural and Physiologic Responses to Stress: Shared Impact of Mood and Sex Across Diagnoses

Jill Goldstein

Harvard Medical School, Boston, Massachusetts, United States

Background: Prenatal stress models posit excess maternal glucocorticoids and immune activation alter fetal stress response circuitry conferring long-term psychiatric vulnerability. Circuitry includes, among others, highly sexually dimorphic hypothalamus [HYPO], amygdala [AMYG], hippocampus [HIPP], and prefrontal cortices [mPFC, OFC]. In clinical and preclinical studies, fetal alterations in these regions have also been associated with adult mood and steroid hormone dysregulation, deficits that are sex-dependent. Here, we show the impact of prenatal immune programming has sex-dependent effects on adult hypothalamic and hippocampal activity deficits and connectivity in response to a stress challenge, that is associated with depressed mood and shared across psychiatric disorders.

Methods: 99 adults participated (48 women/51 men; cases with major depressive disorder, psychoses, and healthy controls), offspring who were part of a prenatal cohort. Their mothers were followed through pregnancy and sera stored at NIH for >50 years, from which prenatal maternal cytokines (beginning of 3rd trimester) were assayed. Cytokines (TNF-α, IL-1β, IL-6, IL-10) were assessed using a multiplexed, bead-based immunoassay on a Luminex 3D detection platform, and run in duplicate. Adult offspring (~45 years old) were assessed clinically and underwent fMRI using a mild visual stress task of negative, neutral and fixation images, along with hormonal and heart rate responses. fMRI data were acquired on a Siemens Tim Trio 3T MRI scanner with a 12-channel head coil using spin echo T2*-weighted sequences, and analyzed using SPM8 (blood oxygen-level dependent (BOLD) signals) and task-related connectivity assessed using generalized psychophysiological interaction. General linear models were used to relate prenatal exposures to BOLD activity and connectivities, controlled for potential confounders.

Results: Severity of dysphoric mood was associated with HYPO and AMYG hyperactivity (FWE-corrected, $p=.006$) and significantly associated with lower connectivity between HYPO-OFC, AMYG-OFC, and AMYG-HIPP ($p=.0001$) among women, but not men. Low HYPO-HIPP connectivity was associated with hypercortisolemia only in women with more severe depressed mood. Increased prenatal exposure to TNF-α was significantly associated with lower BOLD HYPO activity ($p=0.03$). Increased TNF-α:IL-10 significantly predicted lower left-HIPP BOLD activity in women ($p=0.01$) but higher in men ($p=0.04$) [interaction $F=10.97$, $p=0.001$], and lower connectivity between HYPO-HIPP in women [interaction with sex ($F=18.91$, $p<0.0001$), independent of diagnosis. Further, increased IL-6 exposure significantly affected right HIPP in women only ($p=0.02$) [interaction ($F=5.93$, $p=0.02$)] independent of diagnosis.

Conclusions: Sex-dependent deficits in stress circuitry were shared across psychiatric disorders and dependent on dysphoric mood. Women with worse dysphoric mood were less able to recruit cortical and hippocampal control over arousal in response to stress compared with men. Maternal prenatal immune dysregulation was significantly associated with sex-dependent neural-hormone deficits in offspring 45 years later. Uniquely, at a human population-level, findings demonstrated prenatal immune programming of stress circuitry that was sex-dependent and independent of diagnosis.

Disclosure: Nothing to Disclose.

7.4 Female-Specific Intergenerational Biomarkers of Fear and PTSD Symptoms, From Neurophysiology to Neuroimaging

Tanja Jovanovic

Emory University School of Medicine, Atlanta, Georgia, United States

Background: Trauma-related disorders, such as posttraumatic stress disorder (PTSD) and depression are psychiatric illnesses whose prevalence in women is more than twice the rate as men. Women are also more likely to experience chronic illness that persists for more than a year and carry a greater burden of this illness on the healthcare infrastructure. The biological mechanisms underlying these sex differences are still largely unknown. In our earlier studies we found a sex difference in dark-enhanced startle, a biomarker of anxiety. Specifically, in women, PTSD was associated with higher dark-enhanced startle compared to traumatized women without PTSD, but we did not observe the same pattern in men. (Kamkwalala et al, 2012). Our work with a single nucleotide polymorphism (SNP) in the ADCYAP1R1 gene, rs2267735, which is located in an estrogen response element, showed that the risk genotype (CC) was associated with increased dark-enhanced startle and fear-potentiated startle generalization between danger and safety signals in women but not men (Ressler et al, 2011). These data suggest female-specific genetic risk for biomarkers of fear and anxiety in PTSD. Our research examining fear-potentiated startle in adult women has shown an interaction between estradiol and PTSD diagnosis, such that those with low estradiol and PTSD have deficits in fear extinction (Glover et al, 2012). In this study, higher levels of estrogen normalized extinction. These findings are particularly important given that anxiety disorders tend to emerge around puberty, when sex organs begin to release hormones that have activational effects on brain activity.
Methods: In a sample of traumatized women recruited from a public hospital in inner-city Atlanta ($n=49$) we used functional magnetic resonance imaging (fMRI) to examine amygdala activation to fearful faces. In addition, we examined fear-potentiated and dark-enhanced startle in pre-pubertal children (ages 8-12, $n=50$). Startle was assessed using electromyography of the eyeblink muscle, either during presentation of conditioned stimuli paired with an aversive airblast, or during a dark phase. Saliva samples were collected from adults and children to extract DNA and assay for the ADCYAP1R1 rs2267735 SNP.
Results: We found that the same SNP rs2267735 in the ADCYAP1R1 genotype that was associated with increased fear-potentiated startle was also associated with greater amygdala reactivity (pcorr <0.05). When each genotype group was examined separately, the CC group showed bilateral amygdala activation to fearful compared to neutral faces (pcorr $<$ 0.05), which provides the neurobiological basis for the startle findings. In the pediatric sample there was a main effect of sex, with boys showing better discrimination than girls ($p<0.05$). Further, we found that the ADCYAP1R1 rs2267735 CC genotype increased dark-enhanced startle in both girls and boys, $F(1,49)=4.52$, $p<0.05$. However, ADCYAP1R1 genotype interacted with sex on fear-potentiated startle, $F(1,49)=5.47, p<0.05$. The risk genotype increased discrimination in male children, but did not affect fear-potentiated startle in girls.
Conclusions: Taken together, these studies suggest development by gene interactions on female-specific risk for trauma-related disorders that impact neurobiological underpinning of fear behavior. In addition, genetic risk may be dependent on activity of cycling hormones beginning at puberty.
Disclosure: Nothing to Disclose.

Panel
8. The Consequences of Adolescent Marijuana Use

8.1 Marijuana Legalization in Colorado: Impact on Adolescents

Christian Hopfer

University of Colorado School of Medicine, Aurora, Colorado, United States

Background: National policy regarding marijuana access is changing dramatically. Dr. Hopfer will review the evolving national marijuana policy landscape as well as discuss the Colorado experience in terms of medical and, more recently, recreational legalization. He will briefly discuss the history of Dutch Cannabis de-penalization and compare and contrast this with the Colorado experience of full legalization in terms of its impact on adolescents.
Methods: Recent reports from the Rocky Mountain High Intensity Drug Trafficking Area, National Household Survey on Drug Use, and Colorado Department of Public Health and Environment will be reviewed as well as clinical data from youth in treatment for Substance Abuse Disorders. Clinical observations regarding challenges posed by low marijuana risk perceptions in terms of treatment and prevention will be discussed.
Results: Marijuana legalization has resulted in a dramatic growth of the marijuana legalization, with industry sales increasing from $700,000 to $1,000,0000 between 2014 to 2105. There are more marijuana dispenseries in Denver than pharmacies, representing the substantial shift in availability of marijuana and increased public acceptance. Multiple indicators suggest increased adolescent marijuana use as well as adverse consequences.
Conclusions: As marijuana increasingly moves to a legal status, physician and public health approaches will be needed mitigate harms from use, particularly for adolescents. Limitations in the current research knowledge about marijuana's effects impact prevention and treatment as well as gaps in the current data collection systems.
Disclosure: Nothing to Disclose.

8.2 THC Exposure Differently Affects Histone Modifications and Gene Expression in the Adolescent and Adult Rat Brain

Tiziana Rubino

University of Insubria, Busto Arsizio, Italy

Background: The regular use of Cannabis during adolescence seems to be associated with an increased likelihood of

deleterious consequences, as reported by several epidemiological studies. However, the molecular underpinnings that make the adolescent brain so vulnerable are not yet fully understood. The emerging role of epigenetics in the development of psychiatric diseases led us to hypothesize that epigenetic modifications could play a part.
Methods: We investigated whether epigenetic mechanisms may contribute to the long-lasting adverse effects triggered by THC exposure in an experimental model represented by adolescent animals. We first performed a time-course study of different histone H3 modifications occurring in the prefrontal cortex (PFC) of female rats exposed to THC during adolescence, and their possible impact on the expression of a set of genes closely related to synaptic plasticity mechanisms. Next, to verify the vulnerability of the adolescent brain, we performed the same analysis after adult THC exposure. Finally, through the administration of a specific epigenetic drug, we investigated the role played by histone modifications in the complex phenotype present in adult animals after adolescent THC exposure.
Results: Adolescent THC exposure induced alterations in selective histone modifications (mainly H3K9me3), in the female PFC, a brain area involved in the modulation of cognitive and emotional behavior. These changes impacted the expression of a set of genes closely related to synaptic plasticity mechanisms. Interestingly, the expression of some of these genes was still impaired at adulthood. The magnitude of the alterations induced by THC exposure was age-dependent: indeed, changes in both histone modifications and gene expression were more widespread and intense after the adolescent treatment in comparison with the adult one. Moreover, adolescent THC exposure significantly increased protein expression of the histone methyl transferase Suv39H1 that could account for the enhanced H3K9me3. This modification played a relevant role in the development of the depressive/psychotic-like phenotype induced by adolescent THC exposure. Indeed, pharmacological blockade of Suv39H1 during adolescent THC treatment was able to prevent THC-induced cognitive deficits at adulthood.
Conclusions: As a whole these data suggest that in the adolescent prefrontal cortex, THC acts through Suv39H1 to affect histone modifications and gene expression. This pathway appears to be relevant for the development of cognitive deficits present at adulthood.
Disclosure: Nothing to Disclose.

8.3 Neuropsychological Impairment Among Adolescent-Onset Cannabis Users

Madeline Meier

Arizona State University, Gilbert, Arizona, United States

Background: Heavy cannabis use is associated with neuropsychological impairment, and some evidence suggests that adolescents may be particularly vulnerable to cannabis-related neuropsychological impairment. In the present study, we investigated the association between adolescent-onset cannabis use and neuropsychological functioning in two longitudinal samples.
Methods: Participants were members of the Dunedin Multidisciplinary Health and Development Study and the Environmental Risk (E-Risk) longitudinal study. The Dunedin Study is a study of a population-representative cohort of individuals born in Dunedin, New Zealand in 1972-73 and followed prospectively to age 38, with 95% retention. Study members underwent neuropsychological testing at age 13 years before the onset of cannabis use and again at age 38 years, after some had developed a persistent pattern of cannabis use. Standard neuropsychological test batteries were administered. Cannabis use was ascertained in interviews at ages 18, 21, 26, 32 and 38 years. The E-risk study is a study of a nationally-representative birth cohort of 2,232 British twins followed from age 5 to age 18 (55% monozygotic and 45% dizygotic same-sex twin pairs). Cohort children underwent neuropsychological testing at age 12 before the onset of cannabis use and again at age 18 years. Cannabis use was ascertained in interviews at age 18.
Results: In the Dunedin Study, persistent cannabis use from ages 18 to 38 was associated with IQ decline even after controlling for years of education and multiple other alternative explanations (e.g., alcohol or drug dependence). IQ decline was concentrated among adolescent-onset cannabis users, with more persistent use associated with greater decline. For example, individuals who began using cannabis in adolescence and continued to use it for years thereafter lost an average of 8 IQ points. Further, cessation of cannabis use did not fully restore IQ among adolescent-onset cannabis users. In the E-risk study, cannabis use at age 18 was not associated with IQ decline from age 12 to 18 after accounting for familial factors.
Conclusions: Findings suggest that long-term cannabis use that starts in adolescence is associated with IQ decline, whereas short-term, low-level cannabis use in adolescence is not associated with IQ decline.
Disclosure: Nothing to Disclose.

8.4 Reduced Brain Cannabinoid Receptor Availability in Schizophrenia

Deepak D'Souza

Yale University School of Medicine, West Haven, Connecticut, United States

Background: Converging lines of evidence suggest a cannabinoid hypothesis of schizophrenia that includes exogenous and endogenous elements. The exogenous cannabinoid hypothesis suggests that exposure to exogenous agonists at the cannabinoid receptor (CB1R) e.g., cannabis and synthetic cannabinoids, especially during early adolescence, is associated with psychosis outcomes. Furthermore, the risk for psychosis is higher when exposure is during adolescence and/or exposure is genetically vulnerable individuals. The endogenous cannabinoid hypothesis, suggests that alterations in the endocannabinoid (eCB) system may contribute to the pathophysiology of schizophrenia. Support for the endogenous hypothesis includes the observation of elevated blood/CSF eCB levels and alterations in CB1R binding (postmortem) in schizophrenia. However, there are limited *in vivo* measures of the eCB system in SCZ.

Methods: 25 male SCZ subjects (SCZs) including 18 antipsychotic treated [SCZ-MED] and 7 antipsychotic free [SCZ-UNMED]) were compared to 18 age- matched male healthy control subjects (HCs). Subjects underwent one positron emission tomography (PET) scan each with the cannabinoid receptor (CB1R) selective radiotracer [11C] OMAR on the High Resolution Research Tomography (HRRT) scanner. Regional volume of distribution (VT) values were determined using kinetic modeling of positron emission tomography data as a measure of CB1R availability. Group differences in mean composite [11C]OMAR VT values were compared between SCZs and HCs. Exploratory comparisons of CB1R availability within 15 brain regions were also conducted. All analyses were covaried for age and body mass index. More recently, we have studied females with SCZ and individuals with a family history of SCZ.
Results: SCZs showed significantly ($p = 0.02$) lower composite [11C]OMAR VT relative to HCs (~12% difference, effect size d = 0.73). [11C]OMAR VT was significantly (all ps < 0.05) lower in SCZs in the amygdala, caudate, posterior cingulate, hippocampus, hypothalamus and insula. Composite [11C] OMAR VT was greater in HCs > SCZ-MED > SCZ-UNMED. Furthermore, composite [11C]OMAR VT was greater in HCs > SCZ smokers ($n = 11$) > SCZ non-smokers ($n = 14$).
Conclusions: CB1R availability is lower in SCZ compared to HCs. Furthermore, antipsychotics and tobacco use may increase CB1R availability in this population. The findings of the study provide further evidence supporting the hypothesis that alterations in the eCB system might contribute to the pathophysiology of SCZ. These findings raise the intriguing possibility that endocannabinoid dysfunction in schizophrenia if present in adolescence may explain why cannabis use in adolescence makes individuals particularly vulnerable to later developing schizophrenia.
Disclosure: Nothing to Disclose.

Panel
9. Emerging Role of Epigenetic Programming in Psychiatric Disorders

9.1 Adolescent Alcohol Exposure and Persistent Histone Methylation Marks: A Vulnerability Factor for Adult Psychopathology

Subhash Pandey

University of Illinois at Chicago, Chicago, Illinois, United States

Background: Adolescence represents an important stage of brain development and epigenetic mechanisms, such as histone and DNA modifications, are known to orchestrate brain maturation. Binge drinking during adolescence appears to be an important risk factor for the development of alcoholism later in life. The persistent changes in histone acetylation in the amygdala due to adolescent alcohol exposure have been shown to play a role in the development of adult psychopathology. Since histone acetylation and methylation dynamically interact, we investigated the role of histone methylation mechanisms in adolescent intermittent ethanol (AIE) exposure-induced anxiety behaviors in adulthood.
Methods: Adolescent male rats were exposed to 2g/kg ethanol (2 days on/off) or intermittent n-saline (AIS) during post-natal days (PND) 28-41 and allowed to grow to adulthood (PND 92-102) to investigate the lasting effects of AIE on histone methylation mechanisms in the amygdala. We measured anxiety-like behaviors using the elevated plus maze test with or without acute ethanol challenge in adulthood after AIE. Quantitative real-time PCR, *in situ* PCR, and chromatin immunoprecipitation (ChIP) assays were used to measure the mRNA levels of various genes and the histone methylation status of brain-derived neurotrophic factor (BDNF) and activity-regulated cytoskeleton associated protein (Arc) genes in the amygdala.
Results: The expression profiling of various lysine demethylases and histone methyltranferases showed that AIE exposure decreased mRNA levels of Lsd1+8a (a neuron-specific splice variant) and Kdm6b in specific amygdaloid structures compared to AIS rats in adulthood. AIE also increased H3K9 dimethylation (H3K9me2) levels in the Bdnf exon IV promoter as well as H3K27me3 levels in the promoter and gene body of Arc in the amygdala. We also found that AIE produced a persistent decrease in CREB binding protein (CBP) levels globally in the amygdaloid brain regions but also at the same sites of the promoter and gene body of Arc where HK27me3 levels were increased in the amygdala. These epigenetic changes and AIE-induced anxiety-like behaviors were normalized by acute challenge with ethanol in adulthood.
Conclusions: These novel results indicate that adolescent alcohol exposure causes enduring effects on specific histone demethylating enzymes and produced increases in repressive histone marks (H3K9me2 & H3K27me3) in the amygdala that may be involved in AIE-induced condensing of chromatin architecture and synaptic changes, as well as adult anxiety-like behaviors.
Disclosure: Funded by NIAAA-NADIA UO1-AA019971, U24-AA024605 and P50-AA 022538 grants as well as VA senior research career scientist award.

9.2 Early Life Stress Enhances Susceptibility to Depression via Long-Lasting Transcriptional and Epigenetic Alterations in the Brain's Reward Circuitry

Catherine Pena

The Icahn School of Medicine at Mount Sinai, New York, New York, United States

Background: Early life stress (ELS) including abuse and neglect tragically impacts > 3 million US children every year, and increases the lifetime risk of major depression and other psychiatric disorders by 2-4 fold. Reduced or impaired social behavior and anhedonia are hallmark symptoms of depression and other related disorders which implicate involvement of the brain's reward and motivation circuitry including the ventral tegmental area (VTA) and nucleus accumbens (NAc). The molecular mechanisms mediating how ELS impacts development of the reward circuitry and vulnerability to depression are largely unknown.
Methods: Male and female mice were standard-reared (Std) or exposed to ELS during a postnatal sensitive window, and half of each group faced chronic social defeat stress (males)

or sub-chronic variable stress (females) in adulthood. Adult depression-like behaviors were quantified and RNA-seq libraries were generated for each of the four groups from male and female VTA and NAc. We performed differential gene expression and biologically-informed upstream driver analysis using voom limma and Ingenuity Pathway Analysis (Qiagen) tools. Histone turnover and a suite of post-translational histone modifications were examined across postnatal development in Std and ELS male NAc and VTA using mass spectronomy. ChIP-seq was performed for histone modifications associated with genomic enhancer regions (H3K4me1, H3K27Ac) in Std and ELS adult male VTA. Target genes were bi-directionally manipulated by site-specific over-expression or knock-out to rescue or recapitulate the effects of ELS.

Results: Male and female mice subjected to ELS in a postnatal-sensitive window exhibit enhanced susceptibility to depression-like behaviors after experiencing additional, chronic stress in adulthood. Interestingly, without adult stress, ELS alone did not alter depression-like behaviors, enabling us to study latent environmentally-induced vulnerability to depression-like behaviors prior to their onset. ELS induces wide-spread transcriptional alterations in VTA and NAc that may prime the brain to respond to additional stress. In male VTA, bioinformatic analysis identified suppression of the transcription factor orthodenticle homeobox 2 (Otx2) as an upstream regulator of these enduring transcriptional changes, although interestingly Otx2 itself was only transiently suppressed by ELS suggesting lasting regulation of its target genes by epigenetic mechanisms. Otx2 has been implicated in genomic enhancer-region activation, and ChIP-seq reveals ELS alters patterns of enhancer-associated histone modifications in regions near Otx2 binding motifs. Viral-mediated over-expression of Otx2 in VTA, either transiently in the juvenile period or in adulthood, rescues the effects of ELS on vulnerability to depression-like behaviors, while local knock-out of Otx2 recapitulates this vulnerability to adult stress. In male NAc, ELS promotes accelerated rates of histone H3.3 variant turnovers, which has been shown to be necessary for activity-dependent gene expression, synaptic connectivity, and cognition. ELS is also associated with altered levels of histone-modifying enzymes in male and female NAc.

Conclusions: Together, we have identified putative epigenetic mechanisms for the long-lasting effects of ELS on transcriptional alterations within the reward circuitry. Moreover, we have identified a novel molecular target in VTA mediating the impact of ELS on long-lasting vulnerability to depression-like behavior.

Disclosure: Nothing to Disclose.

9.3 MECP2 and the Regulation of Chromatin Plasticity During Psychostimulant Exposure

Anne West

Duke University School of Medicine, Durham, North Carolina, United States

Background: Chronic exposure to psychostimulants can induce epigenetic changes to neuronal chromatin that are thought to contribute to the development and expression of addictive-like behaviors by inducing long-lasting changes in gene transcription. Our previous studies have shown that exposure to both cocaine and amphetamine can induce the phosphorylation of the methyl-DNA binding protein MeCP2 (pMeCP2) selectively in parvalbumin (Pvalb) positive GABAergic interneurons of the nucleus accumbens (NAc). Consistent with a functional role for pMeCP2 in neuronal adaptations to these drugs, mice bearing a phosphorylation site mutation knocked into the Mecp2 gene show enhanced expression of addictive-like behaviors following chronic amphetamine or cocaine. However, the cellular and molecular mechanisms of these behavioral changes have remained largely unknown.

Methods: To first determine what role GABAergic interneurons of the NAc play in the development and expression of addictive-like behaviors, we used an intersectional genetic strategy to selectively silence these neurons in adult mice. We stereotaxically injected an AAV expressing a Cre-inducible tetanus toxin light chain (TeLC) into the NAc of transgenic mice expressing Cre recombinase knocked into the Pvalb gene. We injected a Cre-inducible GFP as control. At the behavioral level we monitored locomotor sensitization and conditioned place preference to amphetamine (3mg/kg i.p.), and at the cellular level we performed microdialysis for dopamine release and measured induced expression of the immediate-early gene Fos. Finally, to determine whether repeated amphetamine induces transcriptional and chromatin changes in these interneurons, we have crossed pMeCP2 knockin and Pvalb-Cre mice to the Cre-inducible RPL22-HA RiboTag and Sun1-GFP INTACT transgene lines, respectively, to selectively isolate RNA and nuclei from Pvalb+ interneurons of the NAc after exposure to amphetamine.

Results: We find that silencing GABAergic interneurons of the NAc prevents the expression of both behavioral sensitization and conditioned place preference following repeated amphetamine. These behavioral changes occur despite normal amphetamine induced dopamine release in the NAc. We see enhanced amphetamine-induced Fos expression in NAc projection neurons of the TeLC mice, suggesting that Pvalb+ interneurons of the NAc have a direct effect on NAc output. At the RNA and chromatin levels, data analyses are ongoing.

Conclusions: Our data show that GABAergic interneurons of the NAc play a key role in regulating the expression of addictive-like behaviors following exposure to chronic amphetamine. Our sequencing data will help to elucidate whether MeCP2-dependent chromatin changes underlie transcriptional and functional plasticity of these neurons following chronic psychostimulant exposure.

Disclosure: Nothing to Disclose.

9.4 Environmental Regulation of Epigenetic and Transcriptional Landscapes

Michael Meaney

Douglas Mental Health University Institute, Montreal, Canada

Background: The childhood social environment influences the risk for psychopathology across the lifespan. Previous findings suggest that such stable influences associate with

chemically stable, epigenetic modifications, including DNA methylation, of genes associated with stress reactivity, and other phenotypes linked to vulnerability for psychopathology. We are examining the tissue specificity and form of the modifications to DNA methylation associated with environmental conditions that modify the risk for psychopathology in humans.
Methods: We use next generation sequencing with single base-pair resolution to examine the influence of environmental enrichment or maternal care on DNA methylation including assessment of both 5-methyl-cytosine (5mC; whole genome bisulfite mapping) and 5-hydroxymethyl-cytosine (5hmC; TET-assisted bisulfite mapping) in rodent brain focusing on the dorsal and ventral hippocampus. We use genome-wide RNA and small RNA sequencing to assess the relation between DNA methylation and transcription.
Results: DNA methylation landscapes between the dorsal and ventral hippocampus differ only modestly in 5mC marks. In contrast, there are profound tissue-related differences in both 5hmC as well as in methylation at non-CpG sites. The results show clear evidence for greater effects of environmental enrichment or maternal care on 5hmC compared with 5mC in both dorsal and ventral hippocampus. The effects are strongly tissue-specific. The greatest affected site for 5hmC in the dorsal hippocampus lies in proximity to transcriptional start sites. In the ventral hippocampus, there are greater effects at the level of 5hmC in gene body regions.
Conclusions: The results show stable variations in DNA methylation across the genome associated with models of 'clinically-relevant' environmental conditions in the form of 5hmC, but less so in 5mC. Sites of variation are enriched for transcriptional factors activated by early experience and associated with synaptic plasticity.
Disclosure: Nothing to Disclose.

Panel
10. Hippocampal Disinhibition in Schizophrenia: A New Target for Treatment?

10.1 Hippocampal Hyperactivity in Schizophrenia

Stephan Heckers

Vanderbilt University, Nashville, Tennessee, United States

Background: The hippocampus is abnormal in schizophrenia. The most robust finding is smaller hippocampal volume, present already early in the disease process. The volume difference is not due to an overall loss of neurons, but is associated with a selective reduction in the number of GABAergic interneurons expressing parvalbumin and somatostatin. Neuroimaging studies have revealed that the hippocampus is hyperactive, with increased blood flow and blood volume at rest and decreased recruitment during memory task performance. In this study we explored regional differences in hippocampal activity along the longitudinal (anterior-posterior) and vertical (CA1-CA4) axes.
Methods: We used 3T and 7T MR to map hippocampal structure and function in schizophrenia. We tested for selective changes in the anterior hippocampus (uncus) and the CA1 subfield. Hippocampal activity was studied with three complementary methods: amplitude of low frequency fluctuations (ALFF), gadolinium-enhanced steady state cerebral blood volume (CBV) and the novel inflow-based-vascular-space-occupancy with dynamic subtraction (iVASO-DS) method, which measures arterial CBV.
Results: Anterior hippocampal volume was smaller, but posterior hippocampal volume was normal in schizophrenia (region-by-diagnosis interaction, $p < .05$). Changes in hippocampal activity preferentially affected the anterior hippocampus. First, ALFF was selectively increased in the anterior hippocampus (region-by-diagnosis interaction, $p < .001$). Second, CBV was increased in the anterior CA1 region, but not other hippocampal subfields. The iVASO-DS method revealed a posterior > anterior gradient of arterial CBV in healthy subjects, but no significant difference between groups.
Conclusions: Here we present initial results of high-resolution mapping of hippocampal volume and from three methods to study hippocampal hyperactivity in schizophrenia. We were able to show that hippocampal volume change in schizophrenia preferentially affects the anterior hippocampus. In addition, two methods revealed hyperactivity of the anterior but not the posterior hippocampus in schizophrenia. We will discuss our findings in light of rodent models of schizophrenia, which have demonstrated interneuron abnormalities in the ventral but not dorsal hippocampus.
Disclosure: Nothing to Disclose.

10.2 Schizophrenia-Relevant Pathophysiology and Behavior in a Transgenic Model of Deficient Cortical Interneuron Development and the Impact of Adding New Interneurons to the Hippocampus in Adulthood

Holly Moore

Columbia University, New York, New York, United States

Background: The evidence for interneuron deficits and abnormal basal activity in the hippocampus in schizophrenia has inspired the use of manipulations of interneuron development and function in rodent models to 1) reveal links between interneuron number and function on one hand, and neuronal and metabolic activity (as assessed with MRI) on the other; and 2) determine the contribution of specific GABA interneuron subpopulations to pathophysiology in other systems, as well as neurobehavioral and cognitive processes affected in schizophrenia. In the current study, how a partial PV interneuron deficit affects hippocampal and basal ganglia circuit function, and behaviors mediated by these circuits. We then present data on the ability of transplantation of interneuron precursors into the adult hippocampus to reverse key phenotypes.
Methods: We focus on a mouse model with a null mutation of the cyclin D2 (cD2) gene, supplemented with data from mice with an MGE-selective knockout of the sonic hedgehog receptor smoothened (Six3Cre;Smo f/f), as well as pharmacological and other developmental models. Transplantation: The medial ganglionic eminence (MGE) was dissected on

embryonic day 17 from mouse embryos with constitutive expression of green fluorescent protein (GFP). Cells were dissociated and suspended as per published methods. The cell suspension was infused into the caudal ventral hippocampus. A suspension of killed cells was used as a control.
GABAergic interneurons were visualized by immunostaining for PV, SST, and/or GABA. Interneurons derived from transplants were identified by GFP. Neurons were quantified using a modified fractionator method. Basal cerebral blood volume (CBV) was measured *in vivo* using gadodiamide-contrast functional MRI. Synaptic activity was measured with whole-cell voltage clamp recordings in hippocampal slices. *In vivo* spontaneous activity was measured with single-cell extracellular recordings in anesthetized mice. Behavioral assays included locomotor activity in an open field before and after amphetamine (i.p.), prepulse inhibition of acoustic startle, and social behavior in a resident-intruder context. The cognitive assays included object- and spatial-based set shifting, discrimination and reversal, and cued and contextual fear learning.
Results: Genetic mouse models showed moderate (40%) reductions in PV interneuron density and GABA-mediated synaptic inhibition in the hippocampus, with other cortical regions less affected in the cD2 model. This corresponded with increased basal hippocampal CBV, increased dopamine (DA) neuron population activity, and a greater responsiveness to amphetamine. The cD2 null mouse also showed cognitive deficits consistent with abnormal hippocampal function. MGE transplants into ventral CA1 in adulthood led to establishment of new PV and SST interneurons throughout the hippocampus, normalized physiological and psychomotor phenotypes, and improved cognition in the cD2 null mice.
Conclusions: These studies suggest that a deficit in functional PV interneurons in the hippocampus similar to reported deficits in schizophrenia is sufficient to produce persistent increases in basal hippocampal activity and an fMRI phenotype homologous to that correlating with psychosis in schizophrenia. This hippocampal dysfunction may augment dopamine system activity and disrupt multiple cognitive processes affected by schizophrenia. Transplanting new interneurons into the adult "diseased" hippocampus has a normalizing effect on hippocampal activity, DA system function, and psychosis-relevant behaviors and cognitive processes. Greater understanding of the local and circuit mechanisms induced by these transplants can lead to new treatment strategies in schizophrenia.
Disclosure: Nothing to Disclose.

10.3 Stem Cell Derived Interneuron Transplants as a Treatment for Schizophrenia: Preclinical Validation in a Rodent Model

Daniel Lodge

The University of Texas Health Science Center at San Antonio, San Antonio, Texas, United States

Background: There is increasing evidence demonstrating hyperactivity in hippocampal subfields at rest in schizophrenia patients. Moreover, this is correlated with clinical measures of psychosis. Based on its connectivity with the midbrain dopamine system, as well frontal cortical regions, the hippocampus may represent a key site of pathology in schizophrenia. This increase in hippocampal activity is thought to be attributable to a deficit in inhibitory interneuron function which is a consistent observation in postmortem studies as well as in rodent models of the disease. Given that these deficits appear to be primarily limited to parvalbumin (PV) and somatostatin (SST) interneuron subtypes, we tested the hypothesis that restoring PV- or SST- interneuron function in the vHipp would reverse behavioral and neurophysiological deficits in a developmental disruption rodent model of schizophrenia (the methylazoxymethanol acetate – MAM rat).
Methods: To produce a schizophrenia-like phenotype in male offspring, pregnant rats were injected with MAM (22 mg/kg, i.p.) on gestational day 17. To generate enriched populations of PV- or SST-positive interneurons, we used a mouse embryonic stem cell line containing dual reporters (Lhx6::GFP and Nkx2.1::mCherry). Cells were grown in culture, sorted by flow cytometry, then injected into the vHipp of MAM or saline control rats on postnatal day 40-45. Thirty days after transplantation, we measured latent inhibition, social interaction, and attentional set-shifting to model positive, negative and cognitive symptoms, respectively. A subset of animals was used for *ex vivo* patch clamp recordings to measure spontaneous inhibitory post-synaptic potentials (sIPSC) from pyramidal cells in the vHipp. With the remaining rats, we performed *in vivo* extracellular recordings to determine firing rate of putative pyramidal cells in the vHipp and spontaneous activity of dopamine cells in the ventral tegmental area (VTA). After recording, all animals were perfused and dual-fluorescence immunohistochemistry was used to confirm the localization and phenotype of the transplanted cells.
Results: We found that both the PV- and SST- positive transplants integrated into the existing circuitry, as evidenced by an increase sIPSC amplitude and frequency in vHipp pyramidal cells. Further, both cell transplants reduced aberrant pyramidal cell firing rate in the vHipp, and normalized dopamine population activity in the VTA. Despite their similar physiological effects, the PV- and SST-enriched transplants had dramatically different effects on behavior. Although both cell types attenuated deficits in reversal learning and restored latent inhibition, only the PV-positive transplants were able to reverse deficits in extradimensional set-shifting and social interaction.
Conclusions: Our results suggest that PV- and SST-positive interneurons in the vHipp differentially regulate schizophrenia-like behaviors. The SST-positive transplants appear to have beneficial effects on behaviors that rely on dopamine signaling, including latent inhibition and reversal learning. PV-positive transplants improved performance in these behavioral tasks, but were also able to normalize behaviors that involve the prefrontal cortex, including social interaction and extradimensional set-shifting. These results suggest that restoring PV interneuron function in the vHipp may be an effective treatment strategy for schizophrenia to improve not only positive, but also negative and cognitive symptoms of the disease.
Disclosure: Nothing to Disclose.

10.4 Human Stem Cell-Derived Cortical Inhibitory Interneurons in the Study and Treatment of Neuropsychiatric Illness

Stewart Anderson

Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States

Background: Cortical inhibitory interneuron dysfunction has been implicated in the pathogenesis of schizophrenia. While animal systems have been invaluable for mechanistic studies of disease, the advent of human stem cells is revolutionizing our ability to study, and even to treat, human illnesses. Inhibitory interneurons of the cerebral cortex originate not in the dorsal region of the neural tube with cortical excitatory neurons, but in the ventral neural tube with GABAergic neurons of the basal ganglia. Since interneuron fate is established at their birth, the separation of regions of origin comes with separation of key fate determining factors. For generating cortical interneurons from stem cells, this separation means that one can enrich for GABAergic forebrain neurons at the expense of cortical excitatory neurons. The generation of cortical inhibitory interneurons from human stem cells has led their use to study and to treat known or suspected "interneuronopathies", including autism, epilepsy, Tourettes disorder, and schizophrenia. In schizophrenia, as noted above transplants of interneuron precursors derived from either embryonic mice or from mouse embryonic stem cells directed to cortical interneuron fates have shown efficacy in mouse and rat models of psychosis-related pathophysiology. Two challenges have existed to using human stem cells in such studies: first, how to isolate the cortical interneurons from mixed cultures of GABAergic cells and second, how to accelerate the normally protracted maturation of these cells. This presentation will address both of these challenges. In addition, I will present evidence from patient-derived stem cells that schizophrenia, in the context of 22q11 deletion syndrome, is associated with mitochondrial deficits which may, in cortical interneurons, result in disinhibition of cortical circuitry.
Methods: First, human stem cells were modified to express the fluorescent reporter citrine within the Lhx6-locus. As Lhx6 is expressed in two major subclasses of cortical interneurons, those that express parvalbumin (PV) and those that express somatostatin (SST), upon their birth within the ventral forebrain, this approach allows us to purify PV and SST cortical interneurons from human stem cell differentiations and to identify them after transplantation into rodent cortex. Second, in the Lhx6-citrine lines we have used CRISPR-CAS9 targeted recombination to flank exon 4 of the PTEN gene with loxP sequences. PTEN is an autism-related gene whose mutation results in upregulation of mTOR pathway signaling, a regulator of cell growth and maturation. Third, 22q11 deletion syndrome (22q11DS) is associated with a 25-fold increase in schizophrenia, and at least 6 of the 40 genes deleted in 22q11DS localize to mitochondria. Using induced pluripotent stem cells (IPSCs) from 22q11DS patients we are deriving forebrain neurons for the study of their mitochondrial function.
Results: Using our Lhx6-citrine interneuron reporter line we find that PTEN loss in postmitotic interneurons greatly accelerates their maturation. Ongoing studies are developing approaches to titrate the level of mTOR signaling activation in order to controllably accelerate interneuron maturation, and are evaluating the effects of PTEN loss on interneuron function. In addition, using the 22q11DS+schizophrenia IPSCs-derived neurons, we find evidence for disruption of mitochondrial protein translation and oxidative phosphorylation.
Conclusions: Human stem cells can be used to study neuropsychiatric illness. Enhancement of mTOR signaling accelerates the maturation of human stem cell derived neurons for studies of disease and for use in cell based therapies. The identification of neuronal mitochondrial deficits in 22qDS schizophrenia suggests a mechanism whereby the highly active interneurons may be vulnerable to dysfunction resulting in disinhibition of cortical circuitry and psychotic symptoms.
Disclosure: Nothing to Disclose.

Panel
11. Computational Psychiatry: A Bridge Between Neuroscience and the Bedside

11.1 Computational Neuroscience Insights Into Glutamate Synaptic Dysfunction and Working Memory Impairment in Schizophrenia

John Krystal

Yale University School of Medicine, New Haven, Connecticut, United States

Background: Glutamate synaptic dysfunction is a central feature of the neurobiology of schizophrenia. However, it is challenging to link the genetic and biochemical evidence supporting this hypothesis to the properties of cortical microcircuits and macrocircuits that might contribute to features of working memory dysfunction in schizophrenia, such as reductions in encoding/early maintenance of information in working memory, reduced precision of working memory, and propensity of memories to be contaminated by distracting information. The purpose of this presentation is to review a series of studies conducted by my collaborators and I that shed light on how experimental and computational approaches may be linked to shed light on these issues.
Methods: This presentation will review results from recent biophysically informed computational neuroscience models conducted in the laboratories of XJ Wang and John Murray, with studies of schizophrenia patients and healthy subjects (+/-ketamine) with fMRI and EEG conducted by Alan Anticevic, Genevieve Yang, Philip Corlett, Gregory McCarthy, Naomi Driesen, Deepak Cyril D'Souza, Jose Cortes-Briones and myself.
Results: We find that reductions in working memory related cortical activation during encoding and early maintenance of information in working memory, as measured by fMRI in healthy subjects and schizophrenia patients are consistent with the role of NMDA glutamate receptors in computational models of prefrontal cortical network activity associated with working memory. We also find that inhibitory deficits emerging from a biophysically informed "bump integrator" computational model give rise to predictions of

reduced working memory precision, "false memories" arising from contamination by distractors, and increased signal variance ("noise"). All of these properties can be demonstrated in spatial working memory studies in healthy subjects administered ketamine, where inhibitory deficits are presumed to arise from reduced excitatory input to interneurons, and schizophrenia, where interneuron deficits are thought to arise developmentally.
Conclusions: This presentation would provide support for the hypothesis that glutamate synaptic deficits associated with schizophrenia contribute to working memory impairment associated with schizophrenia by impairing the "primary infrastructure" of working memory (i.e., the NMDA receptor-dependent recurrent excitation in layer III of PFC) as well as "detuning" working memory circuits by impairing excitatory drive to interneurons, making cortical function and working memory inefficient and error prone. These findings will be interpreted in the context of a developmental model that may have implications for novel therapeutics for schizophrenia.
Disclosure: Part 1: Lilly Pharmaceutical, Consulted, **Part 2:** Janssen, Licensed IP, Biohaven Medical Sciences, Licensed IP.

11.2 Model-Free Learning, Dopamine and Alcoholism: Review and Test

Quentin Huys

University of Zurich, Zurich, Switzerland

Background: Dopamine potentially unites two important roles: one in addiction, being involved in most substances of abuse including alcohol, and a second one in a specific type of learning, namely model-free reinforcement learning. Theories of addiction have thus long suggested that drugs of abuse may usurp dopamine's role in learning. Preclinical data strongly suggests two different accounts of how model-free learning biases could contribute to the development and maintenance of alcohol and other substance addictions are discerned. On the one hand, sign-tracking data suggest that an overall bias towards model-free learning could function as a risk factor for the development of substance abuse. On the other hand, addictive substances do themselves cause a shift towards model-free habitual learning. We here first present a review of the imaging evidence for model-free learning in alcohol. As this is inconclusive, the Learning in Alcohol Dependence (LeAD) study tested this directly using two tasks during fMRI. The two-step task is designed to measure both model-free and model-based decision-making. A Pavlovian-Instrumental-Transfer (PIT) task measured the impact of Pavlovian stimuli on choice behaviors.
Methods: The LeAD study tested a) young social drinkers (age 18 years) and b) detoxified patients with alcohol use disorders (AUD) and matched controls. All performed both tasks during fMRI. Two-step behavior was fitted with a computational reinforcement-learning model. Correlations between parameter estimates and measures of drinking and relapse were tested. Based on the parameter estimates, individual regressors were constructed to separately estimate model-free and model-based learning correlates in the BOLD data. These were in turn correlated with measures of drinking and relapse.
Results: The review of the imaging literature in alcohol addiction revealed a dearth of studies able to test a contribution of model-free biases in general learning, or a model-free bias specific to the drug or drug context. Two-step behavior was well captured by a computational model with both model-free and model-based components, outperforming models with only one of the two components. This was true in both adult and young groups, though among the young group model-based reasoning was far more prominent. Pavlovian-Instrumental Transfer was stronger among AUD patients than controls, and its BOLD correlates were higher in those patients who went on to relapse early on. Furthermore, higher individual PIT effect sizes correlated with impaired model-based decision-making in the two-step task in both young and healthy adult groups. However, in the young at-risk group, two-step behavior and model parameters did not correlate with drinking measures. Analyses of BOLD data also showed no correlates of either model-free or model-based signals with alcohol consumption. Exploratory analyses revealed a correlation between earlier drinking onset and model-free BOLD signals in the posterior putamen.
Conclusions: Preclinical data point to alterations in reinforcement-learning mechanisms as a potential risk factor or mediator for the development of substance use disorders. The relationship of (likely general) PIT effects with early relapse in detoxified patients, and the negative correlation of PIT effects with model-based reasoning hint at the involvement of model-free mechanisms. The fact that current drinking severity among a large group of socially drinking youth did not covary with either model-based or model-free indices in brain or behavior suggests that the imbalance may emerge later in the course of the disorder.
Acknowledgement: Analyses performed in collaboration by the LeAD team.
Disclosure: Nothing to Disclose.

11.3 Quantitative Models Reveal Dissociable Effects of Instructions and Feedback on Pain and Aversive Learning: Implications for Placebo Effects and Clinical Outcomes

Lauren Atlas

National Institutes of Health, Bethesda, Maryland, United States

Background: Expectations – predictions about the future based on conscious beliefs and previous experience – play a central role in emotion, cognition, and well-being. Expectations shape perception across nearly all psychological domains, tuning responses even in the earliest stages of sensory processing. Expectations also directly influence clinical outcomes in the form of placebo effects (expectations about treatments). Despite their profound influence, we know little about the mechanisms by which expectancies actually modulate affective experience and clinical outcomes. In this talk, I will present a series of studies that used quantitative models to characterize the relationship between explicit beliefs, affective learning, and conscious pain experience. We asked whether expectations induced through verbal instruction have dissociable effects from those learned through experience, and how these in turn shape

physiological, neural, and subjective responses to clinically relevant outcomes, such as pain.

Methods: In our initial work (Study 1; Atlas et al, 2016, eLife) we measured the effects of instructions during aversive reversal learning. One group of participants was instructed about stimulus contingencies and reversals, while a second group learned from feedback alone. We introduced a new computational model that flexibly captures effects of instructions on feedback-driven learning, and fit this model to autonomic responses from both groups. Responses fit to the Uninstructed Group capture learning from feedback-alone, while responses fit to the Instructed Group isolate learning that updates with instructions. We then used these responses to isolate neural correlates of feedback-driven and instructed aversive learning. We have since extended this work to measure how these two factors influence subjective decisions about pain (Study 2; Atlas et al, In progress) by crossing this paradigm with a thermal pain task designed to measure expectancy effects on pain (adapted from Atlas et al, 2010, JNeurosci).

Results: Study 1 revealed dissociable effects of instructions on the neural systems of aversive learning. The striatum and orbitofrontal cortex tracked aversive learning that updated with instructions, and correlated with the prefrontal cortex response to instructions. However, the amygdala learned from feedback irrespective of instruction. Study 2 reveals related dissociations between expectancy effects verbal decisions and autonomic responses. Specifically, verbal pain reports are biased toward initial learning, whereas autonomic responses update in response to contingency changes throughout the task. Finally, individual differences in anxiety moderate the effects of initial learning on autonomic responses, suggesting links between anxiety and dissociations in expectancy-based processing.

Conclusions: Expectations have profound effects on subjective, neural, and autonomic outcomes. Quantitative models allow neuroscientists to isolate different factors that give rise to expectations and identify neural processes that support potential dissociations. The dissociations we observe are consistent with evolutionary theories of biological preparedness in the amygdala, and may be linked to the important role of experiential learning for successful treatment of anxiety disorders and PTSD. As placebo effects are thought to depend on the joint combination of explicit instruction and prior experience, this mechanistic approach holds promise to further isolate the mechanisms that underlie placebo effects and thereby harness them to improve clinical outcomes.

Disclosure: Nothing to Disclose.

11.4 Computational Modelling of Emotion Regulation and Application to Depression and PTSD

Amit Etkin

Stanford University School of Medicine, Stanford, California, United States

Background: Individuals monitor the consequences of their actions to adjust their behavior to a changing environment – a phenomenon that includes learning from reward/punishment and dynamic control of emotional processing. While computational modeling has greatly advanced the study of learning, the computational mechanisms underlying emotion regulation remain unknown.

Methods: Drawing on computational of value-based decision-making and reinforcement learning, I will first outline the rationale behind viewing emotion regulation from the perspective of value-based decision-making, and how this may then be implemented computationally. Next, I will report empirical data on the modeling of one form of emotion regulation – conflict-induced emotional control – using a temporal difference algorithm. Internal performance monitoring was modeled by fitting behavior on a trial-by-trial basis, using a sequential monte carlo method, to both accuracy and reaction time (as unlike reinforcement learning, no external reinforcer occurs). Decision weights (i.e. value) were determined for the response to the target and distracter separately, and prediction errors were calculated based on the discrepancy between these weights and observed behavior.

Results: The computational model fit trial-by-trial behavioral data well, and better than alternative models examined. By correlating model parameters at each trial with fMRI activity, we found that activity in the ventromedial prefrontal cortex (vmPFC) correlated positively with the decision weight associated with response to the target stimulus ($p<.05$; FWE-corrected). Prediction error was negatively correlated with a network of regions including the dorsal anterior cingulate, anterior insula and parietal cortex ($p<.05$; FWE-corrected). Moreover, individuals with lesions in the vmPFC failed to update decision weights after either incongruent or congruent trials, while healthy individuals and those with lesions outside the vmPFC did so normally. Test-retest reliability of model-correlated fMRI activity also supported the stability of these relationships. In a large sample of depressed patients ($N=182$) we found stronger prediction error signals in the dorsal anterior cingulate and dorsolateral prefrontal cortex), consistent with prior suggestions that depression is associated with hypersensitivity to errors. Finally, results will be reported on a large cohort of PTSD patients ($N=150$) using the same experimental task and computational model.

Conclusions: These findings mechanistically unify parallel areas of investigation (learning and emotional control) that have long described similar brain circuitry (i.e. vmPFC) but have not converged under a common conceptual model, namely value-based decision-making. Our data also show the applicability of this computational perspective within psychiatry, specifically with relevance to depression and PTSD.

Disclosure: Part 1: Otsuka, consulting, Takaeda, consulting, Acadia, consulting, **Part 4:** Brain Resource, research grant.

Panel
12. Stress, Anxiety and Decision-Making: New Insights From Animal Models

12.1 Neurobiological and Hormonal Modulation of Risky Decision-Making in Rats

Caitlin Orsini

University of Florida, Gainesville, Florida, United States

Background: Substance abuse is associated with maladaptive decision-making and elevated risk-taking behavior.

A potential treatment strategy for addiction could therefore be to attenuate such maladaptive choice behavior so as to mitigate drug-seeking and potential relapse. To realize this goal, however, a thorough understanding of the neurobiology underlying normal risk-taking is required. Much of what we currently know originates from studies in male subjects, but there is growing evidence for sex differences in some forms of risky decision-making, with females generally being characterized as more risk-averse than males. To begin to understand these sex differences, we used a rodent model of risky decision-making to determine the hormonal and neurobiological contributions to risk-taking in males and females.

Methods: Experiment 1: Male and female rats were trained in a "Risky Decision-Making task" (RDT) in which they chose between a small, "safe" food reward and a large, "risky" food reward accompanied by ascending probabilities of mild footshock. Vaginal swabs were taken from females after each session to determine estrous phase. The effects of systemic amphetamine administration on choice behavior were also assessed in both sexes.

Experiment 2: Separate cohorts of rats were trained in the RDT and the effects of prefrontal cortical manipulations on choice behavior were assessed. In the first two groups, the medial prefrontal cortex (mPFC) was inactivated prior to testing in the RDT with ascending or descending probabilities of punishment. In the last group, the insular cortex (INS) was inactivated prior to testing in the RDT.

Results: Experiment 1: Females chose the large, risky reward significantly less (decreased risk-taking) than males. Importantly, female performance in the RDT was not modulated by estrous cycle. Further, this sex difference could not be accounted for by differences in shock reactivity. Amphetamine dose-dependently decreased risk-taking in both males and females, but this effect was more pronounced in female rats.

Experiment 2: Inactivation of the mPFC in male rats caused an increase in choice of the large, risky reward when probabilities of punishment ascended across the session. However, in the descending RDT, inactivation of the mPFC decreased the choice of the large, risky reward. In contrast to mPFC inactivation, inactivation of the INS decreased choice of the large, risky reward in the RDT with ascending probabilities of punishment.

Conclusions: Experiment 1 showed that females are more risk averse than males and that this difference may be due to greater sensitivity in dopaminergic signaling in females. Performance in females was not modulated by estrous phase, and ongoing studies are directly testing whether ovarian hormones play a critical role in task performance.

Experiment 2 showed that different prefrontal cortical subregions make unique contributions to risky decision-making. While the mPFC appears to be necessary for the behavioral flexibility required for shifting choice behavior, the INS is critical for promoting risk-taking behavior. Ongoing studies are investigating the role of each of these subregions in risky decision-making in females to determine whether contributions of these regions differ between sexes.

Disclosure: Nothing to Disclose.

12.2 A Neural Basis for Sex-Dependent Fear Response Selection

Rebecca Shansky

Northeastern University, Boston, Massachusetts, United States

Background: An individual faced with a threatening stimulus may respond in many different ways. The selection of either an active or passive response can predict long-term outcomes in clinical populations, and an understanding of what leads to the selection of one over the other could improve therapeutics. Although there is evidence for sex differences in fear response selection, the neurobiological basis for these discrepancies is unknown. The rostral and caudal mPFC send neuroanatomically distinct projections to the ventrolateral and dorsolateral periaqueductal gray (dl/vlPAG), which respectively mediate active and passive fear responses. However, the possibility that this circuit is sexually dimorphic has not been investigated.

Methods: Using a classic rodent model of Pavlovian cued fear conditioning, we found that female rats were four times as likely as males to exhibit an active, escape-like "darting" response to the conditioned stimulus, even though escape was not possible. To investigate the neural substrates of darting, we examined c-fos activity in the medial prefrontal cortex (mPFC) and periaqueductal gray (PAG) after fear conditioning in darting and freezing subpopulations.

Results: Darters exhibited greater activity in the dorsolateral PAG, which is consistent with active response strategies. Freezers exhibited greater rostral mPFC activity compared to the ventral mPFC, and the rostral: caudal ratio for each animal was tightly correlated to freezing in both Freezers and Darters.

Conclusions: In summary, we have identified and quantified a novel, active conditioned response that primarily occurs in females. This behavior is associated with discrete patterns of neural activity in the mPFC and PAG that implicate a shift in rostral-caudal mPFC control over response selection. These findings provide insight into the neural basis of sex-dependent fear behavior.

Disclosure: Nothing to Disclose.

12.3 The Effects of Stress and Glucocorticoid Receptor Blockade on the Propensity to Attribute Incentive Salience to Reward Cues

Shelly Flagel

University of Michigan, Ann Arbor, Michigan, United States

Background: Cues in the environment can guide behavior, leading us towards valuable resources; but cues can also guide behavior in maladaptive ways. For example, cues previously associated with drug-taking behavior can instigate relapse. Reward cues gain inordinate control over behavior when they are attributed with incentive salience, but this only occurs for some individuals. Following Pavlovian conditioning, some rats, termed sign-trackers, approach and engage a discrete reward-associated cue; whereas others, goal-trackers, approach the location of reward delivery upon cue

presentation. Thus, for sign-trackers, but not goal-trackers, the cue attains incentive motivational value. Previous studies have shown that the acquisition of a sign-tracking, but not goal-tracking, response is dopamine-dependent. In addition, different neural circuits are engaged in sign-trackers vs. goal-trackers upon reward-cue presentation. Surprisingly, relatively few studies have examined the role of the hypothalamic-pituitary-adrenal (HPA) axis in mediating sign- and goal-tracking behaviors. However, it has been shown that, relative to goal-trackers, sign-trackers exhibit a greater increase in corticosterone in response to repeated pairings of a cue and reward. Here we further examine the relationship between stress, glucocorticoids, and the propensity to attribute incentive salience to reward cues.
Methods: Selectively bred high-responder (bHR) and low-responder (bLR) rats that are known to differ in anxiety- and addiction-related behaviors were used. bHR rats are almost always sign-trackers, and bLR rats are goal-trackers. Thus, this animal model allows us to examine the effects of prior manipulations on the propensity to acquire a sign- or goal-tracking response. In one study bHR and bLR rats were exposed to a social defeat paradigm to examine the effects of stress on the inherent tendency to sign- or goal-track. In a second experiment the glucocorticoid receptor (GR) antagonist mifepristone was administered prior to each Pavlovian conditioning session to examine the effects of this drug on the acquisition of sign- and goal-tracking behavior. In a series of ongoing experiments, we are using outbred rats to examine the effects of systemic and local mifepristone administration on the acquisition and expression of these behaviors.
Results: Exposure to social defeat caused a delay in learning the sign- and goal-tracking response in bHR and bLR rats, respectively. In addition, defeated bHRs showed greater goal-tracking behavior relative to controls. Mifepristone administration prior to each Pavlovian training session attenuated sign-tracking behavior in bHRs, but did not affect goal-tracking behavior. When taken off of the drug, bHR rats continued to show an attenuation of sign-tracking behavior. In agreement, in outbred rats, mifepristone administration decreased the expression of sign-tracking behavior, while leaving goal-tracking intact.
Conclusions: These studies demonstrate a relationship between stress, glucocorticoids and the propensity to attribute incentive salience to reward cues. It appears that glucocorticoids, like dopamine, may be specifically involved in regulating the sign-tracking, but not goal-tracking response. Ongoing studies will examine the interaction between dopamine and glucocorticoids in regulating these behaviors and attempt to identify which parts of the motive circuit may be mediating these effects.
Disclosure: Nothing to Disclose.

12.4 Activation of Oxytocin Receptors Induces Defeat-Induced Social Withdrawal in Female California Mice

Brian Trainor

University of California, Davis, Davis, California, United States

Background: Oxytocin (OT) is often considered as pro-social and anxiolytic, but recent evidence suggests that the effects of OT are context-specific. It has been proposed that OT increases the salience of social cues, which can explain why OT can either enhance or inhibit social behaviors. We previously discovered in the California mouse (Peromyscus californicus) that three episodes of social defeat stress reduce social interaction (SI) behavior in females and that this coincides with hyperactivity of OT neurons in the bed nucleus of the stria terminalis (BNST). We also showed that intranasal OT administered to females naive to defeat showed reduced social interaction behavior, a phenotype similar to stressed females. Here we test whether this effect is mediated by oxytocin receptors (OTR) and develop tools for manipulating BNST OT neurons.
Methods: Males and females were randomly assigned to defeat stress or control conditions. Two weeks later mice were randomly assigned to receive an IP injection of saline or OTR antagonist (L-368,899; 3.0 or 5.0 mg/kg) 30 minutes before social interaction testing. In a separate group of control and stressed females, mice received three intra-BNST infusions of antisense morpholinos targeting OT or a scramble sequence. One week later females were tested in a social interaction test.
Results: OTR antagonist dose-dependently increased social interaction behavior in stressed females and had no effect in control females or in males. In females OTR antagonist had no effect on behavior in the open field test or on locomotor activity. Antisense morpholinos significantly reduced the number of OT immunoreactive neurons within the medio-ventral BNST. As predicted, OT knockdown had no significant effect on social interaction behavior in control females. Knockdown experiments on stressed females are in progress.
Conclusions: Oxytocin is usually considered to have anxiolytic properties, but there is growing evidence for anxiogenic effects of oxytocin. Our results suggest that in these contexts, OTR antagonists may have unanticipated anxiolytic properties. This hypothesis could have important implications for conditions such as PTSD and depression, in which elevated OT levels in women have been reported. Further study of neural circuits using OT should provide important insights for novel therapeutic approaches.
Disclosure: Nothing to Disclose.

Panel
13. A Cross-Species Understanding of Developing Corticolimbic Connectivity and Communication During Adolescence

13.1 Development of a Unique Amygdala Subregion in Primates: Implications for Early Circuits

Julie Fudge

University of Rochester Medical Center, Rochester, New York, United States

Background: Brain development in humans is protracted, with neural development continuing in the first years of life. A trade-off for this relatively long period of development is a large brain to support the wide repertoire of social skills that all primates require. The amygdala is crucial for the development of

adequate social-emotional responses and is highly interconnected with the prefrontal cortex. Both the amygdala and prefrontal cortex elaborate connections over post-natal development. Recently we examined a specific subregion of the amygdala known as the paralaminar nucleus (PL). This subregion is not found in rodents. Recent work indicates that it is the last amygdala subregion to contain immature neurons at birth, which gradually mature over the first years of life. Little is known about the connectivity of the PL, or the particular type of neurons that are developing in young animals. We hypothesize that this region is a site where early experience can shape neural connections. Our study sought to characterize the PL in terms of its connections, cellular phenotypes, and vulnerability to early stress.
Methods: We performed tract-tracing studies to examine PL connectivity in late-adolescent to young adult animals using a combination of anterograde and retrograde techniques. In a separate cohort of infant animals, we used laser capture and microarray analyses to specifically compare PL gene expression to other nearby amygdala regions. Finally, we examined PL-specific gene changes in infant animals that been raised in a maternal deprivation paradigm involving group rearing.
Results: In young adult animals immature neurons in the PL persist, and are richly and specifically innervated by the anterior hippocampus. The PL in the infant is enriched in gene transcripts associated with developing glutamatergic neurons in the fetus, including doublecortin, neuroD1, bcl2, and tbr1. We next examined brains of infant monkeys that had been maternally deprived in the first months of life. tbr1, which encodes a transcription factor that is required for maturation and specification of a glutamatergic phenotype, was specifically and significantly downregulated in the PL of maternally deprived animals. Because tbr1 has been identified as a high risk autism gene, we analyzed the relationship between tbr1 expression and typical social behaviors. Across all animals, the time spent in typical social behaviors in the group pen was positively correlated with tbr1 levels in the PL.
Conclusions: The PL is a unique region of the amygdala seen in higher species. In young adult monkeys, the connectivity of the PL indicates an important system that is strongly shaped by the hippocampus. Changes in the early social environment has an impact on development of glutamatergic neurons in the primate PL amygdala, which correlates with development of a normal social repertoire in young animals. Results will be discussed in the context of circuits influencing, and influenced by, the emerging PL.
Disclosure: Nothing to Disclose.

13.2 Adolescent Changes in PFC Innervation and Glutamate Receptivity After Maternal Separation in Male and Female Rats

Heather Brenhouse

Northeastern University, Boston, Massachusetts, United States

Background: Exposure to early life stress increases vulnerability to psychiatric disorders, including depression, anxiety and schizophrenia. Importantly, these disorders often first emerge in adolescence and manifest differently in males and females; therefore, intervening variables found in clinical studies make the role that early life stress plays in these diseases difficult to interpret. Our recent data using maternal separation (MS) as a model of early life stress in rats supports growing evidence of effects in the prefrontal cortex (PFC). The PFC is a relatively late-maturing region and subserves all higher-order cognitive and emotional functions, largely through its communication with limbic regions such as the amygdala. PFC innervation from the basolateral amygdala continues to develop through adolescence, and this increasing glutamatergic innervation co-occurs with changes in glutamate receptivity in the PFC. We have found that MS alters the trajectory of PFC glutamate receptor expression as well as innervation from the basolateral amygdala, in a sex-specific manner.
Methods: Male and female rats were either reared under control conditions or were separated from their mother and littermates (MS) for 4h/day from postnatal days (P) 2-20. The anterograde tracer BDA was microinjected into the basolateral amygdala in juvenility (P21), adolescence (P35) or adulthood (P120). Seven days later, rats were assessed for anxiety-like behaviors using the open-field or elevated plus maze tasks, and terminals in the PFC were stereologically counted in the infralimbic PFC. In a separate cohort, control or MS rats were sacrificed in adolescence for membrane fractionation of medial PFC tissue. Membrane fractions and cytoplasmic fractions were measured for levels of AMPA and NMDA glutamate receptor subunits using Western blot.
Results: We observed MS effects on PFC innervation and glutamate receptor expression in a sex-specific manner. Specifically, MS females displayed increased basolateral innervation to the PFC and increased anxiety-like behaviors by juvenility compared to controls, and MS males displayed effects in adolescence. We also found that in adolescence, PFC NMDA and AMPA receptors expressed at the neuronal membrane display different subunit composition in males and females. Male, but not female adolescents exposed to MS showed lower membrane expression of the AMPA subunit GluR2 than controls, which has been shown to yield increased vulnerability to excitotoxicity. Additionally, both male and female adolescents exposed to MS displayed higher membrane levels of the NMDA subunit NR2A, which is also found after other developmental insults and can affect channel kinetics and cell excitability.
Conclusions: These data show for the first time that adolescent amygdaloid-PFC connectivity is altered by early life stress on a different trajectory in males and females. This may shed light onto the earlier age of onset for anxiety and depression observed in girls than in boys. Altered innervation may also explain the changes in glutamate receptivity in the PFC, which could lead to new targets for intervention in individuals with a history of early life stress.
Disclosure: Nothing to Disclose.

13.3 Afferent Regulation of Prefrontal Cortex Maturation During Adolescence

Kuei Tseng

The Chicago Medical School, North Chicago, Illinois, United States

Background: According to the NIMH Council's Report, most major psychiatric disorders can only be understood as

an interaction between brain development and susceptibility to risk factors. However, a comprehensive understanding of the mechanisms that regulate the normal developmental trajectory of neural circuits involved in these disorders is lacking. Thus, more research is needed to fully grasp how the trajectory of specific neural circuits becomes dysfunctional after a developmental insult, especially during adolescence when disorders such as schizophrenia and affective disorders arise. As a key structure involved in the acquisition of mature cognitive abilities, the prefrontal cortex (PFC) displays a protracted maturation during adolescence, which makes it susceptible to developmental insults during this stage. Data from our recent studies reveal that the PFC undergoes massive functional remodeling during adolescence, yet it is the local GABAergic system that renders the PFC labile during this developmental period.

Methods: Transient inhibition of PFC afferent drive and GABAergic activity will be induced at 2 non-overlapping adolescent periods within postnatal days (P) 35-50 and changes in PFC output function will be determined in adulthood (P75-95) by means of electrophysiological measures.

Results: We found that the characteristic facilitation of prefrontal GABAergic function during adolescence is accompanied by a functional strengthening of the ventral hippocampus-to-PFC connectivity in tandem with an increased glutamatergic drive onto prefrontal fast-spiking interneurons. A similar developmentally-regulated strengthening of basolateral amygdala drive might contribute to maturation of prefrontal GABAergic function. This raises the interesting possibility that specific glutamatergic inputs drive the development of specific populations of GABAergic interneurons in the PFC, enabling the mature PFC output functioning observed in adults. Thus, I will summarize and discuss recent findings obtained using DREADD + available shRNA technology to reveal how PFC maturation during adolescence requires coordinated activation of specific prefrontal interneurons and glutamatergic afferents originated from the ventral hippocampus and amygdala.

Conclusions: Overall, these studies provide for the first time a mechanistic understanding of the requirements at both cellular and circuit levels necessary for sustaining the normal trajectory of PFC maturation that could also explain why it is rendered susceptible during adolescence. Such knowledge is expected to have a positive impact in the development of age-specific interventions aimed at ameliorating the incidence of PFC dysfunctions in mental disorders within the adolescent/young adult population by either strengthening or diminishing specific afferents.

Disclosure: Nothing to Disclose.

13.4 At Risk of Being Risky: The Relationship Between "Brain Age" Under Emotional States and Risk Preference

Damien Fair

Oregon Health and Sciences University, Portland, Oregon, United States

Background: Adolescents and young adulthood are unique periods of development where an increase in risky behaviors, higher degrees of sensation seeking and impulsivity, greater sensitivity to rewards, and heightened reactivity to threat and punishment occur. A particular locus of concern during this period of development pertains to the decision-making and associated functional neuroanatomy during socio-affective environments known to have a profound impact on cognition and behavior. In addition, in all aspects of development, a great deal of heterogeneity exists amongst typically and non-typically developing populations. In other words, particular characteristics may predispose certain subgroups of individuals more than others with a greater inclination toward risk. Within this context, the current research examines the influence of sustained emotional contexts (neutral, negative, and positive) on residual patterns of functional connectivity (pseudo-resting state, RS). We test whether an individual's predicted/functional "brain age" deviates under emotional influence (emotional brain age) and whether or not this deviation from one's true age in a given context is related to a propensity toward, or aversion to risky behaviors.

Methods: As part of a large, ongoing study, 212 healthy right-handed 10 to 25 year olds (118 Females) were examined. Participants completed the rapid event-related emotional go/nogo impulse control task to transient social cues under sustained negative (threat: anticipation of an aversive noise), positive (excitement: anticipation of a reward), and neutral (no anticipation of an aversive noise or reward) emotional contexts. All participants also completed the Benthin Risk Assessment. To examine functional connectivity under emotional influence, task-related BOLD responses were modeled using the general linear model (GLM) and removed by regression prior to functional connectivity preprocessing. We use PLSR to assess a participant's predicted age in neutral contexts and the emotional contexts.

Results: Using functional connectivity data in a neutral brain-state we first were able to identify the "brain age" of a given individual, We then show that both positive and negative contexts can alter an individual's "brain age" based on changes in functional connectivity patterns of cortico-limbic circuits. In the teen years, on average, the "brain age" across the group has the propensity to look younger in emotional contexts relative to neutral contexts. Importantly, these two distinct phenotypes across age (i.e., a subgroup of participants whose emotional brain age was predicted 'older' versus a subgroup whose was predicted 'younger' during emotional contexts) were related to risk perception and preference as measured by the Benthin Risk Assessment – a pattern exemplified greatest in young-adults (ages 18-21).

Conclusions: In the present study we demonstrate that differences in individuals predicted age in specific emotional contexts relates to certain metrics assessing awareness of and preference for risky behavior. The findings also suggest that contextual settings have an impact on underlying functional neurophysiology, in this case an individual's "emotional brain age," and that some individuals are more at-risk than others. In other words, the results are suggestive of a specified functional brain phenotype that relates to being at "risk to be risky.".

Disclosure: Nothing to Disclose.

Panel
14. Novel Mechanisms of Rapid Acting Antidepressants

14.1 Blockade of Tonic Firing GABA Interneurons in the Prefrontal Cortex is Required for the Rapid Antidepressant Actions of Ketamine and Scopolamine

Ronald Duman

Yale University School of Medicine, New Haven, Connecticut, United States

Background: Recent basic and clinical studies demonstrate novel, rapid, and efficacious treatments for depression. This includes the NMDA receptor antagonist ketamine, NR2B selective agents, the metabolite hydroxynorketamine, the glycine site partial agonist GLYX-13, mGlu2/3 receptor antagonists, the muscarinic receptor antagonist scopolamine, and M1-AChR selective agents that produce rapid and long-lasting antidepressant actions. Interestingly, the antidepressant effects of these diverse agents have in common a requirement for increased glutamate-AMPA receptor activity. However, the exact cellular trigger underlying the convergent effects of these rapid agents on glutamate-AMPA activity, whether indirect on GABA interneurons or direct on glutamate neurons, has not been determined.
Methods: To avoid general behavioral alterations caused by whole brain gene deletion we are using a region and cell specific viral shRNA strategy to knockdown NR2B or ACh-M1R on GABA vs. glutamate neurons in the medial prefrontal cortex (mPFC). For cell specificity NR2B or M1-AChR shRNAs were expressed in a Cre-recombinase dependent manner by infusion into CaMKII-, GAD67-, parvalbumin (PV)-, and somatostatin (SST)-Cre mouse lines. Receptor knockdowns are confirmed by double-immunolabeling and functional, electrophysiological recordings in recombined cells. Mice are examined in the forced swim test (FST) novelty suppressed feeding, (NSF), female urine-sniffing test (FUST) and open-field test (OFT) before and after ketamine or scopolamine administration.
Results: Viral-mediated knockdown of NR2B or M1-AChR on GABA interneurons in the mPFC of GAD67-Cre mice blocked or occluded the antidepressant actions of ketamine or scopolamine, respectively in the FST and NSFT, with no significant actions on the OFT. M1-AChR knockdown on GABA interneurons produced a similar blockade of scopolamine in the FUST; NR2B knockdown in this test had not yet been examined. In contrast, knockdown of NR2B or M1-AChR on pyramidal neurons in CaMKII-Cre mice did not significantly block the antidepressant response to ketamine in the FST or NSFT. Further studies of GABA interneuron subtypes demonstrate that knockdown of NR2B or M1-AChR in SST- but not PV-Cre mice blocks or occludes the effects of scopolamine and ketamine, respectively in the FST and NSFT.
Conclusions: The results indicate that tonic firing GABA interneurons in the mPFC act as the "cellular trigger" for ketamine and scopolamine, with acute blockade of NR2B or M1-AChR leading to disinhibition of pyramidal neurons and stimulation of glutamate-AMPA receptor activity. This drives increased synapse number and function and increased network connectivity that blocks or reverses the synaptic deficits caused by chronic stress and depression. Studies are being conducted to determine if other rapid agents, including GLYX-13 and hydroxynorketamine also act via GABA interneurons or if these agents produce direct effects on pyramidal neurons. The results also suggest that rapid acting antidepressants, via actions at SST interneurons, may correct dysregulated excitatory-inhibitory balance that contributes to chronic stress and depression related behaviors. This is supported by evidence of disrupted SST/GABA interneuron function in stress models and in postmortem brains of depressed subjects.
Disclosure: Part 1: Taisho, Consultant, Lundbeck, Consultant, Johnson & Johnson, Consultant, Lilly, Consultant, Forest, Consultant, Sunovion, Consultant, Naurex, Consultant, Psychogenics, Consultant, **Part 2:** Taisho, consultant, **Part 3:** Taisho, consultant, **Part 4:** Lilly, Consultant, Forest, Consultant, Taisho, Consultant, Sunovion, Consultant, Naurex, Consultant, Lundbeck, Consultant, **Part 5:** Yale, employee.

14.2 Hydroxynorketamine Metabolites of Ketamine Exert NMDA Receptor Inhibition-Independent Antidepressant Actions

Todd Gould

University of Maryland School of Medicine, Baltimore, Maryland, United States

Background: Major depressive disorder affects approximately 16 percent of the world population at some point in their lives and is among the leading causes of death. Despite a number of available monoaminergic-based antidepressants, these drugs require long-term administration to be effective, and many patients never attain sustained remission of their symptoms. Although the non-competitive glutamatergic N-methyl-D-aspartate receptor (NMDAR) antagonist, (R,S)-ketamine (ketamine), exerts rapid and sustained antidepressant effects, other NMDAR antagonists do not manifest identical actions, suggesting a distinct ketamine mechanism of action. Ketamine is stereoselectively metabolised into a broad array of metabolites, including norketamine, hydroxyketamines, dehydronorketamine and the hydroxynorketamines (HNKs).
Methods: Tissue distribution and clearance measurements of ketamine and ketamine metabolites were conducted with achiral liquid chromatography-tandem mass spectrometry. Antidepressant efficacy in mice was assessed with the forced swim test, novelty suppressed feeding test, learned helplessness test, social interaction following social defeat, and reversal of anhedonia (sucrose preference and female urine sniffing preference) following chronic corticosterone. Side-effect profiles were assessed with the open field test, pre-pulse inhibition, drug discrimination, rotarod, and intravenous drug administration. Electrophysiology assessing α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and NMDA receptor responses was conducted using field and whole-cell patch-clamp recordings from rat hippocampal slices complemented by mouse *in vivo* EEG recordings. Binding assays were used to assess NMDAR binding, and western blots to assess activity of intracellular signaling pathways.

Results: (R)-ketamine, which has lower affinity for the NMDAR, exerted superior antidepressant responses compared to (S)-ketamine. MK-801 (another NMDAR channel blocker) did not induce the sustained effects observed following ketamine administration. We showed that production of the (2S,6S;2R,6R)-HNK metabolite is essential for ketamine's antidepressant effects, and the (2R,6R)-HNK enantiomer exerts behavioral, electroencephalographic, electrophysiological and cellular antidepressant actions *in vivo*. These effects are NMDAR inhibition-independent but they involve early and sustained AMPA receptor activation and increase in gamma EEG power. (2R,6R)-HNK did not exert ketamine-associated self-administration, sensory dissociation or stimulant side effects.
Conclusions: Administration of (2R,6R)-HNK induces an acute increase in glutamatergic signalling (as supported by our EPSP, EPSC and EEG measurements), followed by a long-term adaptation involving the upregulation of synaptic AMPARs, as evidenced by an increase in GluA1 and GluA2 in hippocampal synapses. Our results indicate a novel mechanism underlying ketamine's unique antidepressant properties, which involves the required activity of a distinct metabolite and which is independent of NMDAR inhibition. These findings have relevance for the development of next generation, rapid-acting antidepressants.
Disclosure: Part 1: Janssen Pharmaceuticals, Consulting, **Part 4:** Roche Pharmaceuticals, Research Grant, Janssen Pharmaceuticals, Research Grant.

14.3 Rapastinel (GLYX-13) Produces Rapid Antidepressant Responses With Key Synaptic and Behavioral Effects Distinct From Ketamine

Joseph Moskal

Northwestern University, Evanston, Illinois, United States

Background: Rapastinel (GLYX-13) is an N-Methyl-D-aspartic acid receptor (NMDAR) modulator that acts as a functional glycine-site partial agonist that produces rapid antidepressant effects in rodents, but without the psychotomimetic-like side effects of ketamine. Rapastinel is a robust cognitive enhancer in multiple learning and memory tasks and is unique among NMDAR modulators in its ability to both enhance the magnitude of LTP and reduce LTD at Schaffer collateral-CA1 synapses. Rapastinel produces robust rapid-acting and long-lasting antidepressant-like actions in multiple animal models of depression, and these effects are associated with enhanced synaptic plasticity. Studies were conducted to further examine the molecular, cellular, and behavioral actions of rapastinel to further characterize the mechanisms underlying this agent.
Methods: The ability of rapastinel to activate the mechanistic target of rapamycin complex 1 (mTOR1) signaling was examined in medial prefrontal cortex (mPFC) synaptoneurosomes at 1 hr and 24 hrs post-dosing and these effects were compared to ketamine. The dependence of mTORC1 activation for the antidepressant-like effects of rapastinel was determined using pretreatment with mPFC injections of rapamycin in the rat forced swim test (FST), novelty suppressed feeding, and female urine-sniffing tests. The effect of rapastinel and ketamine on hypocretin and 5-HT induced excitatory postsynaptic currents in layer V pyramidal cells was examined along with their effects on dendritic spines and c-Fos expression in the mPFC. The ability of rapastinel to reverse chronic unpredictable stress (CUS) induced deficits in mPFC metaplasticity and mPFC-dependent positive emotional learning was also evaluated. The effects of rapastinel and ketamine on attention were measured using the 3 Choice Serial Reaction Time Task (SRT) and 5-HT2A activity was measured using the DOI-induced head twitch model.
Results: A single dose of rapastinel rapidly increases the mTORC1 pathway in the mPFC and infusion of the selective mTORC1 inhibitor rapamycin into the mPFC blocks the antidepressant behavioral actions of rapastinel. Rapastinel also rapidly increases the number and function of spine synapses in the apical dendritic tuft of layer V pyramidal neurons in the mPFC, and reversed CUS-induced deficits in metaplasticity in the mPFC and behavioral deficits in the positive emotional learning test. Rapastinel significantly increased the synaptic responses to hypocretin but not to 5-HT whereas ketamine increased both hypocretin and 5-HT responses. Consistent with this observation, rapastinel and ketamine increased attention in the SRT task, but only ketamine increased impulsivity and DOI/5-HT2A receptor-induced head twitches.
Conclusions: Rapsastinel appears to induce its antidepressant-like effects via mTORC1 activation in the mPFC and increases mature dendritic spine formation and thalamo-cortical hypocretin induced responses. The physiological and behavioral differences between rapastinel and ketamine may be related to the lack of psychotomimetic side effects of rapastinel compared to ketamine (i.e., 5-HT2A effects), as well as the therapeutic actions of these agents (hypocretin responses).
Disclosure: Part 1: Aptinyx Inc., Founder, Stock, Salary, Naurex Inc., Founder, Stock, Consultant, **Part 2:** Aptinyx Inc., Founder, Stock, Salary, Naurex Inc., Founder, Stock, Consultant, **Part 3:** Aptinyx Inc., Founder, Stock, Salary, Naurex Inc., Founder, Stock, Consultant, **Part 5:** Aptinyx Inc., Founder, Stock, Salary.

14.4 Bidirectional Homeostatic Regulation of Depression-Related Brain States by Defects in GABAergic Inhibitory Synaptic Transmission and Ketamine- or Genetically-Induced Enhancement of GABAergic Synaptic Inhibition

Bernhard Luscher

Penn State University, University Park, Pennsylvania, United States

Background: Major depressive disorder (MDD) is increasingly recognized to involve functional deficits in both GABAergic and glutamatergic synaptic transmission. Conversely, antidepressant drug treatments lead to normalization of such deficits. To elucidate the relationship between these phenotypes we made use of GABA-A receptor γ2 subunit heterozygous (γ2+/-) mice, which we previously characterized as a model animal with construct, face and predictive validity for MDD. In addition, we examined the role of

GABAergic inhibition by dendrite-targeting, somatostatin-positive GABAergic interneurons in the regulation of depression-related brain states.
Methods: To assess the consequences of GABAergic deficits on glutamatergic transmission we quantitated the cell surface expression of NMDA- and AMPA-type glutamate receptors and the function of excitatory and inhibitory synapses in the hippocampus and medial prefrontal cortex of γ2+/- mice. In addition, we analyzed the lasting effects of an acute dose ketamine on all these parameters in γ2+/- vs. wild-type mice. Lastly, we generated mice in which GABAergic synaptic transmission of principal cells was chronically enhanced by disinhibition of somatostatin (SST)-positive GABAergic interneurons, using cell-type-specific KO of the γ2 subunit in SST cells. We quantitated the neuronal excitability, the synaptic and tonic currents of SST and principal cells, and the anxiety and depression-related behavioral and biochemical phenotypes of these mice.
Results: Modest defects in GABAergic synaptic transmission of γ2+/- mice resulted in a strikingly prominent homeostatic-like reduction in the cell surface expression of NMDA- and AMPA-type glutamate receptors, along with prominent functional impairment of glutamatergic synapses in the hippocampus and medial prefrontal cortex. A single subanesthetic dose of ketamine normalized the glutamate receptor expression and synaptic function of γ2+/- mice to wild-type levels for a prolonged period, along with antidepressant-like behavioral consequences selectively in γ2+/- mice. Importantly, along with restoration of glutamatergic synapses ketamine also resulted in marked potentiation of GABAergic synapses, suggesting that the sustained antidepressant effects of ketamine required enhanced GABAergic synaptic transmission, and not just restoration of glutamatergic transmission. Consistent with a key role of GABAergic transmission in antidepressant mechanisms, chronic enhancement of GABAergic inhibition of principal cells by genetically-induced disinhibition of somatostatin-positive GABAergic interneurons was sufficient to reproduce anxiolytic- and antidepressant-like behavioral and biochemical endpoints of ketamine treatment.
Conclusions: Glutamatergic deficits associated with depressive disorders may be secondary to GABAergic deficits and involve homeostatic-like reductions of glutamatergic transmission. Sustained restoration of glutamatergic transmission by antidepressant therapies involves potentiation of GABAergic synaptic transmission. Consistent with this interpretation, genetic enhancement of GABAergic synaptic transmission by disinhibition of somatostatin-positive GABAergic interneurons has enduring antidepressant-like consequences independent of altered glutamatergic transmission. Our data merge the GABAergic and glutamatergic deficit hypothesis of MDD and describe MDD as a homeostatic maladaptation to chronic hyperexcitability that can be reversed by enhancement of GABAergic inhibitory synaptic transmission.
Disclosure: Nothing to Disclose.

Panel
15. Novel Mechanisms in Affective Disorder Risk Across the Lifespan: From Synapses to Behaviors

15.1 Childhood Adversity: Risk and Resilience for Affective Disorders During Peri-Menopause

C. Neill Epperson

University of Pennsylvania, Philadelphia, Pennsylvania, United States

Background: Stress exposures are likely to have differential impact on risk and resilience for depression depending upon their timing across development. We sought to determine whether adverse childhood experiences (ACE) and their onset with respect to puberty contributes to the increased risk observed for first episode of major depressive disorder (MDD) during the menopause transition.
Methods: Participants were from the Penn Ovarian Aging Study (POAS) cohort, which is comprised of racially diverse group of women from Philadelphia County who underwent behavioral, cognitive, and endocrine evaluations approximately yearly between 1996-2012 and completed the Adverse Childhood Experiences Questionnaire (ACE-Q) at study endpoint ($n = 243$). ACEs that first occurred 2 or more years before menarche were considered pre-pubertal. Incident menopause MDD was defined as first observed onset of the disorder in the peri- to post-menopause transition using the Structured Clinical Interview for Diagnosis-III-R and PRIME-MD.
Results: In this cohort with roughly 3000 observations over 16 years, incident menopause MDD occurred in 48% of the 100 women who reported lifetime MDD. Women reporting 2 + Total ACEs were at significantly greater risk for lifetime (aOR = 2.05, $p = 0.034$) and incident menopause MDD (aOR = 2.58, $p = 0.03$) compared to those reporting 0 ACEs; women with 2+ Post-pubertal ACEs were 2.3. times more likely to experience incidence menopause MDD ($p = 0.024$) after controlling for race, smoking, body mass index and employment. Experiencing only one ACE in the pre-pubertal window, regardless of additional ACEs post-puberty, was associated with reduced risk for lifetime and incident menopause MDD.
Conclusions: Timing and number of adverse experiences with respect to puberty differentially impacted risk and resilience for MDD across the female lifespan and during the menopause transition in this community cohort.
Disclosure: Part 1: Forest Laboratories, Expert opinion, Sage Therapeutics, Research Grant, Asarina Pharma, Consulting.

15.2 Plasticity of Excitatory Synapses in the Genesis and Treatment of Depression

Scott Thompson

University of Maryland School of Medicine, Baltimore, Maryland, United States

Background: There is increasing evidence of a weakening of a weakening of subsets of excitatory synapses in the nuclei of the forebrain mesolimbic reward circuitry in response to

rodents subjected to chronic stress- a strong predictor of depression in humans. Conversely all major antidepressant drugs, including SSRIs and ketamine, strengthen excitatory synapses in these same brain regions. We have hypothesized that these changes in excitatory synapses underlie the anhedonia that is a common symptom of human depression and its treatment.
Methods: Male rats and mice were subjected to various chronic stress protocols (chronic restraint stress, chronic unpredictable stress) over 2-3 weeks. Endpoints included behavioral assays of reward (sucrose preference, social interaction, female urine preference), electrophysiological measurements of synaptic strength (hippocampal temporammonic - CA1; hippocampus - NAc), and Western blot assays of protein and phospho-protein expression levels.
Results: 1. The strength of temporoammonic - CA1 synapses is weakened by chronic stress. SSRIs act slowly and novel antidepressants (Hydroxynorketamine, alpha5 subunit selective GABA negative allosteric modulators) act quickly to strengthen TA and Schaffer collateral synapses. 2. Hippocampal projections to the NAc display activity-dependent long-term potentiation that is induced by CaM kinase activity and mediated postsynaptically by a change in AMPA receptor function. These synapses are weakened by chronic stress and their plasticity is altered.
Conclusions: These data offer support for the hypothesis that major depression is caused by a weakening of specific subsets of excitatory synapses in multiple brain regions that are critical in the determination of affect and reward, including the hippocampus, prefrontal cortex and nucleus accumbens. Many of the characteristic changes in behavior that define the symptomatology of human depression, such as anhedonia and depressed mood, result because impaired excitatory synaptic transmission leads to reduced activity in the cortico-mesolimbic reward circuitry and impaired dopamine release in response to normally rewarding stimuli. Restoration of excitatory synaptic strength is the critical action of effective antidepressants, including both conventional agents, such as SSRIs, and newer compounds, such as ketamine.
Disclosure: Nothing to Disclose.

15.3 Oxytocin Modulation of Reward Circuitry

Robert Malenka

Stanford University School of Medicine, Stanford, California, United States

Background: Social withdrawal and social anxiety are associated with many psychiatric disorders including affective disorders. Oxytocin has been implicated in playing an important role in a variety of prosocial behaviors but its detailed actions on critical brain circuits are poorly understood. Here, recent results on the modulation of reward circuitry by oxytocin and how such modulation contributes to a variety of prosocial behaviors will be presented. The therapeutic implications of the findings will also be discussed.
Methods: A variety of methods were used in these studies of mouse social behaviors. These include viral tracing approaches to map oxytocin inputs to key nodes of reward circuitry, *in vivo* optogenetic manipulations to test the behavioral roles of these inputs, and electrophysiology assays in brain slices.
Results: Evidence will be presented that oxytocin neurons in the paraventricular nucleus of the hypothalamus send projections to the nucleus accumbens and ventral tegmental area. The actions of activating or inhibiting oxytocin release in these two structures in the context of social behaviors will be described. The synaptic actions of oxytocin in these two structures as assayed by whole cell voltage clamp recordings in brain slices will also be presented.
Conclusions: The actions of oxytocin on two key nodes of classic mesolimbic dopamine circuitry, the nucleus accumbens and the ventral tegmental area, are critical for its role in social behaviors.
Disclosure: Nothing to Disclose.

15.4 Epigenetic Editing of the CDK5 Locus for the Study of Stress and Depression

Elizabeth Heller

University of Pennsylvania, Philadelphia, Pennsylvania, United States

Background: Depression, a chronic and highly heritable disorder, exacts an enormous toll on the global population. Over the past several decades it has become clear that changes in gene expression likely underlie this disorder, yet no human gene polymorphisms have been associated with depression. Such gene expression changes my thus be conferred by epigenetic modifications, rather than gene mutations. Because epigenetic modifications occur across thousands of genes in both human patients and animal models of depression, it has been difficult to relate such regulation at a single gene to the behavioral consequences of chronic stress. Our current approach takes advantage of recent advances in epigenome editing technology to manipulate one particular gene implicated in depression, Cdk5, which has been shown to underlie stress responsiveness in various brain regions in rodent models of depression.
Methods: This project examined the role of Cdk5 in the context of social-defeat stress in mice, a behavioral model that mimics that chronic nature of depression and has been used as a correlate of stress-related pathophysiology for over a decade. The regulation of expression and epigenetic modifications to the Cdk5 gene following social-defeat stress in several brain regions were examined using quantitative RT-PCR and chromatin immunoprecipitation (qChIP). To directly examine the causal function of such changes, engineered transcription factors were introduced into the mouse brain. These factors directly modify the epigenome specifically at the Cdk5 gene, in order to assess the effect on Cdk5 expression and stress-evoked behavior. In this way it is possible to probe the exact molecular mechanisms by which epigenetic remodeling contributes to depression, an approach that can be applied broadly to myriad genes in the context of depression and other neuropsychiatric diseases.
Results: Cdk5-ZFP-p65 efficiently and robustly activate Cdk5 expression in NAc neurons, while Cdk5-ZFP-G9a represses expression. Cdk5-ZFP-G9a deposits H3K9me2 specifically at the Cdk5 gene *in vivo*, while Cdk5-ZFP-p65 activates Cdk5 via H3K9/14 acetylation. In addition, we

found epigenetic remodeling at the Cdk5 promoter, including enrichment of of related histone modifications. Repression of Cdk5 in NAc by Cdk5-ZFP-G9a sensitizes animals to subthreshold defeat stress, while activation has a protective effect.
Conclusions: Using engineered transcription factors, we have identified a direct molecular mechanism for stress-mediated epigenetic remodeling of the Cdk5 gene, and have efficiently manipulated behavioral responses to social defeat stress. This approach allows a functional analysis of chromatin modifications that underlie affective disorders.
Disclosure: Nothing to Disclose.

Mini Panel
16. tDCS, TMS and EEG: What can one Tell the Other About the Brain?

16.1 Direct Current Stimulation Accelerates Synaptic Models of Learning in Animals

Marom Bikson

The City College of New York, New York, New York, United States

Background: Learning and sensory processing in the brain relies on the effective transmission of information across synapses. The strength and efficacy of synaptic transmission is modifiable through training and can be modulated with noninvasive electrical brain stimulation. Transcranial direct current stimulation (tDCS), specifically, induces weak intensity and spatially diffuse electric fields in the brain. Despite being weak, electric fields modulate spiking probability and the efficacy of synaptic transmission. These effects critically depend on the direction of the electric field relative to the orientation of the neuron and on the level of endogenous synaptic activity. tDCS has been used to modulate a wide range of neuropsychiatric indications, for various rehabilitation applications, and cognitive performance in diverse tasks. How can a weak and diffuse electric field, which simultaneously polarizes neurons across the brain, have precise changes in brain function? Designing therapies to maximize desired outcomes and minimize undesired effects presents a challenging problem.
Methods: A series of experiments using rat brain slices and computational models are used to define the anatomical and functional factors leading to specificity of tDCS.
Results: Anatomical specificity may derive from guiding current to targeted brain structures and taking advantage of the direction- sensitivity of neurons with respect to the local electric field. Functional specificity may originate from preferential modulation of neuronal networks that are already active. Diffuse electric fields may recruit connected brain networks involved in a training task and promote plasticity along active synaptic pathways. *In vitro*, electric fields are able to boost endogenous synaptic plasticity and raise the ceiling for synaptic learning with repeated stimulation sessions. Synapses undergoing strong plasticity are preferentially modulated over weak synapses. Therefore, active circuits that are involved in a task could be more susceptible to stimulation than inactive circuits. Moreover, stimulation polarity has asymmetric effects on synaptic strength making it easier to enhance ongoing plasticity.
Conclusions: These results suggest that the susceptibility of brain networks to an electric field depends on the state of synaptic activity. Combining a training task, which activates specific circuits, with tDCS may lead to functionally-specific effects. Given the simplicity of tDCS and the complexity of brain function, understanding the mechanisms leading to specificity is fundamental to the rational advancement of tDCS.
Disclosure: Nothing to Disclose.

16.2 Motor Cortical Excitability as a Biological Predictor and Mediator of Depression Improvement

Andre Brunoni

University of Sao Paulo, Sao Paulo, Brazil

Background: Motor cortical excitability (MCE) is a non-invasive technique that uses single and paired transcranial magnetic stimulation (TMS) pulses to indirectly index the activity of brain GABA and Glutamate. Previous findings showed that major depressive disorder (MDD) patients present deficits in GABAergic activity as well as, for some forms of depression, Glutamatergic activity. We hereby explored the role of MCE as a predictor and mediator (correlate) of depression improvement in a subsample of the ongoing double-blinded trial titled "Escitalopram vs. Electric Current Therapy for Treating Depression Clinical Study (ELECT-TDCS)".
Methods: Eighty-five depressed patients, who were antidepressant-free and moderately depressed at baseline, were randomized to receive bifrontal tDCS ($n = 31$), 10mg/d escitalopram ($n = 37$) or sham tDCS/placebo ($n = 17$). MCE measures were collected in both hemispheres at baseline and after 3 weeks of treatment and included Cortical Silent Period (CSP), Intracortical Inhibition (ICI), and Intracortical Facilitation (ICF), which are indexes of GABAB, GABAA and Glutamate receptor-mediated activity. Change in depression severity was evaluated using the Hamilton scale (HDRS).
Results: There were no differences between left and right MCE measures at baseline and at week 3. After controlling for age and gender, depression improvement was significantly associated with ICI and ICF measures in the right hemisphere at baseline ($F = 5.96$, $p < 0.01$ and $F = 6.42$, $p < 0.01$, respectively), with lower ICI values (increased inhibition) and lower ICF (decreased facilitation) values predicting improvement. Moreover, an increase in right ICF during the trial was also associated with greater depression improvement ($F = 3.98$, $p = 0.049$). In patients receiving escitalopram, lower ICF and lower CSP values in the right hemisphere at baseline were associated with greater response ($ps < 0.05$). For patients receiving tDCS, there was a trend to lower ICF and lower ICI values in the right hemisphere at baseline to be associated with greater response ($ps = 0.06$). Finally, no association between MCE measures and depression improvement was observed in the placebo group.
Conclusions: MCE measures of the right but not left hemisphere were associated with depression improvement. Decreased inhibition and increased facilitation at baseline –a

pattern observed in MDD patients - was associated with overall non-response. Moreover, an increase of ICF (i.e., of glutamatergic activity) between baseline and week 3 predicted clinical response. Moreover, only CSP (indicative of GABAB activity) predicted escitalopram response. These findings indicate that MCE measures at baseline can predict depression improvement and suggest that changes in glutamatergic activity are associated with improvement of depressive symptoms.
Disclosure: Nothing to Disclose.

16.3 Theta-Gamma Coupling as a Mechanism for Working Memory in Individuals at Risk for or With Alzheimer's Dementia

Michelle Goodman

University of Toronto, Toronto, Canada

Background: Working memory impairments among individuals with Alzheimer's dementia (AD) are common. These impairments are also observed among two populations that are at high risk for AD: individuals with late-life depression (LLD) and individuals with mild cognitive impairment (MCI). Still, little is known about the mechanisms that underlie these impairments across these three populations. Theta-gamma coupling (TGC) – the modulation of gamma oscillation amplitude by theta oscillation phase – is a neurophysiologic process that supports working memory function in healthy individuals and is dependent on robust synaptic plasticity. Thus, the overall aim of this study is to evaluate TGC as a mechanism underlying working memory deficits across older healthy individuals and individuals with AD, LLD, or MCI. Further, through Paired Associative Stimulation (PAS), a neurophysiologic technique that uses transcranial magnetic, and peripheral nerve stimulation to assess frontal lobe plasticity, we will explore the relationship between frontal lobe plasticity and TGC in the healthy individuals and those with AD.
Methods: Four groups of participants performed the N-back working memory task combined with electroencephalography (EEG) to assess TGC during N-back performance: AD group ($N=$ 18, Mean Age = 75.8, SD = 6.7); LLD group ($N=$ 17, Mean Age = 72.8, SD = 4.0); MCI group ($N=$ 19, Mean Age = 74.6, SD = 6.0); and Healthy Control (HC) group ($N=$ 16, Mean Age = 75.1, SD = 4.8). Participants with AD and HC also received PAS to assess plasticity in the dorsolateral prefrontal cortex following the N-back-EEG session.
Results: There was a significant group effect on 2-back performance ($F(3, 66) = 27.3$, $p < 0.001$) with post-hoc analyses showing that the AD group performed worse than all other groups. Consistently, there was a significant group effect on TGC during 2-back performance ($F(3, 66) = 9.6$, $p < 0.001$) with post-hoc analyses showing that the AD group experienced less TGC than all other groups. Further, across all groups, there was a strong association between 2-back performance and TGC (Pearson's $r = 0.40$, $p = 0.001$). On plasticity, the AD group was impaired on the PAS-measure of plasticity compared to the HC group ($t(26) = 2.89$, $p = 0.008$) and across these two groups plasticity was associated with 2-back performance (Pearson's $r = 0.44$, $p = 0.018$). Finally, mediation analysis demonstrated that there was a significant indirect effect of plasticity on 2-back performance through TGC (ab = 21.5, BCa CI [0.52 – 72.9]) with TGC accounting for 28.5% of the total effect.
Conclusions: Our results suggest that TGC is a mechanism of working memory, which is common across individuals with AD or at risk for AD. When this mechanism fails, as seen in those with AD, it leads to working memory deficits. Results also suggest that deficits in frontal lobe plasticity in AD lead to impairments in working memory through impairments in TGC. Future work is needed to confirm these findings in larger samples. We will also discuss current work assessing whether TGC and plasticity are targets for interventions such as tDCS to improve working memory in these populations.
Disclosure: Nothing to Disclose.

Mini Panel
17. Neurochemical-Functional Correlations in Health and Disease

17.1 What Glutamate in the Brain's Spontaneous Activity can Tell Us About Childhood Trauma and Self-Relatedness

Georg Northoff

University of Ottawa Faculty of Medicine, Ottawa, Canada

Background: Previous studies demonstrated neural activity in specifically the cortical midline structures including medial prefrontal cortex (MPFC) to be implicated in childhood trauma and self-relatedness. While any neural activity is based on the excitation-inhibition balance (EIB), it remains unclear whether its underlying biochemical correlate namely Glutamate and GABA modulate childhood trauma and self-relatedness. Recent fMRI-MRS studies focused mainly on GABA and its relation to stimulus-induced or task-evoked activity (Duncan et al, 2014 for review). In contrast, the role of Glutamate in specifically modulating spontaneous or resting state activity remains unclear.
Methods: We conducted two studies. The first study combined rest fMRI and aversion-task fMRI with MRS in MPFC and anterior insula in 20 healthy adult subjects. The aversion task consisted in a mild electric shock. Psychologically, the childhood trauma questionnaire was applied. fMRI resting state analysis focused on spatiotemporal patterns as analyzed with Multiscale entropy.
The second study combined MRS in MPFC with EEG during a task where subjects had to decide upon the degree of self-relatedness (high or low self) of visually presented emotional pictures (as based on the International Picture System/IAPS). EEG analysis first focused on event-related potentials (ERP) and then in pre- and post-stimulus event-related spectral perturbation (ERSP) in relation to low and high self-related stimuli.
Results: First study. Our data show significant correlation between resting state entropy and childhood trauma: the higher the MSE in MPFC in the resting state, the higher the degree of early traumatic childhood events (CTQ). Moreover, we observed correlation of both MPFC entropy and traumatic

childhood events/CTQ with the level of glutamate in MPFC: lower levels of glutamate in MPFC mediated higher levels of entropy and childhood trauma/CTQ. This, in turn, was related to task-evoked activity as induced by aversion.
Second study: Our data show significant difference in early and late ERP between high and low self-related trials. Moreover, we observed that pre-stimulus differences in alpha power (-600 to -400ms) predicted whether the subsequent trial was assessed as high or low self-related. Finally, we observed significant correlation of MPFC glutamate levels with pre-stimulus alpha power difference between high and low self-related trials.
Conclusions: Taken together, our data show (i) relationship between early childhood trauma and adult levels of glutamate and resting state entropy in MPFC which in turn impacts subsequent aversion-related task-evoked activity; (ii) MPFC levels of glutamate are related to pre-stimulus alpha that predicts assessment of subsequent stimuli as high or low self-related.
These data show a clear role of Glutamate in mediating resting state activity and its relation to mental features like childhood trauma and self-relatedness. Since both childhood trauma and self-relatedness are central in various psychiatric disorders like schizophrenia or depression, our data carry important implications for the clinical realm.
Disclosure: Nothing to Disclose.

17.2 Relationship Between Bold Activation During Response Inhibition and Glutamate in the Anterior Cingulate Cortex (ACC) in Healthy Volunteers and Patients With Schizophrenia

Stefano Marenco

National Institute of Mental Health, Bethesda, Maryland, United States

Background: Previous investigations by our group have demonstrated that patients with schizophrenia (SCZ) show diminished blood oxygenation level dependent (BOLD) activation in the dorsal anterior cingulate cortex (dACC) during a response inhibition task. Here, we confirm these findings in another sample and assess the role of dACC neurochemistry measured with magnetic resonance spectroscopy (MRS) in these findings. Specifically, we tested the relationship between gamma-amino-butyric-acid (GABA) and Glx (an index reflecting glutamate and glutamine) and inhibition-related BOLD response in the dACC and the dorsolateral prefrontal cortex (DLPFC).
Methods: 102 controls (mean age = 30 ± 9.3, 30 males) and 52 SCZs (mean age = 31 ± 10.2, 36 males) were studied. GABA and Glx were measured in the dACC with j-edited MRS at 3T. BOLD activation during the Flanker task (no-go vs. congruent contrast) was compared betwen groups with ANCOVA in SPM5, using sex, age and performance as covariates. The same statistics were used to assess correlations of dACC GABA and Glx with BOLD activation in the dACC and DLPFC. Corrections for multiple comparisons were applied within predefined regions of interest derived from the AAL atlas (dACC, left and right middle frontal gyrus). In order to assess the directionality of the findings, the BOLD activation data were extracted from a sphere of 5 mm radius around the peak of statistical significance and correlation analyses were run in Statistica. We investigated ratios of metabolites to Creatine (Cre) as our primary MRS variables. If significant findings emerged, we sought confirmation by examining ratios to water and expected no association of Cre/water as a negative control.
We also wanted to link differences found in our primary analyses to cognitive performance, since this is ultimately a target for intervention in schizophrenia. In order to do this, we used no-go task performance during the scan and intelligence quotient (IQ), estimated by four subtests of the WAIS, as dependent variables in backward stepwise multiple regression, with MRS metabolites, age, sex, education and BOLD activation as the independent variables. We allowed all 2-way interactions in the models, and removed those that were collinear with the main effects.
Results: Task performance was lower in SCZ (91 ± 0.07% correct) compared to controls (0.94 ± 0.06), but we used first level analyses where only correct trials were included. BOLD activation in the dACC ($p = 2.2e-3$) and left DLPFC ($p = 7.8e-4$) was greater in controls than SCZ during response inhibition. There was a significant between-group difference in the relationship between Glx and BOLD response in the dACC (x,y,z = 9, 9, 36; $p = 3.8e-5$, Family-wise error [FWE] corrected), in the left DLPFC (x,y,z = -33,48,0; $p = 1.23e-5$, FWE corrected), and in the right DLPFC (x,y,z = 30, 30, 27; $p = 1.2e-4$, FWE corrected), such that Glx/Cre was positively correlated to BOLD activation in controls (range of r values: $0.22 < r < 0.32$) but negatively correlated to BOLD activation in SCZ ($-0.42 < r < -0.26$). Similar interactions were found for Glx/water in the DLPFC and the dACC ($4.04e-5 < p < 3.21e-4$). The relationships of GABA/Cre with BOLD activation had similar directionality as Glx/Cre but lower effect size. As expected, no significant associations of BOLD signal and Cre/water were found.
Task performance was unrelated to metabolites or BOLD activation extracted from the dACC or the DLPFC. Glx/Cre ($p < 5e-4$, partial eta2 = 0.09), age ($p < 5e-5$, partial eta2 = 0.12), education ($p < 0.001$, partial eta2 = 0.08) and diagnosis ($p < 5e-6$, partial eta2 = 0.16) each explained a significant portion of the variance in IQ (overall adjusted r2 = 0.39 ($p < 1e-6$).
Conclusions: Our findings support previous studies showing that BOLD activation in the dACC during response inhibition differs between patients and controls. Our data also suggest that abnormal glutamatergic regulation of BOLD activation in the prefrontal cortex might contribute to altered activation patterns between groups, and that Glx in the dACC contributes to cognitive ability. Work is ongoing to better characterize the impact of metabolite variation on functional connectivity and cognition in schizophrenia.
Disclosure: Nothing to Disclose.

17.3 Glutamate and GABA Relate to Auditory ERP and Visual fMRI Measures in Healthy and Schizophrenia Volunteers

Laura Rowland

University of Maryland School of Medicine, Baltimore, Maryland, United States

Background: Research indicates that patients with schizophrenia have altered auditory and visual sensory

mechanisms that likely contribute to higher order cognitive dysfunction common to the illness. It is not fully known if glutamate and GABA relate to altered sensory processing in schizophrenia. Findings from two multimodal neuroimaging studies focused on auditory and visual processing mechanisms in schizophrenia will be presented. The first study investigated the relationship between auditory mismatch negativity (MMN) and magnetic resonance spectroscopy (MRS) measures of GABA and glutamate. The second study investigated if glutamate and GABA predict visual plasticity as assessed with fMRI.

Methods: Forty-five patients and 53 controls completed MMN, MRS, and neuropsychological testing in the first study. The second study is ongoing with 18 controls and 12 patients having completed fMRI/MRS and neuropsychological testing. MR scanning was conducted on a 3T Siemens Tim Trio. Spectra were acquired from the medial frontal (study 1) and the visual cortex (study 2) using a STEAM sequence optimized for glutamate and MEGA-PRESS with macromolecule suppression for GABA. The fMRI visual plasticity paradigm consisted of a low-frequency visual stimulation for visual cortex activation, followed by a high-frequency stimulation to induce visual plasticity, and another low-frequency stimulation for visual cortex activation. Changes in BOLD activation are thought to reflect long-term potentiation.

Results: Findings of study 1 indicated that MMN amplitude and glutamate were reduced in schizophrenia. Smaller MMN amplitude was significantly related to lower GABA, lower glutamate, and higher glutamine/glutamate ratio, and poorer working memory in schizophrenia. These relationships were not significant in the control group. Further modeling of these measures revealed that GABA and glutamine/glutamate were tightly correlated, and that glutamine/glutamate strongly related to MMN, which in turn was strongly related to working memory in schizophrenia. Findings of study 2 indicated that visual cortex activation was significantly enhanced following high frequency stimulation in the control group, indicating that visual plasticity was induced. This effect was not significant in patients. Both glutamate and GABA were positively correlated with visual plasticity in the control group. There was a suggestion that the relationship between glutamate and GABA and visual cortex activation change were in the negative direction in schizophrenia, although not statistically significant.

Conclusions: These data provide evidence that support glutamatergic and GABAergic regulation of auditory MMN and verbal working memory function in schizophrenia. Results also support that *in vivo* glutamate and GABA predict visual plasticity in controls but not in schizophrenia. Altered glutamate and GABA regulation of visual plasticity in schizophrenia may explain these results. These overall findings provide strong support for the contribution of glutamate and GABA to electrophysiological and fMRI sensory measures in healthy and schizophrenia samples.

Disclosure: Nothing to Disclose.

Panel
18. Hormonal Regulation of Neural Circuit Organization and Function: Implications for Neuropsychiatric Illnesses

18.1 Nature and Nurture: Control of Innate Sexually Dimorphic Social Behaviors

Nirao Shah

University of California, San Francisco, San Francisco, California, United States

Background: The relative contribution of nature and nurture to the neural control of social behaviors is poorly understood. Insights into the roles of developmentally programmed hard-wiring and social experience have implications not only for a fundamental understanding of how the brain controls interpersonal interactions but also for judicial institutions and therapeutic approaches to mental illness. Our research program focuses on the molecular and neural circuit control of sexually dimorphic social behaviors. Sexually reproducing species exhibit developmentally hard-wired sex differences in behaviors that enhance reproductive success of the individual and survival of progeny. Indeed, sexually dimorphic social behaviors such as territorial aggression are instinctual in the sense that they can be displayed without prior training. Territorial aggression is flexible and purposive such that males are territorial in their home range but not when intruding in novel environments, and the aggression is directed to other males but not females. Previous work from our group has identified a molecularly specified population of neurons in the hypothalamus that is essential for male territorial aggression. Here we will present recent findings from our studies aimed at understanding how social context and experience influence these neurons to control territorial behaviors.

Methods: We use genetically engineered mice bearing a progesterone receptor (PR) allele into which Cre recombinase has been inserted via homologous recombination (PR-Cre). This approach permits Cre expression in PR-expressing cells, including PR-expressing neurons in the hypothalamus that we have shown to be essential for male territorial aggression. We manipulate the activity of these neurons using virally delivered optogenetic or pharmacogenetic channels and determine the effects on behavioral outcomes such as territorial aggression.

Results: Solitary males are aggressive to males that intrude in their home cage. Strikingly, we find that activation of PR-expressing hypothalamic neurons enables solitary males to attack males independent of pheromone signaling or even in absence of gonadal sex hormones. Moreover, such solitary males exhibit aggressive displays to females, other species, their own image in a mirror, and even when they are inserted as intruders in the cage of a solitary male. By contrast, activation of these neurons is not sufficient to activate aggression in socially housed males that are inserted as intruders in the cage of a solitary male. In other words, social housing with other males suppresses territorial aggression in a dominant manner.

Conclusions: We find that gonadal sex hormone and pheromone signaling are functionally upstream of PR-expressing neurons whereas social context is functionally

downstream in of these neurons in the circuit underlying male territorial aggression. More generally, we find that the social context (nurture) has a profound influence on a male's ability to display aggression and can suppress the behavior even though the underlying neural pathway is active. Ongoing studies are aimed at uncovering the mechanisms whereby social context suppresses aggression.
Disclosure: Nothing to Disclose.

18.2 Reproductive Steroids Gate Encoding and Function of a Hypothalamic Reward Circuit

Jenna McHenry

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

Background: Women are more than twice as likely to be afflicted by affective disorders and such disparity is evident during times of hormonal flux. Clinical and preclinical evidence link reproductive hormone flux with affective regulation and dysfunction, yet no studies have identified the precise neural circuitry involved. The medial preoptic area (mPOA), a steroid responsive, sexually dimorphic area interconnected with motivational circuits and essential for reproductive behavior in humans and rodents alike. Here, we investigate the behavioral and physiological actions of steroids in molecularly defined mPOA circuits that underlie motivational states in female mice.
Methods: First we optogenetically manipulate a molecularly defined mPOA population containing neurotensin (mPOA Nts) and assess reward behavior and motivation for a reproductive stimulus across hormonal states in intact cycling female mice or ovariectomized females with or without estradiol (E2). Next, we express a calcium indicator in mPOA Nts neurons and perform *in vivo* 2-photon microscopy in awake behaving females. We image activity dynamics from individual mPOA Nts cells across hormonal states and during timelocked odorant delivery. Neuroanatomical tracing reveals dense mPOA Nts fiber expression in the ventral tegmental area (VTA), thus we examine reward behavior during photostimulation of mPOA-VTA Nts fibers. Lastly, we photostimulate mPOA-VTA Nts fibers during fast-scan cyclic voltammetry and measure *in vivo* striatal dopamine release in the presence or absence of E2.
Results: *In vivo* photoactivation of mPOA Nts neurons induced a real-time place preference and self-stimulation, most robustly in proestrus versus estrus, compared to controls ($F(3,27) = 11.37$, $p < 0.001$). In ovariectomized mice, E2 priming enhanced the rewarding effects of mPOA Nts photoactivation ($F(12,99) = 7.630$, $p < 0.001$) and male preference ($F(2,13) = 7.363$, $p = 0.007$). Photoinhibiton of mPOA Nts neurons reduced male preference ($F(1,12) = 7.92$, $p = 0.016$) and male odor investigation ($F(1,11) = 8.07$, $p = 0.016$). *In vivo* imaging revealed that individual mPOA Nts neurons adjust their spontaneous activity following E2 administration ($t(32) = 2.141$, $p = 0.040$) and preferentially encode opposite-sex chemosensory cues. Photoactivation of mPOA-VTA Nts fibers induced similar reward phenotypes as seen with mPOA Nts somatic stimulation and this was enhanced by E2 ($F(1,18) = 15.35$, $p = 0.001$). Lastly, mPOA-VTA Nts stimulation evoked larger dopamine responses in E2 females compared to Veh controls ($t(9) = 3.437$, $p = 0.007$). All statistical measures reported above are interactions derived from two-way ANOVAs or t-tests.
Conclusions: Collectively, these studies indicate that steroid hormones, such as E2, gate the activity dynamics of individual mPOA Nts neurons to generate appropriate behavior tuned to the reproductive state, in part through midbrain reward systems. These are the first findings to functionally identify a molecularly defined estrogen-gated reward circuit within the mammalian brain. Elucidating the role of sex-specific neural circuits in motivational states will provide important insights for development of sex and reproductive based treatments for psychiatric disorders, such as reproductive mood disorders.
Disclosure: Nothing to Disclose.

18.3 Sex Differences and the Role of Estradiol in Behavioral and Neurochemical Responses to Cocaine

Jill Becker

University of Michigan, Ann Arbor, Michigan, United States

Background: Men currently constitute the majority of cocaine users; however, use among women is rapidly increasing and in the 13-18 year age range there is no sex difference. Furthermore, women begin using cocaine and enter treatment at earlier ages than men and have more severe cocaine use at intake. Thus, the progression to dependence may differ between the sexes, with women progressing from initial use to dependence at a faster rate. Part of this difference relates to sex differences in the reinforcing effects of cocaine and the loss of interest in natural rewards. Using a novel choice self-administration paradigm, in which animals develop a preference for either cocaine or tasty food pellets, we have previously demonstrated that a greater proportion of females develop cocaine preferences (CP) compared to males. CP's were associated with increased motivation for cocaine and reduced motivation for food in both sexes. Furthermore, acquisition of cocaine-taking behavior and cocaine-induce dopamine release in dorsolateral striatum (DLS) are enhanced by estradiol in female rats.
Methods: Female and male rats were tested in our choice self-administration paradigm where animals self-administer either cocaine or pellets in independent 30 min sessions and then are allowed to choose whether to self-administer cocaine or pellets. In one experiment, additional control animals were given cocaine and/or pellets on a non-contingent schedule, and feces were collected at the end of each week to analyze changes in the microbiome across preference formation. In a second experiment, the anti-estrogen ICI-182780 was delivered bilaterally to DLS or nucleus accumbens of male and female rats.
Results: We found that the fecal microbiota communities of CP and pellet-preferring (PP) male rats were significantly different from each other. Differences may be the result of changes from PP to CP preference, or may be driving the change in preference. The female CP and PP rats did not differ from each other, but did differ from the non-self-

administering rats overall. The fecal bacteria communities of male and female rats were significantly different from each other. The anti-estrogen experiments suggest that estradiol is important for initiation of cocaine taking in female rats.
Conclusions: The results of the gut microbiome studies indicate there are sex differences in the microbiota of rats. Furthermore, males exhibit a shift in fecal bacterial communities with CP, but females do not. Possible sex differences in the gut-brain axis and the actions of estradiol in modulating cocaine-taking will be discussed.
Disclosure: Nothing to Disclose.

18.4 Neural and Genetic Mechanisms of Sex Differences, Extended Amygdala, Fear and PTSD

Kerry Ressler

Harvard Medical School, Belmont, Massachusetts, United States

Background: Posttraumatic stress disorder (PTSD) affects 5-10% percent of the US adult population with a higher prevalence among women compared to men. While it remains unclear how biological sex associates with susceptibility to PTSD, one mechanism may involve a role for estrogen in a gene by environment interaction. We previously demonstrated a sex-dependent association between the pituitary adenylate cyclase-activating polypeptide type 1 receptor (PAC1) and PTSD, where carriers of a C allele at SNP rs2267735 within the PAC1 receptor gene (ADCYAP1R1) have increased symptoms of PTSD. This SNP is located within a predicted estrogen response element (ERE), which regulates gene transcription when bound to estradiol (E2) activated estrogen receptor alpha (ERa). This presentation will both review recent GWAS findings differentiating male and female findings in PTSD, as well as expand upon our understanding of this specific functional SNP within the ADCYAP1R1 gene as a mediator of sex x genotype effects on stress responsiveness.
Methods: In the current study, we examined E2 regulation of ADCYAP1R1 *in vitro*, in cell culture, and *in vivo* in mice and humans. We will utilize a combination of Genome wide association studies (GWAS), human targeted SNP validation studies, rodent behavior, and rodent neural circuitry studies including optogenetics, to delineate the role of sex-specific effects on fear and fear-related behaviors.
Results: Several recent GWAS studies have found sex differences related to stress and PTSD (at the GWAS $p < 10e\text{-}7$ level). Furthermore, we and others have replicated the sex-specific effect of ADCYAP1R1 in both humans and animal models. Notably, we find in mice that fear conditioning and E2 additively increase ADCYAP1R1 expression (main effects and interactions, $p < 0.05$). *In vitro*, we show that E2/ERa preferentially binds to the ADCYAP1R1 ERE, with less efficient binding to an ERE containing the C allele of rs2267735 ($p < 0.05$). In women with low serum E2, the CC genotype associates with lower ADCYAP1R1 expression ($p < 0.05$), which further associates with higher PTSD symptoms. Finally, we will report additional unpublished data recording from cells within amygdala that express the estrogen E2 receptor and ADCYAP1R1 mediating stress and fear responses.
Conclusions: These findings 1) suggest that the biology leading to differential stress responses in males and females may be notably different; 2) a number of GWAS studies are leading to different targets in males and females, particularly in stress and trauma-related disorders; 3) a previously identified target, based on convergent human and rodent genomics, ADCYAP1R1, is differentially induced by both stress and estrogen in an additive manner; 4) these findings lead to a model in which E2 induces expression of ADCYAP1R1 through binding of ERa at the ERE as an adaptive response to stress. These data suggest that inhibition of E2/ ERa binding to the ERE containing the rs2267735 risk allele results in reduced expression of ADCYAP1R1, diminishing estrogen regulation as an adaptive stress response and increasing risk for PTSD.
Disclosure: Nothing to Disclose.

Study Group
19. Shared Solutions for Prescription Opioid Abuse and Pain

Nora Volkow*, David Thomas, Christopher Jones, Richard Nahin, Gary Franklin, Sean Mackey, Tom McLellan

National Institute on Drug Abuse, Rockville, Maryland, United States

Prescriptions for opioids increased from 76 million in 1991 to 207 million in 2013, which were associated with parallel increases in opioid-related morbidity and mortality. Currently, close to 100 million Americans suffer from chronic pain and may be given prescription opioids, along with close to two million Americans who abuse opioid analgesics, with over 19,000 overdose deaths attributed to prescription opioids in 2014. This situation reflects both the challenge to effectively treat the complex condition of chronic pain and the lack of understanding about opioid abuse potential and the risk of addiction in healthcare settings. This study group will discuss the intertwined prescription opioid and pain epidemics gripping the United States, both of which are exasperated by the overreliance on opioids in the treatment of pain. The panel will also discuss shared approaches for improved treatment of pain and diminishing the prescription opioid epidemic. Two major federal strategies which were released in tandem in March 2016 which address the overreliance on opioids will be described. One is the CDCP Guideline for Prescribing Opioids for Chronic Pain which recommends limits on opioid prescribing and the employment of various safeguards. The second is the National Pain Strategy which promotes comprehensive pain treatment, where opioids, if prescribed, are part of a much broader and integrated pain treatment program. Speakers will then discuss an array of approaches which can mitigate the diversion and abuse of prescription opioids and reduce the risks of opioids to pain patients who use the opioids as prescribed. Further, opioid use disorder and pain are not independent, and strategies treat both pain of opioid use disorders will be discussed. Abuse/misuse deterrent formulations, prescription data monitoring plans and non-opioid pain treatment approaches (both pharmacological and non-pharmacological) will be discussed. And lastly, clinician

education will be considered. Both pain and problematic opioid use are common issues, and often intertwined. Coordinated treatment approaches based on the best available science to treat individuals with various degrees of both problems needs careful consideration.
Disclosure: Nothing to Disclose.

Panel
20. Neurogenomics From the Darker Side

20.1 Modeling Brain Mosaicism With hiPSC-Based Neurogenesis

Michael McConnell

University of Virginia School of Medicine, Charlottesville, Virginia, United States

Background: Brain somatic mosaicism, now revealed and tractable due to advances in single cell 'omic approaches, has emerged as an intriguing and unexplored aspect of neuronal diversity. A major contributor to brain somatic mosaicism is copy number variants (CNVs). Neuronal CNVs, like most CNVs, are likely brought about by DNA repair mechanisms acting on transcription- or replication-induced DNA damage.
Methods: We performed single cell genome analysis on primary human neurons and human induced pluripotent stem cell (hiPSC)-derived neural progenitor cells (NPCs) and neurons.
Results: Sequencing of 110 frontal cortex neurons from three young adult neurotypical individuals identified 148 CNVs in 45 neurons. CNVs ranged in size from 2.9 to 75 megabases, with a preponderance of deletion CNVs relative to duplication CNVs. Furthermore, CNVs were rare in human fibroblasts or hiPSC-derived NPCs; however, hiPSC-derived neurons harbored CNVs at rates and sizes similar to primary neurons.
Conclusions: Megabase-scale CNVs are found in as many as 41% of primary human neurons. hiPSC-based neurogenesis recapitulates this basic finding and provides an *in vitro* system to study the mechanisms that bring about somatic mosaicism in human neurons.
Disclosure: Nothing to Disclose.

20.2 The Changing Neural Genome: LINE1-Associated Somatic Mosaicism and Environmental Stressors

Jennifer Erwin

Salk Institute for Biological Studies, La Jolla, California, United States

Background: Mental illnesses, such as schizophrenia, are complex traits that result from both genetic and environmental influences. How environmental stressors, such as maternal immune activation, contribute to a long-lasting alteration of the way the human brain processes information from the environment remains unknown. Growing evidence implicates somatic mosaicism and transposons as sensors of environmental stress in the brain. Previous studies demonstrate that 1) genetic variation occurs not only as variation in the germline but also within the somatic cells of an individual, termed somatic mosaicism and 2) stressful environmental experiences, such as maternal immune activation, drives Long interspersed element-1 (L1) activity during embryonic neurogenesis. L1 is an active mobile endogenous element capable of de novo insertions into new genomic locations, leading to somatic mosaicism in the human hippocampus and other regions.
Methods: Herein, we investigate the role of L1 in the creation of somatic mosaicism in the healthy and diseased brain. We developed a high-throughput sequencing method, to specifically capture Somatic L1 Associated Variants in bulk tissue and single nuclei from post-mortem human tissue, which we refer to as SLAV-seq. We also use a mouse maternal immune activation model combined with attenuation of L1 retrotransposition to specific attenuate L1 retrotransposition during embryonic neurogenesis. Finally, we use a combination of molecular, immunohistochemistry and genetic studies to investigate the mechanism of somatic mosaicism and retrotransposition during neural development.
Results: We found that somatic events occur at a similar rate in both glia and neurons, at a rate of ~0.45-0.98 events per cell, and affect at least 36% of the cells in the healthy brain. These variants are present in crucial synaptic genes, such as PSD93. Somatic events occurred during a variety of neural development stages, including in an early progenitor cell that contributes to both hippocampus and frontal cortex. Other events occurred late in development and could only be detected in a single cell. We demonstrate that a subset of SLAVs are, in fact, somatic deletions generated by L1 endonuclease cutting activity and resolved by homology-mediated mechanisms independent of retrotransposition.
Conclusions: Our data suggest that L1 sequences are prone to homology-mediated mechanisms of DNA repair in the soma and can generate somatic CNVs in the brain. By identifying a heritable component that contributes to somatic mosaicism, we reveal the potential for features of somatic mosaicism to be subjected to natural selection, suggesting a heritable, genetic state with regions of the genome predisposed to somatic CNVs in the brain. In addition, our data suggests that somatic mosaicism may mediate neuropsychiatric relevant gene-environment interactions.
Disclosure: Nothing to Disclose.

20.3 Developmental and Genetic Regulation of the Human Frontal Cortex Transcriptome in Schizophrenia

Andrew Jaffe

Lieber Institute for Brain Development, Baltimore, Maryland, United States

Background: Recent genome-wide association studies (GWAS) have identified over 100 loci that confer risk for schizophrenia, but potential mechanisms of risk for many individual loci are largely unknown. We hypothesize that many of these risk variants - regardless of the specific genomic regulation - will "read out" in the transcriptome and point towards the genes and their underlying specific transcripts.

Methods: We performed RNA sequencing in the human frontal cortex from 495 subjects across the lifespan, including 175 diagnosed with schizophrenia and 50 during the second trimester of prenatal life. We performed three main differential expression analyses - developmental regulation, differential by diagnosis, and genetic control via expression quantitative trait loci (eQTL) - centered around the latest GWAS risk loci and their underlying genetic variants.
Results: We identify widespread developmental regulation of transcription (15,603 genes with convergent signal at genome-wide significance), including preferential isoform usage across brain development (8,803 genes), which further associated with genetic risk for schizophrenia ($p < 1e\text{-}5$), neurodevelopment (FDR < 1e-8), and signaling (FDR < 1e-8). We identify extensive genetic regulation of nearby expression levels at genome-wide significance, including extensive transcript specificity, and find many schizophrenia risk variants associate with specific genes and transcripts. Lastly, using a novel algorithm for RNA quality adjustment, we identify 237 genes with significant (at FDR < 0.1) and replicated differential expression signal between cases and controls that implicate disrupted signaling processes (FDR < 0.01) that further converge on developmental and genetic regulation.
Conclusions: The convergence of human brain development and subsequent dysregulation in schizophrenia both appear to relate back to genetic risk for the disorder. Our data and approach therefore offer new insights into genetic mechanisms underlying schizophrenia, and create opportunities for new pathogenic models that may lead to new treatments for this debilitating disorder.
Disclosure: Nothing to Disclose.

20.4 Molecular Dissection of Schizophrenia GWAS Significant Loci

Daniel Weinberger

Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Background: Genome-wide association studies (GWAS) have identified many genetic variants associated with risk for schizophrenia but the underlying molecular mechanisms are largely unknown. Here we investigate gene expression in brain in the 11q24.3 and 10q24.32 GWAS significant loci, two regions highlighted in several recent "expression quantitative trait loci" (eQTL) studies.
Methods: We performed deep polyA enriched RNA sequencing (RNAseq) (median 80M 100bp paired end reads/sample) on 658 dorsolateral prefrontal cortex (DLPFC) samples from the Lieber Institute repository, and tested association between genotypes at schizophrenia risk variants and nearly expression (eQTL analysis). We surveyed multiple expression features beyond counting reads mapping to annotated genes, including exon and novel junction counts.
Results: rs10791098 genotype (an LD r-squared = 1 proxy for GWAS significant SNP rs10971097) at the 11q24.3 locus was associated with expression of SNX19 ($p = 0.0014$), in a discovery sample of 108 adult Caucasian controls. However, a more granular analysis revealed that the GWAS risk "A" allele was much more strongly associated with expression of the junction between exons 8 and 10 in our entire sample ($n = 658$; Junc8.10 1.63E-35), which measures predicted transcript XM_005271546, and not with the expression of the junction between exons 8 and 9, which measures the full gene NM_014758 (Junc8.9 $p = 0.538$). Interestingly, only 46% of the samples had Junc8.10 reads > 0; this expression threshold was dramatically related to genotype ($p = 2.39 \times 10\text{-}43$). qPCR confirmed these results in DLPFC ($n = 79$; Junc8.10 $p = 2.23e\text{-}7$, Junc8.9 $p = 0.240$) and hippocampus ($n = 44$; Junc8.10 $p = 8.96e\text{-}8$, Junc8.9 $p = 0.710$). GTEx RNAseq data showed similar association with Junc8.10 expression in cortex ($n = 95$; Junc8.10 $p = 2.05e\text{-}8$) but not with Junc8.9, with a similar pattern in frontal cortex ($n = 90$; Junc8.10 $p = 6.56e\text{-}5$, Junc8.9 $p = 0.172$) hippocampus ($n = 85$; Junc8.10 $p = 3.74e\text{-}4$, Junc8.9 $p = 0.756$), and 10 other brain regions. A prior report of a strong eQTL association with expression of exon 8a is based on a SNP that is not GWAS significant, suggesting that 8a expression is not about schizophrenia risk. In the 10q24.32 locus, risk alleles are associated in the human brain with a previously uncharacterized, human-specific arsenite methyltransferase (AS3MT) isoform lacking exons two and three (AS3MTd2d3) ($p = 1.15x10\text{-}27$), which is more abundant in individuals with schizophrenia than in controls ($p = .007$). The full-length transcript shows none of these associations. AS3MTd2d3 protein lacks arsenite methyltransferase activity and is more abundant in brain than other tissues (Li et al, Nature Medicine 2016). GWAS risk SNPs across this region are linked with a VNTR polymorphism in the first exon of AS3MT that is associated with expression of only AS3MTd2d3 in both Caucasians and African Americans ($p = 1.99x10\text{-}30$). VNTR genotype predicts promoter activity in luciferase assays, as well as DNA methylation within the AS3MT gene ($p = 9.31x10\text{-}39$). AS3MTd2d3 is expressed in adult human neurons and astrocytes, and is upregulated during human stem cell differentiation toward neuronal fates. Neither the novel AS3MTd2d3isoform nor the VNTR are found in the chimpanzee genome/transcriptome.
Conclusions: We identify a human unique and recent evolutionary isoform encoded at the AS3MT locus of unknown function as a molecular mechanism of risk underlying the 10q24.32 GWAS locus and a specific SNX19 isoform not previously identified as the underlying molecular mechanism of risk in the 11q24.3 locus. High level gene expression analyses that do not resolve brain relevant isoforms will miss some molecular mechanisms of genetic associations with schizophrenia.
Disclosure: Nothing to Disclose.

Panel
21. New Advances in Targeting Large-Scale Neurocircuits of Depression With Brain Stimulation

21.1 Network Targets for Invasive and Noninvasive Brain Stimulation

Michael Fox

Harvard Medical School, Boston, Massachusetts, United States

Background: Both invasive and noninvasive brain stimulation propagate beyond the stimulation site to impact

distributed brain networks. Brain connectivity imaging may allow us to predict these network effects, relate different stimulation sites to one another, and predict the efficacy of stimulation sites in individual patients.
Methods: Resting state functional connectivity from a large cohort of normal subjects was used to identify brain networks associated with effective invasive or noninvasive stimulation sites across 14 different neurological and psychiatric diseases. Networks associated with brain stimulation targets for the same disease were compared. This network topography was then used to prospectively predict individual responses to transcranial magnetic stimulation (TMS) for depression.
Results: Invasive and noninvasive stimulation sites for the same disease were part of the same resting state network. In depression, the location of each patient's TMS site with respect to this network topography predicted the patient's antidepressant response.
Conclusions: Therapeutic targets for both invasive and noninvasive brain stimulation are likely distributed brain networks, not single brain regions. Brain connectivity imaging can predict individual responses to stimulation and may identify optimal stimulation sites to impact a target network.
Disclosure: Part 1: Mint Labs, Consulting, Neuroelectrics, Intellectual Property.

21.2 Neurocircuit Functional Connectivity in the Antidepressant Mechanism of TMS

Marc Dubin

Weill Cornell Medical Center, New York, New York, United States

Background: Repetitive transcranial magnetic stimulation (TMS) is an effective treatment for major depressive disorder (MDD). TMS targeting the dorsolateral prefrontal cortex (DLPFC) modulates functional connectivity of the default mode network (DMN) in depressed patients and pre-treatment functional connectivity in the DMN predicts treatment response to TMS. Functional connectivity of both ventral and dorsal frontostriatal networks have been found to be altered in MDD and this may underlie deficits in reward processing and anhedonia (ventral) as well as deficits in psychomotor and executive function (dorsal). Here, we investigated the hypothesis that functional connectivity of frontostriatal networks is modulated by TMS and that pre-treatment functional connectivity in these networks predicts treatment response.
Methods: 27 patients with treatment-resistant depression, having failed two antidepressant trials of adequate dose and duration in the current episode, and 27 healthy controls, underwent resting state fMRI. A seed-based analysis of functional connectivity used bilateral limbic, executive, and motor striatal seeds based on tractographic connectivity to bilateral ventromedial (limbic), dorsolateral (executive) and rostral and caudal (motor) frontal cortex (Cerebral Cortex 24 (5):1165-77). Patients underwent a 5-week course of 10Hz TMS over the left dorsolateral prefrontal cortex. HAMD-24 quantified depression severity and treatment response. Patients also were imaged with post-TMS resting state fMRI. Functional connectivity was compared between patients and controls using ANCOVA and between pre and post TMS using a repeated measures ANCOVA. Cluster thresholding was used to correct for multiple comparisons.
Results: The mean reduction in HAMD-24 after TMS was 34.4%. 15 subjects showed a $>$ 30% reduction in HAMD24, which we defined as response. Compared to healthy controls, depressed subjects showed functional hypoconnectivity in several regions of interest (ROIs) in the executive and motor frontostriatal networks: R and L DLPFC, L Frontopolar cortex, and R Dorsal Anterior Cingulate, R and L Supplementary Motor Area and R Premotor Area. No regions of altered connectivity were observed in the limbic frontostriatal network or caudal motor frontostriatal network. No regions of hyperconnectivity were found. Pre-treatment functional connectivity of the Left DLPFC ROI and its associated striatal seed correlated with the % reduction in HAMD-24 from pre to post TMS ($R2 = 0.58$, $p = 0.0015$). Additionally, the responder group had higher functional connectivity at baseline. This finding was unique to this frontostriatal connection; it was not observed for the frontostriatal connections corresponding to the other 6 ROIs. There were no significant changes in frontostriatal functional connectivity from pre to post TMS within the 7 ROIs.
Conclusions: These results confirm previous findings that depression affects executive and motor frontostriatal circuits. Further, it suggests that a necessary condition for the antidepressant effect of TMS is that TMS has access to basal ganglia circuitry via a functionally intact frontostriatal connection originating from the target site. It also raises the question of whether a similar predictive relationship would exist for right DLPFC and left Frontopolar targets and their associated frontostriatal connections. These results are discussed in the context of their relationship to the findings in the default mode and central executive networks.
Disclosure: Part 1: Tal Medical, Grant, **Part 2:** Tal Medical, Grant.

21.3 Complexity of Brain Temporal Dynamics in Explaining the Cognitive and Antidepressant Effects of Seizure Therapy

Faranak Farzan

University of Toronto, Toronto, Canada

Background: The variability in the brain temporal dynamicity observed through electroencephalography (EEG) reflects richness of information processing across distributed brain networks subserving affective and cognitive processes. One biological phenomenon that impacts this dynamicity is seizure. Seizure therapy remains the most effective treatment in major depressive disorder (MDD), while its biological target remains unclear. Furthermore, the invasive nature and cognitive adverse effects of seizure therapy have restricted its widespread use. We investigated the hypothesis that seizure therapy impacts mood and cognition by modulating the brain temporal dynamicity.
Methods: We obtained resting-state EEG from thirty-four patients receiving two types of seizure treatments - electro-convulsive therapy (ECT) or magnetic seizure therapy

(MST). We quantified the complexity of brain's temporal dynamics before and after seizure therapy.
Results: Reduction of complexity in fine time scales underlined successful response to both seizure treatments. Greater reduction in complexity of fine time scales in midline frontal and posterior brain regions was linked with greater improvement in mood. In ECT only, complexity of coarse time scales was significantly increased. Greater increase in complexity of coarse time scales was associated with greater decline in cognition. Region and time-scale specific changes in complexity classified both antidepressant and cognitive response to seizure therapy with good (80%) and excellent (95%) accuracy, respectively.
Conclusions: Region and time-scale dependent changes in complexity of brain temporal dynamics is a novel mechanistic marker of response to seizure therapy that explains both the antidepressant response and cognitive changes associated with this treatment. This marker has tremendous potential to guide design of the new generation of antidepressant treatments.
Disclosure: Nothing to Disclose.

21.4 Transcranial Magnetic Stimulation Affects Intrinsic Connectivity Networks in Depression

Stephan Taylor

University of Michigan, Ann Arbor, Michigan, United States

Background: Repetitive transcranial magnetic stimulation (rTMS) is an effective therapy for major depressive disorder (MDD), with unknown mechanisms of action. Recent work has shown that MDD is characterized by aberrant interactions between intrinsic connectivity networks (ICN), and we sought to test the hypothesis that rTMS treatment changes ICNs using functional MRI to probe ICNs before and after rTMS therapy.
Methods: Thirty-two patients (45.5 yrs, 21 females) with major depressive disorder entered a sham-controlled, double-blinded, randomized trial of rTMS (10 Hz, 3000 pulses/session) to the left dorsolateral prefrontal cortex (dlFPC). fMRI scans at baseline and after 20 rTMS sessions were obtained during rest (8 min, eyes open). Activation during an n-back task identified the target for left dlPFC stimulation via neuronavigation. After the second fMRI scan, subjects either completed 5 additional rTMS treatments (active arm, $n=16$) or went on to receive 25 active treatments (sham arm, $n=16$). Standard techniques were used to clean and normalize BOLD scans. Seed voxels were used to identify an affective network (subgenual anterior cingulate cortex [sgACC], amygdala), default mode network (DMN; posterior cingulate cortex [PCC]) and the fronto-parietal network (dlPFC stimulation site).
Results: At the end of the blinded phase, there was a trend effect of TMS on MADRS scores (groupXtime interaction, $F=2.1$, $p=0.16$), but there was a significant effect on QIDS-SR scores after 25 treatments (groupXtime interaction, $F=11.5$, $p=0.002$). Analysis of the effect of rTMS (sham vs active) on connectivity revealed that sgACC connectivity with the left superior parietal lobule decreased from positive to negative (more normative pattern) in active, but not sham subjects ($k = 119$, $p<0.05$corr). There were no significant effects of rTMS on connectivity with the dlPFC seed. In a moderator analysis, we analyzed the relationship between baseline ICN activity and treatment response ($>$ 50% drop in MADRS), including subjects who started with sham but went on to active treatment (13 responders, 14 non-responders; 5 placebo responders excluded). This analysis found that greater DMN positive connectivity to the anterior insula (aIns – normal DMN connectivity is negative) was associated with a poorer treatment response ($k = 364$, $p<$ 0.002corr).
Conclusions: These results provide evidence that rTMS moves network connectivity between the affective network to a more normative pattern of negative correlation with a node of the fronto-parietal network. Altered connectivity of DMN with aIns, a node of the salience network involved in integration of internal states and behavioral control, may reflect a type of patient less likely to respond to dlPFC rTMS therapy. Overall, the results, while preliminary in a small sample, provide clues as to the mechanism of rTMS interacting with ICNs in the treatment of MDD.
Disclosure: Part 4: St. Jude Medical, research support, Neuronetics, Research support, Vanguard Research group, research support.

Panel
22. Synaptic Connections and Plasticity in Neuropsychiatric Disease

22.1 Topography and Dynamics of the Synaptic Cleft

Thomas Biederer

Tufts University, Boston, Massachusetts, United States

Background: Synaptic integrity is disrupted in neurodevelopmental disorders as well as in neurological diseases. Moreover, excitatory synapse structure and function are altered by addictive drugs. Defining the pathways that induce and coordinate synapse development is therefore of high importance to human health. Trans-synaptic interactions are now known to guide synapse development. Their important roles are underlined by the genetic link of synapse-organizing adhesion proteins like SynCAMs and the functionally related neurexins and neuroligins to autism-spectrum disorders and schizophrenia.
Methods: Our experiments apply the superresolution imaging approaches STED and 3D dSTORM, electron microscopy, cryo-electron tomography, and single particle tracking of adhesion proteins and neurotransmitter receptors at a superresolution level in live neurons.
Results: We have mapped the molecular and structural organization of the cleft of excitatory synapses at unprecedented resolution using superresolution imaging and EM approaches. Four key findings about the organization of excitatory synapses have emerged from these studies. First, the synaptic cleft is structurally patterned. Second, the cleft is molecularly organized, with different synapse-organizing proteins marking distinct compartments. Third, select synaptic adhesion complexes shape the edge of the cleft,

which can affect synaptic remodeling. Fourth, synaptogenic proteins can undergo activity-dependent re-distribution.
Conclusions: Our results begin to define the topography and molecular makeup of the synaptic cleft and we demonstrate that synaptic adhesion complexes mark distinct nanodomains within the cleft. Further, single particle tracking of synaptic adhesion proteins demonstrates that the cleft is a dynamic compartment of synapses. This progress provides new insights into the properties of synapse-organizing proteins and enables to better understand disease-linked aberrations in synaptopathies.
Disclosure: Nothing to Disclose.

22.2 Synaptic Organizers: Structural Features and Plasticity

Gabrielle Rudenko

University of Texas Medical Branch, Galveston, Texas, United States

Background: Synaptic organizing molecules or 'synaptic organizers' play a role in the formation, stabilization and maintenance of synaptic connections. These molecules are typically tethered to the pre-synaptic or post-synaptic membranes and their large extracellular domains protrude out into the synaptic cleft. Synaptic organizers can form macromolecular bridges spanning the cleft and they can organize protein interaction networks promoting synapse development and stabilization. Members of the neurexin superfamily of synaptic organizers form a wide portfolio of trans-synaptic bridges with their many partners, and strikingly, some of these proteins localize selectively to excitatory or inhibitory synapses. In addition, neurexin superfamily members and their partners are implicated in a wide variety of neuropsychiatric diseases including autism spectrum disorders and schizophrenia.
Methods: We have used a combination of structural, biochemical, and biophysical methods to gain insight into the structure and function of a number of synaptic organizers. By applying a combination of x-ray crystallography and electron microscopy we are able to reveal the architecture, dimensions and conformational variability of the molecules under study. In addition, by applying biophysical methods including surface plasmon resonance and solid phase binding assays, we are able to probe their protein: protein interactions.
Results: Using a panel of molecules, we reveal elegant structural mechanisms employed by members of the neurexin superfamily and their partners to control protein interactions in the synaptic cleft. These include 1) architecture and organization of domains in space, 2) molecular hinges, 3) modification of binding sites through alternative splicing, and 4) portfolios of competing binding sites.
Conclusions: Synaptic organizers use a portfolio of molecular and structural features which enable them to form plastic synaptic connections and to regulate protein interaction networks in the synaptic cleft, thereby promoting the formation and maintenance of functional neuronal circuits. Our data provide a molecular framework to understand the roles that synaptic organizers play in the synaptic cleft; this is important because lesions in many of these proteins are implicated in the pathogenesis of neuropsychiatric disease.
Disclosure: Nothing to Disclose.

22.3 How Do Astrocytes Shape Synaptic Circuits?

Cagla Eroglu

Duke University Medical Center, Durham, North Carolina, United States

Background: Proper establishment of synapses is critical for constructing functional circuits. Interactions between presynaptic neurexins and postsynaptic neuroligins coordinate the formation of synaptic adhesions. An isoform code determines the direct interactions of neurexins and neuroligins across the synapse. However, whether extracellular linker proteins can expand such a code is unknown.
Methods: We used a combination of *in vitro* and *in vivo* cell biological approaches utilizing primary neuron and astrocyte cultures as well as genetically engineered mice. Our analyses methods included histological and electrophysiological analyses of synaptic connectivity in cultured neurons or in mouse visual cortex.
Results: Using a combination of *in vitro* and *in vivo* approaches, we found that hevin, an astrocyte-secreted synaptogenic protein, assembles glutamatergic synapses by bridging neurexin-1 and neuroligin-1B, two isoforms that do not interact with each other. Bridging of neurexin-1 and neuroligin-1B via hevin is critical for the formation and plasticity of thalamocortical connections in the developing visual cortex.
Conclusions: These results show that astrocytes promote the formation of synapses by modulating neurexin/neuroligin adhesions through hevin secretion. Our findings also provide an important mechanistic insight into how mutations in these genes may lead to circuit dysfunction in diseases such as autism.
Disclosure: Nothing to Disclose.

22.4 Dissecting Synaptic and Circuitry Mechanisms of ASD

Guoping Feng

Massachusetts Institute of Technology, Cambridge, Massachusetts, United States

Background: Recent genetic studies have identified a large number of candidate genes for autism spectrum disorder (ASD), many of which encode synaptic proteins, suggesting that synaptic dysfunction might be a key pathology in ASD. In addition, recently, genetic studies have revealed a significant overlap of risk genes for ASD and schizophrenia. However, it is not clear how different mutations of the same gene could contribute to the manifestation of different diseases. One such example is the Shank3 gene. The Shank3 gene encodes a postsynaptic scaffolding protein critical for the development and function of glutamatergic excitatory synapses. Disruption of the Shank3 gene is thought to be the cause of the core neurodevelopmental and neurobehavioral deficits in Phelan-McDermid Syndrome, an autism spectrum

disorder. In addition, rare Shank3 mutations have been found in schizophrenia patients. Using various Shank3 mutant mice as a model system, we investigated neurobiological mechanisms of ASD-related phenotypes and mutation-specific defects.
Methods: Genetically engineered mice containing various Shank3 mutations were used as animal models for ASD. Electrophysiological recordings were performed on acute brain slices to examine synaptic function. GCaMP6 was used as calcium indicator to image neuronal activity in head-fixed awake animals to examine network activity. Standard behavioral paradigms were used to characterize behavioral abnormalities.
Results: Shank3 knockout mice exhibit oversensitivity to whisker stimulations, which we use as a model to study sensory overload phenomenon frequently seen in ASD patients. We found that pyramidal neurons in the somatosensory cortex of Shank3 knockout mice are more active than those in wildtype mice. On the contrary, inhibitory interneurons in the somatosensory cortex of Shank3 knockout mice are much less active than those in wildtype mice. Selective deletion of Shank3 in inhibitory interneurons leads to overactive pyramidal neurons in the n the somatosensory cortex. These data suggest that defects in inhibitory interneurons is the cause of hyperactivity of pyramidal neurons which might be linked to whisker oversensitivity in Shank3 knockout mice.
We also characterized two lines of mutant mice with Shank3 mutations linked to ASD and schizophrenia. We found both shared and distinct synaptic and behavioral phenotypes. Mice with the ASD-linked InsG3680 mutation manifest striatal synaptic transmission defects before weaning age and impaired juvenile social interaction, coinciding with the early onset of ASD symptoms. On the other hand, adult mice carrying the schizophrenia-linked R1117X mutation show profound synaptic defects in prefrontal cortex and social dominance behavior. Furthermore, we found differential Shank3 mRNA stability and SHANK1/2 upregulation in these two lines. These data demonstrate that different alleles of the same gene may have distinct phenotypes at molecular, synaptic and circuit levels in mice, which may inform exploration of these relationships in human patients.
Conclusions: Our data suggest that defects in inhibitory interneurons is the cause of hyperactivity of pyramidal neurons which might be linked to whisker oversensitivity in Shank3 knockout mice. In addition, our results from the studies of ASD- and schizophrenia-linked mutations demonstrate that different alleles of the same gene may have distinct phenotypes at molecular, synaptic and circuit levels in mice, which may inform exploration of these relationships in human patients.
Disclosure: Part 2: Rugen Therapeutics, equity, **Part 4:** Roche, Research grant.

Panel
23. Suicidal Behavior: Using Multimodal Data to Arrive at Biosignatures of Pathology and Response to Pharmacologic Interventions

23.1 Neurodevelopment of Suicidal Behavior in Adolescents With Bipolar Disorder

Hilary Blumberg

Yale University School of Medicine, New Haven, Connecticut, United States

Background: Individuals with bipolar disorder (BD) are at high risk of suicide, estimated at 15-20%. Adolescence is a critical period during which suicide behavior often manifests, including in BD, implicating neurodevelopment differences underlying the emergence of suicide behavior in adolescents. However, there has been little study of the brain circuitry that is associated with the development of suicide behavior in adolescents with bipolar or other disorders; and data that may help to elucidate the neural circuitry associated with risk for future attempts, and associated neurodevelopmental trajectories, are rare.
Methods: Multimodality magnetic resonance imaging (MRI) (structural MRI, diffusion tensor imaging and functional MRI) was performed for 68 adolescents with BD (38% who had made at least one suicide attempt), and 45 healthy comparison (HC) adolescents. Approximately 3 years later on average, a subset of the adolescents, with and without baseline suicide history, were reassessed for interim suicide-related symptoms and behaviors and participated in a second neuroimaging session. Baseline multimodality neuroimaging data were compared between adolescents with BD who had made an attempt, compared to those who had not, and between adolescents with BD who went on to make future attempts and those who did not. Longitudinal brain changes over time were also compared between the latter two groups. Results were compared to data in the HC group. Relationships between brain differences and suicide-related symptoms and behaviors were assessed.
Results: Analyses of baseline scanning data supported brain differences in the adolescents with BD and a history of suicide attempt, compared to the adolescents who had not made an attempt. These included decreases in ventral frontal and other frontotemporal corticolimbic regional gray matter volume and structural integrity, as well as decreases in amygdala-ventral prefrontal functional connectivity ($p<0.005$). Analyses of future attempters supported greater magnitude of these decreases at baseline in adolescents who made a future suicide attempt following their baseline assessment, compared to those who did not ($p<0.005$). In assessing longitudinal within-subject repeat scans, adolescents with interim suicide attempts showed altered development of ventral prefrontal gray matter as well as structural integrity of ventral prefrontal white matter over time, as compared to adolescents without interim attempts. Regional brain circuitry differences were associated with symptoms, such as suicidal ideation severity, and behaviors, such as lethality of attempts, and also showed influence of early adversity ($p<0.05$).

Conclusions: Taken together, these findings suggest that there are ventral frontotemporal corticolimbic system abnormalities, and developmental trajectory differences, in adolescents with BD that are associated with risk for and the development of suicide-related symptoms and behaviors. Implications for understanding the development of suicide behavior and for the identification of early targets and brain circuitry-targeted interventions to reduce risk and prevent suicide will be discussed.
Disclosure: Nothing to Disclose.

23.2 Ketamine and Potential Biomarkers of Suicidal Ideation Response

Elizabeth Ballard

National Institute of Mental Health, Bethesda, Maryland, United States

Background: There are currently no FDA approved medications for active suicidal thoughts and existing antidepressants can take weeks to months for efficacy. Consequently, the 400,000 Americans who present to the emergency department each year with suicidal thoughts may not receive the help that they need. Ketamine, a glutamate modulator, has been associated with rapid reductions in suicidal thoughts within minutes to hours in depressed patients. Identifying biomarkers of ideation response can aid in understanding ketamine's mechanism of action and may provide insights into the neural underpinnings of suicidal thoughts.
Methods: Data from 78 participants with suicidal ideation and treatment-resistant MDD or BP in ketamine clinical trials will be presented. Patients underwent clinical ratings, positron emission tomography (PET) scans and polysomnography as part of their participation. Potential biomarkers of ideation response to ketamine were evaluated in four areas: 1) baseline predictors of ideation response; 2) clinical correlates of ideation change; 3) imaging-related biomarkers of response on FDG PET imaging, and; 4) sleep-related biomarkers of response on polysomnography. Ideation response was measured using the suicide item from Hamilton Depression Rating Scale. The timeframe of interest was one day after ketamine infusion.
Results: Baseline predictors of suicide ideation change at one day after ketamine infusion included personal as well as family history of alcohol dependence ($p < .05$). Clinical correlates of ideation response include depression and anhedonia; results suggest that the relationship between change in suicidal thoughts and change in anhedonia symptoms may be independent of antidepressant change (Adjusted Beta = 0.40, $p = .003$). Initial imaging biomarkers from PET highlight the role of glucose metabolism in the infralimbic cortex (BA 25) in change in suicidal thoughts, but not antidepressant response (r = .54, $p = .02$). Lastly, nocturnal wakefulness, as defined by polysomnography, was associated with suicide ideation response; improvement in sleep quality was demonstrated in individuals with a reduction in suicidal thoughts after ketamine infusion ($p = .04$).
Conclusions: A number of potential biomarkers of suicide ideation response to ketamine were identified across family history, clinical rating, neuroimaging and sleep modalities. It is likely that suicidal ideation response to ketamine is multifactorial, reflecting the complexity of the acute suicide crisis. Results underscore the importance of a translational approach to suicide research. Future directions to identify promising treatment targets for suicide risk as well as identify patients most likely to respond to ketamine will be discussed.
Disclosure: Nothing to Disclose.

23.3 Brain Functional Genomic Approaches Followed by Coherent Anti-Stokes Raman Scattering Spectroscopy Associate Neurodevelopmental Changes With Suicide

Gustavo Turecki

McGill University, Montreal, Canada

Background: A history of childhood maltreatment (CM) significantly increases lifetime risk of suicidal behaviors. Recently, epigenetic regulation has emerged as a long-term genomic plasticity mechanism that has the potential to explain how early-life experiences may drive behavioral dysregulation.
Methods: To investigate possible neurobiological mechanisms contributing to this risk, we performed a genome-wide analysis of DNA methylation (RRBS) and gene expression (RNA-Seq) in the anterior cingulate cortex, a region that acts as a hub of emotional regulation that is sensitive to early-life experiences, in depressed suicides with ($N = 27$) or without ($N = 25$) a history of CM, and psychiatrically normal individuals ($N = 25$). We then applied seterology and Coherent anti-Stokes Raman Scattering (CARS).
Results: Our results demonstrate that CM associates with significant DNA methylation adaptations in 3 myelin-related genes (UIDs: 645191; 18991 and 3688; q-values: 3.08E-11; 6.47E-04; 7.45E-04, respectively) as well as a global down-regulation of genes responsible for oligodendrocyte lineage specification, myelin synthesis, and myelin integrity. We further investigated whether these molecular changes manifested in cellular changes of the ACC. The density of oligodendrocytes, as assessed by stereology, was significantly decreased in maltreated individuals ($P < 0.05$). We then applied Coherent anti-Stokes Raman Scattering (CARS), a spectroscopy technique that allows for high resolution imaging of myelinated fibers. Our results demonstrate that CM specifically associates with decreased myelination of axons (g Ratio; $P < 0.0001$).
Conclusions: Altogether, these complementary approaches converge to uncover global and long-term impairments of oligodendrocytes in the ACC following a history of CM. We propose, therefore, that healthy patterns of early-life development are essential for epigenetic programming of myelination in the maturing brain, and disruption of these processes by early-life adversity may represent a key mechanism contributing to psychopathology and lifetime suicide risk.
Disclosure: Nothing to Disclose.

23.4 Delineating Different Suicidal Phenotypes With Distinct Biosignatures

Maria Oquendo

Columbia University, New York, New York, United States

Background: Suicidal behavior (SB) is complex, heterogeneous, and linked to neurobiological, cognitive and behavioral factors. Thus, its underlying pathophysiology may be more tractable if SB sub-types can be identified.
Methods: Sample 1 (mood + personality disorders $n=134$) had PET and [11C]WAY-100635, a 5-HT1A antagonist, Stroop Task and Continuous Performance Test (CPT). We measured continuous suicidal ideation (SI) (vs Intermittent or None) [Scale for SI (SSI) item 6] and planning SI [sum of SSI items 12-18]. SB lethality scores range: 0 (no damage) - 8 (death) scale. Sample 2 (personality + mood disorders $n=98$), had Trier Social Stress Test (TSST); fMRI (recalling aversive personal memories; instructed to immerse or distance selves); and provided 7 days of ecological momentary assessment data.
Results: Sample 1: 13 made a suicide attempt and 2 suicided during a 2 yr follow-up. Future lethality was associated with 5HT1A BPF in dorsal raphe nucleus (DRN) and many cortical regions. Lower Stroop Interference scores (better cognitive control) and conservative CPT response bias (efforts to manage interference) predicted higher 5HT1A BPF in DRN and many cortical regions suggesting more damaging SB results from focused, well-planned behaviors. Sample 2: Those with childhood trauma had more aggression and reacted to disagreements or rejections with SI. Aggressive/impulsive subjects had the most cortisol reactivity, which predicted >5-point SI increases during follow-up. Compared to attempters, when distancing during the fMRI task non-attempters showed more precuneus and oPFC recruitment, implicated in perspective taking and integrating information about potential rewards/punishments to inhibit inappropriate affective responses.
Conclusions: We find 2 SB subtypes associated with distinct SI patterns. Continuous SI may be linked to blunted serotonergic function and better cognitive control, a constellation leading to planned, lethal SB, not necessarily in response to stress. Variable SI in response to life stress may be seen in those with childhood trauma, reactive aggression, and an exaggerated cortisol response to psychosocial stress, perhaps due to inability to harness neural pathways to manage distressing affect. Delineation of distinct biological and clinical features associated with SI subtypes may identify different risk patterns. Studies could examine the merits of pharmacologic or psychological interventions targeting affect regulation for variable SI, versus antidepressants or cognitive treatments for continuous SI.
Disclosure: Part 1: Bristol Myers Squibb, salary and stock (spouse), C-SSRS, royalties, **Part 2:** Bristol Myers Squibb, salary and stock (spouse), C-SSRS, royalties.

Panel
24. Understanding Network-Level Interactions Across Large-Scale Limbic Circuits

24.1 The Role of Hippocampal Input in the Construction of Task-Relevant Representations in the Medial Prefrontal Cortex

Joshua Gordon

Columbia University, New York, New York, United States

Background: The hippocampus and prefrontal cortex act cooperatively to perform both working memory and avoidance-based anxiety tasks. Prefrontal cortical neurons, meanwhile, represent task-relevant information. We hypothesized that information about task-relevant stimuli is conveyed from the hippocampus to the prefrontal cortex via direct hippocampal inputs, which we proposed might be required for prefrontal cortical representations.
Methods: To test this hypothesis, we used optogenetic terminal inhibition combined with multi-site neuronal recordings in both wild-type mice and mice carrying a mutation that predisposes to schizophrenia (the 22q11.2 microdeletion model). We used light to inhibit the terminals of Archeorhodpsin-expressing ventral hippocampal (vHPC) neurons within the medial prefrontal cortex (mPFC) while measuring the effects of this inhibition on neuronal representations of spatial goals in a working memory test, and of aversive aspects of the environment in an anxiety task.
Results: We found that inhibition of the vHPC terminals within the mPFC dramatically reduced task-relevant spatial representations within the mPFC, without consistent effects on overall firing rates. Synchrony between neural activity in the hippocampus and prefrontal cortex was also disrupted in both tasks, though this occurred in different frequency ranges in the two tasks. Inhibition of mediodorsal thalamus inputs into the mPFC had no such effects. In the 22q11.2 microdeletion model, both vHPC terminal structure and task-relevant representations in the mPFC were disrupted, consistent with the optogenetic results.
Conclusions: These data demonstrate the necessity of hippocampal input into the prefrontal cortex in the construction of neural representations of task-relevant information.
Disclosure: Nothing to Disclose.

24.2 Dopamine as an Aversive Signal in the Medial Prefrontal Cortex

Kay Tye

Massachusetts Institute of Technology, Cambridge, Massachusetts, United States

Background: Dopamine has long been implicated in reward-related processes, but more recently, evidence has arisen that suggests that dopaminergic input to the medial prefrontal cortex (mPFC) is also involved in aversion. The mPFC is involved in higher order cognitive functions and coordinates the execution of motivated behaviors though its projections to downstream targets. However, how mPFC neurons that project to different downstream targets represent and

coordinate motivated behaviors is unclear. Our goal is to understand how mPFC neuronal subpopulations encode stimulus valence and where this information is communicated downstream to instigate adaptive behavioral responses, such as approach or avoidance.
Methods: We use a combination of electrochemical (fast-scan cyclic voltammetry), optogenetic and imaging (GCaMP6) approaches to elucidate the circuit mechanism by which dopamine acts as an aversive signal in the mPFC.
Results: Here, we show that dopamine release in the mPFC occurs upon the presentation of an aversive stimulus using fast-scan cyclic voltammetry. We also find that different projection-defined populations of neurons in the mPFC have different encoding properties during behavior. Finally, we identify distinct downstream targets of the mPFC and show that they play separable functional roles in motivated behavior with projection-specific optogenetic manipulation.
Conclusions: Dopamine is released during both rewarding and aversive events, and dopamine released in the mPFC may act upon heterogeneous populations of neurons that have diverse downstream targets. A projection from the mPFC to the brainstem reveals at least one possible route by which dopamine is used as an aversive signal in the mPFC.
Disclosure: Nothing to Disclose.

24.3 How You Think: Structural Network Mechanisms of Human Brain Function

Danielle Bassett

University of Pennsylvania, Philadelphia, Pennsylvania, United States

Background: Cognitive function is driven by dynamic interactions between large-scale neural circuits or networks, enabling behavior. Fundamental principles constraining these dynamic network processes have remained elusive. I will discuss a recent application of network control theory to human neuroimaging data that provides new insights into the structural network mechanisms of human brain function.
Methods: Using diffusion spectrum imaging data, we build a structural brain network with 234 nodes (brain regions) connected by weighted edges (number of white matter streamlines linking brain regions). We employ a simplified noise-free linear discrete-time and time-invariant network model of neural dynamics in which the state of brain regions depends on the connectivity between them. We interrogate this model to determine the role of brain regions in different control strategies.
Results: Our results suggest that densely connected areas, particularly in the default mode system, facilitate the movement of the brain to many easily-reachable states. Weakly connected areas, particularly in cognitive control systems, facilitate the movement of the brain to difficult-to-reach states. Areas located on the boundary between network communities, particularly in attentional control systems, facilitate the integration or segregation of diverse cognitive systems.
Conclusions: As a whole, this body of work suggests that structural network differences between the default mode, cognitive control, and attentional control systems dictate their distinct roles in controlling brain network function. More generally, our results support the view that macroscale structural design underlies basic cognitive control processes via the fundamental mechanism of network controllability.
Disclosure: Nothing to Disclose.

24.4 Prefrontal Network Interactions Underlying Behavioral Flexibility

Vikaas Sohal

University of California, San Francisco, San Francisco, California, United States

Background: Prefrontal cortex regulates sensory responses, behavioral flexibility, and action selection. However, it remains unclear whether dissociable vs. largely overlapping prefrontal circuits mediate these functions.
Methods: To address this question, we measured auditory evoked response potentials (ERPs) and delivering targeted optogenetic inhibition in mice performing a task involving switching between auditory and visual cue-based rules.
Results: Bilateral optogenetic inhibition of medial prefrontal cortex disrupted auditory-visual switching and also reversed task-induced changes in auditory ERPs. By contrast, inhibiting prefrontal projections to mediodorsal thalamus disrupted rule switching without affecting ERPs. Finally, whereas performance on a single rule (no switching) was intact following bilateral prefrontal inhibition, unilateral inhibition elicited a near-complete preference for choices towards one side. Inhibiting callosal projections reproduced this phenotype without affecting auditory ERPs.
Conclusions: Thus, distinct prefrontal circuits subserve behavioral flexibility, top-down modulation of sensory processing, and left vs. right action selection. Furthermore, these processes can be dissociated within a single task.
Disclosure: Nothing to Disclose.

Mini Panel
25. Drug Discovery for Neuropsychiatric Disorders Using Human Pluripotent Stem Cell - Derived Cells

25.1 The Promise and Challenges of Using Reprogrammed Cells for Biological and Therapeutics Discovery in Mental Illness

David Panchision

National Institute of Mental Health, Bethesda, Maryland, United States

Background: The United States National Institute of Mental Health (NIMH), a component of the National Institutes of Health (NIH), seeks to improve the understanding and treatment of mental illness through basic and clinical research, paving the way for prevention, recovery and cure. Through its extramural program, NIMH supports more than 2,000 research grants and contracts at universities and other institutions across the country and overseas. NIMH has increasingly supported using human embryonic stem cells, induced pluripotent stem cells (hiPSCs), and similar patient-derived reprogrammed cells as surrogates for patient brain

tissue, which is rarely if ever accessible as biopsies from living patients. These reagents permit discovery-based analysis of human functional genomics, psychiatric disease mechanisms and developing assays to screen candidate small molecule therapeutics.
Methods: In 2008, NIMH was among the first NIH institutes to launch an initiative specifically to develop and use patient-derived hiPSCs. In addition to its continuous support of investigator-initiated hiPSC and related research, in 2011 NIMH established the NIMH Stem Cell Center to support the banking and distribution of these cells to the broader investigator community. As part of the ongoing integration of stem cell research with genomics research, the Center is contained within the NIMH Repository and Genomics Resource (RGR), which coordinates multi-project clinical/genetic data and biomaterial sharing (from ~150,000 subjects) among qualified investigators in the wider scientific community. In 2013, NIMH launched a public-private partnership (PAR-13-225) called the National Cooperative Reprogrammed Cell Research Groups (NCRCRG) to use patient-derived reprogrammed cells to develop validated platforms for identifying novel targets and developing new therapeutics or diagnostic tools.
Results: Early investment in exploratory hiPSC studies has yielded progress in several areas: (1) the generation of hundreds of patient and matched control hiPSC lines that are being made available through the NIMH RGR, (2) the improvement of differentiation protocols to generate neurons and glial cells with relevance to mental illness, (3) the development of novel assays that may distinguish normative from disease states, and (4) early evidence of mechanistic differences between cells from patient versus control hiPSC lines. Many of these assays and findings are now being optimized for rigor, reproducibility and predictive value through the NCRCRG program.
Conclusions: These initiatives promise to accelerate research toward the goal of developing robust and reproducible assays for discovering new therapeutics to reduce the burden of mental illness.
Disclosure: Nothing to Disclose.

25.2 High-Throughput Assays for Phenotypic Analyses and Drug Screening of hPSC-Derived Neurons: Balancing Throughput With Relevance

Anne Bang

Sanford Burnham Prebys Medical Discovery Institute, La Jolla, California, United States

Background: Development of patient specific hPSC based disease models to study the cellular and molecular bases of neuropsychiatric illness offers an opportunity to identify improved treatments, and to better stratify patients according to pathological processes. The biology and genetics underlying mental illness are complex and will require the examination of multiple cell types from many patient hPSC lines to identify and validate phenotypes. Development of procedures to interrogate hPSC derived neural cells in miniaturized higher-throughput formats will be advantageous not only for drug screening, but also for phenotype validation and discovery, allowing testing of multiple lines and variables, such as timing and dose response to therapeutic agents, pathway and immune modulators, and stress inducers. Here we describe the development of a suite of foundational assays in higher throughput formats to monitor neuronal morphology, mitochondrial function, and electrophysiology.
Methods: Assays were developed with hPSC-derived cortical neurons produced in-house using a protocol from Shi and Livesey (2012), and iCell and iDopa neurons (Cellular Dynamics International). Image based high content screens (HCS) to monitor neurite growth and multiplexed readouts for mitochondrial membrane potential (MMP) and reactive oxygen species (ROS) were performed on 384-well plates with an Opera (Perkin Elmer) and analyzed with Acapella software. Physiology assays utililized 48- and 96-well multi-electrode array (MEA) plates on the Maestro (Axion Biosystems), and were analyzed with Axis and Neuroexplorer softwares.
Results: We have performed pilot screens to validate our suite of assays. For neuron morphology, we screened a reference set of ~4000 compounds comprising known drugs, modulators of signaling pathways, and human metabolites, at multiple doses, ~26,000 wells in total, We evaluated 11 parameters incorporating neurite length, number, and branching, and nucleus-based measures of cytotoxicity. 41 compounds that enhanced and 38 compounds that decreased neurite growth, were identified including approved antipsychotic drugs. For the MMP/ROS live cell HCS we screened an 80 compound set of neuro-active pharmaceuticals and neurotoxins, at multiple time points in dose response, ~1000 wells in total, resulting in the identification of a panel of mitotoxins. Finally, we also screened the 80 compound set in dose response against our MEA assay, which performed with highly consistent well-to-well activity level and synchronous network bursting. Analysis of network behavior in response to pharmacological manipulation revealed dynamic plasticity of network output, with modulation of excitatory and inhibitory neurotransmission consistent with physiologically relevant network formation, a step towards development of models to evaluate drugs on networks comprised of neuropsychiatric patient hPSC-derived neurons.
Conclusions: We have demonstrated feasibility of developing robust high throughput assays, based on hPSC-derived neurons, key to realizing the potential of hPSC in drug discovery for neuropsychiatric diseases. These data establish assay platforms to interrogate fundamental aspects of neuronal morphology and physiology, providing a basis for further development of more complex phenotypic readouts and compound screens based on neuropsychiatric patient hPSC-derived neurons.
Disclosure: Nothing to Disclose.

25.3 High-Throughput Phenotyping of Human Pluripotent Stem Cell Derived-Neurons

Ajamete Kaykas

Novartis Institutes for Biomedical Research, Cambridge, Massachusetts, United States

Background: The majority of neuroscience drug discovery in the last 3 decades has yielded very few new drugs. One of the

major challenges we face is rodent models of neuropsychiatric and neurodegenerative disorders have shown limited success in identifying compounds that are effective in clinical trials. Traditionally, industry has relied on transformed or genetically-engineered heterologous systems to develop cellular assays for drug discovery. Stem cells offer distinct opportunities to establish genetically and functionally faithful neurological disease models, potentially bridging a significant gap in new target identification, validation and drug discovery. However, a major challenge in the field is establishing standardized differentiation protocols that yield human neurons with disease-relevant phenotypes at a truly industrial scale, in both quantity and screening format.
Methods: To this end, we established a fully automated human pluripotent stem cell (PSC) maintenance and excitatory cortical neuronal differentiation platform that enables parallel phenotyping of many different lines at once. We have generated over 100 iPSC lines from normal and psychiatric disease patients using non-integrating reprogramming technology. We are currently running these lines through automated differentiation platform and collecting genomic & phenotypic data. We have subjected all patients to whole genome-sequencing and are collecting RNA-seq at the pluripotency, neuronal precursor and neuronal phases of differentiation. We are collecting high content imaging data on the neurons to capture neuronal morphology and synaptic formation and FDSS/calcium imaging as a proxy for network activity. This large data set will allow us to look at reproducibility and technical noise at unprecedented levels and allow us to correlate the clinical phenotypes of patients, genotype, transcriptional networks and cellular phenotype. Further analysis of this large dataset may yield new targets for psychiatric disease and/or cellular phenotypes that could be used for high-throughput screening.
Results: We have generated a library of over 100 iPSC lines from normal controls and patients with schizophrenia. We have differentiated some of these lines into cortical excitatory neurons using an automated cell culture platform. We have collected a battery of genomic and phenotypic data on these lines in hopes to develop a cellular model for schizophrenia.
Conclusions: This human disease-modeling platform is being integrated into Novartis' lead discovery pipeline to identify new targets, molecules, and to shed light on the cellular aspects of human neuronal biology.
Disclosure: Nothing to Disclose.

Mini Panel
26. PACAP: A New Regulator of the Stress Response?

26.1 Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) Disrupts Motivation, Attention, and Social Interaction

Rachel Donahue

Harvard Medical School, Belmont, Massachusetts, United States

Background: The PACAP system has been proposed to play a major role in mediating behavioral responses to stress. While the effects of PACAP on anxiety-like behaviors are well characterized, stress related disorders also involve other behavioral domains. The present studies were designed to investigate how PACAP affects behaviors (motivation, attention, social interaction) that reflect these core cognitive features of stress-related disorders. Little is known about the mechanisms that underlie PACAP's effects, but PACAP is known to activate adenylate cyclase. Therefore, PACAP's behavioral effects may involve the activation of the transcription factor cAMP response element binding protein (CREB), which is known to induce neuroplasticity and regulate stress-related behaviors. Thus, we investigated the role of the CREB in mediating the effects of PACAP on social interaction within the nucleus accumbens shell (NAc), a brain area involved in encoding reward and aversion.
Methods: Reward function was assessed using the intracranial self-stimulation (ICSS) test in rats implanted with an intracerebroventricular (ICV) cannula and a lateral hypothalamic electrode. Following training, rats were infused with vehicle or PACAP (0.25-1.0 μg) and tested immediately for 90 min and each day for 8 days' post-treatment. Attention was assessed using the 5-choice serial reaction time task (5CSRTT) in rats food deprived to 85% free-feeding weight. Rats received an ICV infusion of vehicle or PACAP and were tested 1 hr later and each day for 8 days. Finally, we examined if PACAP alters social interaction (SI) together with corresponding molecular adaptations. A third cohort of rats received an ICV infusion of vehicle or PACAP and were placed in an interaction chamber 1 hr later with an untreated partner rat, and with a new partner rat 8 days later. For immunoblotting (Western) studies, NAc tissue was collected 80 min after VEH or PACAP infusion and processed for protein expression of CREB phosphorylated at Ser-133 (pCREB). For viral mediated gene transfer studies, rats with NAc bilateral cannula received infusions of herpes simplex virus (HSV) expressing wild-type CREB, a dominant-negative form of CREB (mCREB), or a virus vector control directly into the NAc (2 μg/side) and were tested for SI 4 days later.
Results: PACAP produced acute dose-dependent decreases in motivation, attention, and social interaction. Performance in the ICSS test and 5CSRTT returned to baseline levels the following day, but effects on social behavior remained dysregulated in tests performed 1 week later, with the initial reductions in social behavior evolving into elevated social behavior. PACAP treatment produced acute decreases in pCREB protein levels in the NAc compared to vehicle treatment. Elevation of CREB produced increases in SI behavior and decreases in anxiety-related behavior compared to disruption of CREB function.
Conclusions: These data show that ICV PACAP administration has profound effects on cognitive behaviors that represent domains often dysregulated in mood and anxiety disorders. The mechanisms underlying the long-lasting effects of PACAP on SI behavior are unclear, although biphasic effects of PACAP on fear expression have been previously described. Given that CREB activation in the NAc has been shown to induce pro-depressive-like behavior, our findings that PACAP decreased NAc CREB activation and that elevation of NAc CREB produced increases in SI were surprising. Ongoing work suggests that CREB may have different effects on behavior depending on localization within different subregions of the NAc shell. This work

may help to devise therapeutics that mitigate specific signs (i.e. social withdrawal) of these mood disorders by affecting the actions of stress peptides.
Disclosure: Nothing to Disclose.

26.2 Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) in the Bed Nucleus of the Stria Terminalis (BNST) Mediates Stress-Induced Reinstatement of Cocaine Seeking Behavior in Rats

Sayamwong Hammack

University of Vermont, Burlington, Vermont, United States

Background: Exposure to stressful stimuli has been argued to play an important role in the etiology of anxiety disorders, and exposure to environmental stressors can elicit anxiety-like behaviors, as well as reinstate previously extinguished cocaine-seeking behaviors in rodents. Activity in the bed nucleus of the stria terminalis (BNST) is critical for both effects. Moreover, the dysregulation of pituitary adenylate cyclase-activating peptide (PACAP) systems has been implicated in several stress-related diseases, and PACAP expression is dense in the BNST oval nucleus. We have previously shown that repeated variate stress upregulates BNST PACAP transcript and protein, and BNST PACAP receptor activation is necessary and sufficient for many of consequences of stressor exposure. Hence, the current set of studies was designed to investigate the role of BNST PACAP systems in stress-induced reinstatement of cocaine-seeking behavior.
Methods: Rats were bilaterally cannulated into the BNST, which was followed by jugular catheterization. One week following surgery, rats were allowed to self-administer cocaine (3mg/ml; 0.5mg/ig/infusion, i.v.) for 1 hr daily for 10 days and were then placed on an extinction schedule in which lever pressing no longer resulted in cocaine reward. Following extinction (21 days), rats were either infused into the BNST with 1 μg PACAP38 (0.5 μl per side) or equivolume vehicle (experiment 1), or 1 μg of the PACAP receptor antagonist PACAP 6-38 (0.5 μl per side) or equivolume vehicle (experiment 2) prior to receiving reinstating footshock (5 sec, 2 mA). In experiment 3, rats were allowed to self-administer cocaine for either 1 or 10 days. One hour following the last infusion session, brains were quickly sectioned and dorsal BNST was dissected with a brain punch set. Total RNA was isolated, reverse transcribed, and underwent real-time Taqman qPCR amplification. Cycle threshold (Ct) data were normalized to the ribosomal protein (18s) reference gene and fold change relative to surgical control animals was determined.
Results: In experiment 1, rats that received PACAP38 infusion into the BNST pressed the lever previously paired with cocaine significantly more than vehicle-infused rats, in the absence of footshock. In experiment 2, BNST PACAP receptor antagonism completely abrogated the footshock-induced reinstatement of cocaine-seeking behavior on the lever previously paired with cocaine. Interestingly, in experiment 3, 10 days of cocaine self-administration (but not 1 day) significantly reduced PACAP transcripts in the dorsal aspect of the BNST.
Conclusions: These studies suggest that BNST PACAP receptor activation is both necessary and sufficient for the stress-induced reinstatement of previously extinguished cocaine-seeking behavior, and that BNST PACAP transcripts can be regulated by the self-administration of cocaine. Current studies are assessing BNST PACAP transcript levels at the time of stress-induced reinstatement. These studies support an increasing literature implicating PACAP dysregulation as a key component of several stress-related illnesses, and suggest that these systems may represent an important treatment target in the prevention and/or treatment of some forms of relapse.
Disclosure: Nothing to Disclose.

26.3 Deletion of PAC1 Receptors From the Medial Intercalated Cells of the Amygdala Enhances Fear Generalization and Decreases Fear Extinction Whereas Deletion From the Basolateral Amygdala Decreases Fear Acquisition

Michael Fanselow

University of California, Los Angeles, Los Angeles, California, United States

Background: Post-traumatic stress disorder (PTSD) involves inappropriate inhibitory control over fear after exposure to life-threatening traumatic experiences. The neuropeptide, pituitary adenylate cyclase activating peptide (PACAP) and its G-coupled receptor PAC1 have been linked to PTSD diagnosis and symptom severity. Using mice expressing green fluorescent protein (GFP) in PACAP containing neurons we found that these neurons in the basolateral amygdala (BLA) project into the medial intercalated cells (mICCs). BLA and mICCs are crucial for modulating fear behaviors and express PAC1 receptors. Therefore, we investigated whether deletion of PAC1 receptors from these two regions alters fear acquisition, generalization or extinction.
Methods: We used mice with a floxed PAC1 gene and infused AAV-driven Cre-recombinase or GFP into the BLA or mICCs. After viral expression, mice went through a fear acquisition protocol (1 trial/day for 5 days) in which 4 minutes after being placed into a conditioning context they were given a 0.65 mA, 1-second shock. Mice were then placed in a different context to test fear generalization and their freezing behaviors measured for 4 minutes. The same mice then went through fear extinction for which they were placed in the acquisition context for 30 minutes every day until their freezing decreased to baseline levels. We measured freezing in the first 4 minutes of each session.
Results: Mice with Cre-mediated deletion of PAC1 receptors or those only expressing GFP (controls) in the mICCs reached an asymptotic level of freezing at the same rate. However, control mice showed an enhancement in fear generalization and decrease in fear extinction, while mice with deletion of PAC1 receptors from the BLA showed a decrease in fear acquisition without alterations in fear generalization or extinction.
Conclusions: While these results are somewhat surprising given the putative role of PACAP in enhancing fear, they indicate that PACAP/PAC1 may play different roles in

modulating fear depending on the site of action in the fear circuitry. The finding that PAC1 receptors in the BLA modulate fear acquisition fits with the known role of BLA in acquisition of fear. Additionally, the finding that the mICCs could play a role in fear generalization is a novel and interesting as studies have so far been focused only on the role of ICCs in fear extinction.
Disclosure: Part 1: Neurovation, I.a.i Board of Directors.

Panel
27. It's the Gatekeeper - Converging Evidence for Impairment of the Thalamic Reticular Nucleus in Schizophrenia Trajectory

27.1 A Key Role for the TRN in NMDA Receptor and DISC1 Models of Schizophrenia-Related Thalamo-Prefrontal Cortex Dysconnectivity

Judith Pratt

University of Strathclyde, Glasgow, Glasgow, United Kingdom

Background: Accumulating evidence suggests that abnormal function of thalamocortical and corticothalamic pathways is central to network dysfunction in schizophrenia and that the thalamoreticular nucleus (TRN) is a functional hub for this disruption (Pratt and Morris 2015, J.Psychopharm 29, 127-137). The TRN is a thin sheet of GABAergic neurones that surrounds other thalamic nuclei and hence occupies an anatomically strategic position to control neural communication between thalamic nuclei and their respective cortical connections. Because of its thin shape the TRN is difficult to delineate in human imaging studies. Hence we have investigated the impact of risk factors for schizophrenia upon TRN function and GABAergic cell markers in relation to thalamocortical circuitry in rodent models.
Methods: To model schizophrenia risk factors we selected a rodent NMDA receptor antagonist and a DISC1 genetic model. We employed 2-deoxyglucose (2-DG) imaging to assess regional rates of cerebral glucose metabolism and we applied partial least squares regression (PLSR) analysis and graph theory analysis to this functional brain imaging data in order to gain insight into altered functional connectivity of brain regions in the context of brain networks. *In situ* hybridisation was used to determine changes in regional expression of parvalbumin (PV) and voltage gated potassium channels (Kv3 subfamily) that confer fast spiking properties of PV positive GABAergic neurones.
Results: Repeated phenycyclidine (PCP) (2.6mg/kg) produced metabolic hypofrontality in rats. Thalamic hypometabolism was evident in the TRN and the centromedial thalamic nucleus Notably, thalamic- prefrontal functional connectivity and TRN functional connectivity to the prefrontal cortex (PFC)was reduced following repeated PCP treatment. Furthermore, graph theory measures showed that the TRN lost its important hub status in functional brain networks after subchronic PCP. PV and Kv3.1 mRNA expression was reduced by ~20-25% in the PFC and TRN after subchronic and chronic PCP treatment. Strikingly the expression changes in the TRN preceded that of the PFC. Although the regional expression profile of Kv3.1 and Kv3.3 were similar, there was an increased expression of Kv3.3 in the TRN and PFC after repeated PCP. The importance of the TRN is corroborated in a Disc1 transgenic mouse model. Disc1tr transgenic mice showed hypofrontality and TRN hypofunction and reduced functional connectivity between the TRN and PFC.
Conclusions: These data suggest that neuroadaptive events are evident in thalamocortical circuitry after repeated PCP treatment and strongly suggest that the TRN may have a prominent role in the dysregulation of neural communication between the thalamus and the PFC in schizophrenia. In keeping with the hypothesis that the TRN plays a central role in driving PFC changes, we found reductions in PV and Kv3.1 expression in the TRN prior to similar changes in the PFC after repeated PCP. Taken together our functional brain imaging data from these two models of disease risk, support a central role for the TRN in driving the long term changes in thalamic – prefrontal cortex connectivity seen in schizophrenia. Targeting this disrupted connectivity may represent biomarkers for symptom development and early intervention therapies.
Disclosure: Part 4: Pfizer, Research Grant, Servier, Research Grant.

27.2 Optogenetic and Cav3.3 Channel Manipulation of TRN Parvalbumin GABAergic Neurons in a Mouse Model of Schizophrenia Spindle Deficit

Robert McCarley

Harvard Medical School, Brockton, Massachusetts, United States

Background: Sleep spindles have been found to be consistently reduced in schizophrenia (Sz) and in their first degree relatives. Our goal in these experiments was to develop an animal model of deficient spindle generation that was congruent with GWAS, post-mortem, and physiological data using optogenetics and reverse microdialysis in mice. Human sleep spindles –12-15 Hz EEG oscillations during NREM sleep—are thought to originate in the thalamic reticular nucleus (TRN), a structure composed of inhibitory GABAergic neurons, 80% 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. Post-mortem data (Beretta) indicate widespread abnormalities of PV TRN neurons in Sz while Steullet reports a redox model of Sz generates PV abnormalities. In our study we model SZ spindle abnormalities resulting from abnormal activity in TRN PV neurons, and also in the Cav 3.3 T channel whose action is essential for spindles and whose CACNA1I gene is a GWAS Sz risk factor. To the best of our knowledge such optogenetic studies of TRN PV neurons and acute pharmacological inhibition of TRN Cav 3.3 channels are novel. Nor have they been published by our group.
Methods: Optogenetics: The TRN was targeted with AAV vectors with either ChannelRhodopsin2 (ChR2) for excitation, or the proton pump ArchT for inhibition in PV Cre mice ($N = 10$), thus specifically transducing TRN PV neurons. Optogenetic and EEG/EMG methods for this

TRN study have been detailed in the context of our basal forebrain study (Kim et al, PNAS Mar., 2015). Reverse microdialysis for acute TRN application of the selective T-type channel inhibitor, TTA-P2 ($N=7$ mice), used methods described in our reverse microdialysis study of the basal forebrain (Zant et al, J. Neurosci., Feb., 2016).
Results: Optogenetics: ChR2 excitation of TRN PV neurons consistently (98%) produced spindles in early NREM sleep, confirming their essential role in spindle production. ArchT inhibition for 10 sec always halted spindle trains of at least 2 spindles for 4 sec and reduced amplitude thereafter, whereas in the no ArchT control a 3rd spindle occurred 65% of the time (Fisher's exact Chi square $P<0.0001$). Prolonged application of ArchT (1 min q 5 min) increased Wakefulness (28%) and reduced NREM (27%); in contrast prolonged ChR2 excitation increased NREM sleep (30% with 1 sec/min 10 Hz stimulation), confirming the role of TRN PV neurons in gating cortical arousal (Pratt). Reverse microdialysis of 1 uM TTA-P2 reduced NREM spindle density (spindles/min) compared with ACSF control (Unilateral TRN hits: -17. 68%, Bilateral: -39.68%). Collectively, uni- and bi-lateral hits ($N=7$ mice) showed a statistically significant decrease ($-27.11 \pm 8.5\%$, $p=0.03$, t-test) without any significant effect on NREM sleep time.
Conclusions: Our model of Sz spindle reduction stemming from as abnormalities of TRN PV neurons is congruent with post-mortem findings and the gene for Cav 3.3 as a schizophrenia risk factor. The absence of NREM sleep time changes with TTA-P2 spindle reduction suggest that Cav 3.3 channel inhibition has a more selective effect on reducing spindles than optogenetic inhibition of TRN PV neurons. It also more congruent with spindle studies in Sz patients.
Disclosure: Nothing to Disclose.

27.3 Oxidative Stress Affects Parvalbumin Interneurons Structure and Function in the Thalamic Reticular Nucleus Early During Development

Pascal Steullet

Lausanne University Hospital, Prilly-Lausanne, Switzerland

Background: The thalamic reticular nucleus (TRN) consists of GABAergic neurons, many of which express parvalbumin (PV). Via their inhibition of thalamic neurons projecting to the cortex, TRN cells modulate the thalamo-cortical communication, which is affected already at early stages of schizophrenia. Anomalies in the TRN of patients are supported by recent post-mortem studies, and by observations of deficit in sleep spindles whose generation depends on TRN cells. Oxidative stress and redox dysregulation is recognized as one pathological mechanism contributing to schizophrenia. In particular, prefrontal and hippocampal parvalbumin-expressing interneurons, known to be affected in the disease, are particularly susceptible to oxidative stress. Here, we examined whether TRN is also vulnerable to a redox dysregulation.
Methods: We assessed the morphological integrity and function of TRN cells in an animal model of redox dysregulation relevant to schizophrenia (GCLM KO mice). These mice have low glutathione content in all tissues, including brain. Morphological integrity of the TRN was evaluated at three different postnatal developmental stages (days 20, 40, and 90) by immunohistology using the lectin Wisteria floribunda agglutinin (to label the extracellular matrix called perineuronal net: PNN), and antibodies against PV and against 8-oxo-2'-deoxyguanosine (to label oxidative stress). Function of TRN cells was assessed in slices of adult mice using extra- and intracellular electrophysiological techniques.
Results: Oxidative stress was present in TRN of GCLM KO mice, from early-life to adulthood. The oxidative stress was more prominent in TRN than in other thalamic nuclei. In all ages, there was a reduced number of PV-immunoreactive cells in KO compared to WT mice (- 21% at day 20, - 22% at 40, - 51% at 90). PNN was also affected at day 40. Supplementation of N-acetylcysteine prevented oxidative stress, PV and PNN deficits. In comparison with PV interneurons in PFC and hippocampus, PV cells in TRN were affected earlier on during the postnatal development of GCLM KO mice. The firing pattern and physiological properties of TRN cells recorded *in vitro* were also altered in KO compared to WT mice. In KO mice, the spontaneous basal activity of TRN cells was high (30 ± 6 spikes/s), mostly due to elevated tonic activity. In WT mice, TRN cells were less active (7 ± 1 spikes/s) but displayed a more regular burst firing pattern. Depolarization-evoked bursts of action potentials were generated at more hyperpolarized membrane potentials in TRN cells of KO (-77 ± 4 mV) compared to WT mice (-65 ± 2 mV). Finally, we observed a deficit in rebound bursts in thalamo-cortical neurons of KO mice following stimulation of TRN.
Conclusions: TRN is highly vulnerable to redox dysregulation and prone to oxidative stress early during development. These data suggest that redox dysregulation is one mechanism by which the TRN could be affected early during the progression of the disease, leading to inappropriate thalamo-cortical communication and affecting for instance sleep spindles as well as "wake-up call" to the cortex for selective attention. Alteration of the TRN could therefore be one early marker of the disease.
Disclosure: Nothing to Disclose.

27.4 Neuronal Deficits in the TRN of Subjects With Schizophrenia Support the Thalamo-Cortical Disconnectivity Hypothesis: Role of Parvalbumin-Expressing Neurons and Perineuronal Nets

Sabina Berretta

McLean Hospital, Belmont, Massachusetts, United States

Background: Growing evidence from imaging studies points to a disruption of thalamo-cortical connectivity as a critical element of the pathophysiology of schizophrenia (SZ) and, potentially, bipolar disorder (BD). Information processed by the cortico-thalamic system is gated by the Thalamic Reticular Nucleus (TRN), which receives massive cortical and thalamic projections and sends inhibitory projections back to thalamic nuclei, thus effectively filtering thalamo-cortical information transfer. TRN GABAergic inhibitory neuronal subpopulations involved in this function express calcium binding proteins, among which parvalbumin (PVB) is prevalent, and peptides such as somatostatin. Many of these neurons are enveloped in perineuronal nets (PNNs),

organized extracellular matrix structures known to affect neuronal maturity, synaptic functions and plasticity. Mounting evidence for the involvement of PVB, somatostatin and PNNs in schizophrenia and bipolar disorder lead us to test the hypothesis that TRN neurons may be affected in the pathophysiology of these disorders. As a first step toward testing this hypothesis we measured numbers of PVB-immunoreactive (IR) neurons and PNNs in subjects with these disorders.

Methods: In these postmortem human studies, a combination of immunocytochemistry and computer-assisted quantitative microscopy was used to measure total numbers and numerical densities of PVB-IR neurons and PNNs (detected with the lectin Wisteria Floribunda agglutinin (WFA)) in the TRN in a cohort of healthy subjects ($n = 20$), schizophrenic ($n = 15$), and bipolar disorder ($n = 15$) subjects. TRN volume for all subjects was measured on adjacent Nissl-stained sections. Dual and triple antigen immunofluorescence combined with confocal microscopy was used in a subgroup of healthy subjects to investigate co-localization ratios between PVB, somatostatin and PNNs. Statistical significance was tested using stepwise linear regression (ANCOVA).

Results: In healthy subjects, subpopulations of PVB-IR and somatostatin-IR neurons are associated with WFA-labeled PNNs. Percentages of PVB-IR neurons associated with these PNNs in human TRN are lower than previous reported in other species. In subjects with schizophrenia, total numbers and numerical densities of PVB-IR neurons were markedly decreased ($p < 0.0001$ for each measure) as compared to healthy controls. Similarly, total numbers and numerical densities of WFA/PNNs were significantly decreased ($p = 0.008$ and $p = 0.006$, respectively). Numbers of PVB-IR neurons and WFA/PNNs significantly and positively correlated to antipsychotic exposure within the last 6 months of life, suggesting that these drugs tend to bring these values toward normality. In subjects with bipolar disorder, total numbers and numerical densities of PVB-IR neurons ($p = 0.001$) and WFA/PNNs ($p = 0.04$ and $p = 0.01$, respectively) were decreased as compared to controls.

Conclusions: PVB-IR neurons in the TRN are markedly affected in the TRN of people with schizophrenia and bipolar disorder. Less robust, yet significant, decreases of PNNs suggest that they may confer a measure of protection, while at the same time being also affected, as suggested by previous findings from the group of Drs. Do and Cuenod. We put forth that, deficits of PVB-IR neurons within the TRN may disrupt inhibitory regulation of thalamo-cortical pathways, leading to altered sensory gating and emotion processing.

Disclosure: Nothing to Disclose.

Study Group

28. The National Neuroscience Curriculum Initiative (NNCI) – A Blueprint for Bringing the Bench to the Bedside

Joshua A Gordon*, Joyce Chung, Jane Elsen, Melissa Arbuckle, David Ross, Michael Travis

Columbia University, New York, New York, United States

Study Group Proposal Psychiatry is in the midst of a paradigm shift due to significant advances in our scientific knowledge. The diseases we treat are increasingly understood in terms of the complex interactions between genetic and environmental factors and the development and regulation of neural circuitry. Yet most psychiatrists have relatively minimal knowledge of neuroscience. This may be due to many factors, including: the difficulty of keeping pace with a rapidly advancing field; the ineffectiveness of traditional, lecture based approaches; and that neuroscience is often presented in a way that seems devoid of clinical relevance and disconnected from the other rich traditions of the field. Regardless of the reason, what has resulted is an enormous practice gap: despite the central role that neuroscience is increasingly assuming in psychiatry, practicing psychiatrists continue to under-represent this essential perspective in their work. This means that it can take years before key findings from the literature are disseminated and can have an impact on individual patients. The National Neuroscience Curriculum Initiative (NNCI) is an NIH-funded collaboration with the mission of developing and disseminating clinical neuroscience teaching resources to practicing psychiatrists and residents in training. The guiding principles for the design of NNCI resources are to: maintain an integrative, patient-centered approach; use evidence-based principles of adult learning, eschewing traditional lecture formats in favor of innovative and experiential exercises and; ensure that materials can be implemented by anyone, anywhere, regardless of content expertise. In this session participants will be introduced to some of the new learning approaches developed by the NNCI to engage trainees in active learning. Our goal with this study group is to foster a discussion around strategies for making neuroscience teaching engaging and relevant to clinical practice in psychiatry.

Disclosure: Nothing to Disclose.

Panel

29. Facing the Complexity of Cannabis Effects on the Brain: Context and Content

29.1 Cannabinoids and Cortical Circuitry: Impact on Development and Schizophrenia

David Lewis

Western Psychiatric Institute and Clinic, Pittsburgh, Pennsylvania, United States

Background: Schizophrenia is associated with alterations in working memory. Working memory depends on the coordinated activity of circuitry in layer 3 of the dorsolateral prefrontal cortex (DLPFC) involving glutamatergic pyramidal cells and parvalbumin (PV)-containing, GABAergic basket cells. Both of these cell types are innervated by cholecystokinin-containing GABAergic neurons whose terminals contain very high levels of the cannabinoid 1 receptor (CB1R). These findings suggest that altered cannabinoid signaling, especially when this circuitry is being refined during adolescence, could contribute to working memory dysfunction in schizophrenia.

Methods: We conducted three sets of studies to address this question. First, we evaluated the integrity of the endocannabinoid system in the postmortem DLPFC from subjects with

schizophrenia and comparison subjects. Second, we studied the impact of repeated exposure to exogenous cannabinoids during adolescence on the maturation of working memory performance in macaque monkeys. Third, we examined the cumulative effects of marijuana use on subclinical psychotic symptoms in 1,009 adolescent boys.

Results: In the DLPFC of subjects with schizophrenia, our findings are consistent with elevated metabolism of the major endocannabinoid 2-arachidonylglycerol (2-AG), greater CB1R receptor binding and lower levels of CB1R mRNA and protein. In adolescent monkeys, repeated exposure to Δ9-tetrahydrocannabinol (THC) blunted the trajectory of accuracy improvements in spatial working memory but did not alter other cognitive functions that mature earlier. These effects were more marked and more persistent in male than in female monkeys. For each year that adolescent boys engaged in regular marijuana use, their expected odds of experiencing subsequent subclinical paranoia or hallucinations rose by 133% and 92%, respectively.

Conclusions: Alterations of the endocannabinoid system in the DLPFC of subjects with schizophrenia may reflect a developmental disturbance that makes these individuals more vulnerable to the adverse effects on working memory maturation or reality testing following repeated exposure to exogenous cannabinoids during adolescence. Apparent sex differences in sensitivity to exogenous cannabinoids may contribute to the tendency for schizophrenia to have an earlier age of onset, greater prevalence and more severe course in males.

Disclosure: Part 1: Pfizer, Grant Support, Sunovion, Consultant, Autifony, Consultant, Bristol-Myers Squibb, Consultant & Grant Support, Concert Pharamaceuticals, Consultant, **Part 4:** Bristol-Myers Squibb, Grant Support, Pfizer, Grant Support.

29.2 Cannabis Use is Associated With Frontoparietal Structural and Functional Abnormalities and Executive Dysfunction in Young Adults With and Without ADHD

Krista Lisdahl

University of Wisconsin-Milwaukee, Milwaukee, Wisconsin, United States

Background: Cannabis (CAN) use is on the rise in youth in the United States, with 23% of high school seniors and approximately 20% of college students reporting past month use (Johnston et al, 2015). This adolescent onset of CAN use coincides with significant neuromaturation, especially in frontoparietal regions (Giedd et al, 1006 Lenroot & Giedd, 2006; Sowell et al, 2002). Given this, the neurotoxic effects of substance use may be more pronounced in adolescents and emerging adults (Lisdahl et al, 2015). This talk will present findings from a series of studies examining the neurocognitive effects of CAN in adolescents and emerging adults.

Methods: Neuroimaging, neuropsychological, and substance use data was collected from regular CAN users and non-using controls (CNT) across three independent NIDA-funded studies (range of abstinence 2-21 days; age range 16-25). For the first two studies ($N=68$; $N=83$), participants were recruited from the community and had to be either a CAN user (>50 uses) or CNT (<5 lifetime uses) with minimal other illicit drug use (<20 occasions). Other exclusionary criteria included independent Axis I psychiatric disorder, major medical or neurologic disorder, premature birth or prenatal drug exposure, left-handedness, loss of consciousness >2 minutes. For the final study, CAN-using and CNT participants ($N=120$) were selected from a national longitudinal multi-site study (MTA) examining the trajectory of Attention Deficit Hyperactivity Disorder (ADHD). 120 participants were recruited for a neuroimaging, neuropsychology, and substance use study session. Exclusion criteria included other illicit drug use, excessive binge drinking, left-handedness, major medical or neurologic disorder.

Results: In the first set of studies, we demonstrate abnormal prefrontal and parietal volumes, prefrontal gyrification, and reduced executive functioning in regular CAN using young adults (Price et al, 2015; Shollenbarger et al, 2015; Lisdahl et al, 2012). In a follow-up study in adolescents and young adults after three weeks of monitored abstinence, we again found reduced selective attention and inhibitory control deficits (p's$<.05$), along with reduced left inferior frontal gyrus (IFG) ($p=.005$), left rostral anterior cingulate (ACC) ($p=.009$), right frontal pole ($p=.03$), and left frontal pole ($p=.003$) volumes (Lisdahl et al, under review). Finally, compared to CNTs, CAN users had abnormal cognitive control of affective stimuli connectivity, including blunted rIFG connectivity with medial prefrontal cortex (PFC), posterior cingulate, and increased connectivity with ACC, insula, precuneus, and parahippocampal regions (Lisdahl et al, under review). In a multi-site neuroimaging study (MTA) that prospectively followed children with and without ADHD into young adulthood, we found that CAN users demonstrated decreased cortical thickness in right superior frontal sulcus, ACC, and isthmus of cingulate gyrus regions and left superior frontal sulcus and precentral gyrus regions (Lisdahl et al., 2016).

Conclusions: Across three independent samples, young regular CAN users demonstrate abnormalities in frontoparietal structure. Further, we recently found abnormal functional brain response while engaging cognitive control of affective stimuli in frontolimbic and parietal regions. Potential limitations, future directions, and clinical implications will be discussed.

Disclosure: Nothing to Disclose.

29.3 Convergent Mechanisms of Cell Type-Specific Genetic Alteration and Vulnerability to Adolescent Cannabis Use for Brain Maturation and Cognitive Behaviors

Atsushi Kamiya

Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Background: Cannabis is an increasingly popular and controversial drug used worldwide. Accumulating evidence suggests cannabis use is a potential environmental risk for the development of cognitive abnormalities and psychoses, including schizophrenia. Given that only some cannabis users display cognitive impairment, genetic predisposition may contribute to detrimental cognitive effects of cannabis.

Nonetheless, the mechanisms whereby genetic risk factors interact with cannabis exposure to produce cognitive dysfunction remain unknown.
Methods: In order to explore the molecular mechanisms of predisposition to cannabis effects, we utilize our mouse model of astrocyte-specific inducible expression of mutant form of Disrupted in Schizophrenia 1 (DN-DISC1). Animals were chronically treated with delta-9-tetrahydrocannabinol (Δ9-THC), a major psychoactive ingredient of cannabis, during adolescence. We then conducted behavioral, biochemical, and molecular assessment in adulthood to examine neurobiological consequences of astrocytic DN-DISC1 and adolescent Δ9-THC exposure as a model of gene and environment interaction.
Results: Adolescent interaction of astrocytic DN-DISC1 and treatment with Δ9-THC synergistically produce cognitive deficits in adult mice. These behavioral changes were accompanied by increased glutamate tissue content that could result in chronic excitotoxicity and neuronal dysfunction. We also found that Δ9-THC treatment and astrocyte DN-DISC1 expression significantly activates NF-kB signaling.
Conclusions: Our results suggest that astrocyte DN-DISC1 and Δ9-THC may interact during adolescence by synergistically activating NF-kB signaling, which could lead to neuronal dysfunction underling long-term cognitive impairments. Our studies will contribute to identify the molecular mechanisms of how adolescent cannabis use leads to cognitive impairment in susceptible individuals and it will facilitate an informed search for preventive treatments of long-term adverse effects of marijuana use.
Disclosure: Part 1: Taisho Pharmaceutical Co., Ltd, Consultancies, **Part 4:** Hitachi, Ltd, Grants for collaboration studies.

29.4 How Does the THC and CBD Content of Cannabis Influence its Effects?

H. Valerie Curran

University College London, London, United Kingdom

Background: Of the roughly 100 unique ingredients we call 'cannabinoids', most research to date has focused on the two most prominent: Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Over the last decade, levels of THC in cannabis have increased markedly whilst levels of CBD have reduced often to negligible amounts. Over the same period, there has been a parallel increase in young people entering drug treatment services for cannabis addiction.
Methods: In this talk I will focus on how variation in THC and CBD may influence the addictive and cognitive effects of the drug. I will summarise recent findings suggesting that CBD may have a protective effect upon some of the harmful effects of THC. I will then present new data from a placebo controlled cross-over study in 18 healthy recreational cannabis users where we directly compared the effects of two types of cannabis containing the same level of THC but differing levels of CBD.
Results: This showed a range of similarities in their effects but also some subtle differences between the two types of cannabis. On an effort-related decision-making task (indexing motivation) cannabis without CBD reduced the likelihood of high-effort choices relative to placebo, and increased sensitivity to expected value compared with both placebo and cannabis with CBD. There were also differences in how the two types of cannabis affected connectivity in the brain's salience network.
Conclusions: The implications of findings with CBD and THC are drawn out for current debates about the changing legal status of the drug.
Disclosure: Nothing to Disclose.

Panel
30. Developmental Perspectives on Aggression and Disruptive Behaviors: Implications for Treatment

30.1 Serotonergic Function and Early Adulthood Outcomes in Children With Disruptive Behavior Disorders

Iliyan Ivanov

The Icahn School of Medicine at Mount Sinai, New York, New York, United States

Background: The relations between abnormal serotonergic function and impulsive aggression has been studied in both animal and human research. Decreased serotonergic function has been demonstrated in individuals with borderline and antisocial personality disorders (ASPD), intermittent explosive disorder, and impulsive individuals with substance use disorders. Thus serotonergic abnormalities have been associated with both categorical (e.g. personality disorder diagnoses) and dimensional (e.g. aggression, impulsivity, hostility) features of adult psychopathology. For this report we evaluated the longitudinal relations between serotonergic functions in clinically referred children ages 7-11 diagnosed with ADHD and clinical outcomes (e.g. ADHD/ASPD diagnoses and symptoms) in early adulthood.
Methods: Our group has conducted a longitudinal follow up of an ethnically diverse cohort of children with ADHD ($n = 155$) who had two waves of follow up assessments. The sample was initially recruited between 1990 and 1997 when the participants were aged 7-11 years; all participants completed a comprehensive clinical evaluation and a subgroup of 110 also completed a fenfluramine challenge test. Measures of prolactin levels and vital signs (heart rate, blood pressure) were obtained at baseline, 60min, 120min, 180min, 240min and 300min.
The second wave assessment included interviews from participant and informant (e.g. parent) for the identification of Axis II psychopathology. A third wave follow-up was conducted between 2006-2011 (average 15 years after the baseline assessment) including interviews with participants to identify adult Axis I and Axis II psychopathology. Of the participants who presented for the third assessment we analyzed 40 adults who completed the fenfluramine challenge procedure in childhood.
Results: Twenty-one participants (52.5%) met criteria for ASPD; of these 11 participants were diagnosed with ASPD during the second follow up and had the diagnosis confirmed at the third follow up. Additional 10 participants

were newly diagnosed with ASPD for the first time as adults. All participants who were diagnosed with ASPD had received an CD diagnosis in childhood. Step-wise logistic regression analyses with ASPD as dependent variable show that childhood CD predicted independently ASPD ($p = .020$) where childhood socio-economic status (SES) was not a significant predictor. Repeated measures ANOVA showed distinct patterns for prolactin response for participants with ASPD vs. no-ASPD. Specifically, the ASPD group exhibited more blunted response to fenfluramine challenge compared to the no-ASPD group especially in the later part of the experiment. For instance, delta prolactin (e.g. change from baseline) was significantly lower for the ASPD group at 240min whereas drug levels were no different for the two groups. Correlation analyses showed significant inverse correlations between prolactin area-under-the curve (AUC) and Child Behavioral Check List (CBCL) scores on aggression ($r = -.45$, $p = .011$), delinquency ($r = -.41$, $p = .028$) and externalizing problems ($r = -.45$; $p = .015$).

Conclusions: The participants in this sample were prospectively studied at two different time points and the relation between childhood prolactin response - a proxi measure for serotonin function - and the development of ASPD was confirmed independently for these two follow-ups. Individuals with ASPD showed significantly lower serotonergic response in comparison to participants without ASPD. These findings further suggest that youth with ADHD who also exhibit low serotonergic activity might be at elevated risk for the development of ASPD.

Disclosure: Part 1: Lundbeck, Honoraria, Self.

30.2 Developmental Neural Markers for Dysfunction in Decision-Making: Implications for Aggression and Irritability

James Blair

National Institute of Mental Health, Bethesda, Maryland, United States

Background: Considerable animal and human literature stresses the importance of the vmPFC, posterior cingulate cortex, anterior insula cortex and striatum for reinforcement-based decision-making (Knutson, Samanez-Larkin, & Kuhnen, 2011; O'Doherty, 2012; Schoenbaum & Roesch, 2005). Patients with Conduct Disorder, Attention Deficit Hyperactivity Disorder (ADHD), irritability, depression and schizophrenia have all been reported to show deficits in reinforcement-based decision-making (Crowley et al, 2010; Finger et al, 2011; Finger et al, 2008; Gradin et al, 2011; Parvaz et al, 2012; Plichta et al, 2009; Rubia, Smith, et al, 2009; S. F. White, Pope, et al, 2013). However, reinforcement-based decision-making is a term that subsumes an array of specific computational processes including responsiveness to reward, prediction error signaling, choice and avoidance responses and expected value signaling. The symptom sets associated with dysfunction in these separable computational processes remain relatively undetermined. The goal of this study is to determine the forms of dysfunction that relate to symptom sets associated with aggression and/or irritability.

Methods: One hundred youth (30 female, mean age = 13.81 [standard deviation = 2.14], mean IQ = 102.34 [standard deviation = 10.99]) from a residential treatment program and the community completed both a passive avoidance task and a novelty decision-making task while undergoing functional MRI. Over 50% of these youth met criteria for Conduct Disorder and over 60% showed clinically significant levels of irritability. Symptom sets corresponding to Callous-Unemotional traits, irritability, antisocial behavior and reactive and proactive aggression were recorded at the time of the fMRI session.

Results: With the exception of callous-unemotional traits, greater levels of symptoms were associated with poorer task performance on both tasks. However, conduct problems were associated with reduced representation of EV when making avoidance responses within bilateral anterior insula cortex/inferior frontal gyrus and striatum. In contrast, irritability was associated with aberrantly increased prediction error signaling of punishments within striatum and periaqueductal gray (i.e., increased responses to punishments that were worse than expected was associated with irritability within these regions). Some of these findings were moderated by participant age.

Conclusions: The current data indicate that behavioral impairment in decision-making is associated with increased levels of a variety of different symptom sets seen in adolescents with externalizing behavior. However, they also indicate that functional impairments at the neural level, underpinning these decision-making impairments, are associated with specific symptom sets. As such, these findings may represent treatment targets that, if successfully addressed, may lead to corresponding reductions in the related symptom sets.

Disclosure: Nothing to Disclose.

30.3 Neuroimaging of Pain Observation and Moral Judgment in Criminal Offenders With Sexual Sadism

Carla Harenski

The Mind Research Network, Albuquerque, New Mexico, United States

Background: Sexual sadism is an unusual and extreme form of aggression in which sexual gratification is derived from inflicting pain on others. While the psychological and forensic aspects of sexual sadism have been well-characterized, little is known about the underlying neurocognitive circuitry. We have previously demonstrated that sexual sadists show aberrant hemodynamic responses within limbic regions such as the amygdala when viewing images of people causing physical pain to others. Here we extend this work and present the following novel data: 1) Whole-brain functional connectivity, analyzed with group independent component analysis (ICA), during pain observation in sadists and non-sadists, 2) Functional brain responses related to moral judgment in sadists and non-sadists, 3) Whole-brain structural connectivity, analyzed using source-based morphometry analysis (SBM), in sadists and non-sadists.

Methods: A mobile magnetic resonance imaging (MRI) scanner was deployed to a secure sex offender treatment facility and state prisons, where 11 sexual sadists and 12 non-

sadists were scanned using a multimodal MRI protocol. All participants were convicted of multiple violent sexual offenses. MRI scans were acquired using a 32-channel ultra-fast multiband imaging sequence. During functional MRI scanning, participants completed two tasks: 1) Pain task: participants made decisions regarding the pain intensity of clips depicting one person inflicting physical pain on another. 2) Moral task: participants made decisions regarding the moral violation severity of pictures that depicted moral transgressions.

Results: Relative to non-sadists, sadists rated pain clips higher in pain intensity relative to non-sadists. They also showed greater hemodynamic responses in the ventral striatum and decreased ventral striatum-orbitofrontal cortex connectivity. The SBM results revealed lower loading weights in sadists relative to non-sadists in two components comprising the temporal poles, temporo-parietal junction, anterior insula, and dorsolateral prefrontal cortex. Both sadists and non-sadists showed increased severity ratings to moral transgressions (relative to thematically-matched pictures without moral transgressions) and increased hemodynamic responses in established moral neural networks including medial prefrontal and anterior temporal cortex. The latter responses were positively correlated with moral violation severity ratings in non-sadists but not sadists.

Conclusions: The results suggest greater sensitivity to pain infliction, and reduced moral sensitivity, in sexual sadists relative to non-sadists. During pain observation sadists showed increased hemodynamic responses, and decreased connectivity, in brain regions implicated in reward, sexual arousal, and emotion regulation. They also showed less congruence between neural and behavioral responses related to moral judgment, and reduced gray matter covariation within fronto-temporal circuits. The results are consistent with prior case studies of brain anatomy in sadists and provide new evidence of aberrant function within emotion and cognitive control networks in sadism-relevant contexts.

Disclosure: Nothing to Disclose.

30.4 ADHD and Disruptive Behavior Disorders: Neurobiology and Response to Stimulant and Non-Stimulant Treatment

Jeffrey Newcorn

The Mount Sinai Hospital, New York, New York, United States

Background: The neurobiological mechanisms which distinguish ADHD from the disruptive behavior disorders (DBDs) have not been fully elucidated. Moreover, the potential differential impact of treatment with stimulant and non-stimulant medications on youth with ADHD with and without DBDs has not been clarified. This presentation will discuss new findings from imaging studies in youth with ADHD with and without DBDs, highlighting the neurobiological substrates which underlie and distinguish aggressive behavior in this population. It will then examine response to treatment in a large, crossover trial of atomoxetine and OROS-methylphenidate. Potential implications of fMRI findings for understanding differential treatment response will be discussed.

Methods: Two data sets will be examined. 1) A large, federally funded two-site crossover trial ($n = 235$) examining comparative effectiveness of atomoxetine and OROS-MPH. Approximately 40-50% of the children have ODD/CD/aggression. We will examine moderating effects of ODD/CD/aggression on ADHD treatment, and response on ODD symptoms and aggression ratings to treatment with atomoxetine and/or MPH. Data analysis uses a multiple regression model controlling for baseline severity, site, age and sex, to test main effects and interactions between treatment and moderators. 2) A composite fMRI data set from two federally funded studies ($n = \sim 80$ by the time of the meeting) and two industry funded studies ($n = \sim 30$) examining BOLD signal activation during fMRI obtained while performing a well standardized go-nogo task. The total sample consists of ~ 110 youth ages 7 – 17 years. Approximately 70% of these subjects were treated with atomoxetine, OROS-MPH, or both drugs. fMRI methods were: a) 3T scanners; b) well accepted go-nogo task, modified for this study; c) analysis of BOLD signal during successful inhibitions (activation during correct nogo minus go task trials) using a multiple regression model with ratings of clinical response as regressors.

Results: Analysis of the composite fMRI data set is ongoing. We will examine the BOLD signal profile in youth with ADHD only vs. those with ODD/CD/aggression. Clinical trial results indicate that youth with ADHD + ODD are more severely affected before and after treatment, with the presence of ODD slightly constraining ADHD response. There is a small but significant moderating effect of ODD, favoring MPH. ODD symptoms responded to both drugs, with no difference between the two classes. The best predictive model incorporated ADHD, ODD and ratings of mood lability. We will conduct new analyses examining whether the findings are similar for aggression, and whether adding aggression to the model increases treatment prediction.

Conclusions: Youth with ADHD and DBDs/aggression represent a more severe variant, with hypothesized unique underlying distinguishing features. The presence of ODD moderates ADHD clinical presentation and treatment response, with the best predictive model incorporating ratings of ADHD, ODD and mood lability. Treatment with both stimulants and non-stimulants yields improvement in both ADHD and ODD/CD/aggression symptoms, with a slightly greater effect for MPH in the presence of ODD. Effects of medication will be discussed in the context of underlying neurobiology as determined in the composite fMRI data set.

Disclosure: Part 1: Alcobra, Advisor/consultant, Cerecor, advisor, Enzymotec, consultant/research support, Ironshore, advisor/consultant, Lundbeck, research support, Neos, advisor, NFL, consultant, Pearson, advisor, Rhodes, advisor, Shire, advisor/lecture/research support, Sunovion, consultant (DSMB), Supernus, advisor, Teva, lecture, **Part 2:** Alcobra, Advisor/consultant, **Part 4:** Lundbeck, clinical trial, Shire, Investigator initiated study, Enzymotec, Investigator initiated study.

Panel
31. Neurobiological and Immunological Mechanisms of Inflammation Effects on the Brain and Behavior: Preclinical Models

31.1 CNS Immune Activation and Effects on Mesolimbic Dopamine in a Non-Human Primate Model of Peripheral Cytokine-Induced Depression

Jennifer Felger

Emory University School of Medicine, Atlanta, Georgia, United States

Background: Peripheral administration of inflammatory cytokines such as interferon alpha (IFNα) or inflammatory stimuli (e.g. endotoxin, vaccination) reliably affects neural activation of reward circuitry in humans in association with depressive symptoms related to reduced motivation and anhedonia. Our previous work in non-human primates has revealed that the effects of peripheral inflammation on reward circuitry and behavior may be due to decreased availability and release of striatal dopamine, which correlated with reduced effort-based sucrose consumption. However, the immunologic and neurobiologic mechanisms by which peripheral cytokines may affect the dopamine system are currently unknown.
Methods: Herein, whole genome-gene expression changes and immune cell activation were assessed in relation to inflammatory cytokine effects on dopamine in the ventral and dorsal striatum of post-mortem brain tissue from rhesus monkeys (aged 10-14 yrs) exposed to chronic IFNα (20 MIU/m2 s.c. for 4 weeks, $n=7$), compared to a reference group administered saline control ($n=4$). Tissue content of monoamines and their major metabolites were measured by HPLC, RNAseq was used to examine immune signaling pathways and gene expression relevant to neurotransmitters, and activation of monocyte/macrophage and microglia was assessed by microscopy.
Results: Significantly less tissue content of dopamine was observed in the nucleus accumbens ($p=0.004$) and putamen ($p=0.009$), while DOPAC was decreased in putamen of IFNα-treated animals. No change was observed for serotonin or norepinephrine. Whole genome analysis revealed that chronic peripheral inflammatory cytokine exposure activated expression of immune pathways in striatum including IFN, nuclear factor of activated T-cells (NFAT) and NF-kB signaling, and decreased genes related to cytoskeleton remodeling and metabolism (oxidative phosphorylation). Furthermore, IFNα increased gene-signaling pathways for neurotransmitter receptors known to modulate dopamine, including the adenosine 2B receptor and NMDA, AMPA and metabotropic glutamate receptor subunits. Interestingly, lower tissue content of dopamine in the putamen of IFNα-treated animals correlated with a decrease in the ratio of protective NR2A to neurotoxic NR2B NMDA subunits ($rs=0.886$, $p=0.019$). Decreased dopamine and the effects of IFNα on neurotransmitter signaling in the striatum were associated with increased MHC class II+ activated monocyte/macrophages in vascular and meningeal compartments, consistent with trafficking of peripheral immune cells to the CNS, as well as restricted activation of neighboring microglia.
Conclusions: These results support our previous findings of decreased striatal dopamine release in IFNα-treated monkeys, and indicate that effects of peripheral IFNα on brain dopamine are likely driven by peripheral immune cell trafficking to the CNS. This work will inform the development of novel strategies to reverse the effects of inflammation on brain dopamine to improve symptoms of reduced motivation and anhedonia in patients with increased inflammation.
Disclosure: Part 1: Proctor & Gamble, Consultantship, Pfizer, Honoraria.

31.2 Prolonged Anxiety-Like Behavior is Associated With Selected Migration of Bone Marrow-Derived, Glucocorticoid-Insensitive Myeloid Cells to the Brain in a Preclinical Model of Repeated Social Defeat

John Sheridan

The Ohio State University, Columbus, Ohio, United States

Background: Neuroinflammation affects mood, cognition, and behavior and is linked with the etiology of psychiatric disorders, including anxiety and depression. In addition, recent studies have demonstrated that peripheral/systemic inflammatory responses may contribute to psychiatric diseases. Repeated social defeat (RSD) is a murine stressor that can be used to study key physiological, immunological and behavioral changes to chronic psychosocial stress. RSD provides a preclinical murine model in which bi-directional responses from the brain to the periphery, and periphery to brain, can be examined. Exposure to RSD activates fear/anxiety neurocircuitry, accompanied by microglial activation and increased pro-inflammatory signaling in the brain. In conjunction, RSD stimulates sympathetic release of catecholamines in the bone marrow (BM) that increases production and egress of primed, glucocorticoid-insensitive Ly6Chi monocytes into circulation. Recruitment of BM-derived Ly6Chi monocytes following RSD occurs in specific brain regions associated with threat appraisal and fear/anxiety responses. Thus, we hypothesized that cross-talk between neurons, microglia, and vascular endothelial cells contributes to brain myeloid cell trafficking via cytokine and chemokine signaling, and vascular adhesion molecule expression.
Methods: Male C57BL/6 (6–8 weeks old) and CD-1 (12 months, retired breeders) mice were purchased from Charles River Breeding Laboratories (Wilmington, MA, USA). Resident C57BL/6 mice were housed in cohorts of 3 and aggressor CD-1 mice were singly housed. Male aggressor mice were introduced into cages of resident C57BL/6 mice from 17:00 to 19:00 (2 h) for one, three, or six consecutive nights. Microglia and monocytes were isolated from whole brain homogenates using Percoll density gradients. Total RNA was extracted from whole brain homogenates using TRIzol and reverse transcribed into cDNA. Quantitative PCR was performed using the Applied Biosystems' TaqMan Gene Expression assay. For immunohistochemistry, brains were collected 14hr after the last cycle of RSD. Fixed brains were frozen, sectioned and stained with antibodies specific for ICAM-1 or VCAM-1.
Results: RSD caused an exposure-dependent increase in the gene expression of ICAM-1, VCAM-1, CXCL1, and CXCL2

in the brain that increased with additional days of stress. RSD-induced ICAM-1 and VCAM-1 protein expression was localized to the vasculature endothelium of brain regions implicated in fear/anxiety responses to the stressor. Expression of adhesion molecules spatially corresponded to previously reported patterns of Ly6Chi monocyte trafficking to the brain. Comparison between isolated CD11b+ cells (microglia/macrophages) and enriched GLAST-1+/CD11b- cells (astrocytes) revealed that RSD increased the gene expression of IL-1β, CCL2, and CXCL2 in microglia/macrophages, but not astrocytes.
Conclusions: Taken together, these data demonstrate that RSD-induce activation of cerebral vasculature facilitates monocyte recruitment to the brain and depends upon dynamic interactions between neurons, endothelial cells and BM-derived Ly6Chi. The results from this study may contribute to the development of novel therapies to treat inflammation-induced behavioral disorders associated with chronic stress.
Disclosure: Nothing to Disclose.

31.3 Sustained Peripheral Inflammation Triggers Central Anandamide Hydrolysis to Promote Anxiety

Matthew Hill

University of Calgary, Calgary, Canada

Background: Many chronic inflammatory disorders exhibit psychiatric comorbidities, particularly in the form of anxiety and depression; however, the neurobiological mechanisms that mediate these changes are not well understood. Systemic inflammation can increase many stress-responsive systems, especially corticotropin releasing hormone (CRH) and glucocorticoid signalling. As CRH and glucocorticoids have been shown to mediate the anxiety-promoting effects of stress through interactions with the endocannabinoid (eCB) system, this set of experiments sought to determine if chronic, sustained inflammation (produced by colitis) can modulate central eCB function, and if this contributes to changes in anxiety.
Methods: Chronic inflammation was induced using a colitis model whereby adult male Sprague Dawley rats were administered trinitrobenzene sulfonic acid (TNBS) to produce colonic inflammation. Seven days after colitis induction, rats were euthanized and biochemical parameters of the eCB system were measured throughout discrete brain regions using mass spectrometry (eCB levels) and enzyme activity assays. The degree of colitis was assessed based on macroscopic tissue damage. Plasma corticosterone and cytokine levels were measured by ELISA. Anxiety behaviors were tested using an elevated plus maze and light dark box.
Results: Colitis produced a significant increase in the circulation of corticosterone and pro-inflammatory cytokines. Similar to what has been seen following chronic stress, tissue levels of the eCB anandamide (AEA) were found to be significantly reduced in the amygdala, hippocampus and medial prefrontal cortex. These changes were met by increased activity of the AEA degrading enzyme fatty acid amide hydrolase (FAAH). The degree of AEA reductions correlated strongly with the degree of colonic inflammation and macroscopic tissue damage. Anxiety-like behavior was found to be increased, with no corresponding changes in locomotor activity. Central administration of the FAAH inhibitor PF4458945 was found to reverse colitis-induced anxiety, indicating that the induction of FAAH and reduction of AEA signalling mediated the anxiety phenotype produced by systemic inflammation.
Conclusions: Using an animal model of colitis to produce a state of sustained, peripheral inflammation, we demonstrated that collateral changes in central eCB signalling mediated the generation of an anxiety-like state. We have previously demonstrated that sustained elevations in glucocorticoids, alone or produced by chronic stress, induce FAAH activity and reduce AEA signaling through recruitment of CRH signalling. Sustained inflammation is known to enhance central CRH signalling, which suggests that a dynamic interplay between inflammatory processes in the periphery and the modulation of central stress-related pathways can compromise eCB signalling to alter emotional behavior. Ongoing studies are defining the role of CRH and central cytokine signalling in these processes.
Disclosure: Part 1: Pfizer International, Consulting Fee, GW Pharmaceuticals, Consulting Fee, **Part 4:** GW Pharmaceutical, Unrestricted operating funds.

31.4 Persistent Changes in Hippocampal Gene Expression and Memory Function After a Systemic Inflammatory Event

Natalie Tronson

University of Michigan, Ann Arbor, Michigan, United States

Background: Neuroimmune signaling plays a number of key roles in normal neural functions, including synaptic plasticity and behavioral and affective changes during peripheral immune activation. After major illness or surgery, these changes in behavior can be severe, including post-operative cognitive deficits, depression, anxiety, and post-traumatic stress disorder. In many patients, the memory deficits and affective dysregulation persists for months or years - long after resolution of the inflammatory signaling. Here we will examine the changes in the brain that persist for weeks and months after resolution of a transient immune challenge.
Recent evidence suggests that immune signaling in the brain triggers changes in histone modifications that may mediate long lasting changes in memory function and affective regulation. In this project, we examined the persistent memory function, epigenetic modifications, and changes in gene expression in males and female mice 8 weeks after a systemic immune challenge.
Methods: Animals: Adult male and female C57Bl/6 mice (Invigo), 8-9 weeks old on arrival, were individually housed, with food and water ad libitum, in a colony room with 12:12h light: dark cycle.
Immune challenges: (a) Five 250μg/kg lipopolysaccharide (LPS) or saline injections over 13 days, were administered i.p. to C57Bl/6 male and female mice. (b) Surgical cryoinjury myocardial infarction was induced in male and female mice, controls were sham surgery and non-operated animals.

Behavioral tasks: Novel object recognition and context fear conditioning were used to assess changes in hippocampal-dependent memory 1 week and 8 weeks after the final LPS injection or surgery. Open field maze and elevated plus maze were used to assess changes in anxiety, and forced swim test was used to assess depression-like behavior.
Histone modifications: Hippocampi were rapidly dissected and flash frozen before subcellular fractionation and western-blotting to determine changes in histone acetylation, methylation, and phosphorylation.
Gene expression: 8 weeks after the LPS challenge, male and female mice were given an acute LPS or saline injection. 6 hours later, hippocampi were rapidly dissected and stored in RNAlater before RNA preparation and Illumina single end RNAsequencing.
Results: In all animals, cytokine signaling in the hippocampus was no longer elevated 8 weeks after immune challenge. Both males and females showed dysregulation of memory at 8 weeks, but not 1 week, after either subchronic LPS or myocardial infarction, with sex differences in the specific deficits observed. In contrast, there were persistent changes in anxiety behavior. Alterations in histone modifications, including acetylation at H3K9 and phospho-acetylation at H3K14ser10 were also evident 8 weeks after LPS or myocardial infarction. Consistent with long-lasting changes in histone modifications, we observed altered gene expression in RNAseq across pathways including inflammatory signaling, neurotrophins, and stress-related signaling.
Conclusions: Transient systemic immune activation causes long lasting epigenetic modifications and changes in gene expression in the hippocampus. These changes may mediate persistent cognitive deficits and affective dysregulation in patients with major illness or surgery. Our data also demonstrate that the underlying mechanisms are sex-specific and challenge-specific, suggesting differential vulnerability of males and female patients to the lasting effects of inflammatory and immune signaling.
Disclosure: Nothing to Disclose.

Panel
32. Predicting Suicidal Behavior; an Integration of Diathesis Traits and Markers of Imminent Risk

32.1 Biomarker Predictors of Short and Longer Term Risk for Suicidal Behavior

J John Mann

Columbia University, New York, New York, United States

Background: A stress diathesis model of suicidal behavior has proven to have heuristic value in descriptive and predictive domains. It may be understood from a clinical, cognitive and neurobiologic perspective. This presentation examines the neurobiologic perspective. There are biologic underpinnings of stress response systems and brain changes due to acute stress and brain changes related to traits that represent the diathesis. The acute stress changes are related to acute or short-term risk for suicidal behavior. The biology of the diathesis related traits are related to the longer term risk. We will describe findings for both short and longer term risk based mainly on brain imaging.
Methods: We have studied medication-free patients with mood disorders during a major depressive episode using PET and MRI. We have quantified both 5-HT1A binding and serotonin transporter binding. We have conducted structural MRI scans as well as DTI and functional connectivity studies. CSF assays of 5-HIAA, MHPG, HVA, GABA, glutamate and CRF were obtained from a subgroup of patients. Genomic assays involving GWAS, transcriptome and DNA methylation have been conducted. Data mainly from PET studies will be presented.
Results: Higher raphe nuclei 5-HT1A autoreceptor binding potential predicted more suicidal ideation at 3 and 12 months and greater lethality of subsequent suicidal behavior. Exploratory analyses showed that insula, anterior cingulate and dorsolateral prefrontal cortex 5-HT1A binding potential were also predictive of lethality. Contrary to our hypothesis, suicide intent was not predicted by 5-HT1A binding potential in any brain region. In contrast, midbrain raphe serotonin transporter binding potential did not predict future attempts, possibly due to lower statistical power.
CSF MHPG proved to be a shorter term predictor of risk of suicidal behavior and of lethality of suicidal behavior. CSF 5-HIAA is a longer-term predictor of suicide.
Conclusions: Greater raphe 5-HT1A binding potential predicts more severe suicidal ideation and more lethal suicidal behavior over a 2-year period. This effect may be mediated through less 5-HT neuron firing and release, affecting mood and suicidal ideation and thereby decision-making. Combined with low CSF 5-HIAA, which may be a consequence of higher autoreceptor binding and less neuronal firing and serotonin release, these are indices of longer term risk. In contrast the oradrenergic deficit may predict suicide risk in the shorter term via hopelessness and pessimism more broadly.
Disclosure: Part 2: Research Foundation for Mental Hygiene, Royalties for commercial use of C-SSRS.

32.2 Adaptive Measurement of Suicidality

Robert Gibbons

University of Chicago, Chicago, Illinois, United States

Background: Current suicide risk screening and measurement is inefficient, has limited measurement precision, and focuses entirely on suicide related items. We have developed a psychometric crosswalk between suicide, depression, anxiety and mania domains which provide a more balanced and complete spectrum of suicidal symptomatology.
Methods: Data from psychiatric outpatients at the University of Pittsburgh and a community health clinic were collected from January 2010-June 2012. 789 participants were enrolled in the calibration phase; 70% were female and 30% were male. The rate of major depression was 47%. The item bank contained 1,008 items related to depression, anxiety, and mania, including 11 suicide items. Data were analyzed using a bifactor model to identify a core dimension between suicidal ideation, depression, anxiety, and mania items. A computerized adaptive test was developed via simulation from the actual complete item responses in 308 subjects.

Results: 111 items were identified that provided a crosswalk between depression, anxiety and suicidal ideation (no mania items were retained). All items had high loadings on the primary suicide dimension (average = 0.67, range 0.49-0.88). Analyses revealed that an average of 10 items (5-20) correlated 0.96 with the 111 item scale, with precision of 5 points on a 100-point scale metric.
Conclusions: The Computer Adaptive Test – Suicide Scale is able to accurately measure the latent suicide dimension with an average of 10 items in approximately 2 minutes. Validation against an independent clinician administered suicide risk assessment and prediction of suicidal behavior in underway.
Disclosure: Part 1: Adaptive Testing Technologies, salary.

32.3 Innovations in the Prediction of Suicidal Behavior

Matthew Nock

Harvard University, Cambridge, Massachusetts, United States

Background: Matthew Nock will present new data on the use of behavioral (reaction time) tests, smartphone data, and wearable biosensors to predict suicidal behavior among adolescents and adults.
Methods: The data presented are from several samples of suicidal participants (and non-suicidal controls).
Results: Results reveal that some of these tests provide novel risk markers for suicidal thoughts and behaviors. Moreover, they are providing new, temporally fine-grained information about dynamic changes in risk factors over time.
Conclusions: This presentation will include a review of how each of these new approaches can be used to enhance the understanding, prediction, and prevention of suicidal behavior.
Disclosure: Nothing to Disclose.

32.4 Ketamine as a Prototype Fast-Acting Anti-Suicidal Medication

James Murrough

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

Background: Suicide is a devastating public health problem and very few biological treatments have been found to be effective in decreasing the intensity of suicidal ideation (SI) or in reducing the risk of suicide. Basic research is illuminating multiple biological pathways that may serve as novel targets for putative anti-suicidal medications. Our group and others have conducted a series of studies examining the antidepressant and anti-suicidal properties of the glutamate N-methyl-d-aspartate (NMDA) receptor antagonist ketamine. Despite promising initial discoveries, many questions remain concerning the utility of ketamine for the treatment of suicidality: the specificity of the effect beyond the antidepressant effect, potential methods of extending therapeutic effects, and defining the biological alterations that underlie rapid anti-suicidal effects in humans.
Methods: The author will review multiple sources of clinical trial data and other clinical research data germane to the question of ketamine as a prototype fast-acting anti-suicidal medication. The talk will focus on two clinical trials conducted by the author. In the first instance, we conducted a randomized, controlled trial of ketamine in patients who presented with clinically significant SI across a trans-diagnostic sample ($n = 24$). Patients received a single infusion of ketamine or midazolam (as an active placebo) in addition to standard of care. Suicidal ideation measured using the Beck Scale for Suicidal Ideation (BSI) 24 hours' post-treatment represented the primary outcome. Secondary analyses included the Montgomery-Asberg Depression Rating Scale–Suicidal Ideation (MADRS-SI) score at 24 hours and additional measures beyond the 24-hour time point. In the second instance, we will present interim or final data (unpublished) from a RCT comparing lithium to placebo in maintaining the antidepressant and anti-suicidal effects of ketamine among patients with treatment-resistant depression (TRD) who demonstrated a rapid therapeutic effect to ketamine.
Results: In the single dose study, the intervention was well tolerated and no dropouts occurred during the primary 7-day assessment period. BSI score was not different between the treatment groups at 24 hours ($p = 0.32$), however a significant difference emerged at 48 hours ($p = 0.047$). MADRS-SI score was lower in the ketamine compared to midazolam group at 24 hours ($p = 0.05$). The study comparing lithium to placebo following ketamine in TRD is ongoing. Interim blinded analyses show that ketamine exerted a rapid effect on SI, as expected. Multiple regressions show that reduction in perceived stress following ketamine is the most robust predictor of reduction in suicidal thinking, among multiple variables examined, including change in total depression severity, anxiety, anhedonia, and emotion regulation. These new unpublished findings have implications for mechanistic models of suicide risk, and for the development of treatment intervention.
Conclusions: Emerging basic, translational, and clinical trial data support the hypothesis that glutamate-acting compounds, and ketamine in particular, may trigger therapeutically relevant rapid anti-suicidal effects. These discoveries may have widespread implications for the medical treatment of suicidal thinking and behavior.
Disclosure: Part 1: Janssen, Consulting, Genentech, Consulting, **Part 4:** Avanir, Research Grant.

Panel
33. A Translational Approach to Refining Molecular Therapeutic Targets Within Glutamatergic Pathways

33.1 NMDA Receptor-Mediated, AMPA Receptor Driven Synaptic Plasticity in Neuropathic Pain and PTSD

Jeffrey Burgdorf

Northwestern University, Evanston, Illinois, United States

Background: JY-505,317 is an N-Methyl-D-aspartic acid receptor (NMDAR) modulator that acts as a functional glycine-site partial agonist. In recombinant human

NMDAR2A-2D –expressing HEK cells, partial agonist activity of JY-515, 317 was demonstrated at all 4 receptor subtypes, with the greatest potency at NMDAR2B. JY-515, 317 also enhanced whole cell NMDA current in CA1 pyramidal neurons in hippocampal slices at concentrations of 100-500 nM but not at 5 μM. Similarly, JY-515,317 enhanced the magnitude of long term potentiation (LTP) at Schaffer collateral-CA1 synapses in rat hippocampal slices at concentrations of 100-500 nM but not 2 μM. JY-515, 317 has approximately 50% oral bioavailability in rats (IV vs PO plasma AUC) and readily crosses the blood brain barrier. JY-515, 317 (1 mg/kg PO) facilitated Morris water maze learning from 1 hr to at least 5 days post-dosing. JY-515, 317 (1 mg/kg PO) also facilitated *ex vivo* hippocampal LTP and significantly increased the number of mature dendritic spine morphologies 24 hrs post-dosing.

Methods: The analgesic effects of JY-515,317 were evaluated in the rat Bennett model (chronic sciatic nerve constriction), the Taxol model of chemotherapy-induced pain, the streptozotocin (STZ) model of diabetic neuropathy, the formalin model of persistent pain, and the tail flick model of acute pain. The therapeutic potential for PTSD was evaluated using a contextual fear extinction paradigm. Potential sedative/ataxic effects were also examined in the rota-rod test.

Results: A single oral dose of JY-515,317 produced a rapid-acting (1 hr post-dosing) and long-lasting (24 hrs and 1-week post-dosing) mechanical and thermal analgesia in the Bennett model (mechanical EC50 = 0.3 mg/kg PO; thermal EC50 = 0.4 mg/kg PO), and mechanical analgesia in the STZ model (EC50 = 0.3 mg/kg PO) and Taxol (EC50 = 5 mg/kg PO) models. In contrast, the gabapentin (150 mg/kg PO) positive control was not analgesic in the Taxol model, and only produced analgesic effect 1 hr but not 24 hr or 1-week post-dosing in the Bennet and STZ models. JY-515,317 also reduced flinching in the late phase of the formalin test 1 hr post dosing (EC50 = 0.2 mg/kg PO) to a similar degree as gabapentin (150 mg/kg PO). The analgesic effects of JY-515,317 (10 mg/kg PO) in the Bennett model were blocked by pretreatment with either the AMPA receptor antagonist NBQX (10 mg/kg IP) or the NMDAR antagonist CPP (10 mg/kg IP). JY-515,317 (1 mg/kg PO) was ineffective in the tail flick model of acute pain. JY-515-317 (0.01 – 10 mg/kg PO) facilitated contextual fear extinction and consolidation 1hr to 14 days after a single dose. Lastly, JY-515,317 (1-100 mg/kg PO) did not induce a sedative/ataxic effect in the Rota-rod test when measured up to 2 hr post-dosing, whereas a therapeutic dose of gabapentin (150 mg/kg PO) did produce sedative/ataxic effects at 1 hr and 2 hrs post-dosing.

Conclusions: These data show that JY-515,317 has therapeutic potential as a rapid-acting and long-lasting therapeutic for the treatment neuropathic pain and PTSD with no sedative or ataxic effects. The therapeutic effects of JY-515,317 are due to a NMDAR triggered, AMPAR dependent enhancement of synaptic plasticity.

Disclosure: Part 1: Aptinyx Inc., Consultant fee and stock, Naurex Inc., Consultant fee and stock, **Part 2:** Aptinyx Inc., Consultant fee and stock, Naurex Inc., Consultant fee and stock, **Part 3:** Aptinyx Inc., Consultant fee and stock, Naurex Inc., Consultant fee and stock.

33.2 Examining the Relationship Between Glutamate Cycling and Rapid Acting Antidepressant Effects

Gerard Sanacora

Yale University, New Haven, Connecticut, United States

Background: Several drugs have recently been reported to induce rapid antidepressant effects in clinical trials and rodent models. Although the cellular mechanisms involved in this action remain unclear, reports suggest that increased glutamate transmission in the region of the mPFC contributes to these effects. In a series of studies using various pharmacological, behavioral and novel MRS methodologies we sought to characterize how changes in glutamate release and cycling are associated with antidepressant-like efficacy. Characterizing the time course and dose response effects of several different pharmaceutical agents on glutamate cycling may provide insight into possible mechanisms of both the antidepressant effects and alterations in cognition and perception that are associated with these agents.

Methods: Ex vivo 1H-[13C]-nuclear magnetic resonance spectroscopy of medial prefronal cortex (mPFC) tissue labeled in awake rats was used to determine glutamate/glutamine and GABA/glutamaine cycling. Rats were acutely pretreated with ketamine, GLYX-13 and a variety of other drugs and their appropriate vehicle (controls). At fixed times after drug injection, animals received an intravenous infusion of [1,6-13C2] glucose for 8 min to enrich the amino-acid pools of the brain with 13C, followed by rapid euthanasia. 13C enrichments of glutamate, glutamine and GABA were measured in mPFC.

Results: We found clear dose- and time-dependent effects of ketamine and other NMDA receptor antagonist drugs on behavior and the percentage of 13C enrichment of glutamate, glutamine and GABA (γ-aminobutyric acid). However, this effect was not seen with GLYX-13. We also found other drugs targeting non-NMDA glutamate receptors were able to alter glutamate/glutamine cycling and modulate ketamine-induced effects on both glutamate cycling and behavior. Further, we also demonstrated that drugs targeting the cholinergic system are also able to rapidly modulate glutamate cycling.

Conclusions: In summary, these studies demonstrate that several pharmacologically distinct classes of drugs, clinically related through their reported rapid antidepressant actions and effects on glutamate neurotransmission, can rapidly alter glutamate cycling. However, this effect was not seen with GLYX-13 at the expected dose range. We conclude that altered glutamate cycling is associated with antidepressant action and other behavioral effects of some NMDA receptor antagonists, and suggest that the rapid change in cycling could be used to predict antidepressant efficacy and side effect profiles of novel agents or to identify doses with unique behavioral properties. However, other drugs may be able to produce these effects by different effects on the glutamatergic neurotransmitter system.

Disclosure: Part 1: Allergan, Consulting, Alkermes, Consulting, AstraZeneca, Consulting, BioHaven Pharmaceuticals, Consulting, Hoffman La-Roche, Consulting, Janssen, Consulting, Merck, Consulting, Naurex, Consulting, Servier Pharmaceuticals, Consulting, Taisho Pharmaceuticals, Consulting, Teva, Consulting, Vistagen therapeutics, Consulting,

Part 2: Vistagen therapeutics, Consulting, **Part 4:** AstraZeneca, Reserach Contract, Bristol-Myers Squibb, Research Contract, Eli Lilly & Co., Research Contract, Johnson & Johnson, Research Contract, Hoffman La-Roche, Research Contract, Merck & Co., Research Contract, Naurex, Research Contract, Servier, Research Contract.

33.3 Relationships Between Brain Glutamate and Age in Schizophrenia: A Glutamate MRS Study in Cortex and Striatum

Ragy Girgis

Columbia University, New York, New York, United States

Background: While there has been much interest in developing new glutamatergic based treatments for schizophrenia, there has been limited progress. One potential reason that recent clinical trials of glutamatergic agents focused on the NMDA receptor have been negative is that glutamatergic abnormalities may be more prominent early in the illness. Prior studies that have suggested a decline in glutamate with age in schizophrenia may have been confounded by antipsychotic medication effects. The objective of this study was to examine the effects of age on glutamate 1H MRS in cortex and striatum in unmedicated patients with schizophrenia.
Methods: We recruited 20 antipsychotic free patients with schizophrenia (age: 31; SD 8) and 18 healthy control subjects (age: 29; SD 10). All subjects received single voxel, glutamate 1H MRS (PRESS TE 80). Voxels were placed in the medial prefrontal cortex (MPFC), hippocampus (HIPP) and striatum (STR). We measured glutamate, glutamine, and Glx (glutamate + glutamine) levels and performed Pearson product moment correlations between age and levels of these neurochemicals.
Results: There were no observed relationships between age and any metabolite for either group in STR (Patients: Glu: r = -0.28, $p = 0.24$; Gln: r = 0.01, $p = 0.97$; Glx: r = -0.23, $p = 0.34$; Controls: Glu: r = 0.01, $p = 0.98$; Gln: r = 0.32, $p = 0.20$; Glx: r = 0.10, $p = 0.70$), MPFC (Patients: Glu: r = -0.11, $p = 0.65$; Gln: r = -0.19, $p = 0.44$; Glx: r = -0.15, $p = 0.54$; Controls: Glu: r = 0.04, $p = 0.87$; Gln: r = 0.18, $p = 0.48$; Glx: r = 0.09, $p = 0.73$), or HIPP (Patients [$N = 11$]: Glu: r = -0.29, $p = 0.38$; Gln: r = -0.37, $p = 0.26$; Glx: r = -0.35, $p = 0.30$; Controls [$N = 10$]: Glu: r = 0.03, $p = 0.94$; Gln: r = 0.22, $p = 0.54$; Glx: r = 0.07; $p = 0.84$).
Conclusions: In contrast to prior studies of individuals with schizophrenia in which all subjects or some subjects were on antipsychotic medications, in our unmedicated sample, age does not appear to be related to 1H MRS measures of glutamate in these cortical and subcortical regions. This was the first study to specifically examine age effects on glutamate in an antipsychotic free sample of individuals with schizophrenia as measured by 1H MRS, and highlights the importance of sample selection in study design (age, medication status) when using MRS as a measure of target engagement of glutamatergic compounds. Unique Data: We present the first data showing that in an antipsychotic free sample of individuals with schizophrenia, there are no observable age effects on MPFC, STR, and HIPP glutamate as measured by 1H MRS. These data are unpublished and will be submitted for publication.
Disclosure: Part 4: Genentech, Research Support, Otsuka, Research Support, PharmaNac, Research Support.

33.4 Pilot Study of GLXY-13, an NMDAR Functional Glycine-Site Partial Agonist, in OCD

Carolyn Rodriguez

Stanford University School of Medicine, Stanford, California, United States

Background: A single intravenous dose of ketamine, a N-methlyl D-aspartate receptor (NMDAR) full antagonist, produces robust and rapid anti-obsessional effects in obsessive-compulsive disorder (OCD), but ketamine's side effects, including dissociation and nausea, may limit clinical use. GLYX-13, an NMDAR functional glycine-site partial agonist, has shown rapid anti-depressant activity without ketamine's side effects, and may be a new therapeutic strategy for OCD. We conducted the first test of the safety and feasibility of GLYX-13 administration in OCD. We also explored the drug's acute effects on obsessive-compulsive symptoms, depression, and anxiety at 90 and 230 minutes' post-infusion and on OCD treatment response at one-week post-infusion.
Methods: Seven unmedicated OCD outpatients (aged 18-55) were recruited (March, 2014 – March, 2015) and provided informed consent. Participants met DSM-IV and DSM-5 criteria for OCD with at least moderate symptoms (Yale-Brown Obsessive-Compulsive Scale [Y-BOCS] score ≥ 16). Exclusion criteria included severe depression (Hamilton Depression Rating Scale [HDRS] > 25), current CBT, and other conditions that made participation unsafe. In an open-label design, participants received a single 3-5 minute IV push of GLYX-13 (dose = 10 mg/kg). At baseline, 90, and 230 minutes' post-infusion, patients self-rated the severity of their obsessions and compulsions (Y-BOCS Challenge Scale [YBOCCS], a 10 item self-report form that assesses OCD symptoms [i.e., time spent, degree of control, severity] over the last 60 minutes [range 0-40]), anxiety (Beck Anxiety Inventory [BAI]), and depression (Beck Depression Inventory [BDI]). Side effects of dissociation, mania, and psychosis were assessed at baseline, 90, and 230 minutes' post-infusion. At baseline and one-week post-infusion, an independent evaluator, blind to study design, evaluated patients using the Y-BOCS, which appraises obsessive and compulsive symptoms over the prior week. Treatment response was defined a priori as $\geq 35\%$ Y-BOCS reduction. At one-week post-infusion, patients additionally self-rated anxiety (BAI) and depression (BDI). Outcomes were analyzed using a non-parametric Wilcoxon signed-rank matched-pairs test ($\alpha = .05$, two-tailed) with no adjustment for multiple comparisons given the exploratory nature of this study.
Results: All seven patients who received GLYX-13 completed the infusion, which was well tolerated and without adverse events. Assessments of dissociation, mania, and psychosis were not significantly changed from baseline. Compared to baseline, YBOCCS, BAI, and BDI scores were significantly lower at 90 and 230 minutes' post-infusion (all p values $< .05$). From baseline to one-week post-infusion, OCD severity,

as measured by the Y-BOCS, was not significantly decreased ($p = .20$), BDI was not significantly decreased ($p = .20$), and BAI was significantly decreased ($p = .02$). No patient met the treatment response criterion (≥35% Y-BOCS reduction) at one-week post-infusion.
Conclusions: The findings suggest that GLYX-13 is well tolerated in unmedicated OCD patients, as it is in patients with depression. Specifically, it did not transiently increase psychotomimetic effects following dosing in this sample of OCD patients, unlike ketamine in prior studies. In this small sample, GLYX-13 had acute effects on obsessions and compulsions, anxiety and depression. However, unlike ketamine, GLYX-13 did not have significant effects on OCD symptoms one-week post-infusion. Differences between ketamine and GLYX-13 in NMDAR engagement (full antagonism vs. partial agonism) or AMPA engagement (via ketamine's metabolite hydroxynorketamine) may account for differences in efficacy; however, this hypothesis needs formal testing in a mechanistic study.
Disclosure: Nothing to Disclose.

Mini Panel
34. Social Cognitive Impairments in Psychiatric Illness: From Trajectory to Treatment

34.1 Origins of Social Deficits in the Psychosis Phenotype

Abraham Reichenberg

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

Background: Social deficits are a core feature of psychotic disorders and are thought to emerge many years before first signs of illness. However, only few studies have been able to elucidate the extent and developmental progression of social deficits in the psychosis phenotype.
Methods: Data from the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort ($N = 4{,}724$) were analysed: social knowledge, practical judgment in social situations, level of social maturation, and the extent of development of moral conscience were measured at ages 4, and, 8; IQ measures were available for the same ages. Psychotic like experiences (PLEs) and psychotic disorder were ascertained at age 18 using a structured interview.
Results: Deficits in social knowledge and social judgment emerged more than a decade before symptoms (Effect size age $4 = -0.4$). Age 4 deficits in IQ were small (Effect size age $4 = -0.18$). Distinct developmental trajectories were found: deficits in social judgment decreased from age 4 to 8, but increased for general intellectual ability (Effect size change $= -0.22$).
Conclusions: Distinct developmental trajectories are evident for IQ and social adjustment. The findings show developmental delay in social judgment, social knowledge and social reasoning present early on in those experiencing psychotic symptoms in adulthood, and no longer evident by age 8. In contrast, intellectual deficits progressively increase during the same period.
Disclosure: Nothing to Disclose.

34.2 Behavioral and Neural Correlates of Social Cognition: A Dimensional Approach in Youth With Mental Illness

Aristotle Voineskos

University of Toronto, Toronto, Canada

Background: Within and across the major mental and neurodevelopmental illnesses, there is a continuum of impairment in social processes that significantly impact social function even after other symptoms have remitted. In order to address this heterogeneity in social deficits, a transdiagnostic approach can be applied to measure social processes along a continuous dimension to reflect the degree of impairment on a performance based task. This approach may facilitate the understanding of the mechanisms of brain circuit dysfunction underlying behavioural impairments. Improved understanding of these neural mechanisms may provide opportunities to develop novel interventions for social function with efficacy from adolescence to adulthood and beyond. The purposes of the present work is to identify brain-behaviour relationships of neural circuit structure/function with social cognitive performance in youth with schizophrenia spectrum disorders (SSDs), high-functioning autism spectrum disorder (HF-ASD), bipolar disorder (BD), and healthy controls.
Methods: A sample of $>$ 150 youth (age range 16-35) with either an SSD, HF-ASD, BD, and healthy controls ($n = 51$) were recruited in the context of existing research protocols at the Centre for Addiction and Mental Health. All individuals were aged 16-35 years (inclusive), were verbal, and had an IQ > 70. The BD subset was clinically euthymic with depression and mania rating scores $\leq$ 10 for 4 weeks preceding study entry. All participants participated in multimodal neuroimaging, neurocognitive, and social cognitive assessments, with the focus being on the 3 parts of The Awareness of Social Inference Test – Revised (TASIT-R) to assess both emotion evaluation and social inference. Social cognitive performance was characterized, and correlational brain-behavior relationships were characterized across the entire group using a median split, and then quartile based analysis. Similarity network fusion (SNF) was then performed to uncover subgroups of participants that demonstrated neural similarities independent of diagnosis that were related to social cognitive performance.
Results: The TASIT data were analyzed using a series of univariate analyses of variance (ANOVAs) to first explore diagnosis-specific impairments. The ASD and SSD groups did not differ from one another on any of the subtests and performed worse than BD and healthy controls in parts 2 and 3. Youth with BD did not differ significantly from healthy controls. Following a median-split based on TASIT subscale performance, (accounting for effects of gender and education), a voxel-wise approach was used, which identified effects largely for TASIT3 (thinner cortex in low performers compared to high performers in a number of frontal and temporal clusters including the caudle middle frontal gyrus and inferior temporal gyrus). Comparisons also revealed reduced surface are for low performers in clusters such as inferior frontal and superior temporal gyri. Interactions showed that certain effects were restricted to high and low performing males with no difference in females. SNF analyses revealed several subgroups. Most notable were two poor performing subgroups comprised exclusively of females

and males respectively. These subgroups were dominated by SSD and ASD participants, but did include a minority of BD and HC participants. Influential neural correlates included intracranial volume, inferior frontal gyrus, superior temporal gyrus, and precuneus.
Conclusions: This is the first study to examine the range of neural circuit and social cognitive impairment across all of these disorders. The wide range of performance across individuals in this study, and the similar neural circuit-behavior relationships across the sample lend credence to Research Domain criteria efforts. They also move us closer to targetable circuit-based interventions at the time of maturation of social cognitive brain circuitry. Initial results from SNF analysis suggest that youth drawn from different diagnostic groups may have brain behavior relationships related to social cognitive performance that in some cases have little to do with diagnostic category, and it may also be critical to include sex as a factor when identifying neural correlates of social cognitive impairment. These results have important implications for social cognitive treatment study design, which may be informed by biological subgroups identified here.
Disclosure: Nothing to Disclose.

34.3 fMRI Biomarkers of Social Cognitive Training in Psychoses

Junghee Lee

University of California, Los Angeles, Los Angeles, California, United States

Background: Impaired daily functioning in interpersonal relations, work, and independent living is a core feature of psychotic disorders, but existing pharmacological treatments have limited benefits for improving daily functioning. Recent efforts have focused on directly targeting determinants of impaired daily functioning with the goal of translating improvements on these determinants into improved daily functioning of patients with psychoses. A key determinant of functional outcome in psychosis is social cognition, and our research group recently developed a 12-week social cognitive skills training program (SCST) and demonstrated the initial efficacy of this program on behavioral measures in a randomized controlled clinical trials with individuals with psychoses. This demonstrated efficacy on behavioral measures suggests that potential biomarkers might capture, at a neural level, the benefits of social cognitive training. Hence, this study aimed to identify biomarkers of social cognitive skills training in individuals with psychoses using functional magnetic resonance imaging (fMRI).
Methods: Twenty-six patients with mixed psychotic disorders participated in a 12-week randomized controlled clinical trials. Thirteen patients received social cognitive skills training (SCST) and 13 patients received illness management training (Control). At baseline and 12-week endpoint, patients completed a Facial Affect Matching Task and a Belief Attribution Task in the 3T Siemens Trio scanner at UCLA. These two paradigms assess constructs that were targeted during the social cognitive training: facial affect perception and mental state attribution. For statistical analyses for each fMRI procedures, multiple regression analysis of FSL was employed.
Results: At baseline, there was no significant activation difference in key brain regions between the SCST and Control group during either task. At 12-week endpoint compared to baseline during the Facial Affect Matching Task neither group showed any activation difference in key brain regions, including the amygdala and orbitofrontal cortex. During the Belief Attribution Task, patients who received SCST showed increased activation in the left temporoparietal junction compared to baseline at 12-week endpoint, but patients in the control group did not.
Conclusions: In this study, we examined whether task-related neural activation can capture the effects of social cognitive skills training in individuals with mixed psychotic disorders. Although we did not observe any neural activation differences during the Facial Affect Recognition, we found increased neural activation in the temporoparietal junction, but only in patients who received social cognitive skills training. These findings suggest that neural activations associated with belief attribution may be a potential biomarker when assessing the benefits of social cognitive training in psychosis.
Disclosure: Nothing to Disclose.

Mini Panel
35. ACNP Ethics Committee Mini Panel

35.1 Recent Advances in Genome-Editing technology: Applications and Challenges

Guoping Feng

Massachusetts Institute of Technology, Cambridge, Massachusetts, United States

Background: The development of new, highly efficient genome-editing technologies, such as CRISPR/Cas9 system, has revolutionized the way of manipulating genomes for studying gene functions, generating animal models, and developing gene therapy approaches.
Methods: Multiple genome-editing technologies including TALEN and CRISPR/Cas9 will be discussed.
Results: Despite great promises, there are several key challenges that need to be solved before these technologies can truly reach their potentials. These challenges include off-target genome-editing, low efficiency of homologous-directed repairs, and mosaicism. New advances in these areas will be discussed.
Conclusions: Significant progresses have been made in addressing these key technical challenges, raising the hope that next generation of technology for precise genome-editing is on the horizon for both research and medical applications.
Disclosure: Part 2: Rugen Therapeutics, equity, **Part 4:** Roche, Research grant.

35.2 Human Induced Pluripotent Stem Cells (hiPSC) in Neuropsychiatric Diseases

Dan Rujescu

University of Munich, Munich, Germany

Background: CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeats) is a new powerful technology

which will is currently transformed to medicine. Already now it is possible to study genes and to turn them on or off.
Methods: The aim is to learn more about their role in human diseases, e.g. neuropsychiatric disorders. Dan Rujescu will talk about patient-derived human induced pluripotent stem cells (hiPSC) which are used to study rare genetic variants recently described in neuropsychiatric diseases including schizophrenia in the context of the patient's genetic background. Complementary, CRISPR-Cas9 technology enable the generation of isogenic hiPSC lines containing only the variant under investigation. These are the starting point for the generation of the desired 2D and 3D (e.g. organoids) cell systems.
Results: Scaling up and miniaturizing these to detect underlying pathobiology and signatures for pharmacological screens are under way.
Conclusions: Ultimately, this may represent an important step towards precision medicine in psychiatry.
Disclosure: Nothing to Disclose.

35.3 Gene Editing: What Are the Ethical Opportunities and Challenges with This Breakthrough Technology?

Jeremy Sugarman

Johns Hopkins University, Baltimore, Maryland, United States

Background: New technologies make it possible to somewhat easily edit the human genome, which could include removing the genes that cause devastating disease. Some of these tools are already being used experimentally to see if they can help with the treatment of diseases such as HIV infection. Others hope that the technology could be used in the future to prevent genetic diseases that are passed on to future generations, but whether it is safe and ethically appropriate to do this is unclear.
Methods: Recent policy and ethics literature was reviewed and analyzed conceptually.
Results: There are ethical concerns related to gene editing related to: mis-targeting causing uncertain and unwanted problems, the specter of eugenics, enhancement, the possibility that alterations may not be reversible, alterations may exacerbate inequalities, and manipulating the germline has long been viewed as morally unacceptable to many. Unfortunately, current ethics oversight mechanisms are variable, which raises concern.
Conclusions: Continued deliberation among a broad range of stakeholders regarding the range of appropriate uses of gene editing technologies is clearly necessary. In addition, guidelines and oversight promise to afford protections to individual and societal interests.
Disclosure: Part 1: Merck KGaA, Consulting, Quintiles, Consulting, Novartis, Consulting, **Part 2:** Merck KGaA, Income and Travel, Quintiles, Income and Travel.

Panel
36. Trace Amine-Associated Receptor 1 as a Target for Neuropsychiatric Therapeutics

36.1 Selective TAAR1 Partial Agonists Modulate Dopaminergic and Serotonergic Neurotransmission and Thereby Regulating Reward Circuits, the Limbic Network, Cognitive Processes, Mood States, Body Weight and Glucose Levels

Marius Hoener

F. Hoffmann-La Roche, Basel, Switzerland

Background: Dysregulation of monoaminergic neurotransmission is a hallmark of major neuropsychiatric disorders. Activation of the trace amine-associated receptor 1 (TAAR1) by specific ligands modulates monoaminergic neurotransmission and represents a novel therapeutic option.
Methods: Through a medicinal chemistry program potent and selective TAAR1 ligands were identified and further optimized for their physicochemical and pharmacokinetic properties in rat, mouse, Cynomolgus monkey and human. TAAR1 agonists have been extensively profiled in nonclinical models predictive of antipsychotic, negative symptoms in schizophrenia, stress response modulating, pro-cognitive, anti-addictive, weight-reducing, and glucose-regulating activities. TAAR1 knock-out as well as Taar1 overexpressing mice and rats along with specific anti-rat and anti-human TAAR1 antibodies have been used to confirm specificity of behavioral effects, to better understand the underlying mechanism of action, and to identify the expression and localization of TAAR1 in different species.
Results: Manipulating TAAR1 activity using optimized ligands and Taar1 knock-out as well as Taar1 overexpressing mice and rats we showed that TAAR1 modulated dopaminergic, serotonergic and glutamatergic neurotransmission. Co-immunoprecipitation experiments supported a functional interaction of TAAR1 with the dopamine D2L receptor (D2R) in heterologous cells and in brain tissue. Interaction of TAAR1 with D2R altered the subcellular localization of TAAR1 and increased D2R agonist binding affinity. Using specific β-arrestin 2 (βArr2) complementation assays we showed that the interaction of TAAR1 with D2R reduced βArr2 recruitment to D2R. In addition, we report that TAAR1 also signals via βArr2. In the presence of D2R, cAMP signaling of TAAR1 was reduced while its βArr2 signaling was enhanced, resulting in reduced GSK3β activation. In rodents, activation of TAAR1 agonists blocked psychostimulant-induced hyperactivity. TAAR1 agonists produced pro-cognitive effects in rodents and monkeys and were active in models indicative for negative symptoms and showed anti-addictive properties in rats. In addition, TAAR1 activation increased glucose-dependent insulin secretion in INS1E cells and human islets and elevated plasma PYY and GLP-1 levels in mice. In diabetic db/db mice, the TAAR1 agonist normalized glucose excursion during an oral glucose tolerance test. Sub-chronic treatment of diet-induced obese (DIO) mice with the TAAR1 agonist resulted in reduced food intake and body weight. Furthermore, insulin sensitivity was improved and plasma triglyceride levels and liver triglyceride content were lower than in controls.

Conclusions: Our results provide extended understanding of TAAR1 signaling pathways and its impact on the monoaminergic neurotransmission supported by the complex formation with D2R. We show that interaction of TAAR1 with D2R increases surface expression of TAAR1 and presumably translocates βArr2 from D2R to TAAR1 which leads to a silencing of the GSK3β signaling. Based on its unique behavioral profile TAAR1 activation represents a novel therapeutic option for neuropsychiatric disorders. In addition, we have identified TAAR1 as a novel integrator of metabolic control, which acts on gastrointestinal and pancreatic islet hormone secretion. Thus TAAR1 also qualifies as a novel and promising target for the treatment of type 2 diabetes and obesity. Given its positive metabolic profile, TAAR1 agonists may provide benefit for the general medical condition of patients with schizophrenia and bipolar disorder with the co-morbidities associated with metabolic syndrome.
Disclosure: Part 1: F. Hoffmann-La Roche, Employed, F. Hoffmann-La Roche, Employed (Spouse).

36.2 Novel Pharmacotherapeutics Based on the Trace Amine-Associated Receptor 1

Juan Canales

University of Leicester, Leicester, United Kingdom

Background: The abuse of psychomotor stimulants, such as cocaine and methamphetamine, remains at epidemic levels worldwide. Currently there are no specific medications to safely facilitate detoxification and promote quicker recovery from chronic abuse of stimulant drugs. Recent evidence suggests that the trace amine-associated receptor 1 (TAAR1) may be a promising target for the development of more effective, new generation therapies in addiction due to its strategic anatomical location of TAAR1 and its unique ability to regulate monoamine neurotransmission and stimulant-induced behaviors.
Methods: To study the therapeutic potential of TAAR1 agonists, we used clinically relevant models of addiction, including stimulant sensitization, intracranial self-stimulation, self-administration and relapse models. To examine TAAR1 regulation of stimulant-induced changes in dopamine transmission we used fast-scan cyclic voltammetry.
Results: The results showed that TAAR1 activation (1) blocked the acquisition and expression of methamphetamine sensitization, (2) prevented the cocaine-induced lowering of intracranial self-stimulation thresholds, (3) reduced the motivation to self-administer cocaine and methamphetamine, (4) blocked relapse to drug seeking after chronic self-administration and (5) prevented stimulant-induced changes in dopamine transmission in the nucleus accumbens. Moreover, TAAR1 displayed low abuse liability in self-administration models.
Conclusions: Taken together, these observations indicate that TAAR1 activate has a unique ability to regulate stimulant-induced behaviours and strongly support the candidacy of TAAR1-based medications as potential substitute treatments in drug addiction.
Disclosure: Nothing to Disclose.

36.3 Trace Amine-Associated Receptor 1 Partial Agonists as Potential Narcolepsy Therapeutics

Thomas Kilduff

SRI International, Menlo Park, California, United States

Background: Trace amine-associated receptor 1 (TAAR1) agonists have been shown to have pro-cognitive, stress-reducing, antipsychotic-like, weight-reducing and glucose-lowering properties and, in rats, to promote wakefulness (Revel et al, 2012, Biol. Psychiatry; Revel et al, 2013, Mol. Psychiatry). To help elucidate a potential role for TAAR1 in sleep/wake regulation, we evaluated the sleep/wake phenotype of Taar1 knockout (KO) and over-expressing (OE) mice and compared the effects of TAAR1 agonists on sleep/wake in these strains to wildtype (WT) mice. We then evaluated whether TAAR1 partial agonists had beneficial effects in two mouse models of the sleep disorder narcolepsy.
Methods: Taar1 KO, OE and WT mice were instrumented for recording of EEG, EMG, body temperature (Tb) and locomotor activity (LMA). In the first experiment, mice were sleep-deprived (SD) for 6h following a 24h recording to characterize basal sleep/wake architecture. In a second experiment, mice were given three doses of the TAAR1 partial agonist RO5263397, caffeine or vehicle p.o. In the third experiment, the effects of the TAAR1 partial agonists RO5263397 and RO5256390 on sleep/wake, LMA, Tb and cataplexy were assessed in two mouse narcolepsy models.
Results: During baseline, OE mice had more wakefulness than WT mice. EEG spectral power in the theta (4.5-8Hz) and low gamma (30-60Hz) bands was elevated in KO mice. During SD, OE mice exhibited longer wake bouts and, during recovery, had increased NREM delta power and more wake bouts, indicative of a strong waking drive. In WT mice, RO5263397 dosing at ZT6 increased waking and reduced NREM and REM sleep, decreased gamma power during wake and NREM, and decreased Tb without affecting LMA; these effects were absent in KO mice and potentiated in OE mice. By contrast, caffeine increased wake time, gamma power during NREM, and LMA in all strains compared to vehicle; this effect was attenuated in KO and potentiated in OE mice. In the mouse narcolepsy models, both TAAR1 compounds mitigated cataplexy, the pathognomonic symptom of this disorder. The therapeutic benefit was mediated through a reduction in the number of cataplexy episodes and time spent in cataplexy.
Conclusions: TAAR1 overexpression mildly increases wakefulness whereas TAAR1 partial agonism causes a profound dose-dependent increase in wake and reduced REM sleep. These results indicate a modulatory role for TAAR1 in sleep/wake and cortical activity. The reduction of cataplexy suggests TAAR1 partial agonism as a new therapeutic pathway for the treatment of this orphan disease.
Disclosure: Part 1: Merck Pharmaceuticals, Consultant, Pfizer, Inc., Consultant, **Part 4:** Sunovion Pharmaceuticals, Contract, F. Hoffman-LaRoche, Contract.

36.4 Investigation of TAAR1 Agonism on Sleep and Cognition in Cynomolgus Macaques

Tanya Wallace

SRI International, Menlo Park, California, United States

Background: Trace-Amine-Associated Receptor 1 (TAAR1) is a G-protein-coupled receptor that has affinity for the trace amines, a subgroup of biogenic amines. Recent studies have shown that brain penetrable TAAR1 agonists have pro-cognitive, antidepressant- and antipsychotic-like properties in both rodent and non-human primates (NHPs). TAAR1 agonism has also been shown to increase wakefulness in rodents (Revel et al, 2012; Molecular Psychiatry). Since rodents are nocturnal and have polyphasic sleep/wake cycles, we investigated the effects of TAAR1 agonists in Cynomolgus macaques, a diurnal species that exhibits consolidated night-time sleep as in humans. In addition, we extended our evaluation of the cognitive-enhancing potential of TAAR1 agonists in macaques by testing the acute effects of a TAAR1 partial agonist in a delayed match-to-sample (DMTS) test of working memory (WM).
Methods: Adult, male Cynomolgus macaques ($n=6$) were implanted with epidural electroencephalograph (EEG) electrodes above left (Fp1) and right (Fp2) frontal cortex referenced to occipital (Oz) cortex, and electromyogram (EMG) electrodes in the trapezius. Animals were kept under LD12:12; all studies were within-subject designs. For the sleep studies, the partial TAAR1 agonist, RO5263397 (0.1, 1 and 10 mg/kg; p.o.) or the psychostimulant, caffeine (10mg/kg; i.m.) was administered just prior to lights off. EEG/EMG was measured by telemetry and both polysomnographic and spectral power measures were assessed during the initial 6h following drug administration. EEG/EMG activity was scored in 30s epochs using guidelines of the American Academy of Sleep Medicine (AASM, 2007) for scoring human sleep but adapted for macaques. For the cognition studies, animals were trained on a visual DMTS test to assess WM using a 5-sec delay between the sample and choice phase. The choice phase contained 2-5 distractor stimuli to increase task difficulty and correct choices were rewarded with a 190-mg flavored food pellet. The TAAR1 agonist was administered orally 1.5 h prior to DMTS or sleep studies.
Results: As expected, caffeine produced significant increases in total time awake as well as wake after sleep onset (WASO), and reductions in overall sleep efficiency, REM and NREM duration, NREM/REM cycles and REM/NREM ratio compared to vehicle. TAAR1-treated groups showed significant reductions in overall REM duration, NREM/REM cycles and REM/NREM ratio and increases in the total number of arousals in comparison to the control group, but did not show significant increases in wakefulness or WASO. Spectral power analysis revealed that caffeine-treated animals showed a reduction in delta (1-4Hz) power during both wake and NREM sleep and increases in theta (4-8Hz) and alpha (8-12Hz) power during NREM sleep in the initial 6h of the dark period. No significant changes in spectral power were observed in any EEG frequency band during wake, NREM or REM sleep following TAAR1 treatment. Evaluation of the effects of the TAAR1 agonist on WM is ongoing.
Conclusions: These results indicate that TAAR1 partial agonists have some effects on NHP sleep that are similar to those previously described in rodents, e.g., REM-suppression.
Disclosure: Supported by: NIH R21NS085757.

Study Group

37. Publicly Funded BRAIN Initiatives From US and EU Perspectives: Will the Brain Yield to the Bucks?

Dean F Wong*, Albert Gjedde, Roberto Barbero, Gregory Farber, Julie Brefczynski-Lewis, Albert Gjedde, Natalie Rasgon, Mark Rasenick

Johns Hopkins Medical Institutions, Baltimore, Maryland, United States

Study Group Proposal: The potential progress of publicly funded brain initiatives in Europe, and more recently in the US, largely will be the result of efforts funded by the public purse. Co-sponsored by the ACNP Liaison Committee, members of this study group will review the intent of the funding agencies to focus on the need for researchers to develop transformative methodology that promises dramatically to reveal brain networks and interactions among brain and behavior that expand the knowledge and impact of brain mechanisms. Often compared to the previous decade of the human genome projects, the hope is that the advent of brain initiatives will bring on a decade of enhanced funding for the pursuit of these lofty goals.
The study group participants include participants from the US BRAIN Initiative, especially focused on translational neuroscience, and the EU Human Brain Project represented by information technology focused on the construction of a human brain model. Participants will include Julie Brefczynski-Lewis of West Virginia University, a recipient of a BRAIN Initiative award for a novel ambulatory, low-dose PET imager, who has worked with fellow awardees to increase networking among colleagues. Dean Wong of Johns Hopkins University will describe the collaborative efforts of four US universities and a Danish university to develop brain imaging approaches that go beyond traditional PET and fMRI. Albert Gjedde of the University of Copenhagen is a co-recipient of the US BRAIN Initiative grant with experience from the EU Human Brain Project, who will contrast the aims and goals of the two programs.
In addition to researchers, several representatives with important roles in the BRAIN Initiative include Greg Farber of NIMH and Robbie Barbero who is Assistant Director of Biological Innovation for the White House Office of Science and Technology Policy. Mark Rasenick, member of the Liaison Committee, and Natalie Rasgon, chair of the Liaison Committee, will discuss how ACNP may support academic-government facilitation of these substantial initiatives for mutual benefit. The participants will discuss how such grants may accelerate research beyond traditional funding mechanisms, by enabling projects that may otherwise have been deemed too risky or conceptually vague for R01 or even R24 grant categories. Recipients of these awards will describe the impact of such funding on their own laboratories, careers, and institutions. Altogether, this study group helps describe the rationale, promises, and progress of the initiatives and

how governmental policy efforts may unfold with public/private partnerships and undergo molding over the coming decade when the initiatives are expected to allow the brain sciences to flourish in the US and EU.

Disclosure: Part 1: Addex, Pharma research grant, Avid, Pharma research grant, Dart Neuroscience, Pharma research grant and consultant, GE Healthcare, Pharma research grant, Intracellular, Pharma research grant, J+J, Pharma research grant, Lundbeck, Pharma research grant, Pfizer, Pharma research grant, Roche, Pharma research grant, **Part 2:** Addex, Pharma research grant, Avid, Pharma research grant, Dart Neuroscience, Pharma research grant, GE Healthcare, Pharma research grant, Intracellular, Pharma research grant, J+J, Pharma research grant, Lundbeck, Pharma research grant, Pfizer, Pharma research grant, Roche, Pharma research grant, **Part 4:** Addex, Pharma research grant, Avid, Pharma research grant, Dart Neuro Science, Pharma research grant, GE Healthcare, Pharma research grant, Intracellular, Pharma research grant, J+J, Pharma research grant, Lundbeck, Pharma research grant, Pfizer, Pharma research grant, Roche, Pharma research grant.

Panel
38. Immune Dysfunction in Psychiatric Disease: Complicated Issues Call for Multifaceted Approaches

38.1 Translational Studies of Gut-Driven Complement Activation in Psychiatric Disorders and Mouse Models

Emily Severance

Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Background: Compromised gut- and brain-associated vascular barriers implicate a role for microbial dysbiosis in the pathogenesis and pathophysiology of psychiatric disorders. We hypothesize that a low-level peripheral inflammation increasingly demonstrated in individuals with schizophrenia may be related to the translocation of gut-based microbes, toxic products and other antigens into systemic circulation. The activation of peripheral immune pathways during the presence of endothelial barrier dysfunction in turn impacts the CNS where classic immune molecules such as complement C1q help to mediate synaptic pruning.

Methods: In updated analyses of our gut-immune-brain interactome model, we investigated the role of gastrointestinal (GI) inflammation and complement dysregulation in clinical biospecimens of people with psychiatric disorders and in mice. In humans, we examined case-control comparisons of serological biomarkers of microbial translocation (sCD14, LPS-binding protein, C. albicans, S. cerevisiae, food antigens) and complement activation (C1q, C4). In mouse models, we evaluated how gut inflammation may precipitate corresponding complement expression changes in the brain.

Results: We found that multiple markers of leaky gut processes were repeatedly elevated in people with psychiatric disorders compared to individuals without a past history of mental health issues. Furthermore, some of these markers co-associated with clinical conditions including inflammatory bowel disorders, endocrine disturbances and cognitive performance. Peripheral complement activation was significantly associated with dietary antigens in adults with psychiatric disorders as well as in mothers whose children went on to develop these disorders as adults. In mouse models of Toxoplasma gondii-generated gut inflammation, infection caused significant elevations of complement components C1q, C1r and C6 in the prefrontal cortex.

Conclusions: Our blood biomarker and microbiome data continue to provide evidence that a disturbed GI equilibrium and associated barrier defects may be inherent to the pathophysiology of disorders such as schizophrenia. This disequilibrium in turn may contribute to the dysregulation of synapse-related immune factors peripherally and in the CNS. By understanding how peripheral system imbalances influence cognition and psychiatric symptoms, we may better design new strategies to effectively treat complex brain disorders.

Disclosure: Nothing to Disclose.

38.2 Joint Evaluation of C-Reactive Protein and Polygenic Risk Scores as Schizophrenia Risk Factors

Vishwajit Nimgaonkar

University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, United States

Background: A multi-factorial polygenic threshold model incorporating environmental and genetic risk factors can satisfactorily explain the etiology of schizophrenia (Scz). Several studies have indicated infectious and immune-related abnormalities in Scz, one of which is abnormal C Reactive protein (CRP), a well-known proxy for infections/immune abnormalities in the serum of Scz subjects. On the other hand, a portion of the genetic risk for Scz can be estimated using the polygenic risk score (PGRS). It is not known whether there is an interaction in the risks traceable to CRP and PGRS. The study thus aimed to estimate risk for Scz attributable to CRP and PGRS.

Methods: Patients with Scz were ascertained and evaluated systematically using structured diagnostic criteria (DSM IV criteria). Individuals screened for absence of psychosis served as controls (total sample, $N=$ 1241). CRP was assayed in the serum using Enzyme linked Immunoassays. Common DNA polymorphisms were assayed from genomic DNA using the Infinium BeadChip PsychArray and PGRS was estimated, based on prior genome wide association studies. Risk attributable to CRP and PGRS was estimated using a logit model also incorporating demographic and clinical covariates.

Results: While CRP levels and PGRS were not significantly correlated, both were significantly associated with Scz risk; CRP: odds ratio (OR) 1.30, (95% confidence intervals, CI, 1.15, 1.47); $p=$ 0.0004; PGRS: OR 1.77, 95% CI 1.56, 2.02; $p=$ 4.21x10-18.

Conclusions: PGRS and CRP are significant predictors for Scz status and their effects are additive. Replicate studies, as well as joint analyses with additional risk factors, are warranted.

Disclosure: Nothing to Disclose.

38.3 Peripheral Markers and Neuropsychological Features of Schizophrenia and Bipolar Disorder

Faith Dickerson

Sheppard Pratt Health System, Baltimore, Maryland, United States

Background: Neuropsychological deficits are a central feature of schizophrenia and bipolar disorder. These deficits include deficits in memory, visual spatial ability, and executive functioning. These deficits are independent of other illness symptoms and a major determinant of social disability. The immune response to infections agents is known to generate cognitive impairments in humans and in animal models of infection. We hypothesize that exposure to infectious agents and systemic inflammation may be important contributors to neuropsychological functioning in schizophrenia and bipolar disorder. We have studied peripheral markers such as antibodies to human herpes viruses including Herpes Virus type 1 (HSV-1). HSV-1 is an enveloped, double-stranded DNA virus that is capable of infecting the central nervous system of otherwise healthy individuals and establishing latency. We have also studied markers of systemic inflammation including those in the pentraxin family such as C-reactive protein (CRP). CRP is a pentameric protein which is generated in the liver and secreted in the blood and which plays a central role in the inflammatory process in humans. The measurement of CRP in the blood provides a reliable marker of inflammation caused by infectious and other inflammatory agents.
Methods: In updated analyses of our model of infectious and immune markers as contributing to neuropsychological deficits, we investigated the role of exposure to HSV-1 and elevated CRP on cognitive performance. We measured HSV-1 exposure by immunoassay and CRP by high sensitivity assay in several cohorts of individuals with schizophrenia or bipolar disorder as well as non-psychiatric controls. All subjects were evaluated on the Repeated Battery for the Assessment of Neurological Status (RBANS), a standard neuropsychological battery.
Results: We found an independent and additive effect of antibodies to HSV-1 and elevated levels of CRP on neuropsychological test performance in the psychiatric populations. Effects were most prominent in memory domains. In some cases, we also found an additive effective with genetic polymorphisms associated with cognitive deficits such as the Val/Val polymorphism of COMT.
Conclusions: Our data from peripheral markers continue to provide evidence that immune dysregulation is associated with the reduced neuropsychological performance in individuals with schizophrenia and bipolar disorder. There is currently no treatment for cognitive problems in these populations that has been established as clearly effective and implemented in routine clinical care. Clinical trials are currently underway to assess the effect of anti-microbial and anti-inflammatory agents in improving neuropsychological functioning in these populations. These studies may lead to new strategies to more effectively treat the cognitive deficits associated with serious mental illness.
Disclosure: Nothing to Disclose.

38.4 Evidence for Immune Dysfunction in Postmortem Brain Samples From People With Schizophrenia

Maree Webster

Stanley Medical Research Institute, Rockville, Maryland, United States

Background: The Stanley Neuropathology Consortium Integrative Database (SNCID) www.sncid.stanleyresearch.org contains over 5,000 neuropathology data sets, and 17 microarray data sets from the same cohort of brains. Recent analysis of this data found most markers that are differentially affected in schizophrenia are down-regulated in cases as compared to controls. These down-regulated markers are related to synaptic transmission, neural development and energy metabolism. Despite increasing evidence showing abnormalities in the immune system in schizophrenia, few data sets submitted to the SNCID are related to immune function. RNA-sequencing (RNA-seq) is a more sensitive technique to assess gene expression, and has been used here in the frontal cortex and hippocampus in an attempt to identify immune changes in the brain of subjects with schizophrenia. The results lead to further RNA-seq of the choroid plexus (CP).
Methods: The mRNA-sequencing was conducted using Illumina Genome Analyser or the HiSeq 2000 platform. All reads were mapped to H. sapien reference genome using TopHatv2.0.0 with UCSC refFlat gene model annotation file (buildhg19) on the -G parameter. The quantification of gene expression was accomplished by HTseq v0.53p9 and edgeR package. Unsupervised Weighted Correlation Network Analysis (WGCNA) was used to build networks of co-expressed genes using the RNA-seq data. Correlation analysis was performed between co-expressed gene networks and neuropathological trait data from the same subjects.
Results: In contrast to previous results, we found that in the RNA-seq data most differentially regulated genes were up-regulated: 123/144 and 25/27 in the hippocampus and frontal cortex, respectively. The genes were enriched for inflammatory response pathways and many of the same genes were up-regulated in both regions e.g. S100A8, S100A9, CHI3L1, CHI3L2, IFITM2, IFITM3. Moreover, many of these genes are expressed in monocytes, pericytes and endothelial cells implicating the blood-brain-barrier (BBB). Subsequent analysis of CP found 23/27 differentially expressed genes up-regulated and these were also enriched for immune/inflammatory response, with several up-regulated genes common to both hippocampus (CD163, TNFSF14) and frontal cortex (SERPINA3). The WGCNA revealed clusters of co-expressed genes, enriched for immune/inflammation related processes, up-regulated in schizophrenia. PVALB (parvalbumin) was one of the few genes down-regulated in the hippocampal data set and validated results from the individual trait data. We found a negative correlation between the immune related co-expression network from the hippocampus and the density of PVALB-containing neurons in the hippocampus of the same subjects. We also found a correlation between the immune related co-expression network from the CP with various immune markers measured in the serum and frontal cortex (TGFβ, IL6, IL1β, CRP) of the same subjects.

Conclusions: Markers of immune processes and inflammation are up-regulated in the brain of people with schizophrenia and many are associated with the BBB. Moreover, individual trait data can be used for internal validation of high-throughput data obtained from the same cases. The inverse correlation between the co-expressed markers of inflammation and the density of PVALB-positive neurons in schizophrenia indicates that neuronal abnormalities may be influenced by immune activation.
Disclosure: Nothing to Disclose.

Panel
39. The Extinction and Inhibition of Drug Seeking and Fear-Related Memories: Mechanisms and Therapeutic Targets

39.1 Recent Insights Into the Infralimbic Cortical Regulation of the Extinction and Inhibition of Cocaine Seeking

Ryan LaLumiere

University of Iowa, Iowa City, Iowa, United States

Background: Previous work indicates that the infralimbic cortex (IL), part of the ventromedial prefrontal cortex, regulates the extinction of cocaine-seeking behavior in rats in a self-administration model. Pharmacological inactivation of the IL following self-administration and extinction training induces cocaine seeking. Similarly, IL manipulations during early extinction of cocaine seeking alter the consolidation of such learning, suggesting a critical role for the IL in both the learning and expression of cocaine-seeking extinction. However, the precise temporal nature of how the IL performs these functions is unknown. Moreover, whether the IL performs a similar function in the inhibition of cocaine seeking in other behavioral paradigms has been unclear. The findings presented used temporally precise optogenetic manipulations of the IL and alternative behavioral paradigms to address these issues.
Methods: Rats were trained in cocaine self-administration, followed by extinction training. Optogenetic inhibition of the IL was given in a manner temporally restricted to the 20-s period immediately following the unreinforced lever presses during the extinction training. Rats then underwent additional extinction training and reinstatement tests to determine whether there were any long-lasting effects of altering IL activity during the early extinction training. To determine whether our results generalized to a long-access self-administration paradigm and to activation, rather than inhibition, of the IL, other rats underwent long-access cocaine self-administration followed by a withdrawal period and different tests of craving. In this case, we used a stable step-function opsin to activate the IL during the craving and reinstatement tests. Finally, in a separate set of experiments, we investigate how IL activity influences cocaine seeking in a discriminative stimulus-based task, in which distinct stimuli indicated the availability of a cocaine infusion, or lack thereof, on a trial-by-trial basis.
Results: Our findings indicate that temporally precise inhibition of the IL immediately following unreinforced lever presses increased cocaine-seeking behavior. Moreover, rats that had received IL inhibition during the early extinction sessions showed higher levels of cued reinstatement when tested later without any inhibition, suggesting a long-lasting effect of altering extinction learning for cocaine seeking. However, similar inhibition of the prelimbic cortex or pseudorandom inhibition of the IL (i.e. not time-locked to the lever pressing) during extinction sessions had no effect. Our results also indicate that IL activation, via the use of a stable step-function opsin, reduced cocaine seeking behavior following withdrawal from long-access self-administration but only after rats had undergone extinction training, suggesting the potential for using such opsins in these investigations. Finally, our results using a discriminative stimulus-based task indicate that IL inhibition increases cocaine seeking when the cue stimulus indicates no cocaine infusions are available and the rat knows that such lever pressing has no consequence.
Conclusions: The present findings provide strong insight into the temporal nature of IL activity with regard to the extinction of cocaine seeking, indicating that IL activity during a limited window following an unreinforced lever press is critical for extinction learning. Moreover, altering extinction learning during this period has long-lasting effects on the reinstatement cocaine seeking. Our experiments using other paradigms and tasks provide further evidence for how the IL regulates cocaine seeking and suggest that the IL has a broader role in suppressing inappropriate or ineffective cocaine-seeking behavior.
Disclosure: Nothing to Disclose.

39.2 Complementary Roles for Accumbens Shell Output Pathways in Promoting Extinction Versus Reinstatement of Alcohol Seeking

Gavan McNally

The University of New South Wales, Australia, Sydney, Australia

Background: Reducing rates of relapse to drug seeking after a period of successful abstinence remains a key treatment goal, Following a period of extinction, drug seeking is determined by a balance between activity in circuits promoting extinction versus those promoting reinstatement. The nucleus accumbens shell (AcbSh), located in the ventral striatum, plays a critical role in both promoting and preventing relapse to drug seeking and motivated behaviours. The circuit level mechanisms for these dual roles has remained unclear. Here we show that these dual roles can be linked to distinct output pathways from the AcbSh.
Methods: We used an ABA renewal (contextual control over extinction and reinstatement) procedure. Rats were trained to respond for alcoholic beer in a distinctive context prior to extinction in a second, distinctive context. Rats were tested for expression of extinction, in the extinction context, for context-induced reinstatement, in the training context, and then for the reacquisition of alcohol seeking. In this way we can model, in the same animal, voluntary abstinence from alcohol seeking (test in extinction context), reinstatement of alcohol seeking (test in the training context), and the transition from lapse to relapse (reacquisition test). We combined this behavioral

procedure with classical anatomical tracing methods, RNA Scope, as well as optogenetic and chemogenetic assisted circuit mapping in normal and transgenic rats to parse the roles of ventral striatal output pathways in extinction versus reinstatement of alcohol seeking.
Results: Using dual retrograde tracing to map the anatomical relationships between AcbSh medium spiny neurons at the origins of the AcbSh – ventral tegmental area (VTA) and AcbSh – lateral hypothalamus pathways, we show that distinct AcbSh neurons are at the origin of these two pathways. Next, combining retrograde tracing with RNA scope to determine the identities of AcbSh neurons at the origins of these two output pathways, we show that D1 receptor expressing neurons project extensively to lateral hypothalamus. We then show that the AcbSh – ventral tegmental area (VTA) pathway mediates renewal and reacquisition of alcohol seeking because optogenetic silencing of this pathway significantly attenuated relapse to alcohol seeking, but we show that different cell types in VTA contribute to these different forms of relapse because chemogenetic silencing of TH neurons prevents reacquisition but not renewal, Next we show that optogenetic stimulation of the AcbSh – LH pathway or chemogenetic silencing of LH prevents renewal and reacquisition of alcohol seeking. This protective effect of stimulation of the AcbSh – LH pathway was behaviorally specific because optogenetic stimulation had no effect on initial or well established alcohol seeking and had no effect on motivation to respond for alcohol.
Conclusions: We show that extinction and reinstatement of drug seeking are linked to activity of distinct ventral striatal output pathways. Extinction, or reduced drug seeking, can be linked to the AcbSh-LH pathway whereas reinstatement is linked AcbSh-VTA pathway. Restoring activity in the former, or reducing activity in the latter, is sufficient to profoundly, and selectively, disrupt relapse to alcohol seeking in an animal model. These findings identify new targets for relapse prevention.
Disclosure: Nothing to Disclose.

39.3 Promoting Extinction of Fear or Drug-Seeking by Inhibition of HDAC3: Implications for Models of the Comorbidity Between PTSD and Substance Use Disorders

Matt Lattal

Oregon Health and Sciences University, Portland, Oregon, United States

Background: Many recent studies have evaluated pharmacological agents that may promote extinction of fear or extinction of drug-seeking. A particularly promising target for these approaches is histone acetylation, which is a key step in the road to transcription and translation, both of which are needed for the formation of long-term memories involving extinction. In previous studies, we have found that nonspecific histone deacetylase (HDAC) inhibitors can promote extinction by increasing transcription of memory-related genes. In this presentation, we will describe several experiments showing long-term changes in drug-seeking behaviors after exposure to a battery of shocks in a distinct context. We will also describe recent evidence from our laboratory that inhibiting a specific HDAC (HDAC3) promotes extinction of fear and extinction of drug-seeking.
Methods: In separate drug-seeking experiments, Long-Evans rats were trained to respond for oral alcohol reinforcers or intravenous cocaine or methamphetamine reinforcers. During extinction, rats were exposed to the self-administration context and allowed to respond on the lever previously associated with the reinforcer. Tests of the persistence of extinction included context-, cue-, and reinforcer-induced reinstatement, as well as reconditioning of the original response. In fear conditioning experiments, rats were exposed to a different context, in which they received a battery of footshocks following procedures previously reported to establish stress-enhanced fear learning. In extinction of drug-seeking or fear, rats received injections of the HDAC3 inhibitor RGFP966 paired with exposures to the context previously associated with drugs or shocks.
Results: The HDAC3 inhibitor RGFP966 promoted extinction of fear and drug-seeking. This was revealed as effects on both the rate and persistence of extinction against post-extinction reinstatement challenges. Further, exposure to a battery of shocks in a specific context caused persistent changes in drug-seeking, measured in reinstatement tests conducted up to 60 days after the stressful experience.
Conclusions: These results demonstrate the extinction-promoting effects of HDAC3 inhibition. Further, they demonstrate this in the context of a behavioral approach that models the comorbidity between post-traumatic stress disorder (PTSD) and substance use disorders (SUDs). These results have implications for theoretical approaches to appetitive-aversive interactions and they open new doors for testing pharmacological approaches designed to promote extinction of fear and drug-seeking behaviors in the context of PTSD-SUD comorbidities.
Disclosure: Nothing to Disclose.

39.4 Vagus Nerve Stimulation Enhances Extinction and Protects Against Reinstatement in a Rat Model of PTSD

Christa McIntyre

The University of Texas at Dallas, Richardson, Texas, United States

Background: Exposure therapy is considered the gold standard of treatment for posttraumatic stress disorder (PTSD). During exposure therapy, patients are repeatedly exposed to cues that remind them of the trauma until they learn healthier responses to those cues. However, exposure therapy demands that patients overcome avoidance symptoms in order to begin treatment, and dropout rates are high. Additionally, people given the diagnosis of PTSD demonstrate impaired extinction of conditioned fear in controlled laboratory studies. An optimal adjunct to exposure therapy would enhance memory consolidation to promote extinction while reducing anxiety in order to lower the emotional demand and prevent reinforcement of negative associations. Many anxiolytic drugs have failed as adjuncts to exposure therapy, possibly because they impair memory consolidation and interfere with the extinction process. Vagus nerve stimulation (VNS) is an FDA-approved treatment for seizure prevention and it enhances memory consolidation in rats and humans and chronic VNS reduces anxiety. We previously reported that VNS facilitated extinction of

conditioned fear in rats. More recently, we have examined the effect of VNS on extinction and reinstatement in the single prolonged stress (SPS) rat model of PTSD. SPS-treated rats exhibit many of the symptoms of PTSD, including impaired extinction of conditioned fear.
Methods: For VNS surgery, a custom-made platinum-iridium wire electrode in a "cuff" made out of microrenathan was wrapped around the left vagus nerve at the cervical level. Wire leads were tunneled subcutaneously to a connector implanted at the top of the skull. Sham-treated controls underwent surgery but the connector was not attached to the cuff electrode. One week after surgery, SPS-treated rats were restrained for 2 hours, followed by 20 min of forced swim and exposure to diethyl ether. They were housed individually for 1 week, then given auditory fear conditioning (AFC). On each of 2 consecutive days, rats were exposed to a 30 sec, 9 kHz tone paired with a 0.4 mA footshock 8 times. On the following day, the tone was played 4 times without footshock and time spent freezing was recorded. This was called a conditioned fear response test (CFRT). On the next day, rats were again exposed to the tone 4 times but each tone was paired with VNS (30 s duration, 0.4 mA, 500 μs pulse width at 30 Hz) or sham stimulation starting 150 ms before the onset of the tone. This was considered an extinction trial, CFRTs and extinction trials were given on alternate days for 11 days, followed by a reinstatement test where a single, unsignaled footshock was administered and the final CFRT followed 24 hours later.
Results: Consistent with previous findings, extinction was impaired in SPS-treated rats and VNS facilitated extinction in both SPS and control rats. Importantly, VNS reversed the SPS-associated extinction impairment. SPS-treated rats given VNS during extinction were not significantly different from non-SPS controls at any timepoint. All groups showed significant reinstatement after the unsignaled shock. SPS-treated rats given VNS did not differ from controls but freezing was significantly elevated in SPS-treated rats given sham stimulation vs. all other groups.
Conclusions: These findings indicate that VNS can enhance extinction of conditioned fear even in a rat model of PTSD that demonstrates extinction impairment. Furthermore, return of fear is a major challenge in treating PTSD and reinstatement is greater in SPS-treated rats but VNS reverses this effect.
Disclosure: Nothing to Disclose.

Panel
40. Mood Disturbance and Addiction: Characterizing the "Dark Side" of Drug Use in Humans and in Animal Models

40.1 Defining the "Dark Side" Phenotype in Epidemiological Samples: Incidence, Comorbidity and Genetic Findings

Cindy Ehlers

The Scripps Research Institute, La Jolla, California, United States

Background: The comorbidity of alcohol use disorders with both independent and substance-induced anxiety and affective disorders has long been documented. However, recently there has been interest in the emergence of a more subtle, subclinical syndrome of mood disturbance associated with the evolution of the clinical course of alcoholism that has been labeled the "dark side" of addiction. The present study sought to operationalize a "dark side" phenotype, estimate its prevalence in individuals with alcohol use disorders, determine its co-morbidity with other psychiatric disorders, and test its heritability and association with genetic sequence variants in three different epidemiological samples.
Methods: Data were available from three studies comprising 2,355 individuals with alcohol use disorders: 1) A Native American sample (NA: $n=612$), 2) A Mexican American sample (MA: $n=295$) and a EuroAmerican sample (EA: $n=1448$). Demographics and alcohol use disorders diagnoses (EA, MA, NA) and other psychiatric disorder diagnoses (in NA and MA only) were assessed using the semi-structured assessment for the genetics of alcoholism (SSAGA). Personality variables in the NA and MA study were assessed using the Maudsley personality inventory and using the Big 5 assessment in the EA study. Two measures from the SSAGA of the "dark side" phenotype were assessed 1) feeling anxious or depressed when cutting down, stopping, or going without drinking after drinking steadily for a while 2) drinking causing feelings of depression or loss of interest in things for more than 24 hours to the point that it interfered with functioning.
Results: Fifty percent of the sample reported "dark side" symptoms and it was significantly more common in those with severe alcohol use disorder. The presence of "dark side" symptoms was not associated with gender, employment, ecostat or education. Significant comorbidity was found in those individuals with dark side symptoms for: anxiety and affective disorders, a history of losing temper/ anger/ resentful, tobacco, stimulant and marijuana dependence, suicidal behaviors, sleep disturbance and neuroticism. A logistic regression revealed that the severity of the alcohol use disorder, neuroticism, and a life time history of affective disorders explained most of the variance in "dark side" symptoms. The heritability of the two dark side symptoms ranged between 5 and 50% depending on ethnicity. A genome wide significant association was found between SNPs in several potassium voltage gated ion channels (KCNC2, KCNJ6) and dark side symptoms in the EA and NA samples.
Conclusions: These findings suggest that alcohol used disorders with mood disturbance ("dark side" symptoms) can be operationalized and assessed and are associated with the severity of the disorder. They are significantly comorbid with a number of other psychiatric disorders and are strongly associated with neuroticism. They are also heritable and appear to be associated with several SNPs in the genome.
Disclosure: Nothing to Disclose.

40.2 Personality-Based Measures of Negative Affective Tendencies as Related to Addictive and Other Psychiatric Disorders

Laura Kwako

National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland, United States

Background: The problem of heterogeneity within alcohol use disorders (AUD) is not new; various attempts to identify

meaningful subtypes have not translated to practice. The Addictions Neuroclinical Assessment project at NIAAA seeks to use neuroscience domains to better understand this heterogeneity. One of these domains, negative emotionality, addresses the negative affective states described by Koob as the "dark side" of addiction. This inquiry uses personality- and DSM-based assessments to explore links between negative emotions and AUD in a diverse sample.
Methods: Study participants included 1,227 individuals participating in research studies at the NIAAA clinical facilities at NIH between 2005 and 2014. Measures collected included the NEO-PI-R, demographics, and Structured Clinical Interview for DSM-IV. We conducted latent class analysis using Latent Gold 5.1; indicators included subfacets of neuroticism, conscientiousness, and extraversion, with age, gender, and race as covariates. Assigned clusters were used to predict DSM diagnoses related to substance use and mood/anxiety disorders.
Results: Results indicated that a five cluster solution provided the best fit. Cluster 1 (28% of sample) included individuals who reported medium levels of neuroticism, positive emotions, and conscientiousness, and were the lowest on the excitement-seeking dimension. They were among the oldest members, and had a high percentage of men. They had the highest rates of all psychiatric disorders, including alcohol and other substance dependence (AD/SD), major depressive disorder (MDD), PTSD, and other anxiety disorders. Cluster 2 (21% of sample) were low on neuroticism, medium on extraversion, and medium to high on conscientiousness. They were among the youngest participants and had a low proportion of men. They reported the lowest rates of all psychiatric disorders. Cluster 3 (19% of sample) reported the lowest levels of neuroticism and highest levels of positive emotions and conscientiousness, and medium levels of excitement-seeking. They were among the youngest participants and had lower proportions of men. They reported high rates of AD/SD, lower rates of MDD, and medium rates of PTSD and other anxiety disorders. Cluster 4 (17% of sample) reported the highest rates of neuroticism and lowest of positive emotions, along with low levels of self-discipline and medium excitement-seeking. This group were among the oldest, and had high proportions of women. They reported low rates of all psychiatric disorders. Cluster 5 (15% of sample) reported medium to high levels of neuroticism and positive emotions, the highest levels of excitement-seeking and low levels of conscientiousness. They were the youngest and had the highest proportions of men. They had medium rates of AD and PTSD, the highest levels of MDD and anxiety disorders, and low rates of SD.
Conclusions: Results suggest a positive relationship between age and negative affect in addiction, as is consistent with development of the dark side as addiction progresses. It is also likely that there are different groups with varying developmental trajectories and relationships between AD/SD and other psychiatric disorders, e.g., Cluster 1 comprises the most severe phenotype for AD/SD and other psychiatric disorders, Cluster 3 is intermediate with predominance for externalizing disorder, Cluster 5 is intermediate with more internalizing, and Clusters 2 and 4 show less comorbidity.
Disclosure: Nothing to Disclose.

40.3 Imaging the Dark Side of Alcohol Self-Administration in a Monkey Model of Heavy Drinking

Kathleen Grant

Oregon Health and Sciences University, Beaverton, Oregon, United States

Background: Anxiety and aggression are temperaments that have a robust limbic and prefrontal cortical circuitry and appear associated with heavy drinking in humans. However, it is unclear if these behaviors and associated corticolimbic networks are pre-existing or consequential of ethanol use.
Methods: A model of voluntary alcohol self-administration capturing detailed intake patterns of alcohol (4% w/v) and water daily (22 hrs/day) for over 14 months provided a dense drinking data set from late adolescent/young adult rhesus monkeys ($n = 12$ ethanol, 8 control). At baseline and after 14 months of alcohol access, monkeys were scored for anxious and aggressive behaviors in the morning using the human intruder test. Resting state functional connectivity (rsFC) MRI was used to assess temporal correlations of spontaneous regional activity in corticolimbic networks under 1% isoflurane anesthesia. Blood ethanol concentrations (BEC) were sampled 7 hours after session start, once a week.
Results: Monkeys that were aggressive at baseline self-administered more alcohol (t10 = 3.7, $p = 0.008$) and attained higher BEC (t10 = 3.3 $p = 0.015$) than non-aggressive monkeys, and higher baseline aggression positively correlated with future alcohol intake (rs = 0.61, $p = 0.033$) and BEC (rs = 0.60, $p = 0.041$). While anxious monkeys did not drink significantly more ethanol (3.1 vs. 2.2 g/kg/d) or attain significantly higher BEC (86 vs. 43 mg%) than non-anxious monkeys, a positive association between higher baseline anxiety and higher future alcohol intake (rs = 0.61, $p = 0.036$) and BEC (rs = 0.60, $p = 0.038$) was observed. Conversely, aggression reassessed after 14 months of alcohol access revealed a negative correlation with ethanol intake (rs = -0.60, $p = 0.040$), and heavy drinkers (intakes > 3.0 g/kg/day) became less responsive (Z = 2.0, $p = 0.042$) compared to non-heavy drinkers. No significant changes in behavior were observed in control subjects across the 12-month period. At baseline, amygdala-dlPFC connectivity was more anticorrelated in aggressive (t10 = 2.4, $p = 0.035$) and anxious monkeys (t10 = 3.2, $p = 0.010$). Further, baseline amgydala-dlPFC rsFC was negatively correlated with higher future alcohol intake (r = -0.60, $p = 0.039$) and BEC (R = -0.60, $p = 0.040$). As a consequence of chronic drinking, increased rsFC from baseline between amygdala and dlPFC was positively correlated with higher ethanol intake (r = 0.84, $p = 0.001$) and BEC (r = 0.69, $p = 0.013$), and decreased aggression from baseline (r = -0.73, $p = 0.008$). Mean change in amygdala-dlPFC rsFC from baseline was only found in heavy drinkers compared to non-heavy drinkers and control subjects (F (2, 19) = 2.8, $p = 0.085$).
Conclusions: The data suggest that monkeys with a "dark side" temperament (aggressive and/or anxious) have a predisposition to drink heavily, whereas chronic heavy drinking increases non-responding in the temperament tests, perhaps due to these observations occurring in the morning prior to the onset of alcohol access (i.e, in a "hungover" state). These relationships are associated with connectivity

between amygdala and dlPFC and suggest both preexisting and alcohol-induced alterations in this circuitry are fundamentally involved in alcohol addiction.
Disclosure: Nothing to Disclose.

40.4 Identification and Control of a Neuronal Ensemble in the Central Amygdala That is Responsible for Compulsive Alcohol Drinking

Olivier George

The Scripps Research Institute, La Jolla, California, United States

Background: Abstinence from alcohol is associated with the recruitment of neurons in the central nucleus of the amygdala (CeA) in rats binge-drinking alcohol (non-dependent) as well as in alcohol dependent rats. However, whether the recruitment of this abstinence neuronal ensemble in the CeA is causally related to excessive alcohol drinking or represents a consequence of the excessive drinking remains to be elucidated. Here we tested the hypothesis that inactivation of the abstinence neuronal ensemble in the CeA is responsible for the excessive alcohol drinking observed in non-dependent binge drinking rats and in dependent rats.
Methods: In order to test our hypothesis, we investigated the effect of the inactivation of neuronal ensembles in the CeA using pharmacogenetic (Daun02 inactivation method in Fos-Lac Z transgenic rats) and optogenetic (Halorhodopsin in CRF-Cre rats) approaches. Non-dependent rats had 30 min daily access to alcohol self-administration. Binge-drinking rats had chronic intermittent access to two-bottle choice. Dependent rats had 30 min daily access to alcohol self-administration and were made dependent using chronic intermittent exposure to alcohol vapor.
Results: We found that inactivation of the abstinence neuronal ensemble by injection of Daun02 in the CeA significantly decreased alcohol drinking in dependent and non-dependent rats with the only difference that in non-dependent rats, the decreased ethanol intake was transient the day of the injection and returned to normal the day after the injection, while in the dependent animals inactivation of the abstinence neuronal ensemble produced a long-term decrease in alcohol drinking that lasted at least 2 weeks. Moreover, a significant reduction of the somatic withdrawal signs in the dependent animals injected with Daun02 in the CeA was also observed. Optogenetic inactivation of CRF neurons in the CeA prevented the recruitment of the CeA neuronal ensemble and alcohol drinking in dependent rats.
Conclusions: These results demonstrate that the recruitment of a neuronal ensemble in the CeA during abstinence from alcohol is causally related to the excessive alcohol drinking observed in alcohol dependent rats, whereas a similar neuronal ensemble only partially contributes to alcohol binge-like drinking in nondependent rats. Moreover, it demonstrates that activation of CeA CRF neurons is required for the recruitment of the CeA neuronal ensemble. These results identify a critical neurobiological mechanism that is responsible for alcohol dependence, suggesting that targeting this CRF-dependent abstinence neuronal ensemble in the CeA may lead to more effective medications for the treatment of alcoholism.
Disclosure: Nothing to Disclose.

Panel
41. Identifying Multimodal Imaging Biomarkers and Treatment Targets for Aberrant, Transdiagnostic Behaviors Associated With Abnormal Reward Circuitry Function

41.1 Multimodal Neuroimaging Reward Circuitry Biomarkers and Treatment Targets for Novel Neurostimulation Interventions for Impulsive Sensation Seeking

Mary Phillips

University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, United States

Background: A common behavioral trait in young adults is impulsive sensation seeking (ISS), the tendency and willingness to seek, and take risks for, novel and intense sensations and experiences. ISS can have deleterious consequences: poor psychosocial function, accidental injury and death. Yet, there are no biological measures to guide interventions for high trait ISS. Identifying neural markers of ISS would facilitate novel treatment developments, including neurostimulation (e.g., transcranial direct current stimulation, tDCS), for disorders with high ISS.
Methods: Study 1: $n = 77$ 18-25 year-olds recruited transdiagnostically from the community across the ISS range ($n = 2$ on SRIs), performed an fMRI event-related number-guessing task examining neural activity during expectancy and receipt of reward/loss. The main stimulus event of interest was uncertain reward expectancy (RE). Participants completed standardized scales for ISS (including Zuckerman Sensation Seeking, Barratt Impulsivity, Behavioral Activation System scales). Participants performed a post-scan decision making task measuring positive bias, the tendency to choose risky possible large win over "sure-thing", smaller win cards.
Study 2: $n = 19$, healthy 19-25 year-olds recruited from the community across the ISS range performed the same reward task as above but during EEG.
Study 3: $n = 20$ healthy 18-25 year-olds recruited from the community across the ISS range performed the same reward task outside the scanner twice: once with cathodal (inhibitory) tDCS to left vlPFC, and once with cathodal tDCS to left somatosensory cortex (control tDCS condition).
Results: Study 1: There were significant positive correlations between trait ISS ($n = 77$; Zuckerman thrill seeking subscale) and activity in: left vlPFC ($r = 0.24$; $p = 0.032$), dorsal ACC (0.25; $p = 0.031$), and right VS (0.27; $p = 0.018$), to uncertain RE, and between trait ISS ($n = 74$; Barratt Motor Impulsiveness) and left vlPFC-left VS functional connectivity to uncertain RE ($r = 0.26$, $p = 0.27$). There was a significant positive relationship between trait ISS $n = 72$ (Barrett Nonplanning Impulsiveness) and positive bias (risky decision making) on the post scan decision-making task ($r = 0.25$; $p = 0.03$).

Study 2: There was a significant positive correlation between trait ISS (Zuckerman total score) and beta power (19-21Hz) over the left vlPFC (electrode F7; r = 0.54; $p = 0.02$) to uncertain RE during the same reward task as in our fMRI data above.
Study 3: Left cathodal tDCS during performance of the reward task reduced positive bias on the post reward task risky decision making task in females (Wilcoxon Signed Ranks Test: $z = 2.65$; $p = 0.008$).
Conclusions: Multimodal neuroimaging data suggest that a potential neural mechanism for ISS is greater activity (BOLD response and beta power) in, and greater positive functional connectivity among, left vlPFC and distributed reward circuitry to potential future reward (ie., to uncertain RE). These measures, in turn, predispose to risky decision making. Left cathodal tDCS may be an effective intervention for individuals with high ISS, to reduce risky decision making and subsequent risky behaviors. These data highlight the importance of multimodal imaging techniques as tools to identify new neural targets, reflecting underlying pathophysiological mechanisms, for novel interventions for abnormal and disabling behaviors in vulnerable individuals.
Disclosure: Part 1: Roche Pharmaceuticals, Consultant in 2015.

41.2 Identifying a Reward Dysfunction Biotype of Anhedonia by Functional Imaging, Neurocognition and Intervention Outcomes

Leanne Williams

Stanford University School of Medicine, Stanford, California, United States

Background: Our brain-based human models of depression tend to focus on heightened attributions about negative information and comparatively less about attenuated processing of positive information. Components of the affective circuits involved in the processing of positive emotion and socially rewarding stimuli are defined by the striatal nucleus accumbens and ventral tegmental areas (collectively referred to as the striatum), the amygdala and projections to the orbitofrontal cortex and medial prefrontal cortex. In a series of studies, we pursued the question of whether distinct dysfunctions in reward circuitry characterize a cohesive subgroup of people with major depression, and if these dysfunctions map on to a particular phenotypic profile. To assess the potential trait-like nature of a reward dysfunction "biotype" we assessed people with depression before and after antidepressant treatment and also assessed unaffected first degree relatives of probands with major depression.
Methods: Across studies, patients with depressive disorder ($n = 204$), first degree relatives of probands with mood disorder ($n = 170$) and 101 matched controls were assessed using functional MRI on the same 3T scanner while viewing facial emotions signaling social reward (happiness) versus threat (fear, anger) and loss (sadness) in masked and unmasked conditions. Self-reported symptoms of anhedonia and emotion regulation as well as behavioral performance on reward-related task tasks and on general cognitive tasks were also assessed. At baseline all participants were unmedicated. Patients randomized to one of three antidepressants and were re-assessed after 8 weeks of treatment. Controls were also re-assessed after 8 weeks to establish a normative frame of reference.
Results: Results suggested a distinct "biotype" of reward-related dysfunction in specific subsets of both patient and first degree relative groups. This biotype was characterized by a profile of striatal and amygdala hypo-activation to happy faces in depressed patients who were non-remitters to treatment ($F = 4.33$, familywise error corrected $p = .001$; Cohen's d effect size = 0.77) and an inability to regulate and reappraise negative emotional states in two-thirds of the relatives ($t = 2.75$, $p = .008$). This profile of hypo-activation was accompanied by a distinct neurocognitive profile ($F = 4.69$, greenhouse-geisser correct $p = .001$), reflected in slowed reaction times for identifying socially rewarding stimuli ($p < .0001$; Cohen's d effect size = 3.33) and under complex task demands ($p = .001$; Cohen's d effect size = .26). Hypo-activation was not correlated with overall symptom severity. By contrast, patients who remitted following antidepressant therapy showed comparative hyper-activation to salient emotional stimuli.
Conclusions: Our observations suggest the presence of a trait-like dysfunction in the processing of socially rewarding stimuli that confers vulnerability for overt depression and that persists even in the presence of antidepressant treatment for overt depression. This putative biotype characterizes otherwise unaffected relatives who have difficulty coping with negative mood states. By contrast, a minority of individuals at familial risk of depression exhibit normal profiles of brain function that might reflect their use of effective strategies for regulating emotion.
Dysregulation in the affective circuits of the brain may be a biobehavior marker of familial risk for depression, and that this dysregulation may be buffered by using reappraisal strategies for regulating negative emotion.
Disclosure: Part 2: Humana, Consultant.

41.3 Dynamic Brain Connectivity: Methods: and Applications to Mood Disorders

Roselinde Kaiser

University of California, Los Angeles, Los Angeles, California, United States

Background: Altered functional connectivity among frontolateral-medial or corticolimbic brain circuits has been observed in depressed individuals both during task performance and in slow-wave frequency bands during a state of rest. However, previous studies examining resting-state functional connectivity (RSFC) have typically focused on "static RSFC", or overarching patterns of correlated activity, without a complementary investigation into "dynamic RSFC", or fluctuations in the strength or spatial organization of functional networks over time. Dynamic RSFC is believed to reflect the flexibility of, or cross-talk among, intrinsic networks, and understanding disruptions to dynamic connectivity in mood disorders may shed new light on the network pathology of depression.
Methods: First, to examine frontolateral-medial RSFC, voxel-wise static and dynamic RSFC of a seed region of medial prefrontal cortex (MPFC) was compared between individuals

with ($n = 100$) or without ($n = 109$) major depression. Dynamic RSFC was operationalized as variability (standard deviation) in RSFC over a sequence of sliding windows. In a subset of the sample, the association was tested between RSFC and individual differences in ruminative thinking. Second, to investigate corticolimbic RSFC in relation to depression and childhood traumatic stress, voxelwise static and dynamic RSFC of seed regions in the amygdala and MPFC were compared between individuals with ($n = 45$) or without ($n = 18$) a history of childhood stress. The association was tested between RSFC and individual differences in ruminative thinking or depressive symptoms severity.

Results: In the first sample, analyses revealed that depression was related to increased dynamic RSFC between lateral and medial prefrontal regions, and individuals prone to rumination were especially likely to exhibit this frontolateral-medial abnormality. In the second sample, analyses showed an amplified negative association (i.e., more extreme antagonism) between lateral prefrontal and limbic regions as a function of childhood trauma, as well as altered dynamic RSFC in overlapping systems. This corticolimbic abnormality was associated with increased rumination and depression, but only for individuals exposed to trauma.

Conclusions: These results show that depression is characterized by altered static and dynamic RSFC in frontolateral-medial and corticolimbic circuits involved in cognitive and emotional regulation. Critically, these findings highlight the potential for RSFC to reveal new insights into the nature of abnormal brain communication in depression, or unique disruption to network development as a consequence of childhood stress. By gaining a better understanding of the network pathology of depression, it may be possible to develop more effective treatments that target network signaling such as transcranial magnetic stimulation.

Disclosure: Nothing to Disclose.

41.4 Multimodal Imaging of Effort-Based Decision Making

David Zald

Vanderbilt University, Nashville, Tennessee, United States

Background: Effort-based decision making has emerged as a translational model of motivation and motivational deficits that provides a key link between preclinical models of neuropsychopharmacology and human psychopathology. Preclinical models initially demonstrated the importance of dopamine for choices to expend effort for rewards. Here, I will describe the convergence of PET imaging studies of the dopamine system, and recent fMRI studies of patients with major depression, which demonstrate the importance of striatal activations. Emerging data from these studies in humans suggest several key insights including the important context of reward probability on when dopaminergic effects are observable and the distinction between striatal modulation by reward magnitude prior to making decisions to expend effort, and responses in anticipation to the effort itself. Potential reverse translational work based on the human data will also be discussed with an emphasis of adapting these techniques for preclinical treatment studies of motivational anhedonia.

Methods: Data on the relation between dopamine function and decisions about effort derive from studies utilizing a dual-scan approach measuring individual differences in dopamine release by contrasting binding potential of the high-affinity D2-like ligand [18F]fallypride following placebo and 0.43 mg/kg amphetamine. Behavioral performance on the Effort Expenditure for Rewards Task (EEfRT) measured outside of the scanner. On each trial of the EEfRT participants chose between a Higher monetary reward/higher Effort option and a lower reward/lower effort option, with the reward level of the higher effort option and reward probability changing across trials. Proportion of higher effort choices was regressed against the PET data. fMRI data derive from a sample of 30 patients with major depressive disorder (MDD) and 26 healthy age-matched controls. Participants completed an MRI-compatible version of the EEfRT during which subjects had to select one of two effort reward magnitude options on each trial, Choices involved selection between high, medium, and low effort options, with the specific pair of options and monetary values varying across trials. Reward values were calibrated for each subject in order to have them select the harder option on 50% of the trials.

Results: Amphetamine-induced dopamine release (an index of the reactivity of the dopamine system to stimulation) was positively correlated with the proportion of hard choice options selected. This relation appeared specific to low probability trials in which the likelihood of reward was low. In the fMRI data, we observed that patients with MDD showed normal striatal responsivity to reward magnitude during the choice phase of the task. However, after selection of effort, healthy individuals showed post-decision activation of the striatum, whereas patients failed to show this preparatory modulation in anticipation of the requirement to perform effort.

Conclusions: The above PET and fMRI data provide increasing evidence for the role of the dopaminergic system and its striatal targets in aspects of effort-based decision-making for reward in decision and disease. In both cases, the data provide specificity in these relations, with neural correlates emerging in specific trial conditions or trial phases. The above PET and fMRI data provide increasing evidence for the role of the dopaminergic system and its striatal targets in aspects of effort-based decision-making for reward. In both cases, the data provide specificity in these relations. The results have implications for the application of effort-based decision tasks for intervention studies aimed at treating motivational deficits.

Disclosure: Nothing to Disclose.

Panel
42. Poverty, Adversity and Neurodevelopment: Pathways to Psychopathology

42.1 Early Family Relationship Risk, Development of Reward Systems, and Adolescent Depression

Erika Forbes

University of Pittsburgh, Pittsburgh, Pennsylvania, United States

Background: Vulnerable brain systems could serve as a mechanism for associations between early social context risk

and the development of adolescent psychopathology. Challenges in family relationships are an influential component of childhood social context risk, with experiences such as maltreatment disproportionately associated with poverty and socioeconomic status. Family relationship risk–for example, family conflict or disruptions in the mother-child relationship, such as physical abuse or low warmth–could alter neural systems involved in reward and social processing. These systems continue to develop into early adulthood, creating a potential foundation for problems in later functioning. Function of dorsomedial prefrontal cortex (dmPFC) and related reward circuitry may be particularly relevant to this line of investigation given its role in social, self, and reward processing. Using detailed social-context measures and fMRI in longitudinal studies of high-risk youth, we are examining the role of neural response to reward in mediating associations between early adversity and adolescent depression. Guided by greater rates of depression in women, we have extended this work to address the role of early family factors across childhood in adolescent girls' development of neural reward function and depression.

Methods: Two longitudinal studies of high-risk, low-socioeconomic status, urban youth are the source of data. Participants in Study 1 were 120 young men at low socioeconomic status followed since infancy. Mothers' history of depression was measured when boys were 42 months, 10 years, and 11 years old, and maternal positive affect was observed during mother-child interactions at 18 and 24 months and at 10 and 11 years. Young men underwent fMRI at age 20 during a standardized monetary reward task. Participants in Study 2 were 232 adolescent girls from a study of risk for depression and followed since age 5. Mothers completed measures of parenting practices, parent-child relationship quality, parent-partner conflict, family climate, and maltreatment throughout childhood. Girls completed diagnostic interviews at ages 9-18 and underwent fMRI with the same monetary reward task at ages 16, 17, and 18 years. Exploratory factor analysis was applied to measures of family context from girls' ages 5-10 years. The scanner for both studies was a Siemens TIM Trio 3T, preprocessing and analyses were conducted in SPM, and the PROCESS macro was used to test mediation.

Results: In late adolescent boys, the combination of maternal depression and low positive affect behavior predicted lower dmPFC response to reward and higher caudate response to loss at age 20. In late adolescent girls, family financial disadvantage during childhood–particularly before age 10–predicted dmPFC response to reward at age 16. Furthermore, dmPFC response mediated the association between financial disadvantage and depression severity. For relationship risk, analyses yielded a factor that was strongly consistent across age 5-10 years and that primarily reflected high punitive discipline and low maternal warmth. This relationship factor, especially during girls' early childhood, was associated with lower dmPFC response to reward in late adolescence.

Conclusions: Neural reward systems could be sensitive to early developmental influences, particularly childhood disadvantage and difficulties in the family domain. Disruption of development in these systems could provide a mechanism for higher vulnerability for depression in young people who are raised in challenging social contexts.

Disclosure: Nothing to Disclose.

42.2 Preterm Birth, Altered Neonatal Functional and Structural Connectivity and Early Childhood Psychopathology

Cynthia Rogers

University of Washington, St. Louis, Missouri, United States

Background: Preterm birth is one of the most deleterious early life stressors and can result in altered brain development in both gray matter and white matter regions. Prematurity is also associated with developmental deficits in multiple domains and increased rates of psychopathology throughout the lifespan including ADHD, Autism Spectrum Disorder, and anxiety disorders. There is evidence that alterations in brain structure, including disruptions in white and gray matter development may contribute to these subsequent developmental deficits in preterm children particularly those born at the earliest gestational ages. Few studies, however, report multimodal imaging results that assess both the structural and functional connectivity of brain networks that may underlie these deficits. Preterm birth is also associated with other sociodemographic and psychosocial risk factors like poverty and maternal psychopathology that can also increase the risk of childhood psychopathology.

Methods: The objective of this study was to compare the structural and functional connectivity of very preterm infants (VPT; (gestational age $<$ 30 weeks; $N=76$) without significant brain injury to that of healthy, full term (FT) infants (gestational age >37 weeks $N=58$). All infants underwent MRI on a 3T scanner at term-equivalent age (36-42 weeks post menstrual age) with diffusion MRI and resting state fMRI sequences acquired. Structural connectivity was measured using tract based spatial statistics (TBSS) and diffusion tractography to assess differences in fractional anisotropy. Functional connectivity was analyzed by computing Fisher z transformed correlations and covariance measures for 7 resting state networks. Children returned at age 2 years and 5 years for assessment of their social-emotional development and symptoms of psychiatric disorders utilizing parent report (age 2 and 5) and teacher report (age 5) questionnaires as well as behavioral assessments. Sociodemographic and maternal psychosocial factors were assessed at all three time points.

Results: Results indicate that compared to full term infants, very preterm infants demonstrated diffuse reductions in fractional anisotropy of white matter tracts with both TBSS and tractography as neonates. VPT neonates also had weaker within network correlations and covariance across all networks. Among the higher order RSNs involved in attentional, social-communicative, and affective processing, the Default Mode ($p=.001$) and Frontoparietal ($p<.001$) networks were particularly affected. Notably, tracts that structurally connect the hubs of these resting state networks (e.g. cingulum, anterior limb internal capsule) were noted to be among the most impaired. VPT children had significantly greater deficits in inattention and social-communication at ages 2 and 5 as well as emergence of greater internalizing symptoms at age 5 years. Preliminary analyses indicate neonatal abnormalities in structural and functional connectivity predict age 2 and age 5 social-emotional deficits and

psychopathology with future analyses planned to be completed by the time of the symposium further exploring this relationship as well as examining the influence of maternal psychosocial and sociodemographic risk factors.
Conclusions: This study suggests that preterm birth represents a significant early adverse exposure that both alters the functional and structural connectivity of brain networks and is linked to the psychiatric disorders that occur at increased rates in the preterm population.
Disclosure: Nothing to Disclose.

42.3 Tracing the Routes of Externalizing Behaviors to Newborn Amygdala Connectivity and Prenatal Influences

Alice Graham

Oregon Health and Sciences University, Portland, Oregon, United States

Background: The interplay between emotionality and cognitive skills is central to understanding multiple forms of psychopathology. In a recent report (Graham et al, 2016), we identified a pattern of amygdala connectivity in newborn infants associated with a subsequent behavioral phenotype characterized by high emotionality (fear) and more advanced cognitive skills at 6-months-of-age. Here, we test the hypothesis that the identified pattern of stronger amygdala-ventral medial prefrontal cortex (vmPFC) connectivity in newborns will also be relevant for emerging internalizing and externalizing behaviors at 24-months-of-age. We further examine maternal biological indicators of stress and inflammation during pregnancy as potential influences on the strength of amygdala-vmPFC connectivity at birth.
Methods: Data from an ongoing prospective longitudinal study of maternal-fetal/infant dyads ($N = 65$ with prenatal and newborn data [31 females]; $N = 36$, with newborn and 24-month data [16 females]) were analyzed. Pregnant women were recruited in early pregnancy. Maternal blood and saliva samples were collected in each trimester to assess multiple indicators of stress and inflammation, including interleukin-6 (IL-6), a pro-inflammatory cytokine. Resting state functional connectivity of the amygdala was examined in neonates (scan age = 25.6+/-11.8 days). Newborn functional connections of interest from the prior study included left amygdala-bilateral anterior insula (associated with fear at 6-months), right amygdala-anterior medial prefrontal cortex (amPFC; associated with cognition) and right amygdala-vmPFC (associated with the fear-cognition phenotype). Internalizing and externalizing behaviors were assessed at 24-months-of-age (Mean age = 23.7+/-.993 months) via parent report on the Child Behavior Checklist. We hypothesized that amygdala-vmPFC connectivity would be most strongly associated with behavior problems at 24-months.
Results: Stronger newborn right amygdala-vMPFC connectivity was associated with higher externalizing problems at 24-monthsage ($\beta = .469$, $p = .007$). Stronger newborn left amygdala-anterior insula connectivity was associated with higher internalizing ($\beta = .373$, $p = .040$) and externalizing behaviors ($\beta = .433$, $p = .014$). Amygdala-amPFC connectivity was not significantly associated with 24-months behavior. When all three connections were included in the same model, only right amygdala-vMPFC connectivity remained a significant predictor of 24-month behavior, and only for externalizing behaviors ($\beta = .430$, $p = .022$). Follow-up whole brain analyses confirmed the specific association between newborn right amygdala-vMPFC connectivity and externalizing behaviors at 24 months-age. Maternal IL-6 concentrations across pregnancy were associated with right amygdala-vMPFC connectivity, such that increasing IL-6 predicted stronger right amydgala-vmPFC connectivity for females ($\beta = .433$, $p = .024$). Ongoing follow up analyses will consider additional potential prenatal influences on this connection.
Conclusions: Extensive research has identified coordinated functioning of the amygdala and vmPFC as playing an important role in emotion regulation and psychopathology. Individual differences in the functional connectivity of these regions may serve as a marker of risk for behavioral difficulties which are evident by the time of birth. Such early markers of risk are sorely needed to increase understanding of pathways from prenatal stress to offspring psychopathology.
Disclosure: Nothing to Disclose.

42.4 Poverty, Maternal Support, Childhood Brain Development, and Psychopathology

Deanna Barch

University of Washington, Saint Louis, Missouri, United States

Background: The tangible effect of early environmental exposures on brain development has been well established in animals. Poverty represents a form of human deprivation that may parallel animal models. Here we present work on the relationship of poverty to the trajectory of hippocampal volume and the mediating role of maternal support. In addition, both the hippocampus and amygdala show negative rfMRI connectivity with bilateral dorsal prefrontal and parietal regions. These 'anti-correlations' are thought to indicate top-down regulation of emotion and stress responsivity supported by hippocampus and amygdala regions, suggesting a critical role for integrity of these connections in mood and affective function. Thus, we also present data on the relationship of poverty to hippocampal and amygdala connectivity, as well as the relationship of poverty to the interrelationships between structural and functional connectivity.
Methods: Subjects were participants in an ongoing 11-year longitudinal neuroimaging study. Behavioral assessments began when subjects were 3.0-6.11 years old and continued annually over 6 waves with scanning starting at age 6.11-12.11 and repeated approximately every 18 months for 3 waves of neuroimaging. We used the longitudinal FreeSurfer pipeline to compute whole brain gray and white matter volumes, as well as hippocampal and amygdala volumes. We analyzed the resting state data used methods validated to reduce motion related artifact. Preschool maternal support was measured at the second annual study wave when subjects were aged 3.11-6.11. Children and their caregivers engaged in a mildly stressful task in the laboratory. Each instance of specific types of supportive caregiving strategies

employed by the parent were counted as 1 unit and summed to give an overall preschool maternal support score.
Results: Lower income-to-needs was associated with reduced volumes of cortical gray matter, white matter, hippocampus, and amygdala. Maternal support mediated the relationship of preschool income-to-needs to hippocampal volume and predicted the trajectory of hippocampal development across childhood and early adolescence. Children with preschool maternal support >1 SD above the mean compared to children with support >1 SD below the mean, had a 2.1–fold increase in total hippocampal volume from the first to the third scan. Individual subject slopes generated from the multilevel linear model of hippocampus volume by preschool maternal support related to responses on Children's Emotion Management Scale – Sadness (CEMS-S) dysregulation and coping subscales. Preschool income-to-needs ratios were associated with reduced anti-correlations between hippocampus and superior prefrontal cortex. Further, income-to-needs from study entry at preschool age negatively predicted depression severity at the time of scanning at school age and connectivity between between left hippocampus and right superior frontal cortex was associated with depression. Importantly, we found that hippocampal and amygdala connectivity significantly mediated the relationship between income-to-needs and depression at the time of scan after controlling for depression in preschool. We also present data on the relationships between hippocampal volume and connectivity and between structural and functional brain connectivity, and the ways in which poverty and maternal support influence these relationships.
Conclusions: Taken together, these findings are consistent with the animal literature documenting the powerful effects of maternal support on hippocampal development and connectivity, and the critical role of these alterations in brain development and in predicting depression and emotion dysregulation.
Disclosure: Part 1: Amgen, consultant, Roche, consultant, Pfizer, consultant, Takeda, consulant.

Panel
43. Signal Outages Disrupting Cellular Networks: Importance of Astrocyte and Neuron Communication to Synaptic Transmission

43.1 Astrocytic Regulation of Cortical Rhythmicity

Kira Poskanzer

University of California, San Francisco, San Francisco, California, United States

Background: The neocortical slow oscillation that defines slow-wave sleep (SWS) is believed to play a critical role in memory consolidation by coordinating cell assembles in areas within and outside cortex. Slow cortical rhythms are also observed during the waking state, as well as during sleep, suggesting widespread functional roles for this oscillation. Although the slow oscillation is cortically generated, the circuit mechanisms that drive the cortex into the slow-wave state remain unclear. Recent studies have examined the effects of neuronal and sensory manipulations on brain state and the effects of brain state on processing, but have not investigated how other cellular circuit components may influence these states.
Methods: We monitor the activity of populations of cortical astrocytes *in vivo* and simultaneously record neurons either electrophysiologically (local field potential [LFP]) or with genetically encoded calcium (Ca2+) imaging. To manipulate astrocytic Ca2+ activity, we use an optogenetic tool, Archaerhodopsin (Arch), to increase intracellular Ca2+ concentration in astrocytic processes in a light-dependent manner. We explore the mechanisms of an astrocyte-generated cortical state shift; we express the genetically encoded glutamate sensor GluSnFR in astrocytes while recording the brain state with LFP.
Results: With simultaneous imaging and recording in sensory cortex *in vivo*, we uncover a temporal relationship between spontaneous astrocyte Ca2+ activity and the shift to a slow-oscillation-dominated state. Moreover, astrocyte-specific optogenetic manipulation shifts the cortical circuit into this state, and increases coactive neuronal firing. We find that spikes in extracellular glutamate around astrocytes co-occur with these shifts to the slow-oscillation state and that Arch stimulation of astrocytes can generate these local extracellular glutamate spikes.
Conclusions: In this work, we identify a previously unidentified cortical mechanism for the shift to the slow-oscillation-dominated regime. Many mechanisms have been described for shifting the brain into a more attentive, desynchronized state. However, far fewer have been found to consistently shift the cortex into the slow-oscillation state, and it has been considered a default state. Our evidence challenges this conception, as we show that the slow-oscillation state may be dynamically controlled by the astrocytic-neuronal network.
Disclosure: Nothing to Disclose.

43.2 Astrocytic Modulation of Synaptic NMDA Receptors

Marta Margeta

University of California, San Francisco, San Francisco, California, United States

Background: A growing body of evidence indicates that glial cells play a critical role in the formation and function of excitatory synapses. In particular, it has been shown that astrocyte-secreted soluble factors promote synaptogenesis and the surface expression of AMPA receptors (AMPARs) during the development. However, little is known about the effect of glia on the expression and function of NMDA receptors (NMDARs).
Methods: Glial modulation of neuronal NMDARs was investigated using physiological, cell biological, and biochemical techniques in primary hippocampal cultures. Hippocampal neurons were obtained from E19 rats and grown for 13-17 days either alone (neuronal cultures) or with a mixed population of glia (mixed cultures); direct or indirect neuron-astrocyte co-cultures were used in a subset of experiments.
Results: Using whole-cell patch clamp recordings, we found that the density of NMDA-evoked currents was significantly

greater in mixed cultures, direct neuron-astrocyte co-cultures, and indirect neuron-astrocyte co-cultures than in neuronal cultures. Both the amplitude and frequency of the NMDAR-mediated mEPSCs (miniature excitatory postsynaptic currents) were significantly larger in mixed than neuronal cultures; in contrast, there was no significant difference in amplitude and frequency of the AMPAR-mediated mEPSCs between different culture conditions, indicating that glial cells preferentially enhance NMDAR activity in hippocampal cultures with mature synapses. The observed doubling of the NMDAR current density could not be explained by an increase in the synapse density or by change in the expression level of major NMDAR subunits (GluN1, GluN2A and GluN2B). In addition, the current fluctuation analysis showed that the mean weighted unitary NMDAR conductance did not significantly differ between mixed and neuronal cultures. The glia-induced potentiation of NMDAR currents was specific for synaptic NMDARs and for the ifenprodil-sensitive, GluN2B-mediated component of the NMDAR current. Finally, the glia-induced increase in the neuronal NMDA current density was blocked in mixed cultures by protein kinase C (PKC) inhibitor GF109203X and increased in neuronal cultures by PKC activator phorbol-12-myristate-13-acetate, indicating that the astrocytic effect was mediated by PKC.

Conclusions: Taken together, our findings indicate that astrocyte-secreted soluble factor(s) can fine-tune synaptic NMDAR activity through the PKC-mediated regulation of GluN2B NMDAR channels already localized at postsynaptic sites, presumably on a rapid time scale. Given the role of GluN2B NMDARs in synaptic plasticity and neuronal survival, our findings suggest that NMDAR modulation is an important mechanism by which glia can impact neuronal circuits in both normal and disease states.

Disclosure: Nothing to Disclose.

43.3 The Effects of Cocaine Self-Administration on Astroglial Modulation of NMDA Receptor Function

Kathryn Reissner

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

Background: Disruptions in glutamatergic transmission and signaling within the nucleus accumbens following cocaine self-administration have been well described. However, the mechanisms responsible for the adaptations remain unclear. We recently reported that astrocytes in the nucleus accumbens exhibit reduced morphometric properties and reduced synaptic colocation following extinction from cocaine self-administration (Scofield et al, 2016). As astrocytes are important modulators of synaptic function, these findings raise the hypothesis that impaired synaptic function associated with cocaine abuse may be mediated in part by impaired astrocyte function and communication. Here we test the hypothesis that NMDA receptor function is impaired following extinction from cocaine self-administration, and that restoration of synaptic colocalization of astroglial processes can restore synaptic strength and function, and decrease cocaine seeking.

Methods: Male Sprague Dawley rats were trained in self-administration and extinction (2h/day) from cocaine. AAV5 expressing a membrane-tagged Lck-GFP was used to assess physical properties of astrocytes by immunohistochemistry and confocal microscopy. In separate experiments, whole cell patch clamp electrophysiology was used to assess NMDA receptor-mediated currents in the nucleus accumbens, also twenty-four hours following the last extinction session. For behavioral analysis, systemic D-serine augmentation was performed using D-serine and D-amino acid oxidase inhibitor sodium benzoate (100 mg/kg each, i.p.) three hours before the last two extinction sessions (or last three days of forced abstinence), and 100 and 200 mg/kg respectively, 3 hours before reinstatement testing.

Results: Extinction from cocaine is associated with a significantly decreased surface area and volume of astrocytes and decreased colocalization with presynaptic marker synapsin I. Among the potential ramifications of this decrease in synaptic colocalization is a decrease in volume transmission of astrocyte-secreted factors which modulate synaptic function, including D-serine. Toward this end, we tested the hypothesis that NMDA receptor function is impaired following cocaine self-administration and extinction, and that D-serine augmentation would reduce reinstatement to cocaine seeking. Three days of D-serine augmentation significantly decreased cocaine plus cue-primed reinstatement, while a single injection on the day of reinstatement was without effect. Preliminary electrophysiology results indicate a cocaine-dependent decrease in NMDA receptor function, as evidenced by a decrease in the stimulus-response relationship of isolated NMDA-mediated currents. In contrast, there is no effect on the current-voltage relationship of NMDA receptor function.

Conclusions: Results presented herein indicate that withdrawal from cocaine use leads to impaired astrocyte-neuron communication, which leads to impaired synaptic processing via NMDA receptors. Accordingly, restored function of NMDA receptors by augmentation of D-serine leads to decreased cocaine reinstatement. We hypothesize that the effect of D-serine augmentation on reinstatement is mediated by NMDA receptor-mediated internalization of AMPA receptors. Ongoing studies are designed to test this hypothesis.

Disclosure: Nothing to Disclose.

43.4 PACAP as a Molecular Bridge Linking Astrocytes and Neurons Into an Integrated Glutamate Network in the Nucleus Accumbens: Implications for Cocaine Seeking

David Baker

Marquette University, Milwaukee, Wisconsin, United States

Background: Compulsive drug use appears to involve, at least in part, persistent changes in excitatory signaling in corticostriatal circuits. Although it is becoming apparent that excitatory synaptic transmission is a product of coordinated activity of glutamate receptors, transporters, and release mechanisms expressed by astrocytes and neurons, the molecular basis of this form of cellular integration is poorly

understood yet may be key to decoding the molecular basis of relapse vulnerability. In these studies, we examined the potential for the neuropeptide PACAP to influence drug seeking by gating the activity of nucleus accumbens efferent projections through the regulation of components of the glutamate network expressed by astrocytes and neurons.
Methods: Transgenic rats were developed by mutating the Slc7A11 gene using Zinc-Finger Nucleases. Rat neuronal and astrocyte cell cultures were generated from freshly dissected striatal tissue obtained from rats aged E15-18 or PN day 3, respectively. System xc (Sxc) activity was assessed by measuring the uptake of 14C-cystine by striatal cultured cells or nucleus accumbens (NAc) tissue. Glutamate transporter activity was assessed by measuring 3H-D-aspartate uptake in NAc tissue. Whole-cell patch-clamp recordings and measures of RNA (using *in situ* hybridization or real-time PCR in FACS tissue) were obtained from distinct NAc efferent projections which were labeled by injecting retrograde fluorescence tracers into the ventral pallidum (VP) or the substantia nigra (SN). Western blotting was used to measure glutamate-related proteins in NAc tissue. Behavioral measures included the rate cocaine self-administration, extinction responding and cocaine-induced reinstatement (3 mg/kg, IP).
Results: Exogenous PACAP altered the activity or expression of glutamate-related proteins present on NAc neurons and astrocytes. These findings included an increase in Sxc activity in striatal astrocytes and a reduction in the levels of GluA1 pT840 on neurons. PACAP also reduced AMPA-mediated EPSCs in medium spiny neurons projecting to the SN (but not the VP) and decreased cocaine-primed reinstatement. Astrocyte and neuronal contributions to PACAP-induced reductions in drug seeking were revealed by blocking GluN2B receptors and by a lack of an effect of PACAP in transgenic rats lacking Sxc activity.
Conclusions: In these studies, we found that PACAP exerts control over glutamate-related proteins expressed by astrocytes and neurons. In doing so, PACAP shapes synaptic transmission in medium spiny neurons projecting from the NAc to the SN in a manner that determines the level of drug seeking. Interestingly, interfering with PACAP's action on astrocytes (Sxc) or neurons (GluN2B-containing NMDARs or AMPA Rs) prevented PACAP-induced blockade of cocaine seeking. Collectively, these data establish the neuropeptide PACAP as a molecular bridge linking astrocytes and neurons in order to regulate NAc efferent projections that give rise to cocaine seeking.
Disclosure: Part 1: Promentis Pharmaceuticals, Consultant, Board Member, Equity Stake, **Part 2:** Promentis Pharmaceuticals, Consultant, Board Member, Equity Stake, **Part 3:** Promentis Pharmaceuticals, Consultant, Board Member, Equity Stake.

Panel
44. Oxytocin and Addictions: Beyond Love Story?

44.1 Direct Evidence of Cerebrospinal Fluid Penetrance of Oxytocin After Intranasal and Intravenous Administration and Effect of Systemic Oxytocin Administration on Methylphenidate-Induced Changes in Accumbal Dopamine Levels and Methylphenidate Self-Administration

Mary Lee

National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland, United States

Background: Diffusion of peptides such as Oxytocin (OT), when delivered intravenously (IV), across the blood brain barrier is restricted. Intranasal (IN) delivery of OT is based on the notion that IN delivery bypasses the BBB. Animal studies report elevated cerebrospinal fluid (CSF) OT levels after IN or IV OT administration. However, actual CSF penetrance of IV or IN delivered OT has not been demonstrated. Additionally, OT alters behaviors related to the administration of drugs of abuse via centrally mediated pathways, however its mechanisms of action are not fully elucidated. Here, we present a set of experiments (Exp) where we investigated: 1) CSF penetrance of labelled OT; 2) the effect of OT on methylphenidate (MPH)-induced increase of dopamine (DA) levels in the nucleus accumbens shell (AcbSh); and 3) the effect of OT on MPH self-administration (SA).
Methods: Exp 1. We developed a LC-MS/MS assay to measure administered [deuterated (D5)] OT and endogenous [nondeuterated (D0)] OT in CSF and plasma. D5 OT was administered IN and IV to rhesus macaques ($N=6$). D5 and D0 OT in plasma and CSF were measured over 60 minutes post-IV and IN administration of 80 IU of D5OT. Exp 2. DA levels were measured via microdialysis in the AcbSh in male rats ($N=10$) who were pretreated with either OT (0 mg/Kg, 1 mg/kg, 2 mg/kg) 10 minutes before IV MPH injections (0.1, 0.32 and 1.0 mg/kg at 0, 30 and 60 min, respectively). Exp 3. Rats ($N=7$) were trained to self-administer MPH at doses of 0.01, 0.03, 0.1 and 0.3 mg/kg/inf. Animals were pre-treated with intraperitoneal OT or vehicle, 20 min prior to MPH SA sessions at doses of 0.1, 0.25, 0.5, 1 and 2 mg/kg. Food SA was used as a control condition.
Results: Exp 1. The LC-MS/MS assay measured D5 and D0 OT with high sensitivity (level of detection and quantification of 10 pg/ml) and specificity. Cmax ($\pm$ SD) D5 OT CSF levels were 636 ± 457 and 82 ± 117 pg/ml after IV and IN D5 OT administration, respectively. There was no significant effect of D5 OT administration on D0 OT levels. Exp 2. MPH injections resulted in a dose-dependent increase in basal DA levels and pretreatment with 2 mg/kg OT resulted in a significantly greater DA stimulation as compared to 1 mg/kg OT and vehicle ($p's < 0.05$) . OT per se did not affect basal DA levels. Exp 3. Pretreatment with 0.25, 0.5, 1 and 2 mg/kg OT resulted in an overall decrease in responses across all doses of MPH while 0.1 mg/kg OT did not alter responses as compared to baseline and vehicle. 0.5, 1 and 2 mg/kg OT also caused a decrease in food SA, while 0.1 and 0.25 mg/kg OT did not have any effect.

Conclusions: The LC-MS/MS assay measures D5 OT in the CSF after IN and IV administration. There is no evidence of a "feed-forward" effect of exogenous OT administration on endogenous OT levels. The IN route does not appear to be a "privileged pathway" for OT delivery over the time course here studied. Systemic OT administration dose-dependently potentiates AcbSh MPH-stimulated DA levels. Most rodent studies to date show a decrease in psychostimulants SA with pretreatment with OT; our microdialysis results suggest that OT would cause a shift to the left in the MPH dose response. However, results from the MPH SA experiment indicate a shift downwards following OT pretreatment, with no dose-dependent effect of OT. Together, these results indicate that OT is involved in the DAergic processes related to drug use and that treatment with OT induces changes with respect to SA behavior.
Disclosure: Nothing to Disclose.

44.2 Oxytocin Blocks Compulsive-Like Alcohol Drinking in Rats

Leandro Vendruscolo

National Institute on Drug Abuse, Baltimore, Maryland, United States

Background: We investigated the potential role of the oxytocin system in alcohol dependence using a preclinical model.
Methods: Wistar rats were trained to lever press for access to alcohol in operant chambers and then either made dependent on alcohol via repeated cycles of alcohol vapor exposure (dependent) or exposed to air (nondependent).
Results: Dependent rats developed increased alcohol drinking compared with nondependent rats. Oxytocin administration selectively reduced compulsive-like alcohol intake in dependent rats. Control experiments indicated that oxytocin does not affect general locomotion, motor coordination, and self-administration of non-alcoholic sweet and caloric solutions, indicating the selectivity of oxytocin in decreasing alcohol drinking during withdrawal.
Conclusions: The results suggest that oxytocin signaling is dysregulated in alcohol dependence and functionally involved in compulsive-like alcohol drinking.
Disclosure: Nothing to Disclose.

44.3 Oxytocin Reduces Alcohol Self-Administration and Stress-Induced Alcohol Relapse Behavior in Mice

Howard Becker

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

Background: A growing body of literature implicates a role for the neuropeptide oxytocin (OXT) in addiction related behaviors. Previous work by others and recent studies from our lab have shown that systemic administration of OXT reduces alcohol consumption in animals under various testing conditions. Clinical and preclinical studies also suggest that OXT has therapeutic potential in ameliorating stress-related behaviors. This presentation will describe results from a series of studies aimed at examining the effects of systemic administration of OXT on alcohol self-administration and stress-induced reinstatement of alcohol responding in adult male C57BL/6J mice.
Methods: Using standard operant conditioning procedures, adult male C57BL/6J mice were trained to lever respond on a fixed ratio (FR4) schedule for 12% (v/v) alcohol or 5% (w/v) sucrose reinforcement during daily 20-min sessions. Once stable responding and intake of alcohol or sucrose was established, mice were injected with OXT (0, 0.1, 0.3, 1 mg/kg; ip) 30 min prior to testing using a Latin-Square design. Subsequently, a subset of mice responding for alcohol or sucrose were injected with 0.3 mg/kg OXT or vehicle 30 min before testing under a progressive ratio (PR) schedule. In a second study, after establishing stable FR4 responding for 12% alcohol, mice were tested under extinction conditions for several days and then injected with OXT (1 mg/kg) or saline given 30 min prior to an injection of yohimbine (0.3 or 0.625 mg/kg), which was given 30 min prior to reinstatement testing. In a separate study, after stable FR4 responding was established, mice were exposed 15 min to a predator odor (TMT) or left undisturbed over 5 consecutive days. Baseline responding was re-established and after extinction testing, mice were injected with OXT (1 mg/kg) or saline 15 min prior to TMT exposure (30 min prior to reinstatement testing).
Results: OXT treatment reduced active lever responding, alcohol intake (g/kg), and resultant blood alcohol levels in a dose related manner. OXT also significantly reduced responding and breakpoint measures in PR testing. In separate mice, OXT reduced responding for sucrose, but failed to reduce PR responding or breakpoint for sucrose. In a second study, yohimbine significantly increased responding under extinction conditions in a dose-related manner. OXT blocked this yohimbine-induced reinstatement of alcohol responding. In the third study, after extinction sessions TMT exposure significantly increased active lever responding. This TMT-induced reinstatement of alcohol seeking behavior was significantly more robust in mice with a history of TMT exposure compared to mice with no prior experience with TMT treatment. OXT given prior to the reinstatement test session significantly reduced stress (TMT)-induced relapse-like behavior, and this effect was more robust in mice with prior TMT exposure.
Conclusions: OXT was shown to reduce operant oral alcohol self-administration in a dose-related manner as well as reduce apparent motivation for alcohol, as measured by PR testing. Additionally, OXT blocked alcohol relapse-like behavior (reinstatement of alcohol seeking behavior) provoked by exposure to different stressors (yohimbine and TMT). Collectively, these results suggest that systemic administration of oxytocin effectively reduces alcohol self-administration as well as attenuates stress-induced relapse-like behavior in mice. Studies are currently being conducted to examine these effects in female mice as well as explore mechanisms underlying this apparent therapeutic effect of oxytocin treatment.
Disclosure: Nothing to Disclose.

44.4 Intranasal Oxytocin Alcohol and Opioid Dependence Treatment Trials

Cort Pedersen

University of North Carolina, Chapel Hill, North Carolina, United States

Background: Current FDA-approved medications are not effective in reducing drinking in most alcohol dependent individuals or simply replace licit for illicit opioids for opioid dependent individuals. Early mouse studies demonstrated that oxytocin (OT) administration inhibits tolerance formation to alcohol and opioids in non-dependent animals and blocks withdrawal in dependent animals. In two small human RCTs, we found that intranasal administration of OT markedly reduces withdrawal and PRN lorazepam requirements during alcohol detoxification (Pedersen et al, 2013, 2nd manuscript in preparation). We are now conducting a NIAAA-funded 12-wk RCT testing the efficacy of intranasal OT on reducing alcohol consumption and craving in heavy drinkers and starting a trial of OT treatment of opioid withdrawal.
Methods: NIAAA-funded 12-wk RCT (R21 AA021927-01A1): Prospective subjects will undergo the MINI-Plus psychiatric interview. 40 subjects will be chosen who: 1) have met DSM-V criteria for alcohol dependence and drink heavily (35 drinks/week for men, 28 drinks/week for women) for at least the past 4 wks but 2) do not meet criteria for other substance use disorders, current major depressive disorder, lifetime psychotic, schizoaffective or bipolar disorder; have no history of dangerous withdrawal (delirium tremens, seizures, psychosis) or unstable medical conditions. Intranasal test treatments begin within 1 wk after initial evaluation: intranasal doses of oxytocin (OT) or placebo (PBO), TID the first 2 days, BID thereafter for the remainder of the trial (10 insufflations/dose; 40IU OT). Clinic evaluations at 1, 2, 3, 4, 6, 8, 10 and 12 wks after starting test treatment each of which include: CIWA-Ar score, breath alcohol concentration measure, Timeline Follow Back (TLFB) interview; Penn Alcohol Craving Scale (PACS); state part of the Spielberger State-Trait Anxiety Inventory; blood and urine samples for lab tests (4, 8, 12 wks). After cessation of intranasal test treatments: TLFB and PACS will be obtained at 4-wk intervals.
Intranasal treatment of opioid withdrawal: Subjects (30) will be selected from those seeking medical detoxification from opioids at the Addictions Detoxification Unit (ADU) at the UNC mental health center at Wakebrook, Raleigh, NC. Subjects must meet DSM-V opioid use disorder criteria for at least 4 wks, have a history of opioid withdrawal and not meet exclusion criteria summarized above. Double blind TID intranasal test treatments (same doses as the outpatient trial above) will begin shortly after admission and completion of baseline TLFB interview, COWS, and visual analog opioid craving scale (VAOCS) and continue for up to 5 days. COWS and VAOCS will be administered q 4 hr for 2 days and then TID.
Results: We anticipate that 1) OT treatment will be superior to PBO in reducing alcohol consumption during the 12-wk trial, and 2) will slow return to heavy drinking after intranasal test treatments are stopped. We hypothesize that OT will be superior to PBO in decreasing opioid withdrawal symptoms.
Conclusions: These will be the first studies testing if OT reduces drinking in heavy drinkers and withdrawal symptoms in opioid dependence. If successful, these studies will justify future animal and human studies into the neural mechanisms of OT anti-addictive effects as well as the role of endogenous OT in alcohol and opioid dependence.
Disclosure: Nothing to Disclose.

Study Group
45. Improving Transparency of Clinical Trial Data

Mark Weiser*, Adam Haim, Joseph Ross, Thomas Laughren, Amir Kalali, Lynn DeLisi, John Krystal, Doris Fuller

Sheba Medical Center, Ramat Gan, Israel

Study Group Proposal: Progress in developing therapeutic interventions is often plagued by publication bias of several sorts, including non-publication of negative studies. Additionally, investigators sometimes report the results of studies improperly, neglecting to differentiate between primary and secondary outcome measures, and/or presenting post hoc and sub-group analyses as primary outcomes. Non-publication or improper publication can be damaging for the field, as treatments which have already been tested with no results published might be tested again, leading to unnecessary exposure of patients, waste of funds that might otherwise for further innovations, as well as resulting in unnecessary delays in treatment development which is badly needed for psychiatric illnesses. This study group aims to address this issue by discussing the rates of publications of studies of two major agencies which fund trials in psychiatry. We will then also highlight an NIMH/Stanley Medical Research Institute initiated effort requiring investigators to submit their unidentified individual patient data blinded to treatment allocation, to a central NIMH data repository as one potential solution to this problem.
Dr. Gogtay will present data on 115 NIMH funded clinical trials completed between 2010-2011, of which 87% were published. Further analyses on rates of publication, time to publish since completion of RCT and impact of those publications will be discussed. He will also discuss the efforts NIMH is putting in place to maximize the dissemination of data to investigators as well as consumer communities.
Dr. Weiser will present data on rates of publications for projects funded by the Stanley Medical Research Institute. Out of 253 studies funded since 2000, 10% were not completed. Mean rates of publication from 2000 to 2009 was 54.6%. Mean time to publication from completion of study was 2.06 ± 0.83 years. Further analyses on the differences between primary outcome measures and enrollment goals reported in the initial protocols and the published papers will be discussed, as well as rates of study replications.
Dr. Ross will present data characterizing the quality and transparency of evidence that supported the approval of novel therapeutics for psychiatric and neurologic diseases by the FDA between 2005 and 2011, including rates of publication and a comparison of the trial results described

in FDA clinical reviews and those described in peer-reviewed medical literature.
Dr. Laughren will discuss this issue with regard to registration trials from a regulatory perspective, including concerns about transparency, access, and interpretation of findings from the diversity of trials that comprise a drug development program.
Dr. Kalali will discuss the broader definition of data transparency including transparency to investigators and study subjects and the impact of technology on clinical trial conduct, data transparency and ownership.
Dr. DeLisi and Krystal will discuss this issue from their points of view as journal editors.
Finally, Doris Fuller, a leader in mental health advocacy with a personal experience of a loss to mental illness, will address the need for these efforts from the perspective of patient advocacy groups and community physicians treating the mental illness.
Disclosure: Nothing to Disclose.

Panel
46. Stress, Habits, Hormones and Trauma: An Endocannabinoid Link in Alcohol-Related Pathologies

46.1 Effects and Interactions of Ethanol and Endocannabinoids in Rat Central and Basolateral Nuclei of the Amygdala

Florence Varodayan

The Scripps Research Institute, La Jolla, California, United States

Background: The central (CeA) and basolateral (BLA) nuclei of the amygdala are critical to the pathophysiology of anxiety-driven alcohol drinking and relapse. Chronic ethanol disrupts stress systems in both the CeA and BLA, leading to aversive withdrawal symptoms.
Methods: In these electrophysiological studies, we investigated the potential disruption of endogenous cannabinoid/type 1 cannabinoid receptor (eCB/CB1) signaling on GABAergic transmission in CeA neurons and BLA pyramidal neurons of rats exposed to 2-3 weeks intermittent ethanol vapor (CIE).
Results: Using the CB1 antagonist AM251, we found a tonic eCB/CB1 control of GABA signaling in the naïve CeA and BLA that was diminished in CIE rats, suggesting a functional impairment of the eCB/CB1 system in these regions after chronic ethanol exposure. In the CeA, CB1 activation (via Win 55,212-2) decreased GABA release and prevented an ethanol-induced increase in GABA release in naïve rats. This per se effect of Win was attenuated in CIE rats, suggesting that chronic ethanol diminishes the regulation of GABA release by the CB1 system in the CeA. In contrast, in BLA pyramidal neurons Win 55,212-2 reduced (without blocking) ethanol's effects in both naïve and CIE rats. Additionally, in both the CeA and BLA, the AM251- and EtOH-induced facilitations of GABA release were additive, ruling out the participation of CB1 in the ethanol effect. We have also evaluated the effects of CB1 receptor activation and blockade on excitatory glutamatergic signaling in the CeA of male and female rats. We found a complex interaction between CB1 receptor manipulations and EtOH-induced alterations in CeA excitatory signaling, with sex exerting robust, though not easily understood influences.
Conclusions: Collectively, these observations demonstrate an important CB1 influence on amygdala nuclei and indicate that these brain regions are particularly sensitive to alcohol-induced disruptions of CB1 signaling.
Disclosure: Nothing to Disclose.

46.2 Chronic Intermittent Alcohol Exposure Produces Sex- and Hormone-Dependent Alterations in Withdrawal-Induced Negative Affect and Corticolimbic Endocannabinoid Signaling

Ryan McLaughlin

Washington State University, Pullman, Washington, United States

Background: Considerable sex differences exist with respect to the symptoms of alcohol use disorders. The endocannabinoid (ECB) system, which is intimately linked to female sex hormones, influences both the motivation to consume alcohol as well as the negative affective symptoms of withdrawal, Despite this important modulatory role, it is unknown whether alcohol differentially alters the ECB system in males and females, and whether this has implications for withdrawal-induced behavioral alterations. Thus, we examined whether chronic intermittent alcohol (CIA) exposure differentially alters escalation of alcohol intake, withdrawal-induced anxiety, and ECB mRNA/content in the basolateral amygdala (BLA), medial prefrontal cortex (mPFC), and nucleus accumbens of male, intact female, and ovariectomized female rats with or without estradiol (E2) replacement.
Methods: Male and female Wistar rats were subjected to 6 weeks of CIA vapor or air exposure (14 h on: 10 h off, 7 d/wk). A separate cohort of female rats were ovariectomized and half were implanted with an E2 capsule while the other half were implanted with a blank capsule. To establish baseline drinking, rats were first trained to drink 10% alcohol in chambers equipped with optical lickometers prior to CIA exposure. Rats were reintroduced to the drinking chambers and intake was assessed periodically over the final 2 weeks of CIA exposure. Rats were then tested for anxiety-like behavior in the elevated plus maze and 22 kHz ultrasonic vocalizations (USVs) were quantified at 6-8 hr into acute withdrawal, Rats were sacrificed at 6-8 hr into withdrawal the following day. The mPFC, BLA, and NAc were dissected for quantification of anandamide (AEA) and 2-arachidonoylglycerol (2-AG) or relative mRNA expression for the primary biosynthetic/catabolic enzymes for these ligands using LC/MS/MS and RT-qPCR, respectively.
Results: Both male and female rats exposed to CIA vapor escalated their alcohol intake post-dependence, but interestingly, air-exposed female rats also showed escalated intake. Male rats exhibited a robust anxiety-like phenotype coupled to decreased expression of DAGL-α and MAGL mRNA in the BLA, reduced NAPE-PLD mRNA expression in the mPFC, and reduced NAPE-PLD, DAGL-α, and MAGL mRNA in the NAc. In contrast, intact females did not show

an anxiety-like phenotype or significant alterations in ECB-related mRNA expression in any brain region examined. However, NAPE-PLD and MAGL mRNA expression in the NAc were significantly elevated in the NAc during proestrus, which was subsequently abolished by CIA exposure. With respect to content, AEA (but not 2-AG) was reduced in the BLA of male, but not female rats exposed to CIA vapor. In the mPFC, AEA content was significantly reduced in female CIA-exposed rats during acute withdrawal, whereas male CIA-exposed rats showed a reduction in 2-AG (but not AEA) content. OVX females exposed to CIA vapor exhibited increased anxiety-like behavior along with reductions in NAPE-PLD mRNA expression in the BLA and decreased expression of all ECB-related genes in the mPFC. E2 produced potent anxiolytic effects in air-exposed female rats, but this was absent in CIA-exposed OVX females. Moreover, E2 replacement failed to prevent the CIA-induced deficits in ECB mRNA expression.

Conclusions: CIA exposure elicits sexually dimorphic alterations in alcohol withdrawal-induced anxiety and ECB mRNA/content. Moreover, CIA exposure abolishes the anxiolytic properties of E2, yet E2 replacement alone is insufficient to prevent CIA-induced reductions in ECB-related mRNA expression. These data provide insight into the neurobiological and hormonal mechanisms that may contribute to the induction of alcohol dependence and withdrawal in female rats, which could be leveraged to identify novel therapeutic strategies for treating alcohol use disorders in women.

Disclosure: Nothing to Disclose.

46.3 Alcohol Effects on Endocannabinoid-Dependent Plasticity at Corticostriatal Synapses Implicated in Habit Formation

David Lovinger

National Institute on Alcohol Abuse and Alcoholism, Rockville, Maryland, United States

Background: Interactions between the associative and sensorimotor cortical-basal ganglia circuits control the balance between goal-directed and habitual instrumental actions. The associative dorsomedial striatum (DMS) is implicated in goal-directed action control, while the sensorimotor dorsolateral striatum (DLS) controls habit learning and habitual actions. Endocannabinoids and CB1 receptors gate habit formation, and one mechanism implicating in this action is presynaptic depression of corticostriatal synapses, although it must be noted that endocannabinoids also modulate GABAergic synapses in striatum. Alcohol exposure promotes habitual behaviors, including habitual alcohol seeking. We are examining the cellular and circuit changes induced by alcohol that may contribute to the habit-promoting effects of alcohol.

Methods: Vapor inhalation was used for chronic alcohol exposure in C57Bl6J mice. Mice were placed in vapor chambers for 16 hours per day, 4 days per week for a total of 2-4 weeks, with average blood alcohol concentrations of 120-150 mg/dl.
Cell-specific knockout of CB1 receptors was accomplished by viral injection into orbitofrontal cortex (OFC), and in some cases DMS, in mice expressing a "floxed" CB1 allele.
Mice were tested for goal-directed versus habitual lever-pressing in a standard food-rewarded task. Reward-specific satiety was used to devalue outcomes to test for goal-directedness versus habitual behavior.
Brain slice electrophysiological and pharmacological experiments were performed 3-6 days after the end of chronic ethanol exposure in coronal brain slices. Whole-cell and field potential recordings were made in striatum. Synaptic responses were evoked with electrical stimulation, or optogenetic activation of channelrhodopsin expressed in specific neuronal subtypes.

Results: Long-lasting synaptic depression at corticostriatal synapses was activated either by high frequency afferent stimulation or application of the CB1 receptor agonist WIN 55,212-2. Knocking out CB1 receptors in OFC projection neurons or specific OFC-DMS projections eliminated CB1 effects on synapses in the DMS. These knockouts also failed to learn habitual lever-pressing for food in the instrumental task, even when mice were trained using a random-interval schedule that normally fosters habit learning.
Chronic exposure to ethanol vapor increased the rate of habit learning in C57Bl6J mice, even when animals were trained using a random ratio schedule that fosters goal-directed learning in control mice. This ethanol exposure paradigm also prevented endocannabinoid-induced long-term depression at corticostriatal synapses. Other forms of corticostriatal long-term synaptic depression were also lost following chronic alcohol exposure.

Conclusions: Alcohol exposure alters long-term synaptic plasticity at corticostriatal synapses, including long-term depression mediated by endocannabinoids and CB1 receptors. Interestingly, other drugs of abuse also disrupt endocannabinoid actions at these synapses. Endocannabinoids and striatal long-term depression have been implicated in action learning involving the striatum, including learning of habitual instrumental action strategies. Our evidence indicates that endocannabinoids and CB1 receptors are crucial for habit learning, especially through their actions at OFC inputs to the DMS. Drugs of abuse, including alcohol, may disinhibit striatal projection neurons, in part by altering long-term depression. This circuit change appears to play a role in drug-facilitated habit formation and habitual drug seeking.

Disclosure: Nothing to Disclose.

46.4 Endocannabinoids Improve Traumatic Brain Injury-Induced Neuropathology: Cellular and Behavioral Effects

Patricia Molina

LSU Health Sciences Center School of Medicine, New Orleans, Louisiana, United States

Background: Traumatic brain injury (TBI), is characterized by neuroinflammation that is associated with lasting neurological and behavioral deficits including increased incidence of anxiety and depression, stress and pain sensitivity, anhedonia and impulse control deficits. TBI, promotes alcohol abuse in humans, but the neurobiological mechanisms underlying post-TBI escalation of alcohol drinking are not known. We have shown that alcohol

exposure following TBI augments and sustains neuroinflammation and excitatory glutamate signaling, and that these changes are associated with impaired neurobehavioral recovery from TBI. A candidate therapeutic target is the endocannabinoid (EC) system. Previously we showed that, in the absence of alcohol, a single post-TBI administration of JZL184, a monoacylglycerol lipase (MAGL) inhibitor that prevents degradation of the endocannabinoid (EC) 2-AG, is sufficient to attenuate neuroinflammation, improve neurobehavioral recovery, and maintain blood brain barrier structural integrity. The aim of this study was to extend these findings to a model including alcohol, so we can test the hypothesis that post-TBI EC degradation inhibition would improve behavioral outcomes including cognition, anxiety-like behavior, and pain sensitivity in rats trained to drink alcohol, and that EC degradation inhibition would also decrease alcohol drinking and ameliorate neuroinflammation and glutamatergic signaling dysregulation following TBI.

Methods: Adult male Wistar rats were trained over four weeks to self-administer alcohol in a 30-minute, free choice two lever FR1 operant paradigm with one press of one lever delivering 0.1 ml water and one press of the other lever delivering 0.1 ml 10% w/v alcohol. After reaching baseline (defined by stabilization of voluntary drinking), rats were counterbalanced into experimental groups based on their baseline alcohol intake: sham, TBI+VEH, and TBI +JZL. All rats underwent a 5-mm left lateral craniotomy, and TBI was induced by lateral fluid percussion. Thirty minutes post-TBI, rats received intraperitoneal injections of vehicle (alcohol, emulphor, and saline; 1:1:18) or JZL184 (16 mg/kg). Rats were allowed to self-administer alcohol for two weeks post-TBI before sacrifice, after which protein levels of glutamate signaling homeostasis were measured. Anxiety-like behavior (open field test), cognitive deficits (Y-maze), pain sensitivity (Von Frey test), and motivated alcohol drinking (progressive ratio operant self-administration; PR) were assessed up to two weeks post-TBI.

Results: Our results show that a single post-TBI injection of JZL184 decreases motivation to drink (PR), attenuates neuroinflammation, rescues dysregulated glutamatergic signaling, and attenuates neuronal hyperexcitability at the site of injury. In addition, JZL184 administered-TBI animals had significantly improved cognitive performance, significantly attenuated pain sensitivity deficits, and significantly attenuated anxiety-like behavior compared to vehicle-injected TBI animals.

Conclusions: Together with our previous data, these results show that EC degradation inhibition 30 minutes post-TBI has potential therapeutic benefits that persist throughout the acute recovery period up to 14 days' post-injury. Based on our findings, we speculate that TBI-induced synaptic hyperexcitability at the site of injury may contribute to the development of negative affective behaviors including anxiety-like behavior and escalation of alcohol self-administration during the early post-TBI period. These results suggest that early treatment with an EC degradation inhibitor may effectively ameliorate the negative outcomes following TBI.

Disclosure: Nothing to Disclose.

Panel
47. Why Adult Psychiatrists Need to Know About Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS)

47.1 Immunomodulatory Therapies: The New "Psychopharmacology" for Acute-Onset OCD

Susan Swedo

National Institute of Mental Health, Bethesda, Maryland, United States

Background: In the past three decades, clinical observations and research investigations have provided evidence that PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections) results from an autoinflammatory response to the molecular mimicry of Group A streptococci (GAS), such that antibodies directed against GAS epitopes cross-react with host antigens and produce inflammation of the basal ganglia. Treatments, such as intravenous immunoglobulin (IVIG) and therapeutic plasmapheresis (TPA), which remove or inactivate the cross-reactive antibodies have been shown to be helpful in PANDAS. Recently, the immunomodulatory therapies have also demonstrated benefits for the larger class of acute-onset neuropsychiatric disorders (PANS), raising the possibility that neuroinflammation may also play a role in their etiology.

Methods: Clinical characteristics, laboratory findings, and results of ancillary studies, (such as electroencephalography, polysomnography and neuroimaging), will be examined to determine their utility as diagnostic or disease biomarkers, and compared against response to blinded and open-label immunomodulatory therapies to determine the factors predicting treatment response. Preliminary results are available for 35 children (25 males; mean age 8.2 yrs; range 3 - 12 yrs) who participated in a NIMH-Yale placebo-controlled trial of intravenous immunoglobulin (IVIG) for treatment of PANDAS.

Results: 27 of 35 children (77%) evaluated at NIMH had lab abnormalities, including low serum IgG levels in 5 (14%); elevated antistreptococcal antibodies (ASO or anti-DNAseB) in 13 (37%) and positive antinuclear antibodies (ANA) in 14 (40%). Eleven with positive ANA's also had positive CaM KII assays ($>$130% activation) and were more likely to show improvements with IVIG therapy than were children with negative ANA/negative CaM KII values. The utility of these potential biomarkers will be explored by examining a new cohort of children with PANS/PANDAS receiving treatment with steroids, IVIG or TPA at one of the 12 sites participating in the PANS/PANDAS Clinical Research Network.

Conclusions: Clinical, paraclinical and laboratory findings will be used as diagnostic biomarkers for PANS/PANDAS, and may predict response to immunomodulatory therapies. If so, the variables will not only prove useful in the evaluation and treatment of children with acute-onset OCD, but may also serve to identify other patients who will benefit from immunologic treatments.

Disclosure: Nothing to Disclose.

47.2 Are Antibiotics the Next Class of Psychopharmaceutical Drugs?

Mark Pasternack

Massachusetts General Hospital, Boston, Massachusetts, United States

Background: Antimicrobial and psychotropic medications often share overlapping mechanisms of actions and pharmacological effects. For example, the isoniazid class of antibiotics has been shown to possess monoamine oxidase activity, while tricyclic antidepressants are reported to have antimalarial and anti-leishmanial properties. Although these shared pharmacological effects may seem to be coincidental, disorders such as neurosyphilis remind us that infectious agents can produce psychiatric symptomatology and antibiotics can play a primary role in the treatment of mental disorders. An emerging literature is demonstrating benefits of antibiotics, such as penicillin, amoxicillin, azithromycin and oral cephalosporins, for post-streptococcal obsessive-compulsive disorder (Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcal infections or PANDAS), as well as the larger class of children without known microbial triggers (Pediatric Acute-onset Neuropsychiatric Syndrome or PANS). Comparing clinical features of responders may provide important clues to the antibiotics' therapeutic mechanism of action.
Methods: The presentation will review the published literature on antibiotic use in PANDAS and PANS, including: a case series documenting improvement in pediatric OCD following open-label antimicrobial treatment (ML Murphy & Pichichero 2002); improvements in symptom severity associated with open-label monthly Bicillin injections (F Falcini et al, 2013); a placebo-controlled trial of penicillin prophylaxis in PANDAS (Garvey et al, 1999); a comparison of azithromycin and pencillin prophylaxis for PANDAS (Snider et al, 2005); and a randomized placebo-controlled trial of cefdinir for recent-onset PANS (TK Murphy et al, 2015). In addition, results of a recently completed double-blind placebo controlled trial of azithromycin for PANS will be presented and discussed.
Results: Taken together, the published literature presents more than 200 children with PANDAS or PANS who have benefited from treatment with antibiotics. Interestingly, the presence or absence of a recognized preceding microbial infection does not appear to influence outcome. However, other clinical features may predict those who will benefit from antibiotic administration, either acutely or as a prophylactic agent.
Conclusions: An increasing number of prospective randomized clinical trials support a therapeutic role for beta lactam agents in the management of PANDAS and the use of macrolides such as azithromycin in the management of PANS and PANDAS. Although azithromycin may have direct anti-inflammatory as well as antibiotic properties, the efficacy of various beta lactam agents in PANDAS patients, which reduce anti-streptococcal antibody titers, support the hypothesis that group A streptococci play a fundamental causative role in the pathogenesis of PANDAS. Analogous dysregulatory immune responses to other childhood pathogens may underlie the development of PANS.
Disclosure: Part 1: Merck, equity, **Part 2:** Merck, equity.

47.3 The Role of Th17 Cells in CNS Autoimmunity in an Animal Model for Pandas

Dritan Agalliu

Columbia University Medical Center, New York, New York, United States

Background: Antibodies against neuronal receptors and synaptic proteins have been associated with both regional and general encephalitic syndromes that produce either movement or psychiatric disorders. While the identification of autoantibodies has facilitated the diagnosis and has offered treatment opportunities for many autoimmune brain disorders, the mechanisms by which autoantibodies enter the brain and promote neurovascular pathology remains unclear. Group A Streptococcus (GAS) infections in children are associated with basal ganglia encephalitis that produces either movement [Sydenham's chorea (SC)] or psychiatric [Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus (PANDAS)] deficits. The humoral adaptive immune response plays an important role in disease pathogenesis in both humans and rodent models for the disease. Autoantibodies that recognize neuronal targets and dopamine receptors (D1R and D2R) are found in sera from acutely-ill children. These autoantibodies promote behavior deficits when infused in the rodent brain or when they are administered intravenously (i.v.) into naïve recipient rodents in conjunction with agents that disrupt the blood-brain barrier (BBB). However, the role of cell-mediated immune response in the neurovascular pathology in these disorders remains unclear.
Methods: To address the role of cell-mediated immune response in the pathogenesis of SC and PANDAS, we developed a novel animal model for the disease that makes use of multiple intranasal infections with GAS. We examined the distribution of Th17 cells in the brain after recurrent infections, blood-brain barrier integrity, the state of neuroinflammation (activation of microglia) and the synaptic changes in the brain.
Results: We found that the normal intranasal (i.n.) route of GAS infections promotes generation and accumulation of Th17 cells, an essential component of the cellular adaptive immune response, into the nose-associated lymphoid tissue of mice and humans. These GAS-specific Th17 cells migrate from the nose toward the olfactory bulb (OB), accumulate and subsequently disperse into other brain regions. Moreover, their presence in the brain correlates with BBB breakdown, extravasation and brain deposition of antibodies, decrease in excitatory synaptic proteins and changes in neuronal activity. However, bacteria are not detected in brains of i.n. infected mice, suggesting that the neurovascular pathology is likely induced by the presence of GAS-specific Th17 cells in the brain. Finally, we have found that two chemokines are induced in the olfactory epithelium (OE) and OB after multiple GAS infections and likely promote migration of GAS-specific Th17 cells from the nose into the brain.
Conclusions: Our findings suggest an important role for both cellular (Th17 cells) in addition to the humoral (autoantibodies) adaptive immune responses in the pathogenesis of SC and PANDAS after multiple i.n. GAS infections.
Disclosure: Nothing to Disclose.

47.4 Immune Cofactors Regulating CNS Access of Autoantibodies in Pediatric Infection-Associated Neuropsychiatric Disorders: Clues From Animal Model and Human Studies

Mady Hornig

Columbia University Medical Center, New York, New York, United States

Background: Growing evidence suggests that some neuropsychiatric disorders may be associated with brain-directed autoantibodies. A diverse set of environmental factors has been implicated as triggers of the production of these antibodies, ranging from toxicants to microbes. The timing of exposure relative to brain maturation is likely to be an important determinant of neuropsychiatric outcomes; however, it remains unclear how autoantibodies produced in the periphery access different regions of the CNS to bind neural epitopes and induce behavioral disturbances.
Methods: Animal models of pre- or postnatal infectious or immune challenges and passive antibody transfer studies were used to examine changes in immune signatures and immunoglobulin Ig isotypes and to determine their degree of association with behavioral disturbances. Regional patterns of blood-brain barrier (BBB) breach and autoantibody binding in CNS were compared following peripheral administration of different types of bacterial products (mycobacterial, E. coli). Human studies interrogated plasma and cerebrospinal fluid (CSF) samples for immune correlates (61-plex immunoassay) of response or non-response to a placebo-controlled trial of intravenous immunoglobulin (IVIg) (before, during or after treatment) in children with Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infection (PANDAS).
Results: Infection-like and immune challenges can provoke altered cytokine signatures and changes in Ig isotypes and subclasses and promote development of anti-neuronal antibodies that correlate with changes in behavior. The BBB that envelops different regions of the CNS appears to be differentially compromised by different types of immune stimuli, ranging from bacterial components and cytokines to stress-related factors such as neurotransmitters. IVIg treatment of children with PANDAS was associated with increased plasma tumor necrosis factor (TNF)α and CSF transforming growth factor (TGF)β; excluding subjects with intercurrent streptococcal infections, inverse correlations were found between the degree of clinical change in the double-blind phase of the trial (Children's Yale-Brown Obsessive Compulsive Scale [CY-BOCS] scores) and CSF levels of interleukin (IL)5, an immune molecule with a role in allergic, eosinophil-mediated, T helper cell (Th)2-type responses.
Conclusions: A growing body of evidence demonstrates a role for anti-neuronal antibodies in the pathogenesis of neuropsychiatric symptoms in PANDAS and related conditions. Regionally-specific BBB compromise induced by different stimuli may underlie regional differences in autoantibody binding and could help provide a neuroanatomically-informed explanation of observed patterns of behavioral abnormalities in immune-mediated models of neuropsychiatric disorder. Peripheral blood and CSF immune profiles may influence access of autoantibodies to CNS targets as well as binding characteristics. Pathologic autoantibodies could provide biomarkers of disease and new opportunities for treatment and prevention.
Disclosure: Nothing to Disclose.

Panel
48. GABA-A Alpha5 Receptors and Tonic Inhibition as Novel Targets in Mood and Cognitive Disorders

48.1 Extrasynaptic Delta GABA-A Receptors: A Potential New Therapeutic Target for Depression

Beverley Orser

University of Toronto, Toronto, Canada

Background: γ-aminobutryic acid (GABA) is the major inhibitory neurotransmitter in the mammalian brain, contributing to nearly 40% of all neurotransmission (Farrant & Nusser, 2005). The major inhibitory receptors are ionotropic GABA type A receptors (GABA-A), that are composed of a pentameric array of subunits (α1-5, β1-3, γ1-3, δ, π, θ, ε, ρ1-3) (Farrant & Nusser, 2005). Evidence suggests reduction and disruption in GABAergic pathways contributes to the pathogenesis of neuropsychiatric disorders. Particularly, patients with major depression have reduced GABA levels in the brain, and the expression levels of extrasynaptic GABA-A receptors are reduced in both suicide victims and anxiety related disorders (Merali et al, 2004; Sequeira et al, 2009). Of particular note is the δ GABA-A subunit (GABRD), which is found extrasynaptically and mediate tonic inhibition in the brain (Whissell et al, 2014). GABRD is implicated in the development of depression, mood disorders, and schizophrenia (Whissell et al, 2014). Extrasynaptic δ GABA-A receptors have unique physiological, pharmacological, and kinetic properties that render them attractive targets for the development of novel antidepressant compounds. The goals of the presentation will to: 1) provide a brief overview of the properties of delta GABA-A receptors; 2) discuss the role of these receptors in cognition, mood and neurogenesis and 3) present new findings that show these receptors are promising targets for the treatment of depression. Specifically, our studies examined whether reduced expression of GABRD is associated with a depressive and/or anxiogenic behavioural phenotype in mice. Mice were also assessed on executive function memory tasks, which have been shown to be impaired in patients with mood disorders (Trivedi & Greer, 2014). We postulated that a decrease in GABRD expression would lead to increases in both depressive and anxiogenic behavior, and lead to impairments in executive function. Further, drugs that selectively increase receptor function would improve memory performance and depression-like behaviors.
Methods: Complementary behavioral, electrophysiological and biochemical methods were used to study the properties of delta GABAA receptors in wild type and delta GABA-A receptor null mutant mice and their role in memory and mood-related behaviors. The contribution of these receptors to the maturation of adult-born neurons, a process referred to as neurogenesis, was also studied.

Results: Our result show that that mice lacking delta GABA-A receptors exhibit depressive-like behaviors, reduced neurogenesis and impaired memory performance for certain hippocampa-dependent memory tasks. Drugs that increase GABA-A receptor function improve cognition and promote neurogenesis. Further, preliminary results will show the effects of such drugs may modify depressive-like behaviors.
Conclusions: Extrasynaptic delta GABA-A receptors are potential new therapies for the development of antidepressant compounds.
Disclosure: Nothing to Disclose.

48.2 Mechanism of Magnetic Seizure Therapy for Treatment Resistant Depression: Insights From TMS-EEG Measures of Plasticity and Inhibition

Zafiris Daskalakis

University of Toronto, Toronto, Canada

Background: Magnetic seizure therapy (MST) is a promising intervention for treatment resistant depression (TRD). MST acts on regulating the balance between excitation and inhibition in the brain. Combined transcranial magnetic stimulation and electroencephalography (TMS-EEG) can also be used to assess neuroplasticity and cortical inhibition, which are expected to improve as a result of successful treatment.
Methods: Twenty patients with TRD were recruited. Clinical symptoms were assessed with the 24-item Hamilton Depression Rating Scale (HDRS-24) and scale for suicidal ideation (SSI). Clinical assessments and TMS-EEG measures were collected at baseline and after a course of MST treatment. All subjects received single and paired pulse stimulation (100 ms interstimulus interval) delivered to the dorsolateral prefrontal cortex (DLPFC). Plasticity was assessed by the overall and theta band cortical evoked activity (CEA) of the single pulse TMS evoked potential (TEP). Cortical inhibition was assessed by the suppression of the paired pulse TEP relative to the single pulse TEP (i.e. long interval cortical inhibition [LICI]). Subjects were divided into responders ($n = 8$, HDRS-24 change $\geq 50\%$) and non-responders ($n = 12$). All results were corrected for multiple comparisons using cluster based statistics.
Results: For CEA, there was a trending main effect of group over the frontal central electrodes (cluster $p < 0.1$), which showed a higher value for responders than non-responders. When the TEP is filtered into the theta (4-7Hz) frequency range, the resulting CEA showed significant effects for both treatment and group, but no significant interaction. Theta CEA was increased after MST treatment for the frontal central electrodes (cluster $p < 0.005$; max at FC3 and C3, t-score = 4.0 and 4.1) and is higher in responders than non-responders over the same region (cluster $p < 0.05$; max at C1, t-score = 3.3). For LICI, there was no main effect of treatment or group, but there is a correlation between the decrease in LICI and the decrease in SSI over the frontal and central electrodes (cluster $p < 0.01$; max at CZ, spearman rho = 0.77).
Conclusions: These results suggest that neuroplasticity and cortical inhibition are affected by MST. While an increase in neuroplasticity as indexed by theta CEA represent an overall neurophysiological effect of MST, individualized changes in cortical inhibition as indexed by LICI underlies the differential treatment response for suicidal ideation.
Disclosure: Part 1: Magventure, Equipment, Sunovion, Advisory board, **Part 4:** Brainsway, Grant.

48.3 Tonic Inhibitory Control of Dentate Gyrus Granule Cells by a5-Containing Gabaa Receptors Reduces Memory Interference

Uwe Rudolph

Harvard Medical School, Belmont, Massachusetts, United States

Background: a5-GABAARs which are strongly expressed in the hippocampus are targets of current drug development efforts in the pharmaceutical industry. As previous studies in animals and humans suggest that a5-GABAARs impair cognitive functions, the focus for drug development is currently on negative allosteric modulation. The role of a5-GABAARs in the management of complex learning and memory functions is however unknown.
Methods: We generated mice lacking the GABAAR a5 subunit in principal cells in CA1 and CA3 hippocampal subregions and in dentate gyrus granule cells (DGGCs). Tonic and phasic inhibitory currents were measured, and mice were tested in a variety of behavioral paradigms assaying learning and memory functions.
Results: Conditional deletion of a5-GABAARs in CA1 and CA3 resulted in apparently improved memory in novel object recognition and conditioning to context, respectively. Conditional deletion of a5-GABAARs in DGGCs led to reduced tonic inhibition without change in phasic inhibition, increased activation of the DG when presented with novel stimuli, and impairments in cognitive tasks characterized by high interference, such as reversal learning in the water maze, context discrimination and extinction of conditioned fear, without any deficiency in low-interference tasks, such as Morris water maze, fear conditioning to tone, and novel object recognition, suggesting specific impairments of pattern separation.
Conclusions: Our results provide empirical support for the computationally derived hypothesis that tonic inhibition of the dentate gyrus is important for pattern separation, provide a molecular target for this inhibition, and provide a mechanism for management of memory interference. Moreover, they suggest that increasing a5-mediated tonic inhibition might ameliorate interference-related cognitive symptoms in some psychiatric disorders, e.g., in schizophrenia, where impaired pattern separation due to dentate gyrus dysfunction has been reported.
Disclosure: Nothing to Disclose.

48.4 SST Neurons and Alpha5 GABA-A Receptors as Novel Targets for Antidepressants

Etienne Sibille

University of Toronto, Toronto, Canada

Background: Deficits in somatostatin (SST) and in SST-positve GABA neurons are common features in neurological

disorders with mood disturbances, but little is known about the contribution or cause of these deficits to mood symptoms.
Methods: The data presented were generated from a combination of postmortem molecular studies in brain samples from MDD and control human subjects, and from genetic and pharmacological studies in mice. All data were analyzed for significance by random intercept models with approapriate covariates in humans and by ANOVA in mice.
Results: Results from human postmortem brains demonstrate molecular changes affecting SST-positive GABA neurons in depression. The mouse genetic studies suggest that low SST and reduced SST-positive GABA neurons have causal roles in generating illness symptoms and are targets for novel antidepressant modalities. Specifically, we show that mice lacking Sst exhibit elevated behavioral emotionality, high basal plasma corticosterone and reduced gene expression that recapitulate behavioral, neuroendocrine and molecular features of human depression. Using laser-capture microdissection, we show that cortical SST-positive interneurons display greater transcriptome deregulations after chronic stress compared to pyramidal neurons. Protein translation through eukaryotic initiation factor 2 (EIF2) signaling, a pathway implicated in neurodegenerative diseases, was most affected and suppressed in stress-exposed SST neurons. We show that activating EIF2 signaling through EIF2 kinase inhibition mitigated stress-induced behavioral emotionality in mice. Finally, as the function of SST-positive GABA neurons is mediated by postsynaptic GABA-A receptors containing the alpha5 subunit, we show that boosting Alpha5-mediated GABA function (through positive allosteric modulation) has antidepressant activity in chronically stress mice.
Conclusions: The data presented suggest that (1) low SST plays a causal role in mood-related phenotypes, (2) deregulated EIF2-mediated protein translation may represent a mechanism for vulnerability of SST neurons, (3) global EIF2 signaling has antidepressant/anxiolytic potential, and (4) boosting postsynaptic SST-positive GABA neuron signaling has antidepressant/anxiolytic potential.
Disclosure: Nothing to Disclose.

Panel
49. New Technologies to Dissect the Serotonergic System

49.1 Mapping Molecular and Functional Subtypes of Serotonin Neurons

Susan Dymecki

Harvard Medical School, Boston, Massachusetts, United States

Background: Cellular heterogeneity within the serotonergic system is proving substantial and likely parallels the wide diversity of effects modulated by serotonin - from sensory processing to cognitive control and motivated behaviors to autonomic responses. Historically, anatomical topography has been used as one means to subdivide groups of serotonin neurons. First into the dorsal, median, and medullary raphe, then further into subnuclei (e.g. B7, B6, B4), and further into subgroups (e.g. dorsal, ventral, lateral wings). Here, genetic tools and findings will be presented that refine further the serotonergic structure-function map, superimposing onto topography new serotonin cell-subtype information. Specifically, our mapping resolution plots (1) developmental lineages of serotonergic neuron subtypes, (2) adult serotonergic cell groups based on unbiased clustering analyses of global gene expression profiles, (3) cellular electrophysiological properties measured in slice preparations and registered within the context of lineage and transcriptomic information, (4) efferent projections of the serotonergic neuron molecular subtypes including terminal and en passant bouton locations, and (5) specific organismal behaviors and physiological processes altered upon *in vivo*, neuron-subtype silencing.
Methods: We combine intersectional (dual recombinase) genetic fate mapping, manual neuron sorting, genome-wide RNA-seq, electrophysiological slice recordings, and neuron subtype-specific activity manipulations in mice to deconstruct the mouse serotonergic neuronal system at multiple levels of granularity.
Results: We reveal principles of serotonergic neuron organization and thousands of genes significantly differentially expressed across identified 5-HT neuron subtypes. Further, we show that these molecularly defined 5-HT neuron subtypes are also functionally distinct, using *in vitro* slice electrophysiology to measure intrinsic properties and drug responses, as well as *in vivo* subtype-specific synaptic suppression and DREADD (hM4Di)-mediated silencing. Our findings support the existence of functionally and molecularly distinct subtypes of serotonergic neurons, with results to date identifying specific kinds of serotonergic neurons related to breathing, chemoreception, thermoregulation, sensorimotor gating, aggression, and depression-related behaviors.
Conclusions: Findings propel forward concepts of functional modularity within the serotonergic neuronal system and the existence of molecularly distinct, disorder-related subpopulations. Findings may facilitate design of novel, targeted therapeutics for specific serotonergic-associated disorders.
Disclosure: Nothing to Disclose.

49.2 New Developments in the Role of 5-HT2A and Other 5-HT Receptors in the Treatment of Schizophrenia, Parkinson's Psychosis, and Related Disorders and the Pathophysiology of Those Disorders

Herbert Meltzer

Northwestern University, Chicago, Illinois, United States

Background: Drugs with potent serotonin (5-HT)2A receptor inverse agonism, e.g. clozapine, quetiapine, risperidone, lurasidone, and aripiprazole [atypical antipsychotic drugs (APDs)], are widely used to treat schizophrenia, bipolar disorder, major depression, Parkinson's psychosis, and other syndromes that do not respond to, or cannot tolerate, more specific therapies. Most, but not all approved atypical APDs, share more potent 5-HT2A inverse agonism than dopamine (DA) D2 receptor antagonism. However, these drugs also have various combinations of direct or indirect effects on other 5-HT receptors, including 5-HT1A, 5-HT7, and

5-HT2C receptors, one or more DA receptors, e.g. D2, D1 and D4 receptors, and various noradrenergic, cholinergic, GABAergic, glutamatergic and other receptors. This complexity adds to the difficulty of understanding the role of 5-HT2A inverse agonism in their mechanism of action as APDs, antidepressants, mood stabilizers, and cognitive enhancers, the latter being the most controversial of their clinical applications, and their influence on side effects, such as EPS and weight gain. The approval in May 2016 of pimavanserin, a highly selective 5-HT2A inverse agonist, for the treatment of Parkinson's psychosis, provides the opportunity for improving our understanding of the role of the 5-HT2A receptor, as well as other 5-HT receptors, in the action of multireceptor psychotropic drugs and their application to neuropsychiatric disorders.
Methods: We will report our own and others' behavioral, electrophysiologic, and microdialysis studies in rodents and clinical trials with monotherapy and augmentation strategies with various selective 5-HT2A inverse agonists, including SR43469B and pimavanserin, a 5-HT1A partial agonist (tandospirone), and a 5-HT7 antagonist (JNJ18038683) in schizophrenia, Parkinson's psychosis, or bipolar depression, or utilizing selective 5-HT ligands and multireceptor agents designed to elucidate the roles of 5-HT2A, 5-HT1A and 5-HT7 receptors, in psychosis, cognition, and depression.
Results: Clinical trial data supports the efficacy of 5-HT2A inverse agonists as monotherapy or a key factor in the antipsychotic or procognitive action of atypical APDs, and the basis for augmentation therapy strategies. Ongoing clinical studies with 5-HT1A partial agonists and 5-HT7 antagonists for cognition will be reported. We will also present data from pharmacogenomic studies to support the importance of the HTR2A in the pathophysiology of psychosis and the mechanism of action of atypical APDs.
Conclusions: These studies have enabled the partial deconvolution of the roles of 5-HT2A receptors, as well as 5-HT1A, 5-HT7, D1 and Ds receptors in pathophysiology and psychotropic drug action. This talk should facilitate the development of novel drugs based upon understanding the effects of targeting 5-HT2A and other 5-HT receptors, and enhance the clinical utilization of drugs that target those receptors.
Disclosure: Part 1: Acadia Pharmaceuticals, Inc., Shareholder, **Part 4:** Sunovion Pharmaceuticals, Inc., Grantee, Sumitomo Dainippon Pharma, Grantee, Eli Lilly, Grantee, Janssen Pharmaceutica, Grantee, Lundbeck, Grantee, Reviva Pharmaceuticals, Grantee, Takeda Pharmaceuticals, Grantee, Otsuka Pharma, Grantee, Forum Pharma, Grantee, Boehringer Ingelheim, Grantee.

49.3 Direct Conversion of Human Fibroblasts to Induced Serotonergic Neurons

Jian Feng

State University of New York at Buffalo, Buffalo, New York, United States

Background: Serotonergic (5HT) neurons exert diverse and widespread functions in the brain. Dysfunction of the serotonergic system gives rise to a variety of mental illnesses including depression, schizophrenia, anxiety, obsessive compulsive disorder, autism and eating disorders. The lack of patient-specific serotonergic neurons significantly hampers research on serotonin-related mental illnesses.
Methods: Based on existing knowledge on the development of serotonergic neurons in the mouse brain, we screened transcription factors for their ability to directly convert human fibroblasts to induced serotonergic (i5HT) neurons under various media conditions. A combination of immunostaining, electrochemical, electrophysiological and transplantation methods were used to characterize the i5HT neurons.
Results: We found that the optimal transcription factor combination for the direct conversion of primary human fibroblasts was Ascl1, Foxa2, Lmx1b and FEV. The transdifferentiation was enhanced by p53 knockdown and appropriate culture conditions including hypoxia (5% O2). The media additives critical for the conversion were ROCK inhibitor Y27632 (10 μM), SMAD inhibitors dorsomorphin (0.5 μM) and SB431542 (5 μM), CDK4/6 inhibitor PD0332991 (1 μM), BDNF (20 ng/ml) and GDNF (20 ng/ml). After 12 days of transdifferentiation, 49.2 ± 2.1% of total cells (DAPI+) were Tuj1+ neurons and 24.4 ± 0.9% of total cells were 5HT+ neurons. The i5HT neurons expressed markers for mature serotonergic neurons, had Calcium-dependent 5HT release and selective 5HT uptake, exhibited spontaneous action potentials and spontaneous excitatory postsynaptic currents. Application of serotonin significantly increased the firing rate of spontaneous action potentials, demonstrating the functional utility of i5HT neurons for studying serotonergic neurotransmission. Furthermore, i5HT neurons transplanted in rat brains produced extensive neuronal processes.
Conclusions: We have found a rapid and efficient method for the direct conversion of human fibroblasts to induced serotonergic (i5HT) neurons that are functional *in vitro* and *in vivo*. The ability to generate patients-specific i5HT neurons will significantly change research and drug discovery on many serotonin-related mental disorders.
Disclosure: Nothing to Disclose.

49.4 How Structural Insights Into Serotonin Receptors Transforms Design of Novel Therapeutics

Bryan Roth

University of North Carolina, Chapel Hill, North Carolina, United States

Background: Serotonin (5-hydroxytryptamine; 5-HT) receptors represent key molecular targets for many, if not most, neuropsychiatric diseases. Of the various 5-HT receptors, 5-HT2 family receptors represent important targets for atypical antipsychotic drugs, anti-obesity medications and atypical antidepressants. Over the past several years my lab has been investigating the molecular mechanism(s) by which drugs interact with 5-HT receptors (see for instance Wacker et al, Science 2013). Here I will provide new and unpublished data regarding 5-HT receptor structure, function and drug design.
Methods: We have solved the structure of a prototypical 5-HT2 family receptor with LSD and related ergoline and non-ergoline molecules (Wacker et al, submitted). High

resolutions crystal structures were solved using the lipidic-cubic-phase and various stabilizing fusion protein partners.
Results: Our findings have identified key motifs and residues essential for arrestin-biased signaling which we have validated via extensive mutagenesis and functional studies. With these results in hand we have been able to create novel 5-HT ligand-receptor pairs with novel functionalities which could ultimately be used for chemogenetic studies *in vivo* and for the design of novel drugs targeting 5-HT receptors.
Conclusions: We have successfully solved several new 5-HT receptor-ligand complexes including the structure of LSD in complex with a serotonin receptor. Our results have provided a potential approach for the structure-based design of novel ligands targeting 5-HT receptors.
Disclosure: Part 1: Pfizer, Consultant, Roche, Consultant, **Part 4:** Merck, Research Support, Asubio Pharmaceuticals, Research Support, Dainippon Sumitomo, Research Support.

Panel
50. Using Human Genetics GWAS and Expression Data to Drive Discovery

50.1 Gene Expression Elucidates Functional Impact of Polygenic Risk for Schizophrenia

Pamela Sklar

The Icahn School of Medicine at Mount Sinai, New York, New York, United States

Background: The CommonMind Consortium (CMC; www.synpase.org/CMC), a public-private partnership, is the largest existing collection of collaborating brain banks with over 1,150 samples generating data including regional gene expression, epigenomics (cell-type specific histone modifications and open chromatin), whole genome sequencing, and somatic mosaicism. We present results from RNA sequencing and ChipSeq demonstrating (a) specific effects on gene expression of SCZ genetic risk variants, (b) genes showing a significant difference in expression and (c) coordinated expression of genes implicated in SCZ.
Methods: DNA and RNA from postmortem human brain samples for SCZ or SCZA ($n = 258$), controls ($n = 279$), and affective disorder ($n = 55$) were extracted from the DLPFC (9/46) from Mount Sinai, University of Pittsburgh, and University of Pennsylvania. Genotyping was by Illumina HumanOmniExpressExome chip, with QC using PLINK. Libraries were constructed from rRNA depleted RNA and Illumina sequenced. The RAPiD pipeline used TopHat for alignment to hg19, with quantification using HTSeq and MISO. The expression matrix was normalized to log (counts per million) using voom, known covariates were adjusted using linear modeling, with voom-derived regression weights. eQTL were detected using MatrixEQTL. eQTL associations were compared using Sherlock to PGC2 SCZ GWAS results. Expression alteration in zebrafish, and in hiPSCs was performed to assess functional effects. Linear regression was used for SCZ differential expression analysis. Gene co-expression networks were constructed using WCGNA.
Results: We generated an eQTL map of 2,154,331 significant cis-eQTLs (FDR $\leq$ 5%) for 13,137 genes. Cis-eSNPs were enriched in genic elements and non-coding RNAs, depleted in intergenic regions and enriched for brain enhancer sequences (KS test: D = 1, $p =$ 4.5 x 10-6). In addition to containing the majority of literature eQTL found, the CMC sample finds a substantial number of genes with previously undetected eQTL. Of the 108 PGC2 SCZ GWAS loci previously reported, 73 harbor cis-eQTL SNPs for one or more genes (FDR $\leq$ 5%). We used Sherlock, a Bayesian approach that prioritizes consistency between disease association and eQTL signatures in GWAS loci, to identify genes likely to contribute to SCZ etiology. GWAS risk and eQTL association signals co-localized for about 20% of loci. Of the 19 GWAS loci with SCZ-associated cis-eQTLs, eight involved a single gene: furin (FURIN), t-SNARE domain containing 1 (TSNARE1), contactin 4 (CNTN4), voltage-sensitive chloride channel 3 (CLCN3), synaptosomal-associated protein of 91 kDa (SNAP91), ENSG00000259946, ENSG00000253553, and the ENST00000528555 isoform of sorting nexin 19 (SNX19) of which 5 are coding. Perturbing 3 of 5 loci, FURIN, TSNARE1 and CNTN4 resulted in altered brain phenotypes in zebrafish and/or hIPSCs. Differential gene expression suggests that approximately 44% of genes are perturbed in SCZ with modest fold changes (mean 1.09; range 1.03-1.33). Co-expression networks revealed 35 modules and only one module is associated with differentially expressed genes and prior genetic associations to SCZ and includes annotations for axon guidance, postsynaptic membrane, transmission across chemical synapses, and voltage-gated potassium channel complexes. Cell type specific ChipSeq data from H3K4m3 and H3K28ac from the same brain sample will be presented with estimates of neuronal specific marks for two brain regions.
Conclusions: By intersecting transcriptomics and genetics, we elucidated important aspects of the genetic control of transcription. This study paves the way for connecting genetic influences on cellular function with changes in macroscopic circuits of the brain that may ultimately lead to disease.
Disclosure: Part 1: Catalytic, Board of Directors.

50.2 Comprehensive Analysis of Gene Expression Reveals Pervasive Genetic Influence on Transcriptome Dysregulation Shared Across Major Psychiatric Disorders

Daniel Geschwind

University of California, Los Angeles, Los Angeles, California, United States

Background: With a few notable exceptions, research in the major mental disorders has not revealed consistent neurobiological findings across these conditions. Many potential reasons are often cited including, phenotypic heterogeneity and multiple comorbid illnesses, and small sample sizes. Genetic studies reveal a high heritability and recent genome-wide association (GWA) and exome sequencing studies have revealed exceptionally heterogeneous contributions from rare and common genetic factors. Many risk alleles lie in non-coding regions of the genome, indicating that gene regulation is likely to be a major mediator of disease. Thus, study of the transcriptome and comparisons across disorders

is likely to provide insight into shared and distinct pathophysiology.
Methods: We performed a combined analysis of available gene-expression microarray and RNAseq studies of cerebral cortex across five major neuropsychiatric disorders and inflammatory bowel disease (IBD, $n = 67$) as a non-neural comparison, comprising more than 1000 post mortem brain samples. We used strict quality control and normalization procedures including re-balancing to remove any confound between diagnosis and biological or technical covariates. Probes were re-annotated using Ensembl v75, and experimental batch effects were corrected both within and between studies. A random-effects meta-analytic approach (restricted maximum-likelihood estimates) was taken to compute disease-specific differential gene expression (DGE) signatures for each disease. Whole genome co-expression analysis (WGCNA) was used to identify disorder related modules. MAGMA was used to assess genetic risk enrichment within speciific modules, and LD regression to partition common variant based heritability.
Results: We identify a robust pattern of shared and distinct gene-expression perturbations across these conditions, including neuronal gene co-expression modules that are downregulated in ASD, SCZ, and BD. We find that ASD, BD and SCZ also share up-regulation of an astrocyte related module, but that microglial up-regulation is significantly more prominent in ASD. IBD also shares up-regulation of some of the inflammatory, microglial component observed in ASD. By comparing the degree of transcriptome sharing with common polygenic risk, we find that the degree of sharing of transcriptional dysregulation is highly correlated (Spearman rho = 0.7) with the polygenic overlap across disorders, indicating a significant genetic component to these transcriptomic alterations, with the exception of IBD, which shares no genetic risk with ASD. This is consistent with this IBD-ASD inflammatory overlap having different pathophysiological origins, which is in contrast to the sharing across the major psychiatric disorders. We also are able to identify modules related to neuronal function that are enriched in both rare and common genetic variation using both MAGMA and LD regression.
Conclusions: By carefully controlling for confounding factors, including the effects of antipsychotic drugs, and balancing cases and controls, we are able to find consistent patterns across microarray and RNAseq studies of major psychiatric disorders. These findings provide a systems-level view of the neurobiological architecture of major neuropsychiatric illness and demonstrate pathways of molecular convergence and specificity.
Disclosure: Nothing to Disclose.

50.3 Coexpression Network Analyses Identify Regulatory Systems Associated With Psychiatric Disorders

Chunyu Liu

University of Illinois at Chicago, Chicago, Illinois, United States

Background: Schizophrenia and bipolar disorder are complex mental disorders, with risks contributed by multiple genes. Recently, genome-wide systemic approaches have been used to reveal the associations of hundreds of SNPs with these disorders. These findings implicate dysregulation of gene expression in disease etiology, but little is known about such regulation systems in the human brain.
Methods: By integrating gene expression data of mRNA and micro-RNA (miRNA) obtained from 51 cases and 24 controls using the weighted gene co-expression network analysis (WGCNA), we built mRNA-miRNA co-expression networks and tested co-expression modules for differential expression in patients with schizophrenia and bipolar disorder relative to controls. In the disorder-associated modules, we identified potential regulators, including transcription factors (TFs) and miRNAs, and their co-expressed protein-coding genes. In silico predicted binding relationships were used to validate the putative regulations suggested by co-expression patterns. We used SNP genotype data to resolve some causal relationships among the regulators and their targets. We further validated the predicted regulations experimentally using RNA interference knockdown.
Results: We identified a module that is differentially-expressed between cases and controls. This module contained five miRNAs and 501 mRNA genes. Six TFs were found to be hub genes in these modules. The regulatory relationships suggested by co-expression were consistent with the binding relationships predicted by databases. Focusing on regulatory relationships involving TFs and miRNAs, we resolved a regulation cascade from SNP variants (rs16853832) to TF (POU2F1) to miRNA (hsa-mir-320e) to target genes (NR2E1) and ultimately, to disease risks.
Conclusions: This study showed that we can utilize multi-dimensional data to construct co-expression networks that are enriched for regulatory relationships. Causal relationships can be resolved among SNPs, regulatory molecules and their downstream target genes through data integration. Novel genes and their corresponding regulations underlying the disease risks could be revealed.
Disclosure: Nothing to Disclose.

50.4 Data Integration for Discovery of Novel Therapies for Neuropsychiatric Disorders

Nancy Cox

Vanderbilt University, Nashville, Tennessee, United States

Background: While genome-wide association studies (GWAS) have provided many highly significant and reproducible associations of DNA variants with common human diseases, it has been more difficult to understand the underlying biology of the signals. We have therefore focused on developing methods for analysis that integrate functional information directly into the association test.
Methods: We use GTEx data (RNA-Seq on up to 50 tissues and genome variation from genotyping arrays and now whole genome sequencing) to build SNP-based predictors of gene expression within and across 44 human tissues for a method we call PrediXcan, for predicted expression scanning (Gamazon et al, Nat Genet 2015). We then test that predicted expression phenotype for association with disease or quantitative phenotypes, yielding a gene-based test that is

mechanistic by design, and has an easy-to-interpret direction of effect. We have applied PrediXcan to BioVU, the biobank at Vanderbilt University with more than 215,000 subjects linked to de-identified electronic health records going back an average of 10-15 years. These studies were conducted on 20,000 subjects with GWAS data (~18,000 after QC).
Results: We find that transcriptome deviance – a tally of the number of genes for which a person has genetically predicted expression +/- 3 SD from the BioVU population mean – is significantly correlated with disease burden – a tally of the number of phenome codes a person has accumulated. In addition, we have been able to validate using model systems a number of the initial discoveries (based on only 5000 BioVU subjects) made. We observe a remarkable association between reduced genetically predicted expression of Mendelian disease genes and the subphenotypes that make up that Mendelian disease. For a number of Mendelian diseases in which children are reported to exhibit behavioral problems, reduced predicted expression of the Mendelian disease gene is highly significantly associated with neuropsychiatric diagnoses. We highlight examples, such as for the gene SLC39A4, in which innocuous therapies, such as vitamin or mineral supplementation might reduce risk of neuropsychiatric disorders in those expected to be vulnerable based on common variant predicted expression of Mendelian disease genes. We will also describe preliminary results suggesting that genes and diseases are aggregating on a number of major axes. Schizophrenia, for example, is among the diseases, also including kidney disease and kidney failure, that appear to aggregate on an axis we characterize as innate immunity/wound healing based on the number of genes driving the axis that have been reported to be "switches" for this biology.
Conclusions: Our observations on the continuum between Mendelian and common disease and on the axes on which genes and diseases are aggregating are possible only for data in which the medical phenome can be studied in its entirety, and offer intriguing possibilities for biomarker and drug development that could impact multiple diseases simultaneously.
Disclosure: Nothing to Disclose.

Mini Panel
51. Cerebellar Circuits in Schizophrenia and Autism: Cognitive, Social and Affective Dysmetria

51.1 Identification of a Subdivision of the Lateral Nucleus of the Cerebellum That Regulates Cognitive, Social and Affective Behaviors

Erik Carlson

University of Washington, Seattle, Washington, United States

Background: The cerebellum is well known for its role in coordinating motor output and adapting involuntary reflexes to support sensory prediction-error based learning. A lesser appreciated function is its role in voluntary, cognitive, social, and affective functions. Brain imaging in humans has identified a region of the dentate nucleus of the cerebellum (DNC), or lateral nucleus in rodents (LNC) that is activated during performance of cognitive tasks involving complex spatial and sequential planning. Numerous neuropsychiatric disorders are associated with alterations in cerebellar function, including schizophrenia and autism. In addition to the cerebellum as a whole, several of these disorders are associated with alterations in DNC anatomy and gene expression, and cognitive and behavioral perturbations occur in the absence of gross motor impairments. Thus, there is a strong desire to understand the relationship between cerebellar anatomy and function and the symptom domains of these disorders. Dopamine and dopamine receptors are broadly implicated in mental illness and localize to microanatomical regions of the cerebellum. These observations suggest that dopamine receptors may identify subdivisions within the LNC that would allow for manipulation and interrogation of LNC function.
Methods: To test this hypothesis, we targeted conditional viral expression to the LNC of mice expressing Cre recombinase (Cre) under the control of the dopamine D1 receptor (D1R) locus (Drd1-Cre). We then utilized molecular profiling, intra-cerebellar projection analysis, and electrophysiological characterization of neurons expressing D1R to identify their anatomical distribution and neurochemical profile. We then utilized chemogenetic silencing of these neurons with the Designer Receptor Exclusively Activated by a Designer Drug (DREADD) receptor system and measured performance of mice in spatial memory, interval timing, social recognition memory, and affective behaviors.
Results: Cell-specific expression of GFP was achieved by injecting a conditional adeno-associated viral vector into the LCN of mice expressing Drd1-Cre. Labeled neurons localized to caudal, medial, and ventral zones of the LNC. Whole-cell patch clamp recordings on fluorescently identified neurons from acute cerebellar slices revealed action potential waveforms similar to established properties of local glycinergic/GABAergic neurons and nucleocortical projecting glycinergic neurons. Translational profiling after isolation of polyribosomal-associated mRNA from D1R+ cells indicated gene expression consistent with a heterogeneous group of cells, largely inhibitory, but also including a small population of glutamatergic cells. Analysis of nucleocortical projections confirmed both rosette-like and bead-like synapses consistent with both excitatory and inhibitory synapses arising from D1R+ neurons. To determine whether these neurons differentially influence motor and cognitive operations in mice, we selectively silenced D1R+ LNC neurons through conditional viral-mediated expression of the inhibitory DREADD, HM4Di (D1R:HM4), and application of the DREADD-specific ligand clozapine-N-oxide. No differences were seen between groups on measures of coordination, stance and stride. On a test for spatial navigation memory, D1R:HM4 mice performed significantly worse than controls. On an operant paradigm of duration-based timing, which reinforces a subject's ability to refrain from responding for a set time period, latencies of non-burst responses were significantly different between groups. D1R:HM4 mice had responses which indicated overestimates of interval timing. We examined social interaction and preference using a three-chamber assay. D1R:HM4 mice failed to discriminate between novel and familiar mice in this task. On the elevated plus maze, D1R:HM4 mice spent significantly less time in the

open arms relative to controls, indicating increased anxiety-like behavior.
Conclusions: We identified a distinct region of the LNC containing a population of D1R+ neurons. The profile of these neurons is consistent with a heterogeneous population of glutamatergic and GABAergic/glycinergic cell types. Specific inhibition of D1R-LNC neurons impairs spatial memory, interval timing, social preference, and alters affective state, thus, demonstrating a functional link between the LNC in cognitive, social and affective behaviors.
Disclosure: Nothing to Disclose.

51.2 Cerebellar Stimulation Can Compensate for Frontal Dysfunction

Krystal Parker

University of Iowa, Iowa City, Iowa, United States

Background: The cerebellum orchestrates fluid, coordinated sequences of thought required for normal cognition. Cognitive impairments are a hallmark of neuropsychiatric illnesses such as schizophrenia. Recently, cerebellar stimulation was shown to alleviate some debilitating and currently untreated cognitive symptoms of schizophrenia. However, the precise cerebellar contribution to cognition is unknown. One mechanism for cognitive impairment in schizophrenia involves aberrant dopamine neurotransmission in the medial frontal cortex. We study cognition in humans with schizophrenia and rodents with disrupted frontal D1 dopamine using an interval timing task. Timing is an essential biological process that serves as a window into cognitive function as it requires executive processes such as working memory, attention, and planning. Here we investigate novel strategies to normalize frontal brain regions by manipulating cerebellar projections.
Methods: Interval timing is a task that requires subjects to make a motor indication of the passage of a specified duration of time following a cue that indicates trial start. The task can be modified to use in rodents yet it remains comparable to humans. In humans with schizophrenia, we combine EEG during performance on the interval timing task and we investigate the influence of pre/post low-frequency cerebellar transcranial magnetic stimulation on frontal cortical activities. In rodents performing the interval timing task we combine neuronal ensemble recordings of the frontal cortex and cerebellum to elucidate the cerebellar and frontal contributions to timing. We then model frontal dopamine disruption using focal infusions of D1 dopamine blocker SCH23390 and we investigate how frontal dysfunction influences timing and cortical rhythms during the timing task. Finally, we stimulate the cerebellum two ways: optogenetically using Channelrhodopsin2 and pharmacologically using GABA antagonist, gabazine.
Results: We report four novel findings. First, patients with schizophrenia and our animal model with disrupted frontal D1 dopamine have dysfunctional delta rhythms in medial frontal cortex. Second, simultaneously recording neuronal ensembles in rodent medial frontal cortex and lateral (dentate) cerebellar nuclei indicate strong modulation during timing tasks. Third, frontal and cerebellar neurons have extensive interactions, and spectral causality analyses revealed strong low-frequency interactions at ~1-4Hz. Further analysis with Granger causality indicates that the cerebellum leads activity in the frontal cortex at low frequencies providing a mechanism for the cerebellum to influence the frontal cortex. Finally, 2Hz optogenetic stimulation of cerebellar-thalamic terminals rescued cognitive performance as well as frontal activity in a rodent frontal dysfunction. We are currently enrolling patients investigate how cerebellar stimulation influences frontal rhythms and cognition in patients with schizophrenia.
Conclusions: Our data are a demonstration of cerebellar compensatory mechanisms and one way in which the cerebellum contributes to cognition. Specifically, we show that the cerebellum can modulate cognitive processing in the medial frontal cortex, and that cerebellar stimulation can reinstate frontal rhythms necessary for timing. Our ability to safely and specifically target the cerebellum *in vivo* demonstrates that there is therapeutic relevance for cerebellar transcranial stimulation in many diseases involving impaired cognition and frontal abnormalities.
Disclosure: Nothing to Disclose.

51.3 Cerebellar Circuits and Sensitive Periods for Treatment of ASD-Relevant Behaviors

Peter Tsai

University of Texas Southwestern Medical Center, Dallas, Texas, United States

Background: Autism is a prevalent neurodevelopmental disorder with recent studies estimated prevalance to be 1:68 in the United States. However, despite this high prevalence, the underlying mechanisms remain poorly understood. Cerebellar dysfunction has been increasingly implicated in the pathogenesis of the disorder with pathology, imaging, and clinical studies demonstrating association of autism with cerebellar abnormalities, lesions, and abnormal function. Using a mouse model of Tuberous Sclerosis Complex, a neurodevelopmental disorder associated with high rates of autism, my lab has recently demonstrated that cerebellar dysfunction is sufficient to generate autism-relevant behaviors. Moreover, we have demonstrated that early treatment with the mechanistic target of rapamycin (mTOR) inhibitor, rapamycin, can prevent the development of these abnormal behaviors as well as anatomic and electrophysiologic dysfunction in mutant Purkinje neurons. Here, we sought to better delineate sensitive periods of treatment efficacy while seeking to better understand the underlying mechanisms that might mediate these developmental time windows.
Methods: Using rapamycin, we examined whether delayed treatment with rapamycin would prove beneficial for behaviors and pathology and to better understand mechanisms that code for these benefits through a combination of pharmacologic treatment, rodent imaging and structural connectivity, behavioral testing, electrophysiology, and cellular imaging.
Results: We demonstrate that adult treatment with rapamycin is sufficient to rescue abnormal social phenotypes in our TSC mutant mouse model while it is unable to rescue repetitive and inflexibility behaviors. To better understand mechanism underlying these divergent sensitive periods, we

evaluated cellular, anatomic, and circuit connectivity and identified mechanisms that may mediate both rescue and inability to rescue the autism-relevant behavioral dysfunction.
Conclusions: These results demonstrate the possible benefits of adult onset therapy for autism related behaviors but also demonstrate mechanisms underlying behavioral rescue, findings which may yield methodologies for development of targeted therapeutics for treatment of autism-related behaviors.
Disclosure: Nothing to Disclose.

Mini Panel
52. Potential of Epigenetic Drugs for Addition Treatment

52.1 MicroRNAs in Cocaine Addiction

Paul Kenny

The Icahn School of Medicine at Mount Sinai, New York, New York, United States

Background: MicroRNAs (miRNAs) are small (~22 nucleotides) RNA transcripts that do not code for protein but instead serve to repress target gene translation. We have found that disruption of the microRNA biogenic machinery in striatum decreases cocaine intake in mice, supporting a key role for striatal microRNAs in regulating the reinforcing properties of cocaine.
Two closely related microRNAs, miR-212 and miR-132, are induced in the striatum of rats demonstrating compulsive-like cocaine-taking behaviors. By contrast, our recent data suggests that miR-132 may enhance the motivational properties of cocaine through, as yet, unclear mechanisms. We hypothesize that the balance between miR-212 and miR-132 signaling influences resilience vs. vulnerability to addiction. That miR-132 should increase the motivational properties of cocaine is unexpected. It shares an identical seed sequence with miR-212, considered critical for determining target specification. As such, miR-132 and miR-212 are expected to share identical functions. Currently, we are investigating these possible mechanisms of differential miR/132/212 action.
Methods: We have successfully constructed mice with loxP sites flanking the miR-212 stem loop (miR-212 floxed) or the miR-132 stem loop (miR-132 floxed), allowing miR-212 or miR-132 to be conditionally deleted in cells expressing Cre recombinase. miR-212fl/+ and miR-132fl/+ mice were crossed with a Cre deleter strain to create a miR-212 and miR-132 constitutive knockout (KO) mice. No gross physical differences are observed in heterozygous or knockout mice. The effects of cocaine on behavior in these two lines of KO mice are currently being assessed. Also, we are conditionally deleting miR-212 or miR-132 in D1 receptor-expressing medium spiny neurons (D1-MSNs) or in D2-MSNs. The effects of these cell-specific microRNA lesions on cocaine self-administration, and other addiction-relevant behaviors, will be being investigated.
Results: To determine if miRNAs in striatum may regulate the motivational effects of cocaine, we deleted Ago2 throughout in D2-MSNs. This was achieved by breeding Agofl/fl mice with mice expressing Cre under the control of the D2 receptor promoter (Drd2-Cre::Agofl/fl mice). We found that cocaine reward was abolished in Drd2-Cre::Agofl/fl mice, as measured by place conditioning. In addition, the mutant animals intravenously (IV) self-administered far less cocaine than control mice. To identify specific miRNAs in striatum may regulate cocaine intake we assessed miRNA expression profiles in rats with a history of extended or restricted access to IV cocaine self-administration. Two miRNAs, miR-212 and miR-132, were found to be induced in striatum of extended access rats relative to control groups. We found that overexpression of miR-212 markedly decreased cocaine intake in rats whereas disruption of miR-212 signaling in striatum, accomplished using an antisense oligonucleotide, increased cocaine intake in rats. Most recently, we found that virus-mediated overexpression of miR-132 in striatum increases cocaine intake in rats, opposite to the inhibitory effects of miR-212. As miR-212 and miR-132 share the same seed sequence, this is an unexpected finding. Considering that D1-MSNs and D2-MSNs exert opposite effects on reward-relevant behavioral processes, it is possible that miR-212 and miR-132 may regulate cocaine intake in an opposite manner by acting preferentially in these different MSN types. Alternatively, both miRNAs could act in the same cell population but exert opposite effects of gene/s that control cocaine intake. To investigate the mechanisms by which miR-212 and miR-132 can exert opposite effects of cocaine-taking behaviors, we generated mice in which these miRNAs can be constitutively or conditionally deleted. We are testing the effects of cocaine in newly created miR-132 and miR-212 KO mice. We have found that miR-132 (but not miR-212) KO mice should a marked anxiogenic response to cocaine, as reflected by decreased time spent rearing and on the open portions of an open arena. Currently, we are generating mice in which miR-212 or miR-132 is conditionally deleted only in D1-MSNs of D2-MSNs.
Conclusions: These findings establish key roles of miRNAs in regulating cocaine intake, but reveal unexpected complexity in the actions of miRNAs. Understanding of the mechanisms by which miRNAs act in striatum to control cocaine intake may reveal novel targets for medications development.
Disclosure: Part 1: Eolas Therapeutics, Inc., Ownership.

52.2 Methamphetamine-Associated Memory is Regulated by a Writer and an Eraser of Permissive Histone Methylation

Courtney Miller

The Scripps Research Institute, Jupiter, Florida, United States

Background: Memories associated with drugs of abuse, such as methamphetamine (METH), increase relapse vulnerability to substance use disorder by triggering craving. The nucleus accumbens (NAc) is essential to these drug-associated memories, but underlying mechanisms are poorly understood. Posttranslational chromatin modifications, such as histone methylation, modulate gene transcription, thus we

investigated the role of the associated epigenetic modifiers in METH-associated memory.
Methods: Conditioned place preference was used to assess the epigenetic landscape in the NAc supporting METH-associated memory ($n = 79$). The impact of histone methylation (H3K4me2/3) on the formation and expression of METH-associated memory was determined by focal, intra-NAc knockdown (KD) of a writer, the methyltransferase MLL1 ($n = 26$), and an eraser, the histone demethylase KDM5C ($n = 38$), of H3K4me2/3.
Results: A survey of chromatin modifications in the NAc of animals forming a METH-associated memory revealed the global induction of several modifications associated with active transcription. This correlated with a pattern of gene activation, as revealed by microarray analysis, including upregulation of Oxtr and Fos, whose promoters also had increased H3K4me3. KD of Mll1 reduced H3K4me3, Fos and Oxtr levels and disrupted METH-associated memory. KD of Kdm5c resulted in hypermethylation of H3K4 and prevented the expression of METH-associated memory.
Conclusions: The development and expression of METH-associated memory are supported by regulation of H3K4me2/3 levels by MLL1 and KDM5C, respectively, in the NAc. These data indicate that permissive histone methylation, and the associated epigenetic writers and erasers, represent potential targets for the treatment of substance abuse relapse, a psychiatric condition perpetuated by unwanted associative memories.
Disclosure: Nothing to Disclose.

52.3 Histone Acetyl-Lysine Reader Proteins Mediate Neurobehavioral Adaptations to Psychostimulants

Gregory Sartor

University of Miami, Miami, Florida, United States

Background: Epigenetic-based therapies are emerging as potential treatments for several psychiatric disorders, including drug addiction. In particular, the BET family of histone acetyl-lysine reader proteins (BRD2, BRD3 and BRD4) represents a novel, druggable class of epigenetic targets. BET proteins bind to acetylated histones and recruit protein complexes involved in transcriptional activation, elongation and super-enhancer activity. Because BET proteins have been implicated in the pathophysiology of several disorders, multiple companies have developed selective, small molecule BET inhibitors that are currently being tested in clinical trials. Given that histone acetylation mechanisms are importantly involved in drug-induced neuroadaptations, we hypothesized that BET proteins may also play a vital role in addiction-related phenomena.
Methods: Using the conditioned place preference (CPP) procedure, male C57BL/6 mice were injected with cocaine (15 mg/kg), amphetamine (3 mg/kg), nicotine (0.5 mg/kg) or morphine (5-10 mg/kg) and confined to one side of the chamber by a solid divider for 30 minutes, or injected with saline and restricted to the other side of the chamber for 30 minutes. During the 3 conditioning days, injections were administered in a balanced fashion in morning and afternoon sessions (at least 4 h apart). The BET bromodomain inhibitor, JQ1 (10, 25, or 50 mg/kg, i.p.), or vehicle was administered before each conditioning session. As a control, the inactive enantiomer of JQ1 (-JQ1, 50 mg/kg) or iBET-151 (a BET inhibitor that does not cross the blood brain barrier, 50 mg/kg) was injected prior to conditioning in a different set of mice. Other animals received intra-accumbal injections of JQ1 or AAV5-BRD4-shRNA prior to conditioning. In additional behavioral studies, JQ1 was administered during acquisition of LiCl-induced conditioned place aversion (CPA) or contextual fear conditioning to determine the effects of JQ1 on other learning and memory procedures. In molecular studies, we measured BRD2, BRD3, BRD4 and phospho-BRD4 protein levels and BRD4 binding to specific promoter regions (ChIP-qPCR) in the nucleus accumbens (NAc) following repeated exposure to drugs of abuse. In additional mice, JQ1 or vehicle was administered prior to an injection of cocaine, amphetamine, nicotine or morphine and addiction-related genes were measured in the NAc, prefrontal cortex and dorsal striatum.
Results: JQ1 significantly reduced behavioral responses to cocaine, amphetamine and nicotine but not morphine. JQ1 did not attenuate LiCl-induced conditioned place aversion or contextual fear conditioning, indicating that BET inhibition does not affect all types of contextual learning. Repeated exposure to psychostimulants increased BRD4, but not BRD2 or BRD3, proteins levels. Psychostimulants increased BRD4 binding to addiction-related genes in the NAc, and JQ1 attenuated psychostimulant-induced gene expression in multiple brain regions.
Conclusions: Together, these studies indicate that the displacement of BET proteins from chromatin may have therapeutic efficacy in psychostimulant addiction.
Disclosure: Nothing to Disclose.

Panel
53. Computational Approaches to Behavior Analysis in Psychiatry

53.1 A Computational Linguistics Approach for Prodromal Psychosis

Guillermo Cecchi

IBM Research, Yorktown Heights, New York, United States

Background: We recently presented a pilot study [Bedi et al, NPJ Schizophrenia, 2015] showing that it is possible to identify linguistic features in clinical high risk (CHR) patients that are strongly predictive of conversion to psychosis (CHR+). These features are: (a) the formalization of the concept of 'flight of ideas' using tools from distributed semantics, specifically a measure we call semantic coherence, and (b) the formalization of the concept of 'poverty of speech' using tools from computational probabilistic parsing to measure morpho-syntactic complexity, specifically length of phrases and density of interrogative word determiners ("what", "which"). Applied to transcripts from a cohort of 34 CHR patients who had a baseline open-ended (OE) interview during which they were asked to talk about themselves (~ 1hr), a machine learning model predicted with 100% accuracy the 5 CHR+ patients, validated statistically with multiple approaches.

Methods: In collaboration with Carrie Bearden, we conducted a validation/follow-up study using existing retrospective data from previously published work. The experimental setting consisted of a semi-structured baseline interview following the Story Game (SG) protocol [Caplan et al, 1989; Bearden et al, 2011], during which participants had to listen to a story, retell it, and then were asked questions about the narrative. A cohort of 59 CHR patients, of which 19 later converted to psychosis, and 21 healthy age-similar controls, was analyzed using a similar set of linguistic features as in the previous study, encompassing semantic coherence and morpho-syntactic complexity measures.
Results: A machine learning ensemble model predicts conversion with a rate of 0.84 for True Positives and 0.17 for False Positives using leave-two-out cross-validation (L2OCV), dropping to 0.81 and 0.18 respectively for L4OCV and L6OCV. This classifier model, applied verbatim to the same linguistic features extracted from the OE data, achieves an accuracy of 0.6 True Positives (3 out of 5, $p<0.03$) and 0.11 False Positives. A simple Logistic Regression (LR) classifier trained on the entire SG dataset achieves an accuracy of AUC = 0.88. Moreover, the LR model applied verbatim to the same linguistic features extracted from the OE data, achieves an accuracy of AUC = 0.71.
Conclusions: The high predictive accuracy of the semantic and morpho-syntactic markers on the OE and SG data are a strong indication that psychosis outcome can be predicted computationally, which can lead to high-frequency and remote evaluations that may include voice, facial expressions and written text. The significant predictive value on the OE data of a model learned on SG, in a very different experimental setting (e.g. low vs. high interviewer intervention), furthermore, supports the idea of a speech- and behavior-based naturalistic assessment of mental states that may extend to many other psychiatric and neurologic conditions.
Disclosure: Nothing to Disclose.

53.2 Computational Analysis of the Face and Speech in Acute Psychosis and Mania

Justin Baker

Harvard Medical School, Belmont, Massachusetts, United States

Background: Computational approaches to measuring naturalistic behavior in clinical settings could provide an objective backstop for mental health assessment and disease monitoring, both of which are costly and unreliable using traditional, mostly subjective methods. In a typical clinical encounter, providers extract information about a patient's mental status from a brief interview as the basis for both record keeping and decision making. We reasoned that if an expert human can extract this information from the encounter, we could in principle design a system to detect similar (or at least highly correlated) information by (1) obtaining high quality video and audio from each participant in the encounter, (2) computing relevant features from each data stream, and (3) training classifiers to map between behavioral features and psychiatric attributes, based on a standardized clinical assessment.
Methods: Patients were recruited from an inpatient psychiatric unit where they were hospitalized for exacerbations of affective or non-affective psychosis, and carried diagnoses of schizophrenia, bipolar disorder, and related conditions. A total of 18 patients participated in 24 interviews lasting 5-15 minutes, which were comprised of two epochs: (1) patient-alone (~2 min), and (2) a 13-question, semi-structured interview performed by a psychiatrist, modeled after the "clinical rounds" assessment. For each participant, synchronized audio and video data were acquired from a 1080p webcam focused on the face and upper torso and a cardioid headset microphone. A standardized clinical assessment, including PANSS, MADRS, and BPRS, was performed after each recorded interview. Feature-level data, including facial action units (AUs), gaze, and speech characteristics (e.g., prosody, pitch, tone, texture) were computed automatically using in-house and publicly available software. To predict clinical scales, we trained a linear kernel support vector regressor (SVR) using features from both the entire session (i.e. global mean) and each experimental epoch (e.g., means during time spent alone and each individual question), leading to 15 predictors for each clinical scale item and scale totals. We used leave-one-out cross-validation on the training data (maximizing the Pearson correlation coefficient) to determine the C parameter for the SVR models; for testing, we used leave-one-subject-out cross-validation (i.e. leaving 17 participants for training/validation in each fold).
Results: Providing evidence of our approach's ability to capture and quantify psychopathology that is clearly visible to clinicians, we found that parameters such as brow furrowing (AU4, R = 0.744) and eye widening (AU5, R = -0.601) were correlated with depression measures on the BPRS. In many cases, these effects were specific to the question or experimental epoch. For instance, unusual thought content was most evident in increased frequency of brow flashes (AU2, R = 0.752) and greater smile variability (R = 0.656) that occurred while participants were alone in the room. Individuals with higher ratings of delusions also showed increased brow flashes in response to a question about their self-confidence (R = 0.739). Many relationships showed a "dose effect," with midrange scores corresponding with moderate psychopathology.
Conclusions: Our experiments show that automatically detected facial action units and speech properties can be used to assess a number of psychiatric symptoms across domains of psychopathology, including both mood and psychosis. We demonstrate the importance of analyzing behaviors in the appropriate context (i.e., while participants are alone or prompted with a specific question) in order to optimally extract clinically relevant information from objective behavioral indices. Thus, quantitative assessment of behavior in naturalistic settings is both feasible and informative as an adjunct to traditional methods of mental status assessment.
Disclosure: Nothing to Disclose.

53.3 Predicting Treatment Outcome in Social Anxiety Disorder and Tracking Major Depression and Parkinson State Using Behavioral Information

Satrajit Ghosh

Massachusetts Institute of Technology, Cambridge, Massachusetts, United States

Background: Our current methods for assessment, treatment, and monitoring of mental health rely extensively on human resources, including expertise, objectivity, and bandwidth. While brain imaging offers critical insight into cognition in health and disease and can evaluate treatment choices (Doehrmann et al, 2013; Whitfield-Gabrieli et al, 2015), it is currently infeasible from a practical perspective to use it for continuous monitoring. Therefore, there is a need to develop scalable information gathering and processing tools that can support, augment, and extend clinical practice towards individualized solutions. One complex motor behavior that engages numerous cognitive and physiological processes is voice, which can serve together with cognitive and clinical assessments as sensors into mental health. This work focuses on the feasibility of machine learning tools to estimate treatment performance or to track mental health state from such voice and behavioral data and introduces an open source, mobile platform to collect and monitor longitudinal data.
Methods: Behavioral data from studies of social anxiety disorder (SAD; $N=126$), major depressive disorder (MDD; $N=55$), and Parkinson disease (PD; $N=940$) were processed using state of the art machine learning tools and neurocomputational models. Clinical, demographic, and cognitive assessment data in individuals with SAD (Leibowitz Social Anxiety Scale - LSAS $>$ 60) prior to cognitive behavioral therapy was used train and cross-validate an Extra Trees algorithm to predict changes in LSAS following treatment. For MDD and PD, features extracted from audio recordings of individual speech were used to train a classifier that predicted either the Beck Depression Inventory-II score (in case of MDD) and the Unified Parkinson's Disease Rating Scale score (in case of PD). Based on the requirements of such algorithms and research studies, an open source, mobile platform was developed to provide a framework for continuous data collection, analysis, and prediction.
Results: The LSAS classifier was able to explain over 30% of the variance in treatment outcome (post cross-validation). This is comparable to performance from task fMRI data (Doehrmann et al, 2013) using emotional faces and neutral scenes. For MDD and PD, features extracted from voice data were able to explain 17% and 13% of the variance respectively (median performance using bootstrapped cross-validation). The VoiceUp mobile platform allows investigators to design their own studies that can be delivered as apps through the Google and Apple stores on mobile devices. The data collected from such studies can be used to test, validate, and improve these prediction models.
Conclusions: A doctor has a finite set of experiences to draw from, is subject to the same cognitive fallacies in assessment and decision making as any human, and often must synthesize information from many specialists well outside of the doctor's expertise in order to make a recommendation. The clinical decision can be supported with informatics solutions. Today's smartphone technologies allow a scalable approach to personal data collection, include voice, gait, and video. This data can be combined with machine learning tools and life outcomes to build models that track and determine individual-specific treatment options for mental health disorders.
Disclosure: Nothing to Disclose.

53.4 New Computational Methods for Childhood PTSD Risk Factor Research

Glenn Saxe

New York University School of Medicine, New York, New York, United States

Background: This presentation details the application of algorithms related to Complex Systems Science/Network Science, Causal Discovery, and Machine Learning Predictive Analytics and Intervention Modeling to understand the emergence and sustenance of Posttraumatic Stress Disorder (PTSD) in acutely traumatized children. There is a great need to develop new computational approaches to understand risk for PTSD given its complex etiology. Our application of these approaches is dedicated to identify children who are at highest risk for PTSD and to identify promising prevention and treatment targets. The research that will be presented examines risk and intervention targets with a longitudinal data set on acutely injured children.
Methods: The data set is comprised of information on 163 children aged 7-18 collected as part of a National Institute of Mental Health funded study (R01 MH063247) on risk factors for PTSD in children hospitalized with injuries. The basic design follows: injured children were assessed within hours or days after their hospitalization and reassessed 3 months and 1 year following discharge. The data set includes variables measured during the hospitalization period and at each follow-up and includes domains such as early childhood development, demographics, school and social function, family stress, parent symptoms and functioning, psychosocial stress, qualities and magnitude of injury, candidate genes, neuroendocrine response, psychophysiologic response, and child symptoms and functioning. PTSD was measured with the UCLA PTSD Reaction Index.
We apply a unique computational approach called the Complex Systems-Causal Network (CS-CN) method designed to discover sets of variables related to psychiatric disorders that together possess well-known properties of Complex Adaptive Systems (e.g. efficiency of information transfer, modularity, power-law scaling, robustness) and, if such properties are demonstrated, the variables that disproportionally contribute to the systems robust qualities are determined. We then apply Machine Learning Predictive Analytics with Causal Discovery Feature Selection and Intervention Modeling (Pearl's 'Do Calculus') to determine if PTSD can be predicted from variables measured around the time of the trauma, if any of the predictive variables have causal influence on the development of PTSD, and the effect on PTSD if intervention is modeled on any of the discovered causal variables.
Results: The CS-CN method revealed a network of 110 variables and 166 bivariate relations that had strong adaptive

properties compared with 1000 permutations of a random network. The variables that most contributed to its adaptive properties were CRHR1 gene, FKBP5 gene, age, socio-economic status, and acute anxiety. Machine Learning analyses revealed an accurate and reliable predictive model for PTSD from variables measured at the time of trauma (AUC = .78) and modeling the influence of change (i.e. Judea Pearl's 'Do Calculus') in several 'remediable' causal variables (e.g. acute pain, pulse rate, anxiety, parent's symptoms of acute stress) led to reduction in PTSD symptoms.
Conclusions: New computational methods can lead to reliable and accurate predictive models for PTSD and identify promising prevention and treatment targets.
Disclosure: Nothing to Disclose.

Panel
54. The Role of Direct and Indirect Striatal Pathways in Anxiety and Altered Response to Salient Stimuli: A New Target for Treatment?

54.1 Bidirectional Dopamine Signaling in Motivated Behaviors

Alain Dagher

McGill University, Montreal, Canada

Background: Dopamine is implicated reinforcement learning. First, dopamine manipulations can make animals and humans impulsive. Second, dopamine has been identified as a learning signal that encodes reward prediction errors. Less clear is its role in learning from punishments. Here we test a bidirectional role for dopamine in learning from positive and negative feedback, acting on the direct and indirect basal ganglia pathways by measuring dopamine receptor levels in healthy subjects using positron emission tomography, and by reducing dopamine levels using a dietary manipulation (tyrosine depletion), in combination with neuroimaging and behavioral tasks.
Methods: 1.Positron emission tomography (PET) studies with dopamine tracers [11C]raclopride (measure of D2 receptors and dopamine release) and [11C]SCH23390 (D1 receptors) in healthy subjects. 2. Functional MRI studies in PD patients and controls at rest and while performing gambling tasks. 3. Tyrosine depletion combined with fMRI in healthy subjects to identify the role of dopamine in brain activation at rest and during task performance. 4. Analysis of the Parkinson's Progression Markers Initiative (PPMI), a large open-source database of imaging and clinical data in de novo PD patients.
Results: 1. D1 receptor binding in humans predicts reward but not punishment learning, while D2 receptor binding has the opposite relationship, supporting a model according to which these two modes of learning are under dopamine control acting on segregated basal ganglia pathways. 2. PD patients with gambling addiction show impaired loss prediction error compared to control PD patients and age-matched healthy controls. 3. Reducing dopamine levels using tyrosine depletion improves punishment learning while reducing risky behavior on a gambling task. This effect is shown to depend on D2 signaling. 4. De novo Parkinson's Disease patients demonstrate atrophy of all mesolimbic projection sites, including striatum, basal forebrain, amygdala, hippocampus, insula and anterior cingulate cortex. The affected network matches regions encoding either stimulus value or "reward" from meta-analyses of fMRI studies.
Conclusions: 1. D2 stimulation inhibits learning from negative outcomes via effects on the indirect pathway. 2. Reduced D2 signaling increases motivation to avoid potential losses (loss aversion). 3. PD patients at risk for ICD demonstrate impaired punishment learning even off medications. The interaction of this vulnerable phenotype with D2 stimulation from dopamine agonist medications promotes addictive behaviours. 4. In the non-Parkinsonian population, D2 anomalies previously described as the "reward deficiency syndrome" more likely represent impaired punishment learning, which may be a feature of the impulsive or externalizing personality type. 5. Early PD is associated with widespread atrophy of the entire mesolimbic system. This may account for the "Parkinsonian personality" consisting of rigid behavior and lack of engagement in potentially rewarding behaviours.
Disclosure: Nothing to Disclose.

54.2 Dopamine D2 Receptors on Striatal Indirect Pathway Neurons Modulate Anxiety-Like Behavior in Mice

Alexxai Kravitz

National Institute on Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland, United States

Background: Classic motor disorders such as Parkinson's disease have a high degree of comorbidity with negative emotional states such as anxiety and depression, which can precede the motor deficits of Parkinson's disease by decades. It is possible that this comorbidity reflects dopaminergic dysfunction early in the progression of Parkinson's disease. Consistently, dysfunction of dopamine D2 receptors (D2R) have been linked to negative emotional states including anxiety and depression independently of motor disorders.
Methods: To investigate the role of D2Rs signaling in anxiety-like behavior in mice, we used cre-dependent genetic approaches to knock D2Rs out of specific cell types in the brain. These included indirect pathway medium spiny neurons (iMSNs, via A2A-cre mice), cholinergic interneurons (via ChAT-cre mice), and dopamine neurons (via DAT-cre mice). We assayed anxiety-like behavior with the elevated zero maze and the open field. We followed up these studies with investigations of the natural dynamics of iMSN activity during anxiety-like behavior by recording calcium transients in iMSNs via freely moving GCaMP6 imaging while mice explored the zero-maze. Calcium recordings were performed with a head-mounted micro-endoscope camera. Finally, as the D2R modulates the output of iMSNs, we tested the sufficiency of altering iMSN output for altering anxiety-like behavior, via optogenetic and chemo-genetic manipulations.
Results: Removing D2Rs from iMSNs, but not other cell types, enhanced anxiety-like behavior in both tasks, demonstrating that D2Rs on iMSNs are particularly important for modulating anxiety-like behavior. Our imaging experiments revealed that the majority of iMSNs fired transients in only

one or two locations on the maze, most commonly around transition points between the open and closed arms on the zero maze, thereby reflecting anxiety-related locations in their firing dynamics. Optogenetic stimulation of iMSNs produced anxiogenic effects at low stimulation powers (<0.2mW), while producing motor freezing at higher power (>1mW), consistent with the hypothesis that heightened anxiety preceding Parkinson's disease may be the result of early dopamine dysfunction. Chemo-genetic inhibition of iMSNs reduced anxiety-like behavior in both tasks.
Conclusions: Our results suggest that D2Rs modulate the output of iMSNs to control anxiety-like behavior, and that the comorbidity between movement and anxiety disorders might share a common etiology of striatal dopamine dysfunction.
Disclosure: Nothing to Disclose.

54.3 Imbalanced Mitochondrial Dynamics in Striatal Pathways Mediates Psychostimulant Behavioral Plasticity

Mary Kay Lobo

University of Maryland School of Medicine, Baltimore, Maryland, United States

Background: Altered brain energy homeostasis is a hallmark adaptation occurring in the cocaine-addicted brain, but the effect of cocaine on the fundamental source of energy, mitochondria, is unknown. Since recent studies demonstrate that mitochondria dysfunction is associated with psychiatric disorders, we sought to examine mitochondrial dynamics in the two main ventral striatal (nucleus accumbens- NAc) projection medium spiny neuron (MSN) subtypes. These MSN subtypes, enriched in dopamine D1 vs. D2 receptors, display critical but antagonizing roles in cocaine-mediated behaviors. Previous studies demonstrate differential plasticity and signaling processes in D1-MSNs vs. D2-MSNs after chronic cocaine exposure. Given these findings it is plausible that these MSNs have different energy demands, which could lead to altered mitochondrial dynamics in each MSN subtype.
Methods: Using D1-Cre and D2-Cre mice combined with a Cre-inducible AAV to express mito-dsRed we examined mitochondrial volume, density, and size in MSN subtypes after cocaine self-administration. We then examined mRNA of mitochondrial fission and fusion genes in NAc from rodents that self-administer cocaine and in postmortem NAc of cocaine dependent individuals. We further examined ribosome-associated mRNA of these genes in D1-MSNs vs. D2-MSNs after repeated cocaine using the RiboTag approach. Additionally, we examined the fission promoting pSer616- dynamin-related protein 1 (Drp1) protein in NAc after repeated cocaine exposure. We then used an inhibitor, Mdivi-1, of pSer616-Drp1 during cocaine mediated behaviors. Finally, we overexpressed Drp1 wildtype (WT) or the fission promoting Drp1(S637A) variant in MSN subtypes, using Cre-inducible AAVs, during cocaine self-administration and a drug seeking test after abstinence from cocaine.
Results: Cocaine self-administration caused an increase in the frequency of smaller mitochondria in D1-MSN dendrites, implicating enhanced mitochondrial fission in D1-MSNs. Consistent with this data we observe an increase in Drp1 mRNA in NAc of rodents that self-administer cocaine and in postmortem NAc of cocaine dependent individuals. Further, we observe an upregulation of ribosome-associated mRNA of Drp1 in D1-MSNs and a decrease in D2-MSNs after repeated cocaine. Additionally, the fission promoting pSer616-Drp1 protein is increased in NAc after repeated cocaine exposure. Blocking mitochondrial fission, with Mdivi-1, blocked cocaine conditioned place preference and the expression of cocaine locomotor sensitization. Finally, overexpression of the fission promoting Drp1(S637A) variant in D1-MSNs increased drug seeking after abstinence from cocaine self-administration.
Conclusions: Our study establishes a direct role of altered mitochondrial fission in striatal MSN subtypes in cocaine abuse. Both human cocaine dependents and cocaine self-administering rodents display increased fission molecule Drp1 in ventral striatum, NAc. Further, fission promoting pSer616-Drp1 is increased in NAc after repeated cocaine. Blockade of fission promoting Drp1 blunts cocaine-mediated behaviors. These effects occur through D1-MSNs since we observe increased Drp1 and overall reduced mitochondrial length, indicative of increased fission, in D1-MSNs after cocaine exposure. This is consistent with enhanced cocaine seeking after abstinence with overexpression of Drp1 promoting fission variant in D1-MSNs. Our data implicate imbalanced mitochondrial dynamics in striatal circuits in psychostimulant mediated behaviors.
Disclosure: Nothing to Disclose.

54.4 Unmotivated to Eat in Anorexia Nervosa: Interactions Between Anxious, Inhibited, Risk Avoidant Temperament and Diminished Reward Response to Salient Stimuli

Walter Kaye

University of California, San Diego, La Jolla, California, United States

Background: How are individuals with anorexia nervosa (AN) able to starve themselves and become emaciated? Normally hunger increases reward valuation of food and other salient stimuli. Individuals with AN tend to have reduced sensitivity to reward, and food generates anxiety. In previous studies, AN individuals had reduced ventral striatum response to food, and exaggerated anxiety and harm avoidance was correlated with striatal or dopamine functional activity. Because these studies were done after eating, it was unclear how being hungry or fed affects motivated behavior in AN.
Methods: Using fMRI, study 1 investigated response to tastes of sucrose or water in neural circuitry that translates taste signals into valuation of food and motivation to eat (the insula, amygdala, and striatum) when hungry and fed. Study 2 used a delay discounting monetary decision task known to discriminate brain regions contributing to processing of immediate monetary reward when hungry and fed. We compared 22 healthy control women (CW) to 26 women remitted from AN to avoid confounding effects of malnutrition.

Results: Study 1: CW showed increased activation when hungry versus fed in the insula, amygdala, and ventral putamen. In contrast, AN had the opposite pattern, with attenuated activation when hungry versus fed. Study 2: Hunger, compared to satiety, significantly increased response to monetary reward in CW in striatal and anterior cingulate circuitry. But hunger did not increase brain reward responses in AN compared to satiety. In both studies, diminished striatal brain response in only AN to both food and money, when hungry and fed, was associated with increased harm avoidance (HA) and anxiety.
Conclusions: CW showed the expected increased response when hungry versus fed in brain regions involved in generating motivated behaviors. AN had the opposite pattern, with attenuated response when hungry versus fed. Moreover, in AN, the more harm avoidant or anxious, the lower the striatal response to sucrose or money, whether hungry or fed, suggesting these constructs interferes with reward signals, regardless of metabolic state. A lack of motivated behavior may explain why individuals with AN fail to eat when hungry. Indirect and direct striatal pathways are essential for survival in animals, by selecting whether to seek rewards or avoid danger. AN may have an intrinsic sensitivity to coding salient stimuli, such as food, as aversive or risky, rather than rewarding, which overrides the influences of hunger or satiety.
Disclosure: Nothing to Disclose.

Panel
55. Reelin Signaling in the Brain and Implications for Neuropsychiatric Disorders

55.1 Advances in the Current Understanding of Reelin Signaling in Brain Development and Function

Gabriella D'Arcangelo

Rutgers University, Piscataway, New Jersey, United States

Background: Reelin is an extracellular protein that controls many aspects of mammalian brain development and function, and has long been implicated in neuropsychiatric disorders such as schizophrenia. Reelin is expressed and secreted as a large glycoprotein by different cell types in the prenatal or postnatal mammalian brain, and it is also cleaved by different proteases to generate multiple fragments. These fragments and the full-length protein can assemble into oligomers, thus generating a variety of Reelin ligands with distinct biological activities and potency. In the prenatal brain Reelin controls neuronal radial migration and directs the formation of cortical layers, whereas in the postnatal and adult brain Reelin promotes dendrite outgrowth, excitatory synapse formation, and enhances synaptic plasticity. I will present and discuss recent advances in our understanding of the Reelin signaling transduction pathways that regulate different aspects of brain development and function.
Methods: Mouse genetics, neuronal culture systems, and classic biochemistry assays were used to elucidate many of the molecular mechanisms underlying the distinct biological functions of Reelin during and after brain development.
Results: The results of these investigations indicate that Reelin signal transduction involves unique and overlapping signaling pathways that are activated following ligand binding to its cell surface receptors. ApoER2/VLDLR lipoprotein receptors mediate all known prenatal functions of Reelin, and also participate in many postnatal activities. Upon Reelin binding these receptors initiate a canonical signaling cascade involving the adaptor protein Dab1 and Src/Fyn kinases. Downstream of Dab1, the Crk/Rap1 pathway and cell adhesion molecules play crucial roles in the control of neuronal migration, whereas the PI3K/Akt/mTOR pathway preferentially affects dendrite and spine development. A specific isoform of ApoER2 also interacts with the NMDA receptor, leading to Ca++ influx and contributing to the potentiation of synaptic plasticity. However, additional Reelin receptors, which have not yet been identified, may also play a role in the control of synaptic activity. I will present evidence that non-lipoprotein receptors activate a non-canonical pathway involving the MEK/Erk1/2 pathway, leading to the expression of immediate-early genes involved in learning and memory.
Conclusions: Reelin activates a multitude of signaling molecules that regulate its diverse functions in the brain. Knowledge of these mechanisms may provide new insight into neurodevelopmental and neurodegenerative disorders that are associated with Reelin dysfunction.
Disclosure: Nothing to Disclose.

55.2 The Role of Reelin at the Synapse

Joachim Herz

University of Texas Southwestern Medical Center, Dallas, Texas, United States

Background: Reelin, a large multimodular oligomeric protein is secreted by Cajal-Retzius neurons at the surface of the developing brain during the embryonic neuronal migration period. Towards the end of this radial migration phase, which lays down the laminated structure of the mammalian cortex, Cajal-Retzius neurons are beginning to be replaced by a new class of Reelin secreting neurons that originate from the medial ganglionic eminence and enter the neocortex and the hippocampus following a tangential migration path. These neurons become part of the inhibitory neuronal network that is essential for maintaining coordinated neuronal network activity.
Methods: We use electrophysiology to monitor how secretion of Reelin by these inhibitory interneurons modulates the function of excitatory synapses that respond to glutamate by regulating their AMPA and NMDA receptor composition.
Results: We show that by signaling through the ApoE receptors Apoer2 and Vldlr, Reelin function is essential for maintaining optimal pre- and post-synaptic Ca2+-homeostasis and plasticity.
Conclusions: Through these mechanisms, Reelin signaling controls the response of the excitatory synapse and protects the brain against amyloid induced synaptic suppression in Alzheimer's disease.
Disclosure: Nothing to Disclose.

55.3 Reelin-Mediated Signaling in Regulation of Neurotransmitter Release

Ege Kavalali

University of Texas Southwestern Medical Center, Dallas, Texas, United States

Background: Dysregulation of neurotransmitter release and alterations in synaptic vesicle trafficking pathways have been strongly implicated in the pathophysiology of several neuropsychiatric and neurological disorders. Sustained impairments in synaptic vesicle trafficking lead to dysfunction in neuronal signaling and synaptic plasticity that ultimately culminate in circuit abnormalities. Key genetic determinants of familial forms of Alzheimer's disease, including ApoE isoforms and presenilin mutations, have been shown to impair synaptic transmission. Recent studies have identified a direct impact of presenilin mutations on the regulation of presynaptic function leading to alterations in synaptic plasticity. In contrast, the impact of ApoE isoforms on presynaptic regulation is unknown. ApoE isoforms exert their effects by binding to ApoE receptor type 2 (ApoER2), which is a dual functional receptor that also mediates acute synaptic effects of the secreted glycoprotein Reelin.
Methods: In this study, we used electrophysiological analysis of neurotransmitter release as well as optical monitoring of presynaptic vesicle recycling and calcium.
Results: In recent work, we examined the presynaptic effect of Reelin and found that Reelin increases spontaneous neurotransmitter release from excitatory as well as inhibitory synaptic terminals without significantly altering the properties of evoked neurotransmission. This effect of Reelin is initiated by the ApoER2 and VLDLR signaling pathway(s) leading to activation of PI3 kinase and an increase in presynaptic Ca2+. Importantly, our results showed that this action of Reelin depends on the function of the vesicular SNARE protein VAMP7 but not synaptobrevin2, VAMP4 or vti1a.
Conclusions: These findings demonstrate a novel example where an endogenous neuromodulator relies on the diversity of synaptic vesicle pool associated SNAREs and selectively mobilizes a subset of vesicles independent of electrical activity. These results suggest that ApoER2 mediated signaling plays a critical role in the regulation of neurotransmitter release. We are currently testing whether ApoE isoforms - ApoE4, ApoE2 or ApoE3 - can mimic the effect of Reelin and augment neurotransmitter release.
Disclosure: Nothing to Disclose.

55.4 Epigenetic Reln Dysfunction in Schizophrenia and Related Neuropsychiatric Disorders

Alessandro Guidotti

University of Illinois at Chicago, Chicago, Illinois, United States

Background: REELEN (RELN) is a large (420 kDa) glycoprotein that in adulthood is mostly synthesized in GABAergic neurons of corticolimbic structures. Upon secretion in the extracellular matrix, RELN binds to VLDL, APOE2, and $\alpha 3\beta 2$ Integrin receptors located on dendritic shafts and spines of postsynaptic pyramidal neurons. Reduced levels of RELN expression in the adult brain induce cognitive impairment and dendritic spine density deficits. RELN supplementation recovers these deficits suggesting a trophic action for RELN in synaptic plasticity.
Methods: RELN mRNA (RT-PCR, *In situ* hybridization, Laser microdissection) and RELN proteins (Western blot, immuno-histochemistry) were measured in cortico- limbic structures of schizophrenia (SZ) and (BP) disorder patients.
Results: We and others have shown that reduced RELN expression in SZ and BP disorder patients is difficult to reconcile with classical Mendelian genetic disorders and it is instead plausible to associate these disorders with altered epigenetic homeostasis. Support for the contribution of altered epigenetic mechanisms in the down-regulation of RELN expression in corticolimbic structures of psychotic patients includes the concomitant increase of DNA-methyltransferases and the increased levels of the methyl donor S-adenosylmethionine. It is hypothesized that these conditions lead to RELN promoter hypermethylation and a reduction in RELN protein amounts in psychotic patients.
Conclusions: The decreased synthesis and release of RELN from GABAergic corticolimbic neurons could serve as a model to elucidate the epigenetic pathophysiological mechanisms acting at pyramidal neuron dendrites that regulate synaptic plasticity and cognition in psychotic and non-psychotic subjects.
Disclosure: Nothing to Disclose.

Panel
56. Antidepressant Ketamines: Racemic, Enantiomeric and Active Metabolites

56.1 Molecular Mechanism of Rapid Antidepressant Response

Lisa Monteggia

University of Texas Southwestern Medical Center, Dallas, Texas, United States

Background: Recent clinical studies have demonstrated that a single subpsychotomimetic dose of ketamine, an ionotropic glutamatergic N-methyl-D-aspartate (NMDA) receptor antagonist, produces a rapid antidepressant response in patients with major depressive disorder, with effects lasting up to 2 weeks. Despite enthusiasm about this unexpected efficacy of ketamine, its widespread use as a fast-acting antidepressant in routine clinical settings is curtailed by its abuse potential as well as possible psychotomimetic effects. However, the ability of ketamine to produce a rapid and long-lasting antidepressant response in patients with depression provides a unique opportunity for investigation of mechanisms that mediate these clinically relevant behavioral effects.
Methods: From a mechanistic perspective, it is easy to imagine how activation of NMDA receptors may trigger cellular and behavioral responses; it is relatively more difficult, however, to envision how transient blockade of one of the key pathways for neuronal communication produces a persistent beneficial effect. We have used

electrophysiological, biochemical, and behavioral experiments to analyze the synaptic basis of the antidepressant-like behavioral effects triggered by acute ketamine application.
Results: Data will be presented showing that ketamine mediated blockade of NMDA receptors at rest deactivates eukaryotic elongation factor 2 (eEF2) kinase, resulting in reduced eEF2 phosphorylation and desuppression of rapid dendritic protein translation, including BDNF (brain-derived neurotrophic factor), which then contributes to synaptic plasticity mechanisms that mediate long-term effects of the drug. Electrophysiological data shows that ketamine potentiates synaptic responses in the CA1 regions of rat and mouse hippocampus. This potentiation requires protein synthesis, BDNF expression, eEF2 function, and increased surface expression of AMPA receptors, in particular GluA1 and GluA2. This potentiation is blocked by AMPA receptor antagonists such as DNQX, but not by NASPM which blocks AMPA receptors lacking GluA2. The requirements for GluA2 are further explored in genetic knockout mice and show an attenuation to ketamine's antidepressant and synaptic effects.
Conclusions: These findings reveal critical determinants of how blocking spontaneous neurotransmission impacts synaptic plasticity, with implications for ketamine mediated antidepressant responses. Moreover, our recent findings linking ketamine's mechanism of action to homeostatic synaptic plasticity processes activated after suppression of NMDA-mediated glutamatergic neurotransmission will be explored.
Disclosure: Nothing to Disclose.

56.2 NMDAR Inhibition-Independent Antidepressant Actions of Ketamine Metabolites: Reexamining the Ketamine Paradigm

Irving Wainer

Mitchell Woods Pharmaceuticals, Washington, District of Columbia, United States

Background: (R,S)-Ketamine is a chiral phencyclidine derivative that produces rapid and short-lived anesthesia via inhibition of the N-methyl-D-aspartate (NMDA) receptor. The anesthetic activity of this drug has been associated with the parent compound and to a lesser degree with the N-demethylated metabolite (R,S)-norketamine, while other (R,S)-ketamine metabolites, in particular (2S,6S;2R,6R)-hydroxynorketamine, were designated as "inactive", the "Ketamine Paradigm". Sub-anesthetic dosing of (R,S)-ketamine and (S)-ketamine are currently used in the treatment of depression and neuropathic pain, and the "Ketamine Paradigm" is invoked as the pharmacological mechanism. In 2010, pharmacodynamic analysis of plasma from patients receiving low dose (R,S)-ketamine in the treatment of Complex Regional Pain Syndrome failed to establish a relationship between pain relief and (R,S)-ketamine and (R,S)-norketamine plasma concentrations. Zarate and co-workers came to a similar conclusion in their studies of the antidepressant effects of low dose (R,S)-ketamine. These observations lead to the initiation of a clinic - to bench - to clinic research program. The data from this program will be discussed.
Methods: The plasma distributions and pharmacokinetics of (R,S)-ketamine and its major metabolites were determined in samples obtained from clinical and animal studies using lc-MS/MS. The major metabolites were synthesized and studied in animal models and in cell-based studies. Metabolic studies were conducted using characterized human liver microsomes. The effects of (R,S)-ketamine, ketamine enantiomers and major metabolites on the expression and function of serine racmase and the ACT2 transporter were determined using standard biological techniques and corresponding concentrations of D-serine were determined using capillary electrophoresis/laser-induced fluorescence detection. Statistical analysis and computational modeling studies were accomplished using standard programs run on a personal computer.
Results: The data from this program established that (R,S)-ketamine is rapidly transformed into multiple metabolites, that these metabolites are weak NMDA receptor antagonists but are active at other receptors and that these metabolites are associated with clinical response. These results laid the basis for the recent report demonstrating that (2R,6R)-hydroxynorketamine produces antidepressant effects in mice and that these antidepressant actions are independent of NMDAR inhibition but involve early and sustained activation of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. The pharmacological effects produced by sub-anesthetic dosing of (R,S)-ketamine were also studied using non-targeted and targeted pharmacometabolomics analysis, which identified a relationship between basal D-serine plasma levels and response in which reduced D-serine plasma levels predict and track a positive antidepressant response. A similar relationship was observed for the positive response to (R,S)-ketamine treatment in patients suffering from Post-Traumatic Stress Disorder (PTSD).
Conclusions: 1. The ketamine metabolites (2S,6S)-hydroxynorketamine and (2R,6R)-hydroxynorketamine contribute to the antidepressant effects produced by the administration of (R,S)-ketamine. 2. (2R,6R)-Hydroxynorketamine is a potentially viable antidepressant agent that is orally bioavailable, readily passes the blood-brain barrier and does not produce the CNS side effects associated with the administration of (R,S)-ketamine. 3. The "Ketamine Paradigm" that described the pharmacological effects of anesthetic doses of (R,S)-ketamine is not viable for the description of the antidepressant effects of sub-anesthetic dosing of the drug. 4. Basal D-serine plasma level is a biomarker of clinical antidepressant response to treatment with (R,S)-ketamine.
Disclosure: Part 1: Mitchell Woods Pharamceuticals, Partial ownership, National Institutes of Health, patent applications, Mitchell Woods Pharmaceuticals, patent application, **Part 3:** Mitchell Woods Pharmaceuticals, salary, **Part 5:** Mitcehll Woods Pharmaceuticals, employer.

56.3 R-Enantiomers of Ketamine and Its Metabolite Norketamine as Rapid-Onset and Sustained Antidepressants

Kenji Hashimoto

Chiba University, Chiba, Japan

Background: The N-methyl-D-aspartate receptor (NMDA-R) antagonist ketamine is one of the most attractive

antidepressants since this drug can produce rapid-onset and sustained antidepressant effects in treatment-resistant patients with major depression and bipolar disorder. Recent meta-analyses show that the antidepressant effect of ketamine is greater potent than other NMDA-R antagonists (e.g., memantine, traxoprodil, lanicemine, rapastinel) in patients with depression. Ketamine (Ki = 500 nM for NMDA-R) is a racemic mixture containing equal parts of S-ketamine (esketamine: Ki = 300 nM) and R-ketamine (Ki = 1,400 nM). Recently, we reported that R-ketamine showed greater potency and longer lasting antidepressant effects than esketamine in animal models of depression, suggesting that NMDA receptor may not have a major role in the long-lasting antidepressant effects of R-ketamine. R-ketamine is metabolized to R-norketamine (Ki = 13,000 nM for NMDA-R). In the present study, we compared the antidepressant and side effects of two enantiomers of ketamine and norketamine in animal models of depression.

Methods: The antidepressant effects of two enantiomers of ketamine and its major metabolite norketamine in the rodent models of depression were examined. Furthermore, side effects profiles (e.g., locomotion, prepulse inhibition (PPI), conditioned place preference, parvalubumin (PV)-immunohistochemistry) of these compounds were examined. Finally, we examined whether esketamine and R-ketamine can increase dopamine release in the striatum using PET study of conscious monkey.

Results: In the animal models of depression, R-ketamine showed greater potency and longer lasting antidepressant effects than esketamine. Furthermore, esketamine, but not R-ketamine, caused behavioral abnormalities such as hyperlocomotion, PPI deficits, abuse potential, Furthermore, repeated intermittent administrations of esketamine, but not R-ketamine, caused loss of PV-immunoreactivity in the medial prefrontal cortex and hippocampus of mice. A PET study showed that s single infusion of esketamine (0.5 mg/kg, 40-min), but not R-ketamine (0.5 mg/kg, 40-min), significantly caused reduction of dopamine D2/3 receptor binding in the striatum of conscious monkey, suggesting that esketamine, but not R-ketamine, can cause marked dopamine release in the monkey striatum. Furthermore, R-norketamine showed greater potency and longer-lasting antidepressant effects than esketamine and S-norketamine. In addition, R-norketamine did not cause behavioral side effects in rodents.

Conclusions: These findings suggest that R-enantiomers of ketamine and norketamine show greater potency and longer lasting antidepressant effects than their S-enantiomers, and that, unlike to S-enantiomers, R-enantiomers of ketamine and norketamine did not cause behavioral side effects. Therefore, both R-ketamine and R-norketamine would be rapid onset and sustained antidepressants without psychotomimetic side effects.

Disclosure: Part 4: Otsuka, research grant, Taisho, research grant, Mochida, research grant, Dainippon-Sumitomo, research grant.

56.4 Efficacy, Safety, Dose Response and Sustained Effect of Esketamine in Treatment Resistant Depression (TRD)

Jaskaran Singh

Janssen, San Diego, California, United States

Background: Currently available antidepressant therapies for major depressive disorder (MDD) have several limitations, such as delayed onset of efficacy and low remission rates. Ketamine and its S-enantiomer, esketamine, exert a rapid onset of antidepressant effect, which is sustained in patients with TRD. Esketamine has a 3 to 4-fold higher affinity for NMDA receptors than arketamine (R-enantiomer of ketamine). Esketamine (JNJ54135419), is under investigation as a treatment for TRD and as a treatment for rapid reduction of symptoms of MDD in patients at imminent risk for suicide.

Methods: This study was a 2-Panel, doubly-randomized, double-blind, dose-response placebo-controlled, multicenter study. Panel A was conducted in the United States and Belgium (84, 56, 28 mg and placebo) and Panel B in Japan (56, 14 mg and placebo). In both panels, each subject participated in up to 4 phases: a screening phase of up to 4 weeks, a double-blind treatment phase which included two 1-week periods (Periods 1 and 2), an optional open-label treatment phase (9-week for Panel A and 2 weeks for Panel B) and an 8-week post-treatment follow-up phase. The primary efficacy endpoint was the change from baseline to Day 8 in each period in the Montgomery-Asberg Depression Rating Scale (MADRS) total score combined.

Results: In Panel A (US, Belgium), the mean change in MADRS total scores on day 8 (Periods 1 and 2 combined; weighted combination test) in all the three esketamine treatment groups were significantly superior to placebo (28 mg: $p = 0.021$, 56 mg: $p = 0.001$, and 84 mg: $p < 0.001$; 1-sided significance level). Mean differences (standard error) from placebo (after 1-week treatment; i.e. day 8) were -4.2 (2.09) for esketamine 28 mg, -6.3 (2.07) for esketamine 56 mg, and -9.0 (2.13) for esketamine 84 mg. In Panel B (Japan), in Period 1, greater improvements in MADRS total score were observed in the esketamine 56 mg group compared with the placebo group (least squares mean treatment difference [SE]: -3.7 [2.81]; $p = 0.096$). Smaller improvements in MADRS total score were observed in the esketamine 14 mg group compared with the placebo group (least squares mean treatment difference [SE]: +1.8 [2.62]). At the end of the OL phase in Panel A, 31.6% (18 of 57) patients were in remission and at the end of follow up phase, approximately 2 months post last dose 14 of of those 18 patients were in remission. In Panel B, at the end of the OL phase 33% (13 of 39) patients were in remission and at the end of follow up phase, 2 months post last dose, 12 of those 13 of those patients sustained that remission. Table 2: Secondary Efficacy Endpoints of Patients who have perceptual changes were dose dependent, began shortly after start of dosing and resolved in $<$ 2 hours, and significantly attenuated with multiple dosing. All adverse events generally resolved $<$2 hours after dosing and overall, esketamine was well tolerated.

Conclusions: Intranasal esketamine doses of 28 mg, 56 mg and 84 mg were efficacious in treating TRD and all doses

evaluated were generally well tolerated. 14mg was not efficacious.
Data suggests that efficacy was sustained over the OL phase, even with a reduced frequency of intranasal treatment dosing session. In addition, efficacy appeared to be sustained for the 8 week follow up phase after cessation of intranasal dosing.
Disclosure: Part 1: Janssen, Employee, **Part 5:** Janssen, Employee.

Panel
57. It's All Your Parents' Fault! Maternal and Paternal Exposure to Drugs of Abuse or Stress Produces Maladaptive Effects on Descendant Behavior

57.1 Paternal Cocaine Exposure Elicits Epigenetic Remodeling and Learning Deficits in Progeny

Mathieu Wimmer

University of Pennsylvania, Philadelphia, Pennsylvania, United States

Background: Cocaine taking is often associated with global cognitive impairments, including deficits in attention and declarative memory. Additionally, paternal environmental perturbations such as chronic stress or drugs of abuse can produce profound effects on the physiology and behavior of offspring via epigenetic changes in sperm. Our recent studies demonstrated that cocaine exposure in sires produced alterations in mood and addiction-like behaviors in progeny. However, it remains unclear whether paternal cocaine taking is associated with cognitive impairments in their progeny. We hypothesized that paternal cocaine taking might reduce cognitive function in subsequent generations.
Methods: Male rats self-administered cocaine daily for 60 days, the duration of spermatogenesis, and controls received yoked saline infusions. Sires were then bred with drug-naïve females resulting in cocaine-sired and saline-sired first generation (F1) offspring. Memory formation was examined in both male and female adult (60 days and older) drug-naïve offspring using a hippocampus-dependent object location memory task. We also measured theta burst-induced long term potentiation (LTP) in the Schaffer collateral pathway of F1 progeny. Glutamate signaling through N-methyl-D-aspartate receptors (NMDARs) in the hippocampus is critically important for memory formation and synaptic plasticity. Hippocampus tissue was collected from a separate cohort of naïve adult F1 offspring to measure NMDARs gene and protein expression. Epigenetic remodeling was also assessed using genome-wide mass spectrometry-based approaches as well as chromatin immunoprecipitation (ChIP).
Results: Male offspring of saline-treated sires spent more time exploring the displaced object during the object location memory test, indicating intact spatial memory formation. In contrast, cocaine-sired male progeny showed memory formation deficits and explored both objects equally during the retrieval test. The observed impairments were sex specific in that object location memory formation was intact in all female offspring. Theta bursts-induced LTP in the Schaeffer collateral pathway was impaired in cocaine-sired offspring compared to controls. Total levels of the endogenous NMDAR co-agonist D-serine, which is critically involved in memory formation and synaptic plasticity, were diminished in the hippocampus of cocaine-sired offspring compared to controls. Consistent with this observation, the D-amino acid oxidase (DAAO) enzyme that breaks down D-serine was over-expressed in the hippocampus of male cocaine-sired progeny. We next measured genome-wide post-translational modifications (PTMs) located on the N-terminal tails of histone proteins that package DNA, which are known to regulate gene expression. Six permissive marks were globally enriched in cocaine-sired offspring, including acetylation of histone H3. We used ChIP to interrogate H3 acetylation near the Dao1 locus, which encodes the DAAO enzyme. Acetylation of H3 was enriched at the transcription start site of Dao1 in the progeny of cocaine-exposed sires. We next attempted to rescue the LTP and memory formation deficits in cocaine-sired progeny using D-serine. Bath application of D-serine restored LTP induction in hippocampal slices from cocaine-sired rats. In addition, hippocampal micro-injections of D-serine prior to training on the object location task restored memory in the male offspring of cocaine-exposed sires.
Conclusions: Our results demonstrate that paternal cocaine exposure elicits learning and synaptic plasticity impairments. These effects are associated with reduced D-serine levels, overexpression of Dao1 and epigenetic remodeling near the Dao1 locus in the hippocampus of male progeny.
Disclosure: Nothing to Disclose.

57.2 Sex-Related F2 and F3 Transgenerational Epigenetic Effects of Paternal Germline THC Exposure History

Yasmin Hurd

The Icahn School of Medicine at Mount Sinai, New York, New York, United States

Background: Marijuana (Cannabis sativa) is now used to a greater extent than cigarettes by adolescents. While most research efforts have focused on the direct impact of marijuana during one's lifetime, the potential multi-generational consequences have only recently been acknowledged. Our previous animal study established that adolescent exposure to the main psychoactive component of cannabis, delta-9-tetrahydrocannabinol (THC) induces a spectrum of behavioral and neurobiological abnormalities in adult F1 offspring. The question remained as to whether true 'transgenerational' transmission had occurred that would be evident in subsequent generations.
Methods: To gain insight into potential transgenerational effects, we utilize a rat model to examine the behavioral and molecular profile of the striatum of male and female F2 and F3 offspring with paternal THC germline exposure history.
Results: Various behavioral differences were noted. For example, reward sensitivity tested with palatable food self-administration and sucrose preference paradigms revealed that adult F2 males with paternal THC germline history had decreased work effort to self-administer palatable food. This behavior correlated with decreased sucrose preference,

suggesting a depression-like phenotype. They also extinguished food-seeking behavior faster, but had elevated reinstatement behavior in response to a previously reward-associated cue. In the F3 generation, males exhibited elevated reward sensitivity even from the first exposure to the paradigm under a fixed ratio-1 (FR-1) schedule (highlighting no major impairment of general learning) and also had increased motivation to obtain the reward in a progressive ratio (PR) paradigm. Intriguingly, none of these behavioral abnormalities were observed in F2 or F3 female offspring. RNA-sequencing and epigenetic studies are being evaluated to provide molecular insights.
Conclusions: These results suggest that cannabis use deriving from the paternal lineage can lead to true transgenerational behavioral consequences and can impact future generations even into F3 adults with greater sensitivity of males for the behaviors studied.
Disclosure: Nothing to Disclose.

57.3 Multi-Generational Impact of Adolescent Opioid Exposure

Elizabeth Byrnes

Tufts University, North Grafton, Massachusetts, United States

Background: The United States is in the midst of an opioid epidemic, with abuse of prescription and illicit opioids increasing steadily over the past decade. As a result, there has been increasing use of opioids in younger populations, including use initiated during adolescence. The current set of studies was designed to test the hypothesis that adolescent exposure to opioids can induce significant effects in future generations. Of particular interest are effects on systems normally regulated by endogenous opioids, such as the hypothalamic pituitary adrenal (HPA) axis and reward-related circuits.
Methods: Adolescent rats were administered morphine at increasing doses (5-25 mg/kg, s.c.) or matched volumes of saline for 10 days (postnatal day 30-39). As adults, all adolescent exposed subjects were bred with drug-naive animals. Their male and female offspring (F1 generation) were then examined as adults. Drug abuse liability was examined using drug self-administration (morphine and cocaine). In addition, HPA axis sensitivity and metabolic function were also determined. Finally, modifications in both gene and protein expression were assessed in the nucleus accumbens and hypothalamus. Some studies were extended to examine effects in the F2 generation.
Results: Molecular, endocrine, and behavioral effects were observed in the F1 offspring of adolescent morphine-exposed animals. Some of these effects were sex-specific and a number of effects could still be discerned in the F2 generation. Significant transgenerational effects on both morphine and cocaine self-administration were observed. In addition, male offspring demonstrated HPA hyporesponsiveness and increased risk for metabolic syndrome under certain environmental conditions. Significant alterations in the hypothalamic proopiomelanocortin (POMC) system were observed and these effects were maintained in the F2 generation.
Conclusions: These findings indicate that even limited opioid exposure during adolescence can have significant effects on future generations. These effects are often sex-specific and appear to involve systems normally modulated by endogenous opioids. Given the broad role of endogenous opioids in regulating homeostatic processes, the potential impact of these transgenerational effects may be far-reaching. Furthermore, the specific nature of any observed effect may be further modified by environmental factors.
Disclosure: Nothing to Disclose.

57.4 Paternal Transmission of Stress Induced Phenotypes Are Transmitted via Male Germ Cells

Deena Walker

The Icahn School of Medicine at Mount Sinai, New York, New York, United States

Background: Depression is a complex disorder caused by a combination of genetic and environmental factors. Therefore, it has been proposed that epigenetic mechanisms may contribute to the risk for depression. Recent evidence suggests that the offspring of males who are susceptible to chronic social defeat stress (CSDS) display increased depressive- and anxiety-like behaviors but only after the fathers have been exposed to stress. The mechanisms by which such parental experience influence stress susceptibility of their offspring are poorly understood. We tested the hypothesis that changes in sperm cells during CSDS are responsible for encoding increased susceptibility to stress in F1 and F2 offspring.
Methods: Male mice were exposed to 10 days of CSDS and subjected to social interaction testing to assess paternal phenotype. Males identified as resilient or susceptible to CSDS, as well as control F0 males, were allowed to mate 30 days after the stress, to allow the sperm exposed to the stress to mature. One week following natural mating, those same F0 males were euthanized and sperm was collected for artificial insemination and snap frozen for epigenetic analysis. At ~P60, 1 male and 1 female in each litter was exposed to sub-threshold unpredictable stress and depression- and anxiety-like behaviors were assessed. Each animal was compared to an unstressed littermate of the same sex to control for litter effects. Additionally, phenotypes of offspring produced via natural mating and artificial insemination were compared between litters sired by the same father to investigate if the phenotype was passed via the paternal germ cells.
Results: Preliminary evidence suggests that F1 offspring of susceptible fathers produced by natural mating or by artificial insemination display altered phenotypes, effects that were not observed in offspring from control or resilient fathers. Finally, opposite effects were observed in males and females. Paternal stress augmented stress susceptibility of F1 males, while reducing it in F1 females. These data suggest that there are changes in sperm during CSDS that encode altered depressive- and anxiety–like phenotypes in their offspring. Transgenerational inheritance is currently being investigated by assessing similar outcomes in the F2 generation, and epigenetic changes are being investigated

in the sperm of the F0 fathers to identify the molecular mechanisms of this paternal transmission.
Conclusions: These data suggest that there are changes in sperm during CSDS that encode altered depressive- and anxiety–like phenotypes in their offspring. Transgenerational inheritance is currently being investigated by assessing similar outcomes in the F2 generation, and epigenetic changes are being investigated in the sperm of the F0 fathers to identify the molecular mechanisms of this paternal transmission.
Disclosure: Nothing to Disclose.

Panel
58. Maternal Immune Activation, Synaptic Function, and Phenotypes of Schizophrenia and Autism

58.1 The Maternal Interleukin-17a Pathway in Mice Promotes Autism-Like Phenotypes in Offspring

Gloria Choi

Massachusetts Institute of Technology, Cambridge, Massachusetts, United States

Background: Human studies suggest that maternal viral infections early in pregnancy correlate with an increased frequency of ASD in the offspring. This observation, coined maternal immune activation (MIA), has been modeled in rodents by inducing inflammation in pregnant dams. However, the immune cell populations critical in the MIA model have not been identified. Th17 cells are CD4+ T helper effector cells that express pro-inflammatory cytokines, including IL-17a. The orphan nuclear hormone receptor (NhR) RORgt (retinoic acid receptor-related orphan nuclear receptor gamma t) is a key transcription factor for the development of Th17 cells in both humans and mice. Several recent studies have proposed that Th17 cells and their cytokine mediators may contribute to autism–related behavioral phenotypes. Importantly, IL-6, a pro–inflammatory cytokine which has been shown to induce the MIA phenotypes in offspring when injected into pregnant mothers, is required for Th17 cell differentiation. These observations suggest that Th17 cells may play a crucial role in the inflammation-induced behavioral changes observed in affected offspring.
Methods: We tested this hypothesis by examining the effect of MIA in pregnant dams selectively deficient for RORgt in T cells. In these animals, the RORgt was selectively removed in cells expressing CD4-Cre. We bred T cell–specific RORgt KO or heterozygous (HET) female littermates with C57BL/6 WT males. Thus, the offspring expressed one copy of RORgt regardless of maternal genotypes, and this allowed us to assess the contribution of maternal RORgt in T cells. In another set of experiments, we pretreated pregnant dams with IL-17a blocking antibody to test whether IL-17a acting downstream of Th17 cells is important for MIA-mediated behavioral phenotypes that are observed in the offspring.
Results: T cell-specific inactivation of RORgt in mothers or blocking activities of IL-17a protected against induction of MIA-dependent behavioral phenotypes in offspring. In addition, we found that maternal inflammation leads to abnormal cortical phenotypes in offspring and this malformation is fully rescued by inhibiting the maternal IL-17a pathway or by removing Th17 cells in pregnant dams. We also found that the receptor subunit for IL-17a (IL-17Ra) is expressed in the developing fetal brain and its expression is increased in the cortex upon MIA. Thus, using both genetic mutants and blocking antibodies targeting their activities, we demonstrated that Th17 cells are critical mediators of behavioral abnormalities as well as brain pathologies in MIA-affected offspring (Choi et al., 2016).
Conclusions: These observations support the hypothesis that uncontrolled activation of IL-17Ra expressed in fetal brain induces abnormal cortical development, and our preliminary results suggest that these structural abnormalities may be an underlying cause of the MIA-dependent behavioral phenotypes.
Disclosure: Nothing to Disclose.
Reference: Choi, G.B., Yim, Y.S., Wong, H., Kim, S., Kim, H., Kim, S.V., Hoeffer, C.A., Littman, D.R. and Huh, J.R. (2016). The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. Science 351, 933-939.

58.2 Maternal Immune Activation Alters a Cytokine-MHCI-Dependent Molecular Pathway in the Developing Brain That Controls the Level of Excitatory Synaptic Connectivity in an Age-Dependent Manner

A. Kimberley McAllister

University of California, Davis, Davis, California, United States

Background: Schizophrenia (SZ) and autism spectrum disorders (ASD) are complex diseases likely caused by a combination of genetic and environmental factors during early development. Recent work points to a central role for immune-related genes and immune responses to environmental stimuli in SZ and ASD, and specifically for maternal infection during early gestation as a risk factor for both disorders. The development of mouse and non-human primate models of maternal infection with strong face and construct validity for SZ and ASD has strengthened the link between maternal immune activation (MIA) and many of the abnormal behaviors and neuropathologic findings characteristic of these disorders. Results from these animal models and recent human studies have converged on the hypothesis that MIA causes a chronic immune-dysregulated state in the offspring that alters brain development and behavior. However, little is known about the nature of the synaptic changes that occur in the frontal cortex throughout development in MIA offspring and whether changes in immune molecules in the brain mediate the MIA-induced changes in connectivity. The aim of our study was to address both questions.
Methods: Pregnant mice were injected with saline or poly(I:C) on E12.5 and postnatal offspring were examined at 3 postnatal ages. Tissue was processed for Golgi staining and immunocytochemistry (ICC). Changes in spine density, dendritic complexity, and levels of several immune molecules, including MHCI and several cytokines, were quantified.

Results: Using a mouse model for maternal infection, we discovered that maternal immune activation (MIA) causes region and age-specific changes in cytokine levels and cytokine receptor expression in the brains of offspring throughout development. In the frontal cortex of MIA offspring, a wide range of cytokines are elevated at birth, dramatically decrease during the first month of postnatal development, and increase again in young adults. New, preliminary results suggest that the expression of MHCI molecules in frontal cortex of MIA offspring follows the direction of change in cytokines with age, but that spine density on pyramidal neurons in superficial layers is altered in a generally opposite pattern to that of the cytokines and MHCI. This is consistent with our findings that MHCI molecules are found on neurons at cortical synapses throughout development, where they negatively regulate glutamatergic and GABAergic synapse density and function, as well as the balance of excitation and inhibition, onto cortical neurons *in vivo* and *in vitro* during early postnatal development.
Conclusions: Our new results suggest the hypothesis MIA alters a cytokine-MHCI-dependent molecular pathway in the developing brain that controls the level of excitatory synaptic connectivity. Our research is currently aimed at testing the hypothesis that MHCI proteins are required for the effects of MIA-induced changes in cytokines on brain development and behavior and determining the mechanism used by MHCI to limit cortical synaptogenesis.
Disclosure: Nothing to Disclose.

58.3 Synaptic Genes Involved in Neurotransmission and Plasticity are Downregulated Following Prenatal Inflammation and Reversed by Environmental Complexity in a Sex-Specific Manner

Amanda Kentner

Massachusetts College of Pharmacy and Health Sciences, Boston, Massachusetts, United States

Background: Prenatal infection is a risk factor for some psychiatric illnesses such as schizophrenia. In laboratory animals, many behavioral and neuropathological outcomes relevant to these developmental disorders can be modeled in the offspring of infected dams. Given its central role in neurotransmission, and clinical reports of its involvement in schizophrenia, we were interested in evaluating the glutamatergic transporter system in our prenatal infection model. Moreover, environmental enrichment (EE; i.e. social enhancement, novelty, physical activity) is well known to facilitate neuroprotection and rescue from a variety of insults, primarily through action on the brain-derived neurotrophic factor (BDNF) system; however, its utility in protecting or reversing the effects of early life inflammation has only recently been evaluated [Connors et al, Brain Behavior and Immunity, 42, 2014; Kentner et al, Brain, Behavior, and Immunity, in press].
Methods: Standard housed Sprague-Dawley rat dams were administered either the inflammatory endotoxin lipopolysaccharide (LPS; 100ug/kg) or equivolume pyrogen-free saline on gestational day (G)15. On postnatal day 50, male and female offspring were housed in either 'novel' EE (groups of 3-4 animals housed in a large multi-level cage with toys, tubes and ramps), Social Enrichment (SE; groups of 4 animals socially-housed in a larger style cage), or Standard Care (SC; animals pair-housed in standard cages). Six weeks later we scored social engagement, performance in the object-in-place task, and evaluated the expression of genes involved in synaptic function, specifically excitatory amino acid transporters (EAAT)1-3, BDNF, and tyrosine kinase receptor type 2 (TrkB), in hippocampus and prefrontal cortex.
Results: Our prenatal infection model resulted in social and spatial discrimination disruptions and reduced excitatory amino acid transporter 2 (EAAT2) gene expression in the prefrontal cortex of males. Both spatial discrimination and EAAT2 effects were reversed by SE housing and returned to levels comparable to saline controls. Spatial discrimination was also disrupted in female LPS rats, but this was not associated with EAAT2, suggesting a separate underlying mechanism for this effect. Interestingly, EE was protective against this impairment. Only in males was G15 LPS associated with a respective downregulation of BDNF and TrkB genes in hippocampus and prefrontal cortex. The LPS induced downregulation of BDNF was abated by SE and EE, while only EE corrected the reduced TrkB in prefrontal cortex to control levels.
Conclusions: Together our findings corroborate evidence that early life infection can interfere with brain development and impart long-term programming effects on genes involved in synaptic functioning that underlie disorders such as schizophrenia. Specifically, prenatal LPS induced a sex-dependent downregulation of EAAT2, BDNF, and TrkB genes, effects which may underlie the dysregulated excitatory/inhibitory balance in the brains of some patients. Notably these synaptic genes regulate glutamatergic transmission which is implicated in disrupted spatial discrimination, an impairment common in schizophrenia and observed in our prenatal inflammatory model. Moreover, our data provide compelling implications for the role of the environment in rescuing and promoting resiliency against adversities such as early life infection.
Disclosure: Nothing to Disclose.

58.4 Examining the Role of Microglia in Schizophrenia Using Developmental Immune Activation Models

Urs Meyer

University of Zurich, Zurich, Switzerland

Background: There is growing interest in the potential role of microglia in the pathogenesis of schizophrenia. It has become a popular idea that abnormal activity of these CNS-resident immune cells might lead to excessive synaptic pruning and cause synaptic deficits, which in turn might represent a core neuropathological basis of behavioral and cognitive symptoms in schizophrenia. However, the current evidence of altered microglia activity and its potential association with synaptic deficits in schizophrenia remains equivocal, In this context, we sought to examine the relationship between microglia activity, synaptic maturation, and behavioral functions using developmental immune activation models in mice. These models are currently

among the most widely used models in preclinical schizophrenia research and capture a wide spectrum of disease-relevant behavioral and cognitive abnormalities.
Methods: Developmental immune activation was induced by treating pregnant C57BL6 mice with the viral mimic poly(I:C) during early (gestation day 9) or late (gestation day 17) gestation. The offspring of immune-challenged and control mothers were first assigned to behavioral testing in pubescence and adulthood, followed by quantification of major presynaptic (synaptophysin, bassoon) and postsynaptic (PSD95, SynGAP) proteins. Detailed analyses of microglia phenotypes using microglia-specific cellular markers (Iba1 and CD68) and morphological assessments (microglia cell soma size and ramification) were conducted to explore whether microglia activity differs between immune-challenged and control offspring. In addition, we quantified brain inflammatory cytokines and translocator protein (TSPO) expression and binding, the latter of which is routinely used to assess microglia activity in human imaging studies.
Results: Prenatal immune activation led to behavioral impairments relevant to schizophrenia, including deficits in prepulse inhibition, social interaction, and working memory. These effects were influenced by the timing of immune activation and were partly dependent on the offspring's age. The infection-mediated behavioral and cognitive abnormalities were associated with impairments in the expression of pre- and postsynaptic proteins. Prenatal immune activation also caused a region- and age-dependent increase in brain inflammatory cytokines and chemokines (IL-1β, IL-5, IL-6, TNF-α, CXCL1) and led to a reduction in TSPO protein levels in various cell types, including endothelial cells, astrocytes, and microglia. In contrast, there were no overt signs of altered microglia activity in pubescent or adult offspring of immune-challenged mothers.
Conclusions: Using well-characterized developmental immune activation models with relevance to schizophrenia, our data suggest that maturation-dependent synaptic deficits, behavioral abnormalities, and increased inflammatory cytokine expression can occur in the absence of overt microglia anomalies. These findings thus challenge the hypothesis that schizophrenia-relevant impairments in synaptic and behavioral development require persistent changes in microglia activity. Furthermore, our findings of reduced TSPO expression indicate that its use as a possible marker of microglia activity and neuroinflammation may be inadequate under mild inflammatory conditions that lack clear microgliosis.
Disclosure: Nothing to Disclose.

Panel
59. Probing Human Neurological Disorders Through Genetic Manipulation in the Rodent: From Mechanisms to Therapeutic Targets

59.1 NRG3 Regulates Glutamate Release

Lin Mei

Augusta University, Augusta, Georgia, United States

Background: Neuregulin 3 (NRG3), a member of the neuregulin family, is associated schizophrenia in a variety of populations. Its SNPs are associated with psychotic symptoms and attention deficits in schizophrenic patients. NRG3 has also been suggested as a candidate gene for ADHD, bipolar disorder, Alzheimer's disease, and nicotine dependence. Although NRG3 is known to be specifically expressed in the brain, its physiological function remains unclear.
Methods: To study the function of Nrg3 in the brain, we generated mutant mice lacking Nrg3 specifically in neurons in the brain. Neurotransmission and morphology of the mutant mice were compared with those of control littermates.
Results: We found that Nrg3 mutation in neurons had no apparent effect on global cortical lamination or spine numbers. However, it enhances EPSC frequency, suggesting a role of Nrg3 in controlling glutamate release. This effect appeared to be independent of ErbB4, a tyrosine kinase that is believed to be a receptor of Nrg3. Mechanistic studies indicate that NRG3 is a presynaptic protein and its mutation led to increased probability of synaptic vesicle release.
Conclusions: These results identify a novel function of NRG3 in regulating glutamate release and provide insight into pathophysiological mechanisms of NRG3 mutation in schizophrenia.
Disclosure: Nothing to Disclose.

59.2 Investigating the Temporal Regulation of the NRG3-ErbB4 Pathway in the Developing and Aging Mouse Brain

Clare Paterson

University of Colorado School of Medicine, Aurora, Colorado, United States

Background: The genes encoding the growth factor Neuregulin 3 (NRG3) and its receptor tyrosine kinase ErbB4 have been associated with a wide range of neurodevelopmental and neurocognitive disorders. Neuregulin-ErbB4 signaling has been demonstrated as an essential modulator of multiple neurodevelopmental processes such as cortical plate development and neuronal migration, as well as postnatal processes including myelination, neuromodulation and synaptic plasticity. These data suggest a key role for NRG3-ErbB4 signaling in mediating normal brain function, consistent with the observations that mice with alterations in NRG3 or ErbB4 display abnormal brain development and altered cognitive function. However, the patterns of gene expression of NRG3 and ErbB4, and their alternative transcripts, across mouse brain development and aging are unknown. To address this, we have performed quantitative profiling of the expression trajectories of multiple components of the NRG3-ErbB4 pathway in mouse prefrontal cortex (PFC) throughout the lifespan.
Methods: PFC tissue was collected from male C57BL/6 mice at 27 time points from postnatal day (PND) 0 through to two years of age (total $n = 92$). Following total RNA extraction and cDNA synthesis, real time quantitative PCR was performed on the tissue samples to examine the expression levels of NRG3 and ErbB4 splice isoforms JMa, JMb, CYT1 and CYT2, and normalized to the expression of two housekeeping genes whose expression did not change over the lifespan.

Results: NRG3 was abundantly expressed in the mouse PFC at all time points examined, its expression positively correlated with age ($p<0.001$) with maximal levels of expression achieved by PND 35 and remaining at that level until 2 years of age, consistent with the timing of peak myelination. ErbB4 expression profiles were isoform-specific, with JMb and CYT2 gradually increasing in expression across the lifespan, being positively correlated with age (both $p<0.001$). Conversely, JMa and CYT1 displayed expression trajectories indicative of neurodevelopmental roles, with expression of both isoforms negatively correlating with age (both $p<0.001$), being most highly expressed prior to PND 10.
Conclusions: These results provide novel evidence that NRG3 and ErbB4 expression is developmentally regulated in the mouse PFC, and that ErbB4 isoforms display non-overlapping expression trajectories. Moreover, these data further support NRG3-ErbB4 signaling in playing an essential role at multiple critical windows of cortical development and maintenance. Additionally, expression profiles of other components of the NRG3-ErbB4 pathway, including splicing factors will be presented, providing mechanistic insight into the developmental regulation of the pathway. Overall, our data, by providing a map of how NRG3-ErbB4 exists in the normal brain across multiple critical developmental periods, will serve as a tool to aid investigators developing transgenic approaches to manipulate these genes. By identifying specific time periods of significance in NRG3-ErbB4 signaling in cortical development we hope to leverage investigations of the pathophysiological involvement of the NRG3-ErbB4 pathway in complex brain disorders.
Disclosure: Nothing to Disclose.

59.3 Mechanisms Underlying Synaptic and Dendritic Alterations in the 16p11.2 Duplication Mouse Model

Peter Penzes

Northwestern University, Chicago, Illinois, United States

Background: Alterations in dendrites and synapses occur in psychiatric disorders, including schizophrenia and autism. These disorders have complex genetic architecture partly explained by rare variants with higher penetrance. The most well characterized forms of rare variations are large genomic regional duplications or deletions known as copy number variations (CNVs), which confer significant risk (OR = 3-30). However, understanding the relationship between genotype and phenotypes and the rational identification of potential targets for reversing pathologically-relevant phenotypes in CNV disorders has been challenging. Recently, much attention has been paid to recurrent microduplication at the 16p11.2 locus which encompasses 29 known protein-coding genes. 16p11.2 CNVs have been linked to schizophrenia, bipolar disorder, and autism. Mouse models of 16p11.2 deletion and duplication have been generated. Here we examined cortical pyramidal neurons from primary cultures from mice heterozygous for the 16p11.2-orthologous duplication (dp/+) and their wild type littermates. The complexity of the genetic architecture of psychiatric disorders poses a serious challenge for the identification of experimentally approachable mechanisms. While causation may be complex and experimentally difficult to approach, we reasoned that reversal may be more accessible experimentally. It has been hypothesized that disease phenotypes are a consequence of altered gene pathways or networks, and the optimal therapeutic targets should be nodes that are highly influential to the function of the entire network. Because use of network-based approaches have been speculated to be able to help to identify major drivers of pathogenesis or candidate therapeutic targets, we further reasoned that use of network analysis might provide an approach to reduce complexity and identify salient mechanisms that can be harnessed therapeutically.
Methods: Dp/+ mice were purchased from JAX. Dissociated cultures of primary cortical neurons were generated according to Neurons expressing GFP were fixed and imaged by confocal and structured illumination microscopy (SIM) as in Smith et al, (2014). For protein-protein interaction network analysis, lists of the proteins encoded by genes within five rare schizophrenia-linked CNVs (1q21.1, NRXN1, 15q13.2, 16p11.2, and 22q11.2) were assembled using DAVID, PINA2, and Cytoscape. iPSCs were generated by Rutgers University DNA and Cell Repository (RUCDR) from lymphocytes and differentiated into glutamatergic neurons.
Results: We identified potential candidate drivers of phenotype reversal in dp/+ mice, and followed up by experimental validation of the hypothesized mechanisms. Dp/+ neurons showed significantly increased dendritic branching (Sholl analysis $p< 0.05$ - 0.001). Network analysis identified MAPK3, encoding Erk1 MAP kinase, as the most topologically important node in the PPI network related to SZ-associated CNV genes, including 16p11.2. Pharmacological inhibition of Erk signaling resulted in a reversal of dendritic alterations ($p< 0.05$), suggesting potential approaches for treatment. In addition, we have generated and analyzed human iPSC-derived neurons from patients with schizophrenia with 16p11.2 duplication and normal controls and compared them with mouse neurons.
Conclusions: Taken together, our data indicate that pharmacological targeting of a topologically central node in psychiatric disease networks can drive the reversal of cellular phenotypes relevant for these disorders, and outline a general strategy for the analysis and reversal of microphenotypes in CNV-related disorders.
Disclosure: Nothing to Disclose.

59.4 Genetic Targeting of the Mammalian Target of Rapamycin (mTOR) Impairs Learning and Memory and Cognitive Processes Relevant to Neurological Disease

Amanda Law

University of Colorado School of Medicine, Aurora, Colorado, United States

Background: The mammalian target of rapamycin (mTOR; 1p36.22) is an essential cellular serine/threonine kinase that acts as a molecular integrator of nutrient, energy, and growth factor signaling, mediated via the PIK3/AKT pathway. mTOR signaling plays essential roles in the development, assembly, maturation and maintenance of cortical circuitry. It is therefore not surprising that mTOR dysfunction has been implicated in the biology of several neurodevelopmental and cognitive disorders, including autism, schizophrenia, epilepsy and post-traumatic stress disorder (PTSD).

Rodent studies of Rapamycin, a potent pharmacological mTOR antagonist, suggest a key role for the protein in learning, memory and synaptic plasticity; with mTOR recently been proposed as a pharmacological target for the treatment of autism and acquired anxiety-like disorders, such as PTSD. Emerging data suggest that antipsychotic drugs, in contrast, increase mTOR activity, a proposed mechanism of their therapeutic action. These data suggest a critical and complex role for mTOR in disorders of the brain. An obvious limitation of existing rodent studies is that they are primarily based on pharmacological inhibition mTOR and data is absent on the genetic and developmental role of mTOR in the brain. Here we sought to examine the neurobiological consequences of genetic deletion of mTOR using a novel floxed, mouse model of mTOR deficiency and assessment of murine behavior profiles relevant to schizophrenia, autism and disorders of learning and memory.
Methods: mTOR male heterozygous mice [B6.Mtortm1.2-Kozfl/CMV-CRE]; $N = 16$-24) were compared to WT littermates ($N = 12$-15). Mice were tested in a comprehensive battery of tasks, including general health, locomotor activity, PPI, temporal order object recognition memory, contextual and cued fear conditioning and fear extinction learning.
Results: mTOR mutant mice demonstrated significantly impaired recency discrimination memory ($p < 0.001$) and profound deficits of context and associative learning, manifest in impairment in acquisition and consolidation of contextual ($p < 0.05$) and cued fear learning ($p < 0.01$) and increased fear extinction ($p = 0.005$). mTOR deficiency, did not cause abnormalities of PPI, locomotor activity in open field or anxiety like behaviors, suggestive of a highly selective role for mTOR in learning and memory.
Conclusions: Our data provide novel genetic evidence in support of a critical role for mTOR in learning and memory, and show that its deletion impacts behavioral profiles relevant to several forms of psychopathology and intellectual disability. Mechanistic investigation of the impact of mTOR genetic deletion on neuronal development and synaptogenesis will be discussed. Overall, these findings suggest that decreased mTOR activity leads to impairments in learning and memory, potentially via altered synaptogenesis (and reminiscent of phenotypes observed in schizophrenia), but also support the tender that inhibition of mTOR may be a useful strategy for treating acquired-anxiety disorders, such as PTSD, mediated via its profound impact on disrupting the acquisition and consolidation of learned memory.
Disclosure: Part 1: Astrazeneca, consultancy.

Mini Panel
60. Understanding Resilience: From Maltreatment to Molecules

60.1 Noradrenergic Control of Resilience to Chronic Stress

Bruno Giros

McGill University, Montreal, Canada

Background: Dopamine (DA) neurons in the ventral tegmental area (VTA) have been shown to play a key role in controlling stress susceptibility and resilience. However, upstream mechanisms responsible for the functional control of these neurons remain unknown. Noradrenergic (NE) neurons in the locus cœruleus, implicated in the pathophysiology of depression, have direct anatomical and functional connections within the VTA.
Methods: We investigated the role of these NE neurons using conditional knockout of the Vesicular Monoamine Transporter type-2 (VMAT2). VMAT2lox/lox mice were crossed with DBH (Dopamine Beta Hydroxylase) cre mice, to generate a specific removal of VMAT2 only in NE neurons; without VMAT2, these NE neurons are not able anymore to accumulate, and therefore release, NE into synaptic space. To generate a "mirror" model, we bi-laterally injected a virus expressing the Channel Rhodosin 2 (ChR2) in the locus Coeruleus of DBHcre transgenic mice. Laser-optical stimulation in the VTA can triggers a direct circuit-specific NE release in these mice.
Results: We investigated the role of these NE neurons in regulating susceptibility to chronic social defeat (CSD) versus resilience via inhibitory control of VTA-DA neurons. Whereas the genetic absence of NE release totally suppressed resiliency, the pharmacological or optogenetical stimulation of NE release in the VTA could promote resilience in vulnerable mice.
Conclusions: We therefore characterized a new neural circuit, from the LC to the VTA, that underlies resilience against chronic emotional stress, providing a rationale for the use of NE releaser in depression or PTSD and future direction to develop therapeutic treatments.
Disclosure: Nothing to Disclose.

60.2 Genome-Wide Association Study of Positive Emotion Identifies a Genetic Variant and a Role for MicroRNAs

Aliza Wingo

Emory University School of Medicine, Decatur, Georgia, United States

Background: Resilience refers to capacity to cope adaptively with stress and trauma and is conceptualized as a multidimensional construct with factors like hardiness, optimism, faith/spirituality, adaptive coping styles, cognitive flexibility, and positive emotion. As an element of resilience, positive emotion facilitates adaptive coping strategies, counteracts physiological effects of negative emotion, buffers against risk for psychiatric symptoms, and fuels thriving. With a heritability of $\leq 64\%$, positive affect is substantially influenced by genetic factors; however, our understanding of genetic pathways underlying individual differences in positive affect is still very limited.
Methods: We here perform a genome-wide association study (GWAS) of positive emotion in 2522 inner-city African American participants and replicate our finding in the Center for Health Discovery and Well Being (CHDWB) cohort. Positive emotion is assessed with the Positive and Negative Affect Schedule and represented by the total score of the 10-item scale for positive emotion (i.e. feeling interested, excited, strong, enthusiastic, proud, inspired, determined, attentive, and active). We next investigate

functional significance of the identified genetic variants using brain and blood gene expression data and behavioral and fMRI imaging data.
Results: The GWAS identifies two single nucleotide polymorphisms (SNPs) significantly associated with positive affect after gender, childhood maltreatment, and principal components (PCs) are adjusted for: rs322931 ($p = 2.59$x10-8) and rs7550394 ($p = 3.84$x10-8). These SNPs reside on chromosome 1 and are in high linkage disequilibrium with each other (D' = 0.988; r2 = 0.844). The minor allele is associated with more positive affect for both. Consistently, the minor allele of rs322931 is associated with having more spiritual wellbeing in the CHDWB cohort after gender and PCs are adjusted for ($p = 0.044$). In follow-up studies we show that rs322931 is a cis-eQTL for microRNAs miR-181a and miR-181b in the brain (Bonferroni adjusted $p = 1.04$x10-4) and influences expression of miR-181b in peripheral blood (Bonferroni adjusted $p = 0.0363$). Further, the minor allele of rs322931 is associated with greater fMRI activation of the nucleus accumbens (NAc) and amygdala to positive vs. neutral stimuli. No group differences are observed in the NAc and amygdala for negative vs. neutral stimuli. Lastly, participants with at least one minor allele for rs322931 have less fear when exposed to the safety cue than those without ($p = 0.025$).
Conclusions: Prior studies have demonstrated that miR-181a/b mediate synaptic plasticity and immune system functioning, as well as participating in the reward neurocircuitry. Our findings suggest that rs322931 mediates positive affect potentially via miR-181 and the nucleus accumbens. Taken together, we identify a novel genetic variant for positive emotion for further study that may also influence synaptic plasticity and immune functioning, consistent with the psychosomatic interplay among affect, neuroplasticity, and physical health observed in epidemiological studies.
Disclosure: Nothing to Disclose.

60.3 Predictors of Resilience: Findings From Army Starrs

Murray Stein

University of California, San Diego, La Jolla, California, United States

Background: There is great interest in factors that can promote resilience to the effects of stress. Nowhere is this need more acute than in the military, where traumatic stressors – many of which are associated with deployment (combat) – have a profound impact on the health of warfighters. The Army Study to Assess Risk and Resilience in Servicemembers (Army STARRS) is a unique series of studies designed to investigate factors – historical, psychosocial, medical, experiential, and genomic – that contribute to mental health and illness within the US Army.
Methods: The New Soldier Study (NSS) of Army STARRS surveyed approximately 38,000 US Army recruits during Basic Combat Training. The computerized survey included assessment of childhood maltreatment, lifetime mental disorders, personality dimensions, and many other psychosocial domains. The Pre-Post Deployment Study (PPDS) of Army STARRS surveyed approximately 8,000 soldiers within 3 Brigade Combat Teams 4-6 weeks prior to their deployment to Afghanistan (T0), and then prospectively reassessed them upon their redeployment to the US (T1), two months later (T2), and 6 months later (T3). Both studies collected DNA, in which over 80% of soldiers participated. Genomewide association analysis (GWAS) was conducted using standard methods and related to (1) a 5-item measure of self-reported resilience in a meta-analysis across studies, and (2) prospective resilience as defined by good mental health outcomes post-deployment in PPDS.
Results: Childhood maltreatment was found to be a robust experiential predictor of low self-reported resilience. Using GCTA to estimate SNP-based heritability, self-reported resilience was found to be significantly heritable (h**2 = 0.135, se = 0.030, $p = 2.02$e-7) in the European American (EA) sample ($N = 11{,}492$). A meta-analysis across cohorts of GWAS results from the EA subjects revealed a genomewide significant locus (4 SNPs in LD; top SNP: rs4260523, $p = 5.654$e-09) on Chr 4 just upstream from DCLK2 (Doublecortin-Like Kinase 2), a gene known to be involved in hippocampal organization. DCLK2 was not significantly associated with prospective resilience in the EA subjects in PPDS, whereas genomewide significant evidence of association was found for a locus on Chr 12 in SLC15A5 (top SNP: rs12580015) in the group exposed to the highest level of deployment stress. Looking at SLC15A5 for self-reported resilience in the EA subjects in NSS, nominally significant association was detected (top SNP: rs1671483, $p = 0.00248$).
Conclusions: This study of US Army soldiers revealed experiential (e.g., childhood maltreatment) and genetic contributions to self-reported resilience. Genomewide significant association with self-reported resilience was found in meta-analysis of EA soldiers for the gene DCLK2. DCX – an important paralog of this gene – is widely involved in neuronal development, and mutations are known to case human disease involving epilepsy and mental retardation. Genomewide significant association for SLC15A5 with resilience to high deployment stress was found prospectively in PPDS. These findings illustrate the promise of being able to use increasingly available large, representative and prospective datasets to understand environmental and genetic pathways to resilience.
Disclosure: Part 1: Resilience Therapeutics, Consultant, Oxeia Biopharmaceuticals, Consultant, Janssen Pharmaceuticals, Clinical Trial Site, Actelion Pharmaceuticals, Consultant, **Part 2:** Resilience Therapeutics, Consultant.

Mini Panel
61. Neural Mechanisms Underlying Aberrant Cognition and Emotion in Pediatric Psychopathology

61.1 Prefrontal Cortical Substrate for Performance Monitoring in Pediatric Obsessive- Compulsive Disorder: Atypical Development and Implications for Treatment Response

Kate Fitzgerald

University of Michigan, Ann Arbor, Michigan, United States

Background: Performance monitoring is a cognitive control function that is abnormal in many pediatric psycho-

pathologies, as evidenced by either hyper- or hypo-activation of dorsal anterior cingulate cortex (dACC) in response to errors and conflict. Hyper-activation of dACC has been noted in pediatric obsessive-compulsive disorder (OCD), but how the functioning of cognitive control circuits changes over development and is affected by medication in young patients remains poorly understood. Thus, functional MRI was used to examine the developing neural substrate for cognitive control in unmedicated and medicated patients with pediatric OCD, compared to healthy youth, across a range of ages and illness severity.

Methods: Performance monitoring functions, including error- and interference-processing, were elicited by the Multisource Interference Task in 69 patients with pediatric OCD (half unmedicated, half medicated) and 72 healthy controls (HC), ages 8-19 years, during fMRI scanning. Contrasts for error- and interference- processing were generated in SPM8, revealing large clusters of activation spanning dACC into pre-supplementary motor area (SMA). Bilateral anterior insula (aI) was a secondary region of interest, given the importance of the salience subnetwork associated with cognitive control (dACC-aI) in detecting salient events (e.g., errors, interference) to mediate ongoing performance. Contrast estimates from these volumes were entered into linear regression models testing effects of group (unmedicated OCD, uOCD; medicated OCD, mOCD; HC), age, performance, and interactions. In patients, the effect of OCD severity on performance monitoring function in dACC and bilateral aI activation was also assessed.

Results: Developmental differences in dACC activation to errors were demonstrated for uOCD (but not mOCD) relative to healthy controls, based on an age-by-uOCD interaction ($b = .43 \pm .19$, $p = .03$). The interaction was driven by increases in dACC activation with age in uOCD relative to decreases in healthy and mOCD participants. In addition, greater dACC activation was associated with faster RTs in healthy and mOCD groups, but not in uOCD patients ($b = .006 \pm .002$, $p = .01$). Controlling for age and performance, greater activation of the dACC ($b = 1.69 \pm .55$, $p = .003$) and left aI ($b = 1.91 \pm .53$, $p = .001$) to errors was observed for mOCD (but not uOCD) compared to healthy youth. Among uOCD patients, greater error-related dACC activation ($b = -.11 \pm .05$, $p = .02$) and bilateral aI activation right aI to errors ($-.06 \pm .03$, $p = .05$) and left aI to interference ($-.03 \pm .01$, $p = .01$) was associated with lower OCD severity.

Conclusions: Compared to healthy youth, unmedicated patients exhibited steeper age-related increase in dACC response to errors. Greater error-related dACC and aI response was associated with both medication status and lower symptom severity in patients. These findings suggest that engagement of the salience network during performance monitoring may occur with maturation and medication to reduce symptom severity in OCD. Developmentally-mediated and medication-related increases in the engagement of this sub-network associated with cognitive control suggests a potential target for novel treatment strategies for pediatric OCD and anxiety in youth.

Disclosure: Nothing to Disclose.

61.2 Deficient Activation of Fronto-Striatal Circuits: A Developmental Biomarker for Bulimia Nervosa

Rachel Marsh

Columbia University Medical Center, New York, New York, United States

Background: Functional abnormalities within fronto-striatal circuits likely underlie the cognitive control deficits that characterize many pediatric psychopathologies. This presentation will describe new machine learning and longitudinal findings showing that deficient activation of these circuits during the resolution of cognitive conflict can classify adolescents with Bulimia Nervosa (BN) early in the course of the illness and contribute to the persistence of BN symptoms into early adulthood.

Methods: Functional MRI data were acquired at baseline from 34 adolescents with BN (16.41 +/- 1.5 years) and 32 age- and BMI-matched healthy control (HC) adolescents (16.13 +/- 2 years) during their performance of the Simon Spatial Incompatibility Task. Follow-up data were then acquired from both groups within 2-year intervals at two follow-up visits over adolescence. First, multi-voxel pattern analyses were used to predict diagnostic classification (BN, HC) based on conflict-related fronto-striatal activations detected in an older, more symptomatic sample of 18 BN adolescents (18.4+/-2.2 years) versus 18 matched HCs. Latent growth curve modeling was then used to assess changes in fronto-striatal activations over time in the younger, longitudinal sample of BN and HC adolescents.

Results: Compared to HCs, BN adolescents in the younger sample displayed reduced activation of bilateral inferior and superior frontal gyri and right putamen during conflict resolution ($p < 0.05$, corrected). Clinical status was accurately classified from these activation patterns; group-specific patterns detected in the older sample accurately classified adolescents as BN or HC in the younger sample (maximal accuracy detected in left inferior frontal gyrus (IFG): 61% accuracy, $p < 0.001$, 59% sensitivity, 66% specificity). Thirty percent of the BNs in the younger sample achieved remission over adolescence, with less than 4 binge-eating and purging episodes in the month prior to their last study visit. Growth curve modeling revealed group x time interactions in fronto-striatal regions ($p < 0.05$), with conflict-related activations increasing over adolescence in the BN group (bilateral anterior cingulate, $b = 0.08$; left caudate, $b = 0.10$; p's < 0.04). Activation of left IFG also increased in the BN group ($b = 0.04$, $p = 0.04$), an effect driven by the BN adolescents who remitted over time (remitters: $b = 0.08$, $p < .001$; non-remitters: $b = 0.01$, $p < .067$).

Conclusions: These new findings suggest that deficient activation of fronto-striatal circuits can be identified early in BN, when binge-eating and purging behaviors are less severe. In addition, the trajectory of functional development within cognitive control circuits seems to normalize with the remission of BN symptoms over adolescence. Thus disturbances in these circuits may constitute a useful biomarker for BN and a tool for understanding its developmental trajectory as well as the design of novel treatments and early interventions.

Disclosure: Nothing to Disclose.

61.3 Neurobehavioral Markers of Aberrant Approach Motivation in Pediatric Depression

Manpreet Singh

Stanford University School of Medicine, Stanford, California, United States

Background: Aberrant functioning of reward circuits likely underlies the deficits in approach motivation described across various pediatric psychopathologies. This presentation will describe new cross-sectional and longitudinal findings showing that dysfunctional activation of reward circuitry during approach motivation characterizes youth with obesity and depressive symptoms across a spectrum of mood diagnoses (unipolar and bipolar) early in their course and contributes to the worsening of depressive symptoms.
Methods: Functional MRI data were acquired at baseline and at 6-month follow-up from 21 children and adolescents 9-17 years of age with significant depressive symptoms while processing food and monetary rewards, and were compared to 14 age matched healthy controls. Baseline and follow up analyses combined youth samples across mood diagnoses, and evaluated dimensional levels of depression symptoms severity using the clinician administered Children's Depression Rating Scale-Revised (CDRS-R) to the parents and youth. We calculated each child's BMI Z score as a well-validated proxy marker of impaired insulin sensitivity and a standardized measure for overweight and obesity that accounts for sex and age norms in children.
Results: Depressed youth with higher compared to lower baseline BMI Z scores showed a trend for increasing nucleus accumbens (NAcc) activation during reward anticipation over time ($r = 0.69$, $p = 0.08$). Further, increasing NAcc activation was associated with greater changes in depression severity over time ($r = 0.54$, $p = 0.212$). A change in BMI Z scores from baseline to follow-up was more strongly associated with increasing NAcc activation over time in depressed ($r = 0.69$, $p = 0.089$) compared to healthy ($r = 0.47$, $p = 0.091$; p-value for interaction term $p = 0.01$) youth; $p = 0.015$ for the overall model including group status, BMI, and their interaction. Strikingly, this model explained 45% of the variance in the increase in NAcc activation (Figure). We conducted a psychophysiological interaction (PPI) analysis to examine network connectivity during reward anticipation by seeding the NAcc at both baseline and follow-up fMRI scans. Higher baseline BMI scores were associated with a trend for increasing NAcc-amygdala connectivity ($r = 0.49$, $p = 0.26$). Further, a change in BMI Z scores from baseline to follow-up was associated with a trend for greater decreases in connectivity between NAcc and amygdala over time in depressed ($r = 0.64$, $p < 0.01$) compared to healthy ($r = 0.11$, $p =$ ns; p-value for interaction $= 0.06$) youth.
Conclusions: These new findings suggest that aberrant nucleus accumbens activation and connectivity can be identified early in the development of depressive symptoms among youth who may be at risk for developing insulin resistance. In addition, the trajectory of functional development within the NAcc may reflect downregulation of D2 receptors leading to decreased reward sensitivity among youth with depressive symptoms, or suboptimal modulation of the affective or emotional aspects of a reward value or an associated cue's motivational salience. This functional disturbance may constitute a useful biomarker for depression and a tool for understanding its developmental trajectory as well as the design of novel treatments and early interventions for the commonly co-occurring conditions of depression and obesity in youth.
Disclosure: Nothing to Disclose.