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Panels, Mini-Panels and Study Groups
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Panel
1. The Molecular Pathology and Dynamics of Spine Loss in Schizophrenia

1.1 Selective Loss of Small Spines in the Auditory Cortex of Schizophrenia

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Background: Dendritic structural features, such as spine density and volume, are dynamically regulated by glutamate signaling to the actin cytoskeleton. Spine growth and retraction result from enhanced polymerization and depolymerization of F-actin. Decreased spine density has been observed in layer 3 of multiple cortical areas in schizophrenia while genetic and pharmacological studies support a role for glutamate signaling to F-actin in disease pathology. However, the mechanics by which spine signaling is impaired and the effect this impairment has on homeostasis of spine formation and retraction is currently unknown. To begin to address this question in human postmortem brain tissue we have utilized orthogonal approaches of confocal microscopy and targeted mass spectrometry to investigate spine density, volume, and protein expression.
Methods: Primary auditory cortex tissue from 20 pairs of SCZ and matched control subjects, in which we have previously evaluated the expression of 155 synaptic proteins, were utilized. Dendritic spines were identified by co-localization of spinophilin and F-actin (defined by binding of phalloidin). This approach is innovative as it allows for an estimate of spine F-actin content and volume in human postmortem tissue.
Results: In line with previous reports we observed a 20% decrease in auditory cortex deep layer 3 spine density ($p = 0.009$). Total F-actin per spine object was increased in disease ($p = 0.03$), but mean F-actin per spine was unchanged, indicating that the assay's ability to measure F-actin signal was unaltered between disease and controls. To gain a more detailed picture of spine alterations in schizophrenia we used F-actin as a measure of spine volume and then calculated the density of spines of different volumes, as defined by bins with 0.15 μm3 increments. Significant decreases in spine density were limited to spines with the smallest volumes ($p = 0.01$). We recently reported the altered expression of glutamate signaling proteins including the AMPA receptor subunits GRIA3 and GIRA4 as well as GNAQ, a modulator of mGLUR5 signaling, in the primary auditory cortex of schizophrenia subjects. Interestingly, only the expression of GRIA3 was correlated with spine density ($r = 0.4$, $p = 0.029$).
Conclusions: We have identified a specific sub-population of spines that are lost in schizophrenia. Two-photon *in vivo* imaging studies reveal that smaller spines are often transient, emerging from and retreating into the dendritic shaft over a period of a few hours if a new synaptic connection is not formed. Thus, this constantly churning population of young spines serves as the substrate for new learning and memory. Our findings show that the density of small, presumptive transient spines is decreased in schizophrenia and that affected cortical areas may be less capable of forming new synaptic connections, impairing cognitive function. However, the dynamics and molecular pathology of this loss is still unknown. A decrease in either the rate at which small/new spines emerge or the duration of their existence could both lead to a decreased total number. Future studies using time lapse and *in vivo* imaging in genetic model culture and mouse systems will likely shed light on this question. These findings further implicate glutamate signaling in spine pathology and support the continuing investigation of compounds that act on glutamate receptors, such as mGLUR5 positive allosteric modulators.
Disclosures: Nothing to Disclose.

1.2 Cellular Functions of Schizophrenia-Enriched Kalirin Mutations

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Background: Our laboratory has been investigating the signal transduction pathways that regulate actin cytoskeletal remodeling in dendritic spines and their impact on spine structure and function. One of the major regulators of spine plasticity is the protein kalirin, encoded by the KALRN gene. Several rare point mutations in human KALRN have recently been found to be enriched in subjects with schizophrenia versus controls. In addition, kalirin mRNA and protein levels are altered in several cortical regions in subjects with schizophrenia, as compared to controls.
Methods: We have employed primary neuronal and heterologous cell cultures, molecular and biochemical approaches, as well as advanced imaging methods including structured illumination microscopy (SIM), a recently-developed superresolution imaging method.
Results: In this symposium, I will present recent and unpublished data from our laboratory on the functional impact of such schizophrenia-enriched rare mutations in KALRN. We examined the effect of such mutation, in content of several kalirin isoforms, on actin regulation, dendrite and spine morphology, and glutamate receptor trafficking. Using SIM imaging, we find that different kalirin isoforms are localized to distinct microcompartments of the dendritic spine. Schizophrenia-enriched point mutations affect the molecular function and localization of individual kalirin isoforms.

Conclusions: These studies shed new light on the roles of protein isoforms and their schizophrenia-enriched mutations in the regulation of dendritic spine structure and plasticity.
Disclosures: Nothing to Disclose.

1.3 Morphological Brain Abnormalities in Serine Racemase Knockout Mice: A Genetic Model of NMDA Receptor Hypofunction

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Background: There are morphological brain changes in schizophrenia, including reductions in cortico-hippocampal gray matter and enlarged ventricles. As there is no neuronal loss in schizophrenia, the volumetric changes are believed to be due in part to reductions in neuronal dendritic complexity and spine density. There is substantial evidence that N-methyl-D-aspartate receptor (NMDAR) hypofunction is a core pathophysiological mechanism underlying schizophrenia, especially the cognitive impairments and negative symptoms. The largest schizophrenia GWAS to date recently identified genes involved in glutamatergic neurotransmission and the postsynaptic density with increased risk for schizophrenia, including serine racemase (SR), the enzyme that produces the NMDAR co-agonist D-serine. SR and D-serine are concentrated in corticolimbic regions of the brain. Genetic and biochemical findings suggest that serine racemase (SR) and D-serine are reduced in schizophrenia. Thus, our laboratory has used SR deficient (SR-/-) mice as a model to elucidate the molecular mechanisms responsible for dendritic atrophy as result of NMDAR hypofunction.
Methods: Adult (3-4 months old) male wild type (WT) and SR-/- mice were used for all studies. Golgi impregnation was used to examine the neuronal architecture and spine density of pyramidal neurons in the medial prefrontal cortex (mPFC) and primary somatosensory cortex (S1), as well as spine density of granule cells in the dentate gyrus (DG) of the hippocampus. Cortical and hippocampal brain volumes from WT and SR-/- were estimated from Nissl stained tissue sections. In vivo ventricular volumes of anesthetized WT and SR-/- mice were quantified using 9.4T magnetic resonance imaging. Molecular and biochemical approaches were used to quantify mRNA levels, protein levels, and promoter occupancy. For drug reversal studies, WT and SR-/- mice were systemically administered either vehicle or drug.
Results: In the mPFC and S1, pyramidal neurons of SR-/- mice displayed significant reductions in dendritic complexity and total dendritic length, as well as reduced spine density. Spine atrophy was also observed on granule cells in the DG of SR-/- mice. All of these neuropil changes in SR-/- mice were associated with reduced cortico-hippocampal volumes and increased ventricular volume. The dendritic abnormalities in SR-/- mice were paralleled by diminished occupancy of the transcription factor CREB to the promoter regions of BDNF, miR-132, and Arc, and therefore reduced expression. SR-/- mice also had reduced Akt/GS3K/mTOR signaling, all of which are regulated by NMDAR activity. Chronic administration of D-serine or a metabotropic glutamate receptor 5 (mGlu5) positive allosteric modulator (PAM) to adult SR-/- mice was able to rescue the structural, neurochemical, and cognitive deficits.
Conclusions: These data demonstrate that SR-/- mice recapitulate the morphological and neurochemical brain abnormalities observed in schizophrenia, which can be rescued by pharmacologic treatment. Furthermore, they provide a mechanism by which NMDAR hypofunction impairs spine formation through BDNF and Akt signaling. Future studies could utilize proteomics to allow for more detailed comparison of this model with schizophrenia, while *in vivo* imaging studies would provide information on the course and dynamics of spine pathology in these mice.
Disclosures: Nothing to Disclose.

1.4 Astrocytic Contributions in Synaptic and Behavioral Abnormalities of Fragile X Syndrome

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Background: Fragile X syndrome (FXS), the most common inherited form of human intellectual disability, is caused by mutations in the FMR1 gene. Like schizophrenia, FXS is a developmental disorder in which patients suffer from cognitive impairments and postmortem studies have identified alterations in spine density. Fmr1 global knock out (KO) mice share many behavioral phenotypes with human FXS patients: they exhibit deficiency in learning and memory, sensory processing, and social behaviors. They also exhibit an abnormally high number of morphologically immature spines along dendrites of neocortical neurons. Despite the prevailing neuron-centric view of brain function, many lines of evidence suggest that astrocytes are important contributors to developmental and degenerative neurological diseases.
Methods: To investigate the contributions of astrocytes to the progression of synaptic abnormalities and learning impairments associated with FXS, we generated astrocyte-specific Fmr1 KO mice. In this study, we used two-photon *in vivo* imaging to follow individual spines along dendrites of neurons in the motor cortex over time, and compared spine morphology and dynamics of WT, global and astrocyte-specific Fmr1 KOs. We also tested the motor-cortex associated skill learning in all these mice.
Results: We found that astrocyte-specific Fmr1 KO mice display impaired motor-skill learning during adulthood, which correlates with the lack of enhanced spine dynamics in the motor cortex that normally occurs in response to the acquisition of a fine motor skill. Live imaging also revealed increased spine formation in adolescent astrocyte-specific Fmr1 KO mice, which preceded the elevated spine density found in adulthood. Furthermore, the behavioral and synaptic phenotypes in astrocyte-specific Fmr1 KO mice recapitulated those observed in the global Fmr1 KO mice.
Conclusions: Our work reveals a significant contribution of astrocytes in FXS etiology. We are currently investigating other behavioral defects of astrocyte-specific Fmr1 KO

mice. We are also exploring the possibility of Fmr1 rescue in astrocytes *in vivo*. Finally, these findings demonstrate the utility of two-photon *in vivo* imaging to investigate the dynamics of pathological spine activity in a mouse model of a developmental neuropsychiatric disease with cognitive impairments.
Disclosures: Nothing to Disclose.

Panel
2. A Multi-Modality Imaging Approach for the Identification of Brain Biomarkers of Clinical Outcomes in Human Addiction

2.1 Using a Multi-Modal Neuroimaging Approach to Track Longitudinal Changes in Brain Function and Structure, and Associated Self-Control and Reward Valuation Functions in Cocaine Addiction

Rita Goldstein

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Background: Persistent deficits in self-control and reward valuation characterize drug addiction, marked by abnormalities in functional and structural integrity of the prefrontal cortex (PFC). In a series of imaging studies, and using a multimodal approach, we aimed to ascertain the impact on brain function and structure of a 6-month follow-up in initially treatment-seeking abstinent individuals with cocaine use disorders (iCUD). We explored functions that are commonly impacted in drug addiction, inclusive of drug-related inhibitory control and attention-bias towards drug-related stimuli as compared to other salient affective cues.
Methods: Initially abstinent treatment-seeking iCUD participated in a magnetic resonance imaging (MRI) study complemented by recordings of event-related potentials (ERPs). The MRI study acquired functional MRI (fMRI) blood-oxygen level dependent (BOLD) activations during performance of a salient drug Stroop-like task and structural T1-weighted images used for quantification of grey matter volumes in select regions. The ERPs were acquired separately while participants passively viewed pleasant, unpleasant, neutral and cocaine-related pictures. The late positive potentials (LPP) component of the ERP was scored to index motivated attention to these pictures. Procedures were performed at baseline and then again after 6-months; iCUD showed substantially reduced drug use during this period, with 57% maintaining complete abstinence.
Results: Compared to healthy controls, in iCUD at follow-up as compared to baseline we report: 1) higher (and more positively correlated) task fMRI BOLD responses in the dopaminergic midbrain and the mediodorsal nucleus of the thalamus; increases in the midbrain correlated with reduced simulated cocaine choice, indicating that heightened (and normalized) midbrain activations may be marking lower approach motivation for cocaine in this context. Enhanced self-control was suggested by a trend for the commonly hypoactive dorsal anterior cingulate cortex to increase response during the drug trials. 2) increased LPPs to the pleasant (vs. neutral) pictures and decreased LPPs to the drug (vs. pleasant) pictures. And 3) increased grey matter volumes in the left inferior frontal gyrus and the ventromedial PFC.
Conclusions: Results show that reduction of drug use (up to and including full abstinence) over a 6-months period is associated with normalization of function (subcortical and cortical responses to a salient cognitive task, and enhancements in scalp-recorded markers of salience of pleasant relative to that of drug stimuli) and structure (PFC grey matter volume). In addition to providing hope to abstaining drug addicted individuals, these results, which highlight brain plasticity, suggest that neuroimaging could be useful in sensitively tracking follow-up outcomes in drug addiction. By focusing on the utility of both MRI and ERP measures as biomarkers of important end-points (e.g., drug-mediated attention-bias and self-control), these studies also set the stage for a comparative effort to identify the best biomarkers to longitudinally track treatment course or efficacy in drug addiction and related conditions.
Disclosures: Nothing to Disclose.

2.2 Pet Imaging of the Kappa Opioid Receptor/Dynorphin System in Cocaine Abuse

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Background: Animal studies have shown that the kappa receptor/dynorphin opioid receptor system plays a crucial role in addiction, particularly stress-induced relapse. Human postmortem studies have shown that cocaine dependence is associated with an upregulation of kappa opioid receptors (KOR) and dynorphin, but these are limited with respect to correlating neurochemistry with behavior. Our goal in this study was to investigate changes in the KOR/dynorphin system in cocaine abuse using Positron Emission Tomography (PET) and the KOR-selective radiotracer [11C]GR103545 and a laboratory model of relapse. Our hypotheses was that cocaine exposure would increase endogenous signaling of dynorphin at the KOR, and that KOR availability would increase the vulnerability to stress-induced relapse.
Methods: Cocaine abusers were admitted to a research unit for a 28 days. They underwent a baseline scan with the KOR selective agonist radiotracer [11C]GR103545 after 7-8 days of inpatient monitored abstinence. Following this baseline scan, subjects performed cocaine self-administration sessions. These sessions began with a modified cold pressor test, which resulted in 3 minutes of pain/stress, followed by nine opportunities to choose cocaine versus an alternative reinforcer (money). A group of matched healthy controls underwent one set of PET scans to compare between group differences in KOR availability.
Subjects were given a 2 day break, followed by 3 days of binge smoked cocaine (300 mg/day, total of 900 mg) in the laboratory. Then the [11C]GR103545 PET scan was repeated following the cocaine binge to investigate the effect of endogenous dynorphin upregulation on [11C]GR103545 binding.

The outcome measure for the PET scans was the volume of distribution (VT) in the regions of interest, which included the striatum and its subdivisions, cortical regions including prefrontal cortex, and medial temporal structures. Between condition comparisons were made with a repeated measures ANOVA and the correlation were investigated with Spearman's rank-order correlation.
Results: The cocaine abusers were heavy, chronic users of smoked cocaine. There was a positive correlation between KOR binding in the striatum and stress-induced cocaine self-administration: higher values of VT were associated with more choices for cocaine following the cold pressor test (Spearman correlation coefficient $= 0.53$, $p = 0.03$). A significant decrease in [11C]GR103545 VT was detected in the striatum (-17.7% $\pm$ 2.3%) when comparing the baseline and post-binge scans, with similar decreases in other brain regions (-12.9% $\pm$ 5.1%). No difference in baseline VT in any of the regions of interest between the cocaine abusers and controls.
Conclusions: These results demonstrate that binge cocaine administration significantly upregulates endogenous dynorphin in the human brain, measured as displacement of the PET radiotracer [11C]GR103545. Additionally, high KOR availability correlates with greater choices to self-administer cocaine in the setting of pain/stress. Taken together, these findings indicate that KOR/dynorphin signaling is amplified in the setting of cocaine abuse, and plays a role in a laboratory model of relapse.
Disclosures: Nothing to Disclose.

2.3 Chronic Alcohol-related Brain Homeostatic and Stress Alterations and the Development of Biomarkers of Treatment and Relapse in Alcoholism

Rajita Sinha

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Background: Growing evidence indicates that chronic alcohol abuse results in multilevel peripheral and brain adaptations in stress and homeostatic pathways that significantly impact cognitive, affective, alcohol craving and reward processes. However, identification of such measures as biomarkers of alcohol relapse and treatment outcome has rarely been assessed.
Methods: Data from multimodal neuroimaging and human laboratory experiments combined with a prospective clinical outcomes design will be presented. Participants included treatment seeking alcohol dependent (AD) individuals who were abstinent for 4 weeks and engaged in inpatient treatment during separate laboratory and neuroimaging experiments. Healthy social drinking volunteers were included as control subjects. Multimodal neuroimaging using structural and functional magnetic resonance imaging (MRI and fMRI) assessing structural gray matter volume and functional neural responses to brief script-driven guided imagery of stress, alcohol cues and neutral relaxing states, and a laboratory experiment assessing autonomic and hypothalamic pituitary adrenal (HPA axis) basal states and responses to stress, alcohol cue and neutral states were conducted in both AD and controls. AD subjects were followed prospectively after inpatient discharge with repeated assessment of alcohol use outcomes over a 90 day period.
Results: Findings indicate hyperactive basal and neutral state autonomic and HPA axis measures (heart rate, cortisol, cortisol/ACT ratio), lower medial frontal brain volume and hyperactive neutral state ventromedial prefrontal (VmPFC) and blunted VmPFC response to stress and alcohol cue, with each predicting future relapse and treatment outcome. Remarkably, there was a significant association between the cortisol/ACTH ratio and VmPFC disruption with VmPFC changes accounting for 33% of the HPA axis disruption in abstinent patients. Using receiver operating characteristics (ROC) to assess future relapse versus abstinence outcome prediction accuracy, we found that VmPFC hyperactivity in neutral state showed the most optimal prediction characteristics across measures in sensitivity and specificity for alcohol relapse and outcome prediction.
Conclusions: These findings support both multimodal neuroimaging to assess addiction-related neuroadaptations in clinical samples and also further biomarker development to validate optimal biomarkers of alcohol relapse so as to improve treatment outcomes.
Disclosures: Supported by UL1-DE019586, R01-AA013892; PL1-DA24859

2.4 Neurophysiological and HPA Axis Measures of Systemic Dysregulation as Biomarkers of Treatment Outcome in Prescription Opiate Dependence

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Background: There is growing evidence for a model of addiction positing that neuroadaptations of the central nervous system (CNS) reward and stress response systems resulting from long-term opiate use render opioid-dependent patients at high risk for relapse. Theoretically, new set-points are established in the HPA axis and brain reward systems that may persist following withdrawal from habitual opiate use. These include: 1) heightened responses to drug-related stimuli; 2) dampened responses to natural rewards (e.g., food, sex); 3) disturbances in the normal homeostasis of the HPA axis; and 4) increased anhedonia and sleep disturbance. This model has not been well characterized in humans, however, and the temporal dynamics of dysregulation following opiate withdrawal are unknown. The current study is evaluating clinical measures hypothesized to mirror elements of allostatic dysregulation in patients who are dependent on prescription opioids with the following goals: 1) to examine the timecourse of re-regulation, and 2) to evaluate the prognostic value of these variables in the prediction of treatment outcomes.
Methods: Recently withdrawn prescription opiate-dependent patients in an upscale, supervised, drug-free, residential care facility are assessed on 1) prefrontal cortical and psychophysiological responses to images depicting a) drug cues and b) natural rewards; 2) diurnal cortisol, and 3) 12 days of sleep actigraphy and subjective measures of sleep, as well as self-reported mood, stress and craving.

Neuroimaging data were collected using functional near-infrared spectroscopy. All patients are assessed 2-3 weeks following initial withdrawal; and a subset of patients who stay in residential treatment for an additional 60-90 days are evaluated again 30 and 60 days after the initial assessment battery. All patients are followed a minimum of 90 days following discharge to ascertain abstinence/relapse using self-report and objective indices of relapse.
Results: Results will be presented linking neurophysiological, sleep and HPA axis measures to treatment outcome. Preliminary results from the cue reactivity paradigm among patients in 30-day residential treatment ($n = 33$) indicate heightened brain responses to prescription opiate pill cues among patients who relapsed to opiates ($n = 5$) relative to those who maintained abstinence ($n = 28$; $t = 2.34$, $p = 0.026$). Neural responses were in right prefrontal cortical areas identified in previous cross-sectional pilot studies to differentiate patients in early versus extended sobriety. Impaired response to natural reward cues, hedonic responses to drug cues, HPA axis dysregulation and sleep disturbances are being analyzed for their contribution to the heightened risk of relapse in the early, drug-free period following withdrawal.
Conclusions: Preliminary results suggest prefrontal cortical response to drug cues may indicate relative vulnerability to relapse in prescription opiate dependent patients. Further analyses will be used to identify the prognostic significance of these measures in combination, leading to more effective individualized treatment strategies. The assessments employed in this study may readily be adapted to a clinical environment, and could be explored as possible biomarkers in clinical efficacy trials.
Disclosures: Part 1: Owns stock in FNIR Devices, LLC. A company that manufactures and sells fNIR devices for research.

Panel
3. Functional Neurogenomics in Schizophrenia: Recent Accomplishments and Future Perspectives

3.1 Exploring 3-Dimensional Genome Architectures and Function in Normal and Diseased Human Brain

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Background: 3-dimensional chromosomal conformations regulate transcription by moving enhancers and regulatory elements into spatial proximity with target genes. However, there is very little information on 'chromosomal loopings', including promoter-enhancer physical interactions, in normal and diseased human brain. This is surprising given that genome-scale mappings of promoter-distal regulatory regions for a large variety of cell lines and tissues, including brain, have suggested that each transcription start site (TSS) could be targeted on average by five different enhancers.
Methods: We undertook deep and integrative analyses of spatial genome architectures across 100Kb-1Mb of sequence surrounding multiple neuronal genes implicated in the neurobiology and genetic risk architecture of schizophrenia. Chromosome conformation capture assays were conducted in postmortem prefrontal cortex, in neuronal cultures in the context of differentiation and activity-dependent regulation, and in the animal models for cognitive disease. Gene expression regulation by loop-bound sequences was assessed with TALE- and CRISPR/CAS based sequence-specific transcriptional activators and repressors.
Results: We identify activity-regulated long-range loopings bypassing up to hundreds of kilobases of linear sequence in the neuronal genome. Neuronal gene expression was altered when loop-bound intergenic and distal intronic cis-regulatory elements were targeted by designer transcription factors. 3D genome organization is partially conserved between human and mouse brain. A subset of loop-bound sequences matched to non-coding risk polymorphisms implicated in psychiatric GWAS.
Conclusions: Our integrative approach provides a roadmap to assign neurological function for a subset of the vast but largely unexplored non-coding sequences in the human genome. The study of 3-dimensional genome architecture and function, including chromosome conformation capture assays in normal and diseased postmortem brain tissue, are likely to provide important insights into the genetic risk architecture and 'genome pathophysiology' of schizophrenia and related disease.
Disclosures: Nothing to Disclose.

3.2 Searching for Potential Mechanisms of Schizophrenia Risk in the Human Brain

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Background: Genetic risk for schizophrenia and related brain disorders has begun to emerge through large genome-wide association studies (GWAS) in hundreds of thousands of unrelated individuals. However, the exact gene(s) and/or transcript(s) that are being regulated by these risk SNPs are largely uncharacterized due to the difficulty in obtaining expression and genotype data in large samples of post-mortem human tissue. Advances in RNA sequencing (RNA-seq) have further permitted flexible and largely unbiased characterization of high-resolution transcriptomes, but the incomplete annotation of the human brain transcriptome can potentially affect the ability to use existing tools that rely on complete gene structure information.
Methods: We have sequenced the transcriptomes of the dorsolateral prefrontal cortex (DLPFC) from 320 non-psychiatric controls across the lifespan at deep coverage, including 50 second trimester fetal samples, and 175 samples from patients with schizophrenia, and completely characterized their expression profiles across five complementary summarizations that capture elements of transcription – genes, exons, junctions, transcripts, and expressed regions. These samples have been further genotyped and imputed to the latest 1000 Genomes reference panel, defining genetic variation across the genome.

Results: Using expression changes across development and simulated data, we show that annotation-agnostic approaches like junction and expressed-region analysis may outperform gene-, exon- and transcript-based approaches when the annotation is incomplete. We further conducted global expression quantitative trait loci (eQTL) analyses across the five expression summarizations in the adult control samples (age $>$ 13, N = 237), and identify hundreds of thousands of expression features that associate with local genetic variation, including extensive genetic regulation of previously unannotated sequence. The eQTLs in junction-level data (N = 53,497 unique junctions annotated to 16,481 genes at FDR $<$ 0.01) showed the largest effect sizes (fold change per allele copy) and identified SNPs as eQTLs with the lowest minor allele frequencies (18.1% versus 23.1-24.2%). We lastly identified eQTLs to specific transcript elements in individual genes in over half of the genome-significant genetic variants for schizophrenia identified genome-wide association studies (GWAS), with a large subset directionally consistent in the brains of patients with schizophrenia, illuminating potential mechanisms of risk for many of these genetic variants.
Conclusions: Leveraging human postmortem brain data can fine map the functional effects of genetic risk variation for schizophrenia identified in large GWAS, and can identify novel targets for drug discovery and more focused biological assays.
Disclosures: Nothing to Disclose.

3.3 Differential Expression and Functional Analysis of MicroRNAs in Schizophrenia: Focus on miR-132

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Background: Epigenetic mechanisms, which encompass DNA methylation, histone modifications, and non-coding regulatory RNAs, coordinate transcriptomic programs in the brain. Recent research has shown that epigenetic mechanisms can contribute to gene expression changes that underlie neuronal connectivity and behavior. However, relatively little is known regarding the potential role of epigenetic mechanisms in psychiatric disorders. We hypothesize that dysregulation of epigenetic signaling pathways in schizophrenia could underlie gene expression changes in the brain, ultimately leading to synaptic plasticity deficits and behavioral abnormalities.
Methods: We used microarrays and Nanostring nCounter analysis to identify microRNAs and epigenetic enzymes that are dysregulated in the dorsolateral prefrontal cortex (dlPFC) of subjects with schizophrenia compared to matched controls. MicroRNAs and epigenetic enzymes of interest were further characterized by determining their expression patterns during neuronal development in mice. Viral-mediated overexpression of microRNAs was used to validate potential epigenetic enzyme targets *in vitro*.
Results: By large scale profiling of miRNAs in the dlPFC of multiple cohorts of human schizophrenic subjects, stringent analysis revealed that miR-132 is selectively downregulated. We found that miR-132 expression inversely correlates with that of its DNA methyltransferase target DNMT3a during development and in schizophrenia. MiR-132 has at least 34 additional bioinformatically predicted epigenetic enzyme targets. In fact, a previous study found that among 78 conserved microRNAs, miR-132 is statistically enriched for target genes with functional roles in chromatin remodeling. Expression analysis of predicted miR-132 epigenetic enzyme targets revealed that a histone methyltransferase EZH1 is upregulated in the dlPFC of two cohorts of schizophrenic subjects known to have miR-132 down-regulation. Both DNMT3a and EZH1 are regulated by miR-132 *in vitro*. Furthermore, miR-132 and EZH1 are discordantly dysregulated by early life stress, an environmental risk factor for schizophrenia.
Conclusions: Our findings show that miR-132 dysregulation in schizophrenia could contribute to changes in epigenetic regulatory networks, particularly though DNMT1 and EZH1. Ongoing studies aim to identify the functional consequences of DNMT1 and EZH1 dysregulation in the dlPFC, including identification of target genes, synaptic plasticity changes, and behavioral deficits. Exploring the identity and consequences of such micoRNA-epigenetic signaling pathways in schizophrenia may help to elucidate the etiology of this complex psychiatric disease, and potentially identify novel therapeutic targets.
Disclosures: Nothing to Disclose.

3.4 Transcriptome Alterations in DLPFC and Genetic Liability Contribute to Risk for Schizophrenia

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Background: The most recent Psychiatric Genomic Consortium GWAS in schizophrenia (SCZ) reported more than a hundred susceptibility loci, which are predominantly found in non-coding regions. Functional understanding of non-coding disease-associated loci is an important next step towards the development of testable hypotheses regarding biological processes that may be involved in the pathogenesis of SCZ. We have developed the CommonMind consortium to generate and analyze molecular data from human post-mortem brain samples including RNA sequencing and epigenome data. In this study, we combined a diversity of informative data (e.g. genomic; expression quantitative trait loci (eQTLs), cis-regulatory elements (CREs) annotations) to study the distribution of risk variants in gene coexpression networks.
Methods: High density eQTLs, differential expression and coexpression network analysis was conducted in 537 human post-mortem samples (258 SCZ samples and 279 controls) from the dorsolateral prefrontal cortex (DLPFC, BA9/46) as part of the CommonMind Consortium (CMC, http://commonmind.org). A variety of publicly available CRE annotations for promoters, enhancers or open chromatin (DNase hypersensitivity regions) were used. Furthermore, in a subset of cases and controls, we obtained cell type-specific (neuronal and glial) annotations for open chromatin.

Results: Differential expression was detected with 199 upregulated transcripts and 267 down-regulated transcripts in the DLPFC at an FDR of 5%. Prior SCZ genetic findings were significantly enriched among differentially expressed genes ($P = 0.01$). Gene coexpression analysis identified a neuronal subnetwork of $\sim$1400 genes subserving functions related to synaptic transmission in the DLPFC that is significantly perturbed in SCZ and is highly enriched for SCZ genetic signal ($P = 1.37 \times 10\text{-}04$). Certain SCZ risk loci are positioned within cis regulatory sequences and affect gene expression.
Conclusions: The analysis presented here has two fundamental goals, to describe differences in gene expression and the mechanisms that underlie genetic risk. Our findings point to a functional link between SCZ susceptibility loci and regulation of gene expression affecting transcripts clustered in specific subnetworks.
Disclosures: Nothing to Disclose.

Panel
4. Opportunities and Challenges for Buprenorphine in Treating Depression

4.1 Pharmacological Mechanisms Underlying the Antidepressant and Anxiolytic Effects of Buprenorphine

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Background: Buprenorphine (BPN) may be effective in alleviating symptoms in treatment-resistant depressed patients. Buprenorphine has mixed pharmacological effects at opioid receptors, acting as a partial agonist at mu (μ-ORs) and nociceptin receptors and an antagonist at kappa (κ-ORs) and delta receptors. Preclinical studies have shown that BPN produces behavioral effects on rodent tests that are also responsive to clinically effective antidepressant and anxiolytic drugs. Using the forced swim test (FST) and novelty-induced hypophagia (NIH) test, two behavioral tests sensitive to conventional antidepressant drugs, we probed the pharmacological mechanisms associated with the antidepressant and anxiolytic behavioral effects of BPN, respectively. In addition, chronic administration of buprenorphine was evaluated in two models of depressive behavior, chronic unpredictable mild stress and chronic social defeat.
Methods: C57BL/6J mice were tested in the FST and NIH test as described previously. The effects of buprenorphine were evaluated after pretreatment with opioid receptor antagonists or in mice with genetic deletion of opioid receptors. The effects of chronic buprenorphine (0.25 mg/kg for 7-10 days) were examined on deficits of sucrose preference and increased anxiety produced in mice exposed to unpredictable chronic mild stress (UCMS) for 21 days. The effect of chronic buprenorphine (0.25 mg/kg for 1-7 days) was also examined on deficits of social approach behavior induced by chronic social defeat, as previous described.
Results: BPN reduced immobility in the FST in C57BL/6J, similar to effects produced by selective κ-OR antagonists, such as nor-BNI (10 mg/kg). BPN did not reduced immobility in mice pretreated with nor-BNI or with genetic deletion of κ-ORs (Oprk1-/-), but was effective in mice with genetic deletion of either μ-ORs (Oprm1-/-) or delta opioid receptors. In the NIH test, BPN reduced approach latencies for peanut butter chips provided in a novel arena in wild-type mice. Oprm1-/- mice took twice as long to train to eat the chips in their home cage as Oprk1-/- and wild-type mice. The response to BPN in the novel arena of the NIH test was blocked in Oprm1-/- mice, but not altered in Oprk1-/- mice. Using UCMS to induce anhedonia and increased anxiety in mice, chronic administration of buprenorphine reversed deficits in sucrose preference and anxiety behavior induced by chronic stress. The behavioral response of stressed animals was associated with changes of κ-OR gene expression rather than μ-ORs. Chronic buprenorphine also reversed deficits in social approach and interaction induced in susceptible mice by exposure to chronic social defeat.
Conclusions: The pharmacological mechanisms associated with the behavioral effects of BPN in measures of affective behavior were test-dependent and involved both μ-ORs and κ-ORs. The reversal of anhedonia and anxiety behavior after chronic stress was associated with κ-ORs.
Disclosures: Nothing to Disclose.

4.2 Effects of Buprenorphine on Negative Affective Stimuli in Healthy Adults

Harriet de Wit

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Background: The opioid system has been implicated in both physical pain and psychological distress. Preclinical studies suggest that low doses of opioid drugs reduce isolation distress and increase play behavior, and clinically, opioid abusers report that these drugs reduce social stress and negative affect. The effects of opioid analgesic drugs on responses to negative affective stimuli have not been thoroughly studied in humans. Here we examined the effects of the μ-opioid partial agonist buprenorphine on three measures of social distress: 1) response to a public speaking task; 2) response to simulated social rejection, 3) attention to negative emotional facial expressions.
Methods: Healthy young adults attended laboratory sessions during which they received either placebo or 0.2mg sublingual buprenorphine, under double-blind conditions. Ninety minutes after drug administration, they completed a standardized public speaking stress task, a social exclusion task (Cyberball) or a task measuring attention to facial emotions using electrooculography.
Results: During the stress task, buprenorphine dampened the increase in cortisol during the task and reduced subjects' appraisal of how threatening the task was. During the Cyberball task, buprenorphine reduced participants' negative mood responses to rejection, and decreased their perception of the degree to which they were excluded. During the attention task, the drug reduced initial attention to fearful facial expressions, without influencing attention to angry, happy, and sad faces. Buprenorphine produced

these behavioral effects at doses that did not increase ratings of 'high' or 'like' drug.
Conclusions: These results suggest that low doses of buprenorphine may have stress-reducing effects in healthy adults. This provides further support for the role of the opioid system in mediating responses to social rejection and stress, and suggests a possible therapeutic role for this type of drug, at very low doses.
Disclosures: Nothing to Disclose.

4.3 Abuse Potential of Buprenorphine (BPN) in Humans under Varying Conditions

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Background: BPN is approved by the Food and Drug Administration for treating pain and opioid use disorders. Although it is effective in treating both disorders and is generally considered to be safe and well tolerated, several studies have demonstrated that BPN has abuse potential. The conditions under which BPN may be abused are complicated, however, and depend on a number of variables including whether or not the user is physically dependent on opioids, the opioid(s) upon which the user is dependent, and the dose and route of BPN administration. In order to mitigate this risk, a formulation of BPN has been developed that contains the opioid antagonist naloxone. This presentation will describe the various parameters under which BPN may be abused.
Methods: Opioid-dependent participants lived in the hospital for the duration of the studies and were either detoxified from opioids or maintained on an opioid (morphine, hydromorphone or BPN). After a stabilization period ($\sim$1 week), behavioral testing began. Primary dependent measures included drug taking behavior, subjective effects (to assess euphoria (drug liking, high), side effects (sedated, itchy), and opioid withdrawal (nausea, anxiety)) and physiological effects.
Results: In recently detoxified participants, intravenous (IV) BPN was self-administered significantly above placebo levels and produced positive subjective effects that were comparable to those of IV methadone, a full mu agonist. However, when participants were maintained on the short-acting opioid morphine, IV BPN was not self-administered at any dose tested, even though it significantly increased positive subjective effects. In comparison, morphine, heroin, oxycodone, and fentanyl self-administration increased in a dose-related manner in these same participants. In BPN-maintained subjects, IV BPN was self-administered and produced positive subjective effects that were comparable to heroin. Intranasal (IN) BPN, however, was not well liked and was self-administered less than heroin. The addition of naloxone dose dependently reduced abuse potential indicators of both IV and IN BPN among a BPN-maintained sample.
Conclusions: The results of these studies suggest that the abuse liability of BPN is complex and depends on a number of different factors including state of dependence, BPN dose, and route of BPN administration. The data also indicate that the combination of BPN and naloxone has lower abuse potential than BPN alone under all of the conditions tested. These results are important to consider for the use of BPN in other patient populations.
Disclosures: Part 1: AstraZeneca, Camarus, Janssen, Mallinkcrodt, Medicinova, Omeros, Pfizer, Reckitt Benckiser, Salix, Shire, **Part 2:** Reckitt Benckiser, **Part 4:** Reckitt Benckiser (investigator-initiated research grant).

4.4 Low-dose Buprenorphine for Late-Life Treatment Resistant Depression: Assessing Effect and Probing Mechanisms

Jordan Karp

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Background: Over 50% of mid-life and older adults with depression fail to respond to traditional antidepressants. When monoaminergic antidepressants are ineffective at eliciting a full response for patients with treatment resistant depression (TRD), augmentation pharmacotherapy using medications with a unique mechanism of action and rapid onset may offer relief. Modulation of the opiate system may be a novel treatment approach for TRD. Buprenorphine is a partial agonist at mu opiate receptors and antagonist of kappa opiate receptors (KOR). It has a favorable safety profile with low risk of respiratory depression, and the pharmacokinetics are not affected by advanced age or renal dysfunction, supporting its use in late-life. These qualities make buprenorphine an intriguing molecule to test for clinical effect in older adults with TRD and to probe the opiate system in the depressed aging brain.
Methods: Two studies will be presented. In the first unblinded project, fifteen midlife and older adults with TRD received 8 weeks of primarily augmentation pharmacotherapy with buprenorphine (average daily dose = 0.40 mg/day). In the second ongoing multisite project, 80 older patients with TRD receive 8 weeks of augmentation pharmacotherapy with buprenorphine dosed at 0.2-1.2 mg/day. The unique methodology of this project, in which different tools of modern neuroscience are used to probe target engagement of buprenorphine will be presented. Mechanism of action (MOA) study methods unique to each site are: 1) Neuroreceptor PET study of KOR before and after exposure to BPN to demonstrate pharmacodynamic MOA (i.e., target engagement, defined as appreciable CNS receptor occupancy) at our dosing range (St. Louis); 2) fMRI study comparing activation in the limbic system and reward circuits before and after exposure to examine neurocircuitry-level MOA (Pittsburgh); and 3) transcranial magnetic stimulation (TMS) study of cortical inhibition deficits (a neurophysiological proxy for dysfunctional GABA-ergic neurotransmission) before and after exposure to examine neurophysiological MOA (Toronto).
Results: In the open-label project, the average depression score (MADRS) at baseline was 27.0 (SD = 7.3), and at week 8 was 9.5 (SD = 9.5). Depression severity sharply declined during the first 3 weeks of exposure (mean delta = -15.0). The trajectories for the two depression-specific items (low mood and anhedonia) matched the trajectory for the total

MADRS score, representing depression-specific clinical improvements. Response, defined as MADRS < 10 at any week, was observed for 10/15 participants (66.7%). Response at the end of 8 weeks was observed for 8/13 participants (61.54%). During discontinuation of buprenorphine, no subjects experienced withdrawal symptoms. After withdrawing the buprenorphine, the average MADRS score increased to 17.8 (SD = 12.9), indicating a relapse of depression.
Conclusions: Low-dose buprenorphine may be a novel mechanism medication that provides rapid and sustained improvement for older adults with TRD. Clinical trials provide a unique infrastructure to both assess target engagement and promote understanding of an intervention's MOA.
Disclosures: Part 4: Provision of medication supplies for investigator initiated trials from Pfizer and Reckitt Benckiser.

Panel
5. Going with your Gut: Appetitive Hormones and the Regulation of Substance Use

5.1 A Novel Microendoscopy System for Functional Imaging of Circuits that Drive Feeding-Reward Behaviors

Yeka Aponte

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Background: Beginning with early lesion and electrical stimulation studies, the hypothalamus has long been considered essential in regulating feeding. Furthermore, it has been hypothesized that the hypothalamus may regulate food reward through its connections to brain regions associated with reward and goal-directed behaviors, such as the nucleus accumbens and the ventral tegmental area. However, in these experiments, it is not clear which cell types and relevant projections are important for regulating feeding behaviors. Moreover, experiments to date have been unable to measure the activity of specific cell types to provide quantitative relationships between neuronal activity and behavior. To address these questions, we are measuring and manipulating the activity of genetically-defined hypothalamic neurons in awake, behaving mice using a combination of optogenetics, electrophysiology, two-photon fluorescence endomicroscopy, and behavioral assays.
Methods: We implemented two-photon fluorescence endomicroscopy to measure the activity of genetically-identified hypothalamic neurons in head-fixed Cre-expressing transgenic mice during behavior. First, we designed a thin-walled polyimide guide cannula which allows for a minimally invasive implantation of the gradient refractive index (GRIN) lenses. Once mice were acclimated to head restraint, endomicroscopic imaging was performed during anesthetized and awake states in mice expressing the genetically encoded calcium indicator GCaMP6 in specific hypothalamic cell types.
Results: During our preliminary experiments in anesthetized and awake head-fixed mice, we were able to a) acquire high quality three-dimensional images of hypothalamic neurons with little brain motion across days, b) resolve neuronal processes such as dendrites and axons, c) image and record calcium transients indicative of action potential firing from lateral hypothalamic neurons that expressed the genetically encoded calcium indicator GCaMP6.
Conclusions: Having established imaging and surgical approaches, we are currently measuring the activity of genetically-identified cell types in the lateral hypothalamus of cre-expressing transgenic mice during different behavioral states, such as hunger, satiety, and feeding. By imaging neuronal activity, rather than using stimulation or inactivation experiments, we will be able to develop a better quantitative understanding of the specific role each cell type plays in controlling feeding behavior.
Disclosures: Nothing to Disclose.

5.2 New Roles for GLP-1 Receptors in Mediating Reward and Drug Abuse

Gregg Stanwood

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Background: Glucagon-like peptide-1 (GLP-1) is both a peripherally expressed incretin and a centrally active neuropeptide that is released in response to food intake. GLP-1 regulates energy homeostasis and metabolism by interacting with its receptors expressed on neurons in the gut and in the brain, and several GLP-1 analogs are approved for clinical use in type 2 diabetes to improve glycemic control and support weight loss. Recent studies have begun to elucidate the central pathways that mediate GLP-1 effects on food intake and reward, including the mesolimbic dopamine system. This presentation will discuss how forebrain GLP-1 receptors can coordinate interdependent food and drug reward circuits with central stress circuitry, at least in part through modulation of brain dopamine homeostasis.
Methods: Animals: Mice were purchased from Jackson Laboratories or bred internally. All experiments were in accordance with the directives of the "Principles of Laboratory Animal Care" and local institutional animal care and use committees. Reporter mice were created by the Vanderbilt Transgenic and Embryonic Stem Cell Core using a BAC clone containing the Glp1r gene.
Behavioral Studies: Some mice were subjected to a 5 day cocaine behavioral sensitization protocol, whereby they were injected with cocaine ± Ex-4 (or vehicle). Mice were then tested for locomotor activity following a ten day abstinence period. For conditioned place preference, mice were tested for initial bias, randomly assigned a drug treatment, and received four pairings of cocaine or saline vehicle (± Ex-4). Mice were re-assessed for preference on day 10. Cocaine self-administration was conducted in the laboratory of Dr. A. Fink-Jensen, using nose-poke responses that result in tail vein injection of cocaine.

Biochemistry and Neuroanatomy: DA uptake assays, slice biotinylation and immunoblotting were all conducted using standard protocols. Glp1r transcript was measured using fluorescent *in situ* hybridization and RNAScope. GLP-1 receptor expressing cells were mapped in BAC transgenic mice using antibody-mediated amplification of mApple.
Results: We have demonstrated that systemic administration of a GLP-1 analog, Ex-4, reduces cocaine reward, as measured both by conditioned place preference and self-administration. GLP-1 receptor activation also alters central DA homeostasis, at least in part through altering DAT expression and function within the lateral septum. In contrast, cocaine-induced locomotor activity and sensitization were not altered by Ex-4. Ongoing circuit mapping studies in a Glp1r-mApple reporter mouse is allowing us to delineate the sites of GLP-1 receptors and GLP-1 responsive pathways.
Conclusions: We have demonstrated new roles for the peptide hormone and satiety signal GLP-1 in cocaine reward and in the regulation of DA signaling. Our studies expose GLP-1 receptors as a potential target for the treatment of drug abuse, and point to mechanisms in which the autonomic and behavioral processes that regulate energy balance may also be recruited in drug addiction.
Disclosures: Nothing to Disclose.

5.3 On the Role of Feeding Peptides in Alcoholism: Recent Clinical Findings

Carolina Haass-Koffler

Brown University Center for Alcohol and Addiction Studies, Providence, Rhode Island, United States

Background: Increasing evidence supports the role of appetite-regulating pathways, including ghrelin and leptin, in alcoholism. Consistent with the opposite orexigenic and anorectic effects of ghrelin and leptin this set of studies tested the hypothesis that intravenous (IV) exogenous ghrelin administration acutely decreases endogenous serum leptin levels, and changes in leptin levels negatively correlate with alcohol craving.
Methods: This was a double-blind, placebo-controlled human laboratory study. Non-treatment-seeking, alcohol-dependent, heavy-drinkers ($n = 45$) were randomized to receive IV ghrelin or placebo, followed by a cue-reactivity procedure, during which participants were exposed to neutral (juice) and alcohol trial cues. To determine the change in hormone levels, blood samples were collected at baseline and during the entire cue-reactivity experiment.
Results: There was a main effect for IV ghrelin administration, compared to placebo, in reducing serum leptin levels ($p < 0.05$). Post hoc analysis showed significant differences in serum leptin levels at the alcohol trial ($p < 0.05$) that persisted at the end of the experiment ($p < 0.05$). By contrast, there were no significant differences in serum leptin levels at the juice trial ($p =$ n.s.). Serum ghrelin level was correlated with the increase in alcohol urge at alcohol trial ($p < 0.01$). By contrast, urge to drink juice craving questionnaire was not significantly correlated with serum ghrelin levels. The reduction of serum leptin level at the alcohol trial negatively correlated with the increase in alcohol urge ($p < 0.05$), while urge to drink juice was not correlated with the leptin change at the juice trial ($p =$ n.s.). This effect was specific for leptin, since there were no significant effects for IV ghrelin on resistin or visfatin serum level.
Conclusions: These novel findings provide preliminary evidence of ghrelin – leptin cross-talk in alcoholic individuals and suggest that their relationship may play a role in alcohol craving.
Disclosures: Nothing to Disclose.

5.4 Nicotine and the Endocrine Modulation of Appetitive Responses

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Background: In animals, nicotine administration has been demonstrated to reduce body weight by attenuating food intake. Moreover, effects of endocrine signals reflecting metabolic status such as leptin and ghrelin are partly modulated by nicotinic acetylcholine receptors. These molecular interactions point to potential interactions in the modulation of appetitive responses via mesolimbic dopaminergic projections, but little is known about potential interactions in humans.
Methods: We investigated food-cue reactivity and cue-induced appetite using functional magnetic resonance imaging during a fasting state and following the administration of an oral glucose tolerance test (OGTT; ~300 kcal). In a sample of healthy normal-weight never-smokers ($N = 26$), we administered either nicotine (2 mg) or placebo gums following a double-blinded randomized cross-over design on separate days. In a second sample, we investigated quitting smokers before smoking cessation and at least two weeks after successful abstinence and matched never-smoking controls ($N = 31$).
Results: In the sample of healthy never-smokers, we have shown that a) fasting levels of ghrelin correlate with cue-induced appetite and the mesolimbic response to food pictures, b) nicotine administration reduces the hypothalamic response to food pictures during the fasting state and decreases the functional coupling with the ventral striatum, and c) nicotine enhances the modulatory effects of leptin and ghrelin on food-cue reactivity in the amygdala and the ventromedial prefrontal cortex. In the sample of quitting smokers, we found that the reduction in hypothalamic food-cue reactivity after being fed was significantly greater in smokers than non-smokers. Critically, reduced food-cue reactivity in the hypothalamus in smokers was correlated with reduced subjective appetite after the caloric load indicating a link to satiation.
Conclusions: Our findings indicate that nicotine may increase the impact of metabolic state on appetitive behavior and brain responses in homeostatic and hedonic circuits. Taken together, this modulation of endocrine signals might hint at a mechanism contributing to nicotine's anorexic potential.
Disclosures: Nothing to Disclose.

Panel
6. Extinction: New Directions from Basic Science to Clinical Interventions

6.1 Persistent Avoidance Depends on Prefrontal-Striatal Interactions

Gregory Quirk

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Background: Anxiety and related disorders are characterized by persistent avoidance of stimuli containing aversive associations. Persistent avoidance often fails to respond to extinction-based therapies. We investigated extinction of active avoidance using pharmacological and immunohistochemical techniques in rats, in a platform-mediated avoidance task (Bravo-Rivera et al, 2014).
Methods: Rats were trained to avoid a tone paired with footshock by stepping onto a platform. While protective, the platform also prevented rats' access to sucrose pellets. After 10 days of conditioning, avoidance responses were extinguished over two sessions. Some rats were infused with a BDNF-neutralizing antibody in the infralimbic (IL) prefrontal cortex prior to extinction training, while others were analyzed for cFos or BDNF two hours after extinction training.
Results: Persistent avoidance following extinction training was associated with elevated activity in prelimbic (PL) cortex, ventral striatum (VS), and basolateral amygdala (BLA), and reduced activity in IL. Combining cFos with a retrograde tracer implicated direct projections from IL to VS. Blocking extracellular BDNF in IL had no effect on the acquisition of extinction ($p = 0.609$) but impaired the recall of avoidance extinction the following day ($p < 0.001$). Extinction training increased neuronal BDNF in ventral hippocampus (vHPC), but unlike fear extinction, BDNF was also increased in mediodorsal thalamus but not BLA.
Conclusions: These findings suggest that extinction of active avoidance involves inhibition of striatal avoidance circuits by IL. The plasticity necessary for avoidance likely depends on BDNFergic inputs to IL from vHPC or MD, but not BLA. Persistent avoidance could result from deficient BDNF in MD-IL or vHPC-IL projections, limiting extinction plasticity in prefrontal-striatal circuits.
Disclosures: Nothing to Disclose.

6.2 Fear Network Reactivity and Communication in Response to Conditioned Threat Across Anxiety Disorders and PTSD

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Background: A network of brain regions including the amygdala, hippocampus, insular cortex, and regions of the medial prefrontal cortex have been implicated in the pathophysiology of anxiety and fear-based disorders. It remains unclear as to what is shared and what is different in terms of the dysfunctional activation or functional connectivity between those nodes, especially within the context of responding to threat-conditioning.
Methods: The sample studied (110 subjects) was composed of patients diagnosed with panic disorder (PD), social anxiety disorder (SAD), generalized anxiety disorder (GAD), and posttraumatic stress disorder (PTSD), along with subjects not diagnosed with any fear or anxiety disorders. All subjects underwent the same 2-day threat conditioning and extinction paradigm while in an fMRI scanner. BOLD signal was compared across disorders, and psychophysiological interactions analyses will be conducted across all studies. In addition, dynamic causal modeling (DCM) will also be conducted to test information flow across the different nodes of the network.
Results: Deficits in extinction of conditioned threat responses were only noted in the PTSD cohort; the remaining disorders showed what appeared to be intact threat extinction, at least as indexed by SCR. Distinct functional activations were noted to each of the disorders within the ventromedial prefrontal cortex, dorsal anterior cingulate cortex, amygdala, insular cortex as well as somatosensory cortex. The hyper and hypo-activation signature per disorder was noted across the different phases of the experiment (i.e. conditioning, extinction, and extinction recall). DCM analysis on over 100 healthy subjects revealed distinct pattern of information flow between the areas noted above; a patter that was found to be impaired in PTSD. DCM and PPI analyses across the remainder of the disorders is currently being analyzed and will be presented during the talk.
Conclusions: The work to be presented will help us continue to shape our thinking of what is similar and what is different across the fear- and anxiety-based disorders.
Disclosures: Nothing to Disclose.

6.3 Translating Fear Extinction Phenotypes: From the Rodent Chamber to the Clinic

Seth Norrholm

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Background: Psychophysiological measures of fear expression provide observable intermediate phenotypes of fear-related symptoms. Negative Valence Systems in the Research Domains Criteria (RDoC) matrix include the construct of acute threat, which can be measured using psychophysiological indices. Extinction impairments have been observed in traumatized individuals who express maladaptive fear-related behaviors. Genomic factors, such as those associated with FK506 binding protein 5 (FKBP5), have been shown to underlie diverse post-traumatic behavioral patterns. As an example, the role of FKBP5 has only been examined in relation to a priori diagnostic categories or in relation to aggregate behaviors.
Methods: Our current program of study has employed the use of translational fear conditioning, extinction, and inhibition paradigms that are well-suited to advance the study of the non-pharmacological (e.g., retrieval + extinction) and pharmacological (e.g., losartan, dexamethasone, d-cycloserine) manipulations that may facilitate

extinction learning. Outcome measures include freezing in rodents and fear-potentiated startle in humans. With these translational tools in hand, we empirically identified heterogeneous trajectories of fear extinction learning in mice (n = 122) and humans (n = 723) using Latent Growth Mixture Modeling. In humans, we determined if risk variants of FKBP5 were associated with abnormal fear extinction trajectories. In mice, we determined: (1) if FKBP5 mRNA expression in the amygdala following extinction learning was associated with abnormal extinction trajectories of freezing behavior and (2) if high dose dexamethasone altered expression and altered trajectory membership.
Results: In humans, three distinct trajectories were identified: a normative trajectory of moderate increase in fear acquisition and complete extinction, a high fear reactive but extinguishing trajectory, and a high fear reactive non-extinguishing trajectory. Risk alleles associated with FKBP5 were significantly associated with the high fear reactivity trajectory. In mice, three trajectories including a trajectory of rapid and complete extinction, a trajectory of slow but complete extinction, and a non-extinguishing trajectory were identified. High dose dexamethasone significantly increased the probability of extinction and altered FKBP5 expression in the amygdala.
Conclusions: Genetic variation in FKBP5 is associated with abnormal phenotypes of extinction learning. High dose dexamethasone temporally alters FKBP5 mRNA expression in the amygdala but permanently alters behavioral responses to the conditioned threat cue. Findings indicate that FKBP5 confers risk for abnormal fear extinction but also represents a target for treatment. These results will be discussed in terms of rescuing impaired extinction learning (ie., changing trajectory "class" membership) using non-pharmacological and pharmacological manipulations.
Disclosures: Nothing to Disclose.

6.4 The Prevention of PTSD with Early Extinction Training

Barbara Rothbaum

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Background: Unlike other psychiatric disorders, the precipitant for adult posttraumatic stress disorder (PTSD) is a known event, allowing for immediate intervention, presenting the potential to prevent, and ultimately eliminate for many, the occurrence of this most serious condition. The evidence from animal studies suggests that immediate extinction training (10 min after conditioning) was more effective on spontaneous recovery, renewal, and reinstatement than later extinction training (72 hours). This knowledge of the precipitant combined with excellent animal models of fear memory consolidation and the ability to interrupt this consolidation in the early post-trauma-exposure period, has led to the exciting possibility that interventions in the immediate aftermath of trauma could potentially prevent the development of PTSD.
Methods: Patients (N = 137) were randomly assigned to receive 3 sessions of an early intervention beginning in the emergency department (ED) compared to an assessment only control group. PTSD symptoms were assessed at 4 and 12 weeks post-injury and depression at baseline and week 4. The intervention consisted of modified prolonged exposure including imaginal exposure to the trauma memory, processing of traumatic material, and *in vivo* and imaginal exposure homework.
Results: In our completed pilot work, an early exposure-based intervention begun within hours of trauma exposure significantly decreased PTSD and depression 1- and 3-months post-trauma compared to those who did not receive the intervention and seemed to mitigate a genetic risk for PTSD.
Conclusions: Evidence supports that this early extinction training/exposure therapy in the immediate aftermath of trauma can reduce indices of fear and PTSD and depression symptoms.
Disclosures: Nothing to Disclose.

Panel
7. As Good as It Gets? New Insights from Genetic and Circuitry-Based Models of OCD and Tourette Syndrome

7.1 Identifying Neural Activity Changes Underlying OCD-Like Behaviors Using *In Vivo* Microscopy

Susanne Ahmari

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Background: Obsessive Compulsive Disorder (OCD) is a chronic, severe mental illness that affects 2-3% of people worldwide, yet the pathophysiology remains unclear. However, multiple lines of evidence indicate that dysregulation within cortico-striato-thalamo-cortical (CSTC) circuits is correlated with OCD. Specifically, in previous work we demonstrated that brief but repeated optogenetic hyperstimulation of projections from orbitofrontal cortex (OFC) to ventromedial striatum (VMS) leads to long-lasting perseverative grooming, a mouse behavior linked to OCD. However, the changes in cortical and striatal cellular activity that occur during the development of perseverative grooming are unknown. We therefore examined changes in cellular activity in both orbitofrontal cortex (OFC) and ventromedial striatum (VMS) during the evolution of perseverative grooming behavior using miniaturized head-mounted microscopes and calcium imaging.
Methods: We injected EMX-Cre or C57/Bl6 mice with the genetically encoded calcium indicator AAV5.syn.GCaMP6f and implanted microendoscopes (6.1mm x 0.5mm GRIN lens) in either OFC or VMS. 2 weeks after virus injection, mice were fitted with a microscope baseplate. After recovery, behavioral experiments were performed. Using a cross-over within subjects' experimental design, mice were treated with either the D1 agonist, SKF38393 to induce perseverative grooming, or vehicle. Both behavior and calcium signaling was monitored for 30 minutes post injection, using 5 minute imaging blocks every 10 minutes. Calcium transient data was extracted from processed videos to analyze event frequency and time locked activity. In a separate set of experiments, mice were injected with the red-shifted channelrhodopsin, AAV-ReaChR, and implanted

with custom combined microendscopes and fiberoptic probes. Data were analyzed using repeated-measures ANOVAs and post-hoc tests ($\alpha = 0.05$).
Results: Perseverative grooming increased after systemic D1 agonist injection in both OFC and VMS implanted mice compared to saline controls ($p < 0.05$). Although average firing rates over the 5 minute imaging blocks in both regions were not significantly different, patterns of firing during individual grooming events were altered by administration of the D1 agonist. Specifically, grooming events were negatively correlated with activity in striatal neurons. Ongoing analysis is delineating the precise relationship between changes in neural activity and bouts of perseverative grooming in OFC. We have also demonstrated effective combined stimulation of ReaChR with 593nm laser light and visualization of GCaMP6f with blue LED light.
Conclusions: As expected, we demonstrated an increase in perseverative grooming following D1-agonist stimulation; however, we did not observe increased striatal firing rates in response to induction of perseverative behavior. Surprisingly, time-locking of neuronal firing with calcium events showed that striatal neurons were less likely to fire during episodes of perseverative grooming, which may provide insights into novel mechanisms underlying perseveration. Analysis of firing patterns in the OFC during grooming induction is ongoing. Combining *in vivo* optogenetics and *in vivo* microscopy as demonstrated here will provide a new powerful approach for the investigation of plasticity mechanisms underlying the development of perseverative thoughts and actions.
Disclosures: Nothing to Disclose.

7.2 Histamine Modulation of Basal Ganglia in a Pathophysiologically Grounded Model of Tourette Syndrome

Maximiliano Rapanelli

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Background: Tourette syndrome (TS) represents the most severe end of the continuum of tic disorders. TS is substantially genetic, but causative have been elusive; this has impeded the development of well-validated animal models in which to study pathophysiology.
Methods: We used two approaches to specifically manipulate histaminergic neurons in the posterior hypothalamus in otherwise normal mice. First, we ablated these cells, using a combination transgenic-viral strategy. Second, we introduced an inhibitory designer receptor (M4 DREADD) into these cells, allowing them to be chemogenetically silenced. To probe the effects of HA in the basal ganglia we pharmacologically manipulated the H3 receptor and examined behavioral and molecular consequences in wild-type and Hdc-KO mice, using transgenic reporter animals to isolate molecular abnormalities in D1- and D2-positive medium spiny neurons (MSNs) in the striatum by immunohistochemistry.
Results: Both ablation and chemogenetic silencing of histaminergic neurons produced a dramatic increase in grooming; mice in which these neurons were ablated developed bald patches from excessive grooming. H3 agonist treatment produced a range of molecular effects, activating the MAP kinase pathway in D1-positive MSNs and the Akt-Gsk3 pathway in D2-positive MSNs, and inhibiting the Akt-Gsk3 pathway in D1-MSNs. Interactive effects with D1 activation using SKF were reflected in alterations in exploratory behavior. These signaling pathways are altered both in Hdc-KO mice and after ablation or silencing of histaminergic neurons.
Conclusions: The fact that ablation or silencing of histaminergic neurons in an otherwise normal adult mouse can recapitulate TS-relevant behavioral phenomenology implies that the relevant pathophysiological consequences of HA deficiency occur in the adult brain, not during development or in the periphery. Behavioral abnormalities are more marked after adult silencing of these neurons, suggesting compensatory affects in the knockout animal. Histaminergic regulation of the basal ganglia is complex but suggests possible nodes for pharmacological intervention.
Authors: Maximaliano Rapanelli, Christopher Pittenger, Yale University.
Disclosures: Nothing to Disclose.

7.3 Characterization of the Putative OCD Risk Gene BTBD3 Using Mouse Models

Stephanie Dulawa

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Background: Obsessive-compulsive disorder (OCD) is a severe anxiety disorder characterized by unwanted and intrusive thoughts, images, or impulses and/or repetitive compulsive behaviors. The first genome-wide association study (GWAS) of OCD identified the gene BTBD3 as genome-wide significant ($p = 3.8E-8$) in the trio portion of the sample. BTBD3 is a transcription factor that regulates dendritic orientation towards active axons; however, no work has examined the role of BTBD3 in modulating phenotypes relevant to OCD.
Methods: We assessed the role of BTBD3 in modulating OCD-relevant phenotypes using male and female BTBD3 wild-type (WT), heterozygous (HT) and knockout (KO) mice. Mice were pair-housed mice by genotype and gender, and assessed barbering behavior over 14 weeks. Barbering refers to the plucking of hair or whiskers using the teeth. In addition, mice were also assessed in the open field, the dig test, the splash-induced grooming test, and in the prepulse inhibition (PPI) paradigm. In another cohort, we assessed the effects of chronic fluoxetine (10 mg/kg/day) or desipramine (20 mg/kg/day) treatment on observed phenotypes. Barbering was assessed each week during the fourteen weeks of drug treatment, while all other phenotypes were evaluated after four weeks of drug treatment. Finally, we compared neuronal activation using Fos mRNA expression in the orbitofrontal cortex, dorsal striatum, dorsal thalamus, and anterior cingulate gyrus of the genotypes.
Results: We found that KO and HT mice barbered their cagemates significantly more than WT mice. In the open field, BTBD3 KO mice showed increased locomotion compared to WT and HT mice, and HT and KO mice exhibited robust reductions in rearing behavior. BTBD3 HT and KO mice also showed less digging behavior. In the

splash-induced grooming test, HT and KO mice exhibited more frequent, but shorter grooming bouts than WT mice. BTBD3 genotype did not affect PPI or startle reactivity. Chronic fluoxetine treatment reduced barbering beginning at four weeks of treatment, whereas desipramine had no effect at any time point. Within genotype, fluoxetine significantly reduced barbering in WT and HT but not KO mice, indicating an interplay between BTBD3 expression and fluoxetine-specific reversal of barbering. No other behavioral phenotypes were altered by chronic drug treatment. Fos mRNA expression did not differ between genotypes in any brain region examined.
Conclusions: In summary, decreased BTBD3 expression reduces exploratory behaviors including digging and rearing, and increases perseverative behaviors such as barbering. The reduction of barbering in BTBD3 WT and HT mice by chronic fluoxetine, but not desipramine, suggests that this phenotype may be relevant to OCD. In sum, BTBD3 expression modulates some OCD-relevant behaviors in mice. We are currently assessing the mechanisms by which BTBD3 regulates these behaviors.
Disclosures: Nothing to Disclose.

7.4 Investigation of EAAT3 Reduction as a Therapeutic Target for Obsessive Compulsive Disorder

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Background: Emerging structural, neurochemical, and behavioral findings point to a significant role for cortico-striatal-thalamic (CST) circuits in Obsessive Compulsive Disorder (OCD). Despite this, our understanding of the molecular pathophysiology of OCD remains inadequate, and our treatment options leave most patients with continued impairment. Cerebrospinal fluid levels and magnetic resonance spectroscopy implicate the glutamate system in OCD, including in CST circuits. Genetic linkage and association studies in OCD point to SLC1A1, encoding the neuronal glutamate/asparate/cysteine transporter EAAT3/EAAC1. No previous studies have investigated EAAT3 in CST circuits or in relation to OCD-related behavior. The most commonly associated SLC1A1 allele leads to increased expression, leading to our hypotheses that 1) increased EAAT3 contributes to OCD susceptibility, and 2) decreasing EAAT3 activity may alleviate OCD-related behavior by modulating CST signaling.
Methods: To test this hypothesis, we developed a STOP-TetO knock-in mouse line that allows us to flexibly manipulate Slc1a1 expression. Initial validation of the model used quantitative RT-PCR and Western blot to examine expression. Functional validation approaches included both glutamate and cysteine uptake in striatal synaptosomes. Amphetamine (AMPH)-induced hyperactivity and stereotypic movements were used as a probe of CST circuitry-dependent behavior. To build upon AMPH-dependent behavioral changes, stereotyped repetitive grooming behavior was assessed following dopamine D1 agonist SKF-38393 administration. Immediate early gene expression (cFos immunohistochemistry) was used to evaluate the regional specificity of these findings. Dopamine receptor D1 and D2 membrane binding were measured in striatum. Dopamine and metabolite levels were used to assess presynaptic effects of EAAT3 ablation within dopaminergic neurons.
Results: Slc1a1-STOP animals show a successful ablation of both mRNA and protein levels, leading to loss of cysteine uptake. Using amphetamine as a probe, we found that EAAT3 loss decreases CST circuitry-mediated hyperactivity and stereotypic behavior. Further, EAAT3 ablation diminishes response to a dopamine receptor D1 agonist, a pharmacologic model of OCD-like grooming behavior. Diminished immediate early gene response to AMPH was observed in the dorsal striatum of Slc1a1-STOP animals compared to wildtype littermate controls. Further, dopamine receptor D1 membrane binding was decreased in the dorsal striatum, with a trend for decreased D2 membrane binding as well. In wildtype animals, dopamine levels in the ventral tegmental area were decreased following AMPH administration, but no such change was observed in Slc1a1-STOP mice.
Conclusions: These are the first findings implicating the most consistently associated OCD candidate gene, Slc1a1, in striatal-dependent repetitive behavior. The constellation of molecular data points to diminished dopamine receptor expression and immediate early gene response in medium spiny neurons within the dorsal striatum. Ongoing experiments are focused on understanding the regional specificity of EAAT3 ablation within CST circuits, as well as the mechanisms underlying these findings. Overall, these data suggest EAAT3 as a potential target for OCD treatment.
Disclosures: Part 1: Consulting: Roche, Novartis, SynapDx, Seaside Therapeutics. Research funding: Roche, Novartis, SynapDx, Seaside Therapeutics, Forest, Sunovion. Editorial funding/support: Springer, Wiley, **Part 4** Research funding: Roche, Novartis, SynapDx, Seaside Therapeutics, Forest, Sunovion. Editorial funding/support: Springer, Wiley.

Study Group
8. Reproducibility and Robustness of Experimental Data in the Neurosciences - Opportunities for Improvements

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Reproducibility and robustness of research data are the pillars of the scientific method. However, reproducibility and robustness of published data in research, including neuroscience, is considered low, which has raised major concerns amongst industrial and academic scientists, editors, publishers and funding organizations. As a scientific community, there is a shared responsibility to address and resolve this issue. This study group will discuss the factors underlying the lack of reproducibility, but in particular will aim to address ways to improve data reproducibility, robustness and data quality in the neuroscience field using a forward-looking approach. Participants will highlight examples from various areas, including

in vivo animal studies, the area of –omics, biomarkers and small explorative clinical studies, provide the different academic, industrial, editorial and funding organization perspectives, give an overview of ongoing initiatives to tackle the problem in the US and Europe, propose possible solutions and provide recommendations for future neuroscience research.
Disclosures: Part 1: Served on Advisory Board for Otsuka 2013/14.

Tuesday, December 8, 2015

Study Group
9. The Future of Sex Difference Research in Neuropsychopharmacology

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A goal of modern pharmacology is personalized medicine, where treatments are tailored to the specific biology of individual patients. Thus, there is an effort to identify factors that explain why patients respond differently to the same treatment. Surprisingly, the sex of the patient is often ignored as a moderating factor in treatment response. This inattention to sex differences begins at the preclinical stage of the pipeline, where the majority of pharmacology studies—from molecular mechanisms up to behavioral outcomes—are conducted only in males. However, the importance of considering sex is becoming increasingly recognized by the scientific community, and the National Institutes of Health (NIH) are promoting efforts to enhance and stimulate research examining the role of sex in health and disease. Yet it is unclear to many new to sex difference research how best to economically and efficiently include both males and females in their research programs. This study group will help address this issue and generate a discussion of the future of sex difference research in the field of Neuropsychopharmacology among the College membership.
Disclosures: Nothing to Disclose.

Panel
10. Behavioral Implications of Adult Neurogenesis and Its Potential as Treatment Target

10.1 Tuning Lineage Homeostasis to Rejuvenate Memory Circuits and Constrain Fear Generalization in Adulthood and Aging

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Background: The generation of adaptive fear responses to ambiguous threats in the environment is critically dependent on how contexts and cue-contingency relationships are encoded. Inefficient encoding of ambiguous threats results in inappropriate retrieval of aversive memories and activation of fear circuits to produce heightened avoidance behavior, overgeneralization of fear, hyper vigilance and arousal, symptoms that characterize anxiety disorders such as post-traumatic stress disorder. Recent studies by us and others has found that adult-born neurons generated from neural stem cells in the hippocampus play a critical role in discrimination of ambiguous threats and modulating generalization of fear. One neural mechanism by which this is accomplished is pattern separation, a process by which interference between similar memories is minimized. We recently hypothesized that impaired pattern separation may result in the re-activation of previously stored aversive memories and aberrant activation of circuits subserving fear and stress responses to produce fear overgeneralization, a hallmark of PTSD.
Methods: Here, we employ a novel inducible genetic system by which we modulate competition for perforant path inputs between adult-born and mature dentate granule neurons. We generated mice in which we reversibly overexpress a negative transcriptional regulator of dendritic spines in mature dentate granule neurons but not young adult-born neurons. Using genetic reporters, immediate-early gene based analysis of population coding, rabies virus based mono-synaptic synaptic tracing, imaging and behavior, we analyzed the neural stem cell and progenitor compartment, connectivity of mature and adult-born dentate granule neurons and network level pattern separation following rejuvenation of the DG with an expanded population of young adult-born dentate granule neurons. We examined the behavioral impact of rejuvenating the DG with an expanded population of young adult-born neurons in adulthood, middle age and aging on contextual discrimination, reversal learning, remote memory precision and pattern separation.
Results: Partial elimination of dendritic spines of mature dentate granule neurons results in a robust increase in stable long-term integration of adult-born dentate granule neurons and activation of neural stem cells without affecting olfactory bulb neurogenesis. Remarkably, complete reversal of elimination of dendritic spines in mature dentate granule neurons restores neuronal competition- and lineage-homeostasis to steady state levels. Genetic expansion of population of age-matched adult-born neurons does not affect anxiety or depression-like behaviors, but enhances contextual fear discrimination, reversal learning and precision of remote fear memories in adulthood. Furthermore, enhancements in contextual fear discrimination and precision of remote fear memories were also seen in middle-age and aging. At a network level, enhancing the population of adult-born dentate granule neurons increases pattern separation through global remapping in the DG in both adulthood and in middle-age.
Conclusions: Our studies suggest that rejuvenation of the DG with adult-born neurons decreases interference as assessed behaviourally and at a network level through increased pattern separation in the DG in adulthood and middle-age. Stimulation of adult hippocampal neurogenesis may represent a novel therapeutic strategy to constrain the overgeneralization of fear in adulthood, middle-age or aging.
Disclosures: Nothing to Disclose.

10.2 Stress, Unpredictability, and the Role of Adult Neurogenesis in Response to Threat

Heather Cameron

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Background: New neurons are born in the dentate gyrus throughout life, but their normal function and potential role in depressive illness are unclear. Stress is an important factor in depression and also interacts with adult neurogenesis, strongly inhibiting production of the new neurons. New neurons, in turn, modulate endocrine and behavioral stress responses, though it is not known how this occurs. Unpredictability is a key feature of stress, so we asked whether adult-born neurons affect behavior differently in response to predictable and ambiguous threats.
Methods: Mice expressing herpes simplex virus thymidine kinase under the control of the GFAP promoter (GFAP-TK mice) and wild type littermate controls were given valganciclovir during adulthood to selectively ablate adult-born neurons in the transgenic mice. Mice with and without new neurons were trained on cued fear conditioning using either a reliable cue or an ambiguous cue, which terminated with a shock in only 50% of trials. Behavioral responses to the cues and to novel situations were tested following conditioning.
Results: Mice lacking new neurons had normal freezing responses to reliable cues but showed diminished response to ambiguous cues. This same pattern was reflected in activation of mature granule cells and CA3 pyramidal cells as measured by immediate early gene expression. In the novelty-suppressed feeding test of anxiodepressive-like behavior, reliable cue conditioning had no effect on normal mice, while ambiguous cue conditioning dramatically increased latency to eat in these mice. Mice lacking neurogenesis showed intermediate increases in latency following both reliable and ambiguous cue conditioning, and their response did not differ according to the predictability of previous conditioning.
Conclusions: These experiments demonstrate that new neurons are important for behavioral responses to ambiguous threat. New neurons enhance hippocampal activation and protective stress-related behaviors toward an ambiguous threat cue. Days after an aversive experience, new neurons enable differential responding in novel situations according to the predictability of previous threats. These changes could bias behavior in ambiguous situations to optimally adapt to safe and stressful environments.
Disclosures: Nothing to Disclose.

10.3 Modes of Division and Differentiation of Adult Neural Stem Cells may Define Long Term Consequences of Therapies

Grigori Enikolopov

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Background: Production of new neurons in the adult brain is important for behavior, pathophysiology, aging, and neural tissue repair in humans and animals. New hippocampal neurons may mediate the action of antidepressants and other drugs and therapies. New neurons are born from neural stem cells which are maintained at specific locations in the adult brain. Neural stem cells are the only source of new neurons in the adult brain. Therefore, our understanding of the features and the role of new neurons depends on the ability to identify adult stem cells and reveal basic mechanisms governing their maintenance, division, differentiation, and death.
Methods: Our main approach is generating animal models that enable visualization of stem cells and their environment and monitoring of their signaling landscape. These animal models are complemented by new methods that we developed to determine parameters of division and differentiation of neural stem cells.
Results: The use of our reporter mouse lines allowed us to develop a new model for the quiescence, maintenance, and division of the hippocampal stem cells. Our results indicate that adult neural stem cells may remain quiescent for their entire postnatal life, but, when activated, rapidly divide several times in quick succession to bud off daughter cells that eventually yield neurons, while the remaining stem cell differentiates into a mature astrocyte, thus leaving the stem cell pool. We found that decrease in the number of new neurons that accompanies aging is driven by the disappearance of stem cells via their division-coupled astrocytic differentiation. This continuous loss of stem cells underlies age-dependent diminished production of new neurons and may contribute to age-related cognitive impairment.
We next applied our approach to determine the classes of stem and progenitor cells that are affected by various pro- and anti-neurogenic factors. We found that stem cell output can be increased in different ways and that each mode of augmented production of new neurons may have different effect on the pool of stem cells, with important implications. For instance, while some antidepressant treatments (e.g., fluoxetine or deep brain stimulation) do not affect stem cells, but instead target rapidly amplifying progenitor population, other antidepressant therapies increase the number of asymmetric divisions of stem cells without recruiting additional stem cells (and therefore leading to an increased number of new neurons without additional loss of stem cells). In contrast, some clinically used compounds increase recruitment of normally quiescent stem cells in division, potentially leading to an increase in new neurons at the expense of premature exhaustion of the stem cell pool.
Conclusions: Our findings highlight the potential clinical relevance of studies of adult neural stem cells and may have direct implications for human therapy. Our results warn that any drug or therapeutic treatment that involves changes in neurogenesis should be investigated for the precise mechanisms of those changes, since seemingly identical outcomes may be induced by different mechanisms and with different long-term consequences.
Disclosures: Nothing to Disclose.

10.4 Molecular Regulation of Hippocampal Neurogenesis in Neuropsychiatric Disease and Treatment

Maura Boldrini

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Background: Major depressive disorder (MDD) presents with inability to disengage from negatively valenced

material and biased memory recall, and depression score is negatively correlated to pattern separation performance. Adult hippocampal neurogenesis is required for pattern separation and adaptations to stress in rodents. Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation in mice. We reported fewer dentate gyrus (DG) mature granule neurons (GNs) in unmedicated MDD, compared with psychiatrically healthy controls. We published that subjects with earlier onset of MDD have fewer GNs in anterior DG vs. subjects with later MDD onset. Although age-related decline of adult neurogenesis is less pronounced in human than in mice, it may still mediate age-related changes in cognitive processing.
Selective serotonin repute inhibitors (SSRIs) treatment increase adult neurogenesis in mice and we found that in MDD is associated with more neural progenitor cells (NPCs), GNs, mitotic cells, and angiogenesis. It is unclear if the neurogenesis cascade in MDD is compromised at the level of cell proliferation, maturation or survival, and which are the mechanism by which SSRIs increase adult neurogenesis. Proliferation of NPCs is affected by growth factors, including brain derived neurotrophic factor (BDNF), but fluoxetine-mediated increase in cell survival is BDNF-independent. Vascular endothelial growth factor (VEGF), through the Flk-1 receptor (VEGFR2), is required for fluoxetine-induced cell proliferation. Serotonin receptors and trophic factors act on kinases modulating downstream intracellular molecules that regulate cell maturation and survival. These include cyclic-AMP response element binding (CREB), a leucine zipper transcription factor increased by antidepressants and expressed during cell maturation, and poly [ADP-ribose] polymerase (PARP), a DNA-binding protein activated by DNA strand breaks that protects survival via enzymatic DNA repair. PARP ribosylation also regulates chromatin histone ribosylation in the hippocampus after memory acquisition, regulating the epigenetic mechanism involved in reprogramming neuronal gene expression in memory consolidation. We aimed to assess molecular pathways involved in the regulation of human DG cell proliferation, maturation and survival in MDD, with SSRI treatment, and aging.
Methods: We performed immunohistochemistry and stereology to quantify DG neurons and glia expressing VEGFR2, CREB and PARP in 22 psychiatrically healthy controls, 22 unmedicated subjects with MDD and 11 MDD subjects treated with SSRIs for at least 3 months before death. Subject age ranged from 19 to 84 years. All subjects received DSM-IV-validated psychological autopsy for diagnosis, brain and blood toxicology, and neuropathology. All subjects died from sudden death and time between demise and autopsy was within 24 hours.
Results: Uncleaved PARP and non-phosphorylated CREB are expressed in more DG cells in untreated MDD than in SSRI-treated MDD and controls. VEGFR2 expression in DG cells correlated with more NPCs and GNs in anterior DG. More lifetime depressive episodes and more severe Global Assessment Scale (GAS) score correlated with fewer GNs in untreated MDD. We did not detect fewer NPCs or GNs with aging, but angiogenesis was decreased.
Conclusions: Findings support the hypothesis that PARP and CREB have a role in regulating GN maturation and survival and that VEGF action may result in more adult neurogenesis in human DG. MDD is a major cause of global burden and its severity shows a relationship with GN number. Intracellular pathways that regulate brain structural plasticity may be drug targets for new classes of antidepressants or cognitive-enhancing treatments.
Disclosures: Nothing to Disclose.

Panel
11. The Role of Epigenetic Mechanisms in the Transition into Alcohol Addiction

11.1 The Role of Epigenetic Mechanisms in the Medial Prefrontal Cortex in the Transition to Alcohol Dependence

Markus Heilig

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Background: In rodent models, a prolonged history of alcohol dependence is associated with persistent escalation of alcohol seeking and intake. We have previously shown that this is in part driven by coordinated and persistent dysregulations of gene expression networks in the medial prefrontal cortex (mPFC). Recent work in our laboratory has identified contributions to these processes from microRNA (Tapocik et al, J Neurosci 2014) and DNA-methyl transferase activity (Barbier et al, J Neurosci 2015), but little is known about a potential role of other epigenetic enzymes in reprogramming the mPFC transcriptome.
Methods: Alcohol dependence was induced using chronic intermittent alcohol vapor exposure. Following recovery, RNA-sequencing was used to screen the transcriptome of the mPFC for persistent differential expression of epigenetic enzymes. PRDM2 was identified as a differentially expressed candidate, and the molecular consequences of its repression were assessed by measuring H3K9 mono-methylation. Functional consequences at the behavioral level were then assessed by knocking down PRMD2 expression in the mPFC of non-dependent rats using a lenti-viral shRNA vector. Chip-Seq was used to identify PRDM2 regulated target genes as downstream mediators. Functional consequences on addiction-like traits were evaluated by assessing operant self-administration, stress-induced reinstatement of alcohol seeking, and aversion-resistant alcohol seeking.
Results: In rats with a history of dependence, both the RNA-seq screen and a focused confirmatory qPCR analysis showed decreased expression of PRDM2. Immunohistochemical analysis indicated that this occurred in neurons. Alcohol-induced PRDM2 repression was reversed by the DNA methyltransferase inhibitor RG108, suggesting that it is driven by DNA methylation. Conversely, PRDM2 knockdown in non-dependent rats induced a set of gene expression changes that overlapped with those found following alcohol dependence. These expression changes were associated with behavioral consequences otherwise seen following a history of dependence. These consequences included escalated alcohol intake, increased resistance to quinine adulteration, and enhanced stress-induced reinstatement. Several genes that exhibited a significant

decrease in H3K9me1 enrichment following dependence were identified in the ChIP-seq study, including synaptotagmin 1 (Syt1). Since neurosynaptic communication was heavily implicated in the gene ontology analysis, we confirmed H3K9me1 enrichment in controls compared to post-dependent rats using ChIP-PCR.
Conclusions: We demonstrate for the first time a role of PRDM2 for behaviors that are critical in alcoholism. Specifically, our findings indicate that DNA-methylation mediated repression of PRDM2 is involved in multiple aspects of alcohol dependence, specifically stress-induced relapse, compulsivity-like behavior and escalation in alcohol intake) and therefore provide a rationale for exploring the potential of targeting PRDM2 for treatment.
Disclosures: Nothing to Disclose.

11.2 A Novel Role for the Histone Demethylase KDM6B in Alcohol Dependence

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Background: Epigenetic signaling pathways oversee DNA methylation and post-translational histone modifications to encode transcriptomic changes in response to environmental cues. Enzymes that catalyze these modifications are increasingly recognized to mediate behaviors associated with drug and alcohol dependence. We hypothesize that alcohol exposure influences long-term gene expression and behavioral abnormalities through changes in epigenetic enzyme activity.
Methods: We used a rat model of alcohol dependence to identify epigenetic enzyme expression changes in the brain that are induced by alcohol exposure. In this model, rats are chronically and intermittently exposed to intoxicating concentrations of ethanol vapors. These rats exhibit behavioral and molecular changes reminiscent of human alcoholics, including long-term voluntary increases in alcohol consumption. Three weeks after ethanol exposure, Nanostring nCounter analysis was used to quantify mRNA expression levels of over 100 epigenetic enzymes in the nucleus accumbens (NAc) of alcohol dependent rats compared to controls. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was used to validate significantly altered genes. Several enzymes of interest were also quantified using qRT-PCR in prefrontal cortex (PFC) tissue from alcohol dependent rats and human alcoholics. Western blot analysis was used to quantify the protein levels and signaling pathways associated with KDM6B, a histone demethylase implicated in the RNA expression analyses. To identify the genes regulated by this epigenetic enzyme, we used chromatin immunoprecipitation to isolate the DNA associated with trimethylated histone H3 lysine 27 (H3K27me3), the histone site regulated by KDM6B. DNA associated with H3K27me3 in the NAc of control and alcohol dependent rats was then subjected to DNA sequencing (ChIP-seq).
Results: RNA levels of a histone demethylase, KDM6B, are dysregulated in both the PFC and NAc of alcohol dependent rats and human alcoholics. KDM6B protein is increased in the NAc of alcohol dependent rats. Upregulation of KDM6B protein is paralleled by downregulation of H3K27me3, consistent with the known demethylase activity of KDM6B. Preliminary analysis of ChIP-seq data implicated H3K27me3-mediated disruption of inflammatory signaling pathways in response to alcohol exposure.
Conclusions: KDM6B is dysregulated in key brain regions involved in reward perception in a relevant rat model of alcoholism as well as in human alcoholics. KDM6B dysregulation is associated with alcohol induced epigenetic changes in inflammatory signaling pathways. Ongoing experiments aim to determine whether genes implicated in the ChIP-seq study are functionally dysregulated by KDM6B. We also aim to study alcohol-seeking behavior in response to *in vivo* viral-mediated manipulation of KDM6B expression. These studies may elucidate how an epigenetic mechanism translates alcohol exposure into the chronic transcriptional and behavioral changes that underlie alcohol dependence.
Disclosures: Nothing to Disclose.

11.3 Breakdown in the Corticostriatal BDNF Pathway Drives the Transition from Social to Compulsive Drinking for Alcohol

Dorit Ron

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Background: Previously we found that the brain-derived neurotrophic factor (BDNF) in the dorsal striatum and specifically in the dorsolateral striatum (DLS) is part of a homeostatic signaling pathway that keeps alcohol drinking of rodents in moderation1-3.
Methods: We used mouse paradigms that model moderate and excessive alcohol drinking, in combination with molecular methods, viral-mediated gene delivery, pharmacology and a transgenic mouse line.
Results: We found that moderate drinking of alcohol leads to an increase in BDNF expression in the DLS, which is abolished in response to repeated cycles of binge drinking and withdrawal. The same excessive drinking paradigm produces a robust reduction of BDNF expression in the prefrontal cortex (mPFC) and the orbital frontal cortex (OFC). We obtained data suggesting that epigenetic modifications as well as microRNAs expression are the mechanisms underlying the breakdown of corticostriatal BDNF expression that in turn drives excessive drinking. Using a transgenic mouse model, we discovered that a single point mutation within the BDNF gene produces compulsive alcohol drinking despite negative consequences. Finally, we found that restoring the normal function of the corticostrial BDNF signaling pathway brings alcohol drinking back to moderate levels.
Conclusions: Malfunction of the corticostriatal BDNF signaling drives the transition from moderate consumption to excessive, compulsive intake. Restoring the function of the BDNF signaling in the corticostriatal pathway shifts alcohol consumption from excessive to moderate levels.
Disclosures: This work was supported by NIH-NIAAA R01 AA016848 (D.R.), NIH-NIAAA P50 AA017072 (D.R.) and by the State of California for medical research on alcohol and

substance abuse through the University of California, San Francisco (D.R.).

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11.4 Transcriptome Sequencing Reveals Novel Splice Variants in Human Alcoholic Brain

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Background: Long-term alcohol abuse and dependence (Alcohol Use Disorder; AUD) alters brain function and is linked to lasting changes in gene expression. AUD is a chronic, relapsing condition that imposes a significant socioeconomic burden by adversely affecting the health of millions of individuals worldwide. Similar to other complex trait disorders, the development and continuance of AUD is influenced by the interaction of multiple genetic and environmental factors that may be distinctly regulated across multiple brain regions.
Methods: To identify novel transcripts that are differentially expressed in brain regions involved in substance abuse, we conducted whole transcriptome sequencing (RNA-Seq) of the central nucleus of amygdala, basolateral amygdala, and superior prefrontal cortex from postmortem brain tissue of alcoholics (N = 30) and matched control (N = 30) subjects.
Results: RNA-Seq analysis permits an unbiased deeply sequenced assessment of the transcriptional profile altered in disease. For example, over 1 million potentially novel exons that neighbor known genes were identified. In addition, transcriptome profiling revealed global effects on gene co-expression networks altered by chronic alcohol abuse in these brain regions. We identified alterations in complex gene networks that may involve multiple splice variants relevant for the neurobiology of AUD. Gene specific sequencing of GABBR1 from postmortem cortex revealed a number of novel transcripts altered in relation to AUD.
Conclusions: The expression of alternatively spliced transcripts uncovered human-specific isoforms that are not present in animal models, underscoring the importance of human postmortem brain analyses.
Disclosures: Nothing to Disclose.

Panel
12. From Animals to Humans: The Role of Neuroinflammation in Psychosis and Psychosis Risk

12.1 Neuroinflammation and Oxidative Stress Precede the Onset of Schizophrenia-Relevant Behavioral Dysfunctions in Mouse Models of Prenatal Infection

Urs Meyer

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Background: Neuroinflammation and oxidative stress have been widely implicated in schizophrenia and related psychotic disorders. It remains a matter of debate, however, whether these pathophysiological processes develop before or subsequently to the onset of full-blown psychotic disease.
Methods: We used infection-based neurodevelopmental mouse models of schizophrenia and related disorders to study the temporal association of neuroinflammation, oxidative stress, and behavioral dysfunctions. In addition, we explored the functional contribution of neuroinflammation and oxidative stress to the behavioral abnormalities by anti-inflammatory and anti-oxidant pharmacological interventions.
Results: Using a two-hit model of combined exposure to mild prenatal viral-like immune activation (polyI:C, 1 mg/kg; on embryonic day 9) and sub-chronic peri-adolescent stress, we found marked microglia activation, pro-inflammatory cytokine expression, and cellular signs of oxidative stress in the hippocampus and prefrontal cortex of adolescent but not adult animals. On the contrary, multiple schizophrenia-related behavioral abnormalities emerged with a delayed onset in adulthood but not adolescence. Treatment with the anti-inflammatory agent minocycline (MINO) or the anti-oxidant N-acetyl-cysteine (NAC) during peri-adolescent stress exposure prevented the subsequent emergence of behavioral deficits in this environmental two-hit model. Similar findings were obtained in a mouse model of intense prenatal viral-like immune activation (polyI:C, 5 mg/kg; on embryonic day 9), which demonstrated a temporal dissociation between the emergence of neuroinflammation (in early adolescence) and schizophrenia-related behavioral dysfunctions (in adulthood).
Conclusions: Our novel preclinical data suggest that neuroinflammation and oxidative stress are manifest before and contribute to the adult onset of schizophrenia-relevant behavioral dysfunctions following infection-mediated neurodevelopmental disruption.
Disclosures: Nothing to Disclose.

12.2 Study of Altered Markers of Oxidative Stress in Patients with Early Stage Schizophrenia

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Background: Aberrant glutamatergic pathways and oxidative stress may underlie the pathophysiology of schizophrenia. The molecular mechanisms underlying aberrant reduction-oxidation (redox) cascades in the pathophysiology of psychosis remain elusive. N-Acetylaspartate (NAA) levels are high in neurons, sensitive to change in mitochondrial function, and metabolically linked to both redox and glutamatergic pathways. Therefore alterations in the homeostatic relationship between NAA and glutamate in the brains of patients may reflect changes in oxidative stress pathways. We hypothesize that early stage schizophrenia involves an underlying noxious cycle with imbalance of key antioxidants, namely superoxide-dimutase-1 (SOD1) and glutathione (GSH), resulting oxidative stress and decoupling of the homeostatic relationship between NAA and glutamate.

Methods: We collected cerebrospinal fluid (CSF) samples from patients with recent-onset schizophrenia (SZ), antipsychotic-naïve patients with first episode of psychosis (FEP), those at high risk for SZ, and matched healthy controls (HC). The CSF concentrations of several key inflammatory and oxidative markers were compared between cases and controls. We also examined the levels of NAA and Glx (the sum of glutamate and glutamine) measured using proton spectroscopy ([1H]MRS), relative to the level of creatine (Cr) as an internal control. The dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) in 25 patients with schizophrenia and 17 matched healthy controls were studied.
Results: We report greatly changed levels of several markers of oxidative processes in CSF from patients with recent-onset SZ, medication-naïve patients with FEP, and those at risk for SZ. Ratios of NAA/Cr and Glx/Cr did not differ significantly between the patient and control groups in either the DLPFC or ACC. The NAA/Cr and Glx/Cr ratios correlated positively ($r = 0.63$, $p = 0.017$) after controlling for age and smoking in healthy controls, but not in persons with schizophrenia ($r = -0.33$, $p = 0.124$). No significant correlation between NAA/Cr and Glx/Cr ratios was observed in the ACC in either group.
Conclusions: Our findings of diminished levels of SOD1, IL6, and other markers of redox imbalance support our proposed oxidative stress model of schizophrenia. Furthermore, decoupling of NAA and Glx in the DLPFC may reflect the interconnection of glutamatergic pathways and oxidative stress in the pathology of schizophrenia, and may prove a biomarker of the disease.
Disclosures: Nothing to Disclose.

12.3 PET Imaging of Microglia in Drug Naive Patients at High Risk of Psychosis and the Effects of Antipsychotics on Microglia

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Background: Converging lines of epidemiological, genetic and biological indicate that altered inflammatory processes play a role in schizophrenia. Evidence from post-mortem and PET imaging studies in patients with schizophrenia indicates microglia activation plays a role in this. However, it is not known when microglial changes occur in the development of the disorder or what the effect of antipsychotic treatment is on microglia.
Methods: To test this people at ultra-high risk of psychosis ($n = 14$) who were all antipsychotic naive and patients diagnosed with schizophrenia ($n = 14$) were studied using a second generation PET tracer that shows high affinity for a marker express on activated microglia and compared to age matched controls ($n = 28$). PET imaging data were acquired using [11C]-PBR28 and controlling for genotypes that may affect binding.
To investigate the effect of antipsychotics on microglia, rats were treated with antipsychotics or vehicle after receiving an inflammatory stimulus (lipopolysaccharide) or vehicle.
Results: Relative [11C]-PBR28 binding was elevated in grey matter throughout the brain in subjects at ultra-high risk of psychosis with a large effect size ($d = 0.9$, $p < 0.05$). Greater elevation in relative [11C]-PBR28 binding was positively associated with greater symptom severity ($r > 0.4$, $p < 0.05$) and negatively with total grey matter volume ($r > 0.4$, $p < 0.05$). [11C]-PBR28 relative binding was also elevated in the patients with schizophrenia with a large effect size ($d = 1.1$, $p < 0.05$).
Antipsychotic treatment reduced absolute density of microglia, and reduced morphological changes associated with activation in the LPS treated rats.
Conclusions: These data suggest that i) microglia activation is present prior to the onset of psychosis and linked to the severity of prodromal symptoms; ii) antipsychotic treatment is not responsible for microglial activation in rat models; and iii) microglial activation persists despite antipsychotic treatment in patients with schizophrenia.
Disclosures: Part 1: Dr. Howes has received investigator-initiated research funding from and/or participated in advisory/ speaker meetings organised by Astra-Zeneca, BMS, Eli Lilly, Jansenn, Lundbeck, Lyden-Delta, Otsuka, Servier, and Roche. Neither Dr. Howes nor his family have been employed by or have holdings/ a financial stake in any biomedical company, **Part 4:** Dr. Howes has received investigator-initiated research funding from and/or participated in advisory/ speaker meetings organised by Astra-Zeneca, BMS, Eli Lilly, Jansenn, Lundbeck, Lyden-Delta, Otsuka, Servier, and Roche. Neither Dr. Howes nor his family have been employed by or have holdings/ a financial stake in any biomedical company.

12.4 Imaging Neuroinflammation in Clinical High Risk and First Episode Antipsychotic Free Psychosis: An in-Vivo Pet Study with [(18)F]-FEPPA

Romina Mizrahi

University of Toronto, Toronto, Canada

Background: Neuroinflammation and abnormal immune responses have been implicated in schizophrenia. Past studies using positron emission tomography (PET) that examined neuroinflammation in patients with schizophrenia in-vivo using the translocator protein 18kDa (TSPO) target were limited by radioligand use, resolution of scanners used, and the potential confounding effect of antipsychotic medications. No previous report investigated the state of the clinical high risk for schizophrenia.
Methods: A cross-sectional study was performed using [(18)F]-FEPPA and a high-resolution research tomograph (HRRT). PET data were analyzed to obtain [(18)F]-FEPPA total volume of distribution (VT) using a 2-tissue compartment model with an arterial plasma input function, as previously validated. All subjects were classified as high-, medium- or low-affinity [(18)F]-FEPPA binders on the basis of rs6971 polymorphism, and genotype information was incorporated into the analyses of imaging outcomes.
Results: 8 (mean age = 23.25, SD = 4.43) CHR and 17 patients with untreated first episode psychosis (FEP) (mean age = 26.53, SD = 7.32), and 15 healthy volunteers (HV) (mean age = 25.07, SD = 4.96) underwent [(18)F]-FEPPA

PET and magnetic resonance imaging. We found a trend for a significant effect in medial prefrontal cortex ($F = 2.62$ $p = 0.08$) with CHR participants having 30.5 % increased [(18)F]-FEPPA binding relative to HV, with no difference with FEP (7.14%). No other significant effects were found, although several regions (temporal, DLPFC and PFC) show >20% increase in CHR. Explorative association analyses found a significant negative association between PANSS total and general scores and [(18)F]-FEPPA binding in hippocampus ($r = -0.573$, $p = 0.026$; $r = -0.637$, $p = 0.011$), and total gray matter ($r = -0.522$, $p = 0.046$; $r = -0.628$, $p = 0.012$). Further analyses with cognitive measures will be presented, together with analyses with pseudo-reference regions (cerebellum/total gray matter) and comparison data in Alzheimer's disease with the same radioligand.
Conclusions: This study addresses a relevant question in schizophrenia research; the role of neuroinflammation in the pathophysiology of the disease, without the confound of antipsychotic medications, using state of the art imaging technology and a second generation TSPO radioligand while controlling for genotype. Understanding the neurobiological changes associated with microglial activation has the potential to identify novel treatment targets (i.e. decrease neuroinflammation) in schizophrenia and in those at clinical high risk for the disease.
Disclosures: Nothing to Disclose.

Panel
13. Genetic Approaches to Delay Discounting: Human and Non-Human Animal Approaches

13.1 Genetic Basis of Impulsive Behavior in Humans Project: Initial Delay Discounting

James MacKillop

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Background: Delay discounting is a behavioral economic index of impulsivity that is robustly associated with addictive behavior and hypothesized to be an endophenotype for addiction. A small number of molecular genetic studies have reported significant associations between loci associated with dopaminergic signaling and DD, but have had a number of limitations. These studies have used relatively small numbers of participants and clinical samples within which genetic and disorder-induced influences on impulsivity cannot be disentangled. Furthermore, the studies to date have largely focused on polymorphisms that are the "usual suspects" within candidate genes. This presentation will report on the initial findings pertaining to delay discounting within the Genetics of Impulsive Behavior project, a study of genetic associations with diverse impulsivity phenotypes that employs candidate genes, candidate systems, and genomewide approaches in a large sample of healthy young adults. The focus is on the latent phenotypic structure of the impulsivity phenotypes and, specifically, the hypothesis that the phenotypes would conform to an orthogonal tripartite model, the three factors being delay discounting, behavioral inhibition, and impulsive personality traits.
Methods: A sample of 1262 healthy young adults (18-25) with limited substance abuse was ascertained from two sites and all participants were evaluated for delay discounting, motor inhibition, and personality indices of impulsivity using multiple measures (15 total phenotypes). A comprehensive assessment of control variables (e.g., income, education) was also conducted. A urine drug screen confirmed no recent drug use. DNA was collected via saliva sample and genotyping using the Illimina Infinium PsychArray BeadChip, developed by the Psychiatric Genomics Consortium to augment coverage of loci associated with psychiatric disorders (515,000 loci). Confirmatory factor analysis (CFA) was used for hypothesis testing.
Results: Preliminary analyses revealed significant site differences in impulsivity, but these were eliminated when other control variables (e.g., education, age) were included and site was not included in subsequent models. The primary CFA provided strong support for the three-factor structure ($CFI = 0.948$, $TLI = 0.936$, $RMSEA = 0.06$, $SRMR = 0.041$). The four indices of delay discounting were highly intercorrelated ($rs > 0.75$) and all substantially loaded on the latent delay discounting factor. Similar patterns were present for behavioral inhibition and impulsive personality traits, although the trait of Sensation Seeking did not substantially load on the personality traits factor. The delay discounting factor exhibited low magnitude associations with the other factors, suggesting it is an independent trait.
Conclusions: Using an a priori hypothesis-testing approach for the first time, these findings reveal the multidimensional nature of impulsivity as a trait. Specifically, the results indicate that the latent phenotypic structure of impulsivity is tripartite in nature and, within that structure, delay discounting is one independent factor. Based on these findings, the individual's latent impulsivity phenotypes will be the focus of genomic dissection.
Disclosures: Nothing to Disclose.

13.2 Genetics of Delay Discounting in Humans: Heritability and Preliminary Evidence for Genetic Association

Andrey Anokhin

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

Background: Delay discounting (DD), preference for smaller immediate rewards over larger but delayed rewards, is an established behavioral model of impulsive choice, a key component of a broader impulsivity construct. Elevated DD has been implicated as a potential intermediate phenotype (endophenotype) for a range of psychopathologies characterized by impulsive decision making, most notably, addictions and psychopathology. DD has been extensively studied in animal models of impulsive behavior, including genetic studies. However, few studies examined genetic influences on DD in humans.
Methods: Adolescent twins participating in a prospective longitudinal study of ($n = 602$, 52% females, ages 16-20) completed a computerized DD test. DD was quantified using both parametric and non-parametric methods (the k-coefficient of a hyperbolic function and area under the

discounting curve, respectively). We investigated age-related changes in DD, long-term stability of individual differences, heritability (i.e. the proportion of inter-individual variability that can be attributed to genetic factors), and examined the role of genetic variation in the 5HT system in the determination of DD.
Results: DD rate showed a modest but significant decrease with age, suggesting a reduction in overall impulsivity from middle to late adolescence. Significant test-retest correlations were observed in the age range from 16 to 20 years ($r = 0.64$ to 0.75, $p < 0.001$) indicating longitudinal stability of individual differences in decision-making behavior during middle and late adolescence. The genetic analysis using the twin design revealed significant heritability of both DD measures, with genetic factors accounting for 45%-64% of inter-individual variability in DD. Furthermore, DD showed significant associations with 5HT receptor genes (HTR1B, HTR2B) and tryptophan hydroxylase gene (THP2), providing preliminary support for possible contribution of genetic variation in the serotonergic system to individual differences in DD and warranting further association studies in larger samples.
Conclusions: In conclusion, converging evidence suggests that DD is a stable and heritable trait in adolescents and emerging adults that can serve as an intermediate phenotype in genetic studies of addictive and impulsive disorders.
Disclosures: Nothing to Disclose.

13.3 Strategies and Results: When using Mouse Models to Identify Commonalities Between a Delay Discounting Endophenotype and Endophenotypes Associated with Alcohol Use Disorder

Suzanne Mitchell

School of Medicine Oregon Health & Science University, Portland, Oregon, United States

Background: High levels of impulsivity (delay discounting [DD], relative preference for smaller but immediate rewards over larger but delayed rewards) are associated with various psychopathologies including alcohol use disorder. Data indicate that there are genetic influences on DD and on the development of alcohol use disorder, but the genetic relationships amongst DD and alcohol consumption and other heritable features of alcohol response are unclear.
Methods: In all studies, male mice were exposed to the adjusting amount procedure (Richards et al. 1997, J Exp Anal Behav, 67, 353-366). This method allows mice to choose between a small, immediate sucrose-solution reward and a larger sucrose-solution reward that is delayed 0, 2, 4, 8 or 12 s on different sessions. An initial within-subject study compared behavior on this procedure and a variant in which delays were presented in within sessions blocks. However, little discounting was seen in the latter procedure, causing behavior on the two task variants to be uncorrelated, and its use was discontinued. In Study 1, behavior for 11 inbred strains was assessed, and genetic correlations with ethanol-associated endophenotypes derived. In other studies, we assessed DD in lines selected for differing levels of ethanol withdrawal symptomatology or ethanol consumption, and on-going studies are examining correlations between DD and responses to passively administered ethanol in a heterogeneous mouse stock to identify novel phenotypic targets.
Results: In Study 1, our data indicated significant strain differences in DD and substantial heritability for DD as a behavioral trait ($h2 = 0.39$), as well as heritability for side bias away from the "delayed" alternative when the delay was absent ($h2 = 0.31$). Further, there were significant genetic correlations between DD and ethanol preference (10%, $n = 10$: $r = 0.72$), though not with other indices of response to ethanol (e.g., chronic withdrawal, $n = 7$: $r = -0.65$; sedation, $n = 10$: $r = 0.11$), nor with sucrose consumption or preference ($n = 11$: $r = -0.04$ and 0.19 respectively). In selected line studies, heightened chronic withdrawal tended to be associated with steeper DD ($p = 0.07$) but short term selection for high and low ethanol drinking was not ($p = 0.12$). Preliminary data from on-going studies suggest that, for heterogeneous stock mice, there are positive correlations amongst DD and various chronic withdrawal measures, including social approach, and chronic exposure measures, including behavioral sensitization.
Conclusions: These data suggest that DD has a heritable component in mice, and is genetically associated with chronic withdrawal and consumption, but that effect sizes are small. Reasons for this, including differences in amount and delay sensitivity that contribute to heightened delay discounting, as well as pleiotropic genetic contributions to these complex behaviors, warrant additional investigation. Further, heterogeneous stock studies suggest addition ethanol-associated behaviors which could be examined for shared genetic contribution in future studies.
Disclosures: Nothing to Disclose.

13.4 Identification of Individual Differences in Delay Discounting by Heterogeneous Stock Rats

Jerry Richards

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Background: In the natural environment important reinforcers such as food or water are often distributed in patches. Staying in a patch may deplete the density of available reinforcers to low levels so that it is better to travel to a new patch. The decision about when to leave a depleting patch in order to maximize overall reinforcement rate depends upon the time required to travel to a new patch. Longer travel delays mean that it is better to stay in the patch longer and deplete the patch to lower densities before leaving. Decisions made in the patch foraging situation are similar to those made in delay discounting (DD) procedures. In each case, choices are made between a delayed larger reinforcers and more immediate smaller reinforcers. Optimal foraging theory predicts that the animal will discount by delay to a degree that maximizes over all reinforcement. Patch foraging differs from laboratory based "self-control" procedures typically used to study DD in non-human animals because repeated choice of the more immediate alternative can lead to greater reinforcer rates, whereas in "self-control" procedures choice of the delayed alternative always produces greater reinforcement due to experimenter imposed inter-trial intervals. It is

arguable that the contingencies of reinforcement imposed by laboratory based "self-control" procedures are unlikely to be encountered in the natural environment.
Methods: A large number of heterogeneous stock rats (n >100) with highly variable genotypes were tested on a sequential choice DD procedure that simulates contingencies of reinforcement that are intended to be similar to those encountered by animals foraging in patchy environments. Delays of 0, 6, 12, 18, 24 s were tested. Lococotor activity in a novel environment was tested prior to DD testing.
Results: Rats were sorted according to how much they discounted by delay. Comparison of the twenty of rats that discounted the most (high discounters) with the 20 rats that discounted the least (low discounters) showed that high DD yielded the greater reinforcement rates. We also observed marked differences in switching between the two water feeders at the 0 s delay with some rats switching after every reinforcer while others stayed at the depleting water feeders for much longer periods before switching. There was no correlation between DD and the tendency to switch patches. Switchers and stayers were equally sensitive to the effects of delay. We found no correlation between loco motor activity and either switching or discounting, indicating that the observed differences were not due to general differences in activity.
Conclusions: These results demonstrate that DD can be advantageous by increasing overall consumption rate. Two behavioral phenotypes, high & low discounters and switchers & stayers were observed. Research is currently underway to identify genotypes that may underlie these patterns. This research may contribute to the identification of genetically determined biases in decision making underlying personality differences related to impulsivity.
Disclosures: Nothing to Disclose.

Panel
14. The Road to Recovery: Delineating the Neural Circuits of Compulsive Drug Use

14.1 The 'Ins' and 'Outs' of the Striatum: Mapping Addiction Circuits

Susan Ferguson

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Background: Addiction is a chronic relapsing disorder characterized by the loss of control over drug intake, high motivation to obtain drug, and a persistent craving for the drug. Accumulating evidence implicates cellular and molecular alterations within cortico-basal ganglia-thalamic circuitry in the development and persistence of this disease. The striatum is a heterogeneous structure that sits at the interface of this circuit, receiving input from a variety of brain regions (e.g., prefrontal cortex, ventral tegmental area) to guide behavioral output, including motor planning, decision-making, and motivation. However, the vast interconnectivity of this circuit has made it difficult to isolate how individual projections and cellular subtypes within this circuit modulate each of the facets of addiction.
Methods: To begin to address these issues, we used novel viral vector targeting approaches to express inhibitory Gi/o-coupled DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) selectively in striatal cells of the indirect or the direct pathways or in prefrontal cortical afferents to the striatum. Activation of DREADDs by the otherwise inert ligand clozapine-n-oxide produces transient decreases in neuronal activity and allowed us to examine the effect of these targeted cellular manipulations on behaviors associated with addiction (drug self-administration, psychomotor sensitization). In addition, we have expressed the genetically encoded calcium indicator GCaMP6m into the striatum to exam calcium signaling following different patterns of cocaine administration (drug self-administration under continuous or spiking access as well as following extended access, acute and repeated experimenter administered) using two-photon imaging in a slice preparation.
Results: We found that decreasing activity of the indirect pathway enhanced the development of psychomotor sensitization whereas decreasing activity of the direct pathway blocked the persistence of this phenomenon. Although decreasing activity of cortical afferents to the striatum had no effect on drug-taking in a self-administration paradigm it did impair the development of sensitization. Interestingly, inhibiting corticostriatal afferent activity during drug use enhanced conditioned responding to the drug-associated context as well as produced slower rates of extinction and increased responding during drug prime-induced reinstatement – an effect that was normalized by inhibiting these corticostriatal afferents immediately prior to the drug prime. Finally, we found that different patterns of drug administration lead to distinct alterations in calcium signaling in the striatum, both at baseline and following drug exposure.
Conclusions: These studies use cell-specific targeting and novel molecular tools to begin to isolate the contributions of specific striatal afferent and efferent projections in behaviors related to addiction as well as to map changes in the activity of striatal neurons following different patterns of drug use. The findings from these studies support the hypothesis that an imbalance between direct and indirect striatal pathway activity may mediate a transition to addiction, and that activity of these pathways is regulated by top-down control from the cortex. However, they also demonstrate that the cortico-basal ganglia-thalamic circuitry is more complex and dynamic than has been revealed previously.
Disclosures: Nothing to Disclose.

14.2 Corticostriatal Mechanisms of Compulsive Cocaine Seeking in Rats

Barry Everitt

University of Cambridge, Cambridge, United Kingdom

Background: Compulsive drug use is defined as the maladaptive propensity to repeat, or perseverate, in drug seeking or consumption in the face of significant aversive or disadvantageous consequences. But not all individuals that initially take drugs develop compulsive drug seeking and this individual vulnerability has been modeled in procedure that requires drug seeking to be performed under the threat or

actual receipt of punishment, enabling investigation of the neural mechanisms underlying compulsive drug seeking.
Methods: Rats were trained in a seeking-taking chained procedure in which responding on one lever (the 'seeking' lever) results in access to a second, 'taking' lever, responding on which results in a cocaine infusion. Randomly on 50% of trials, seeking responses result not in access to the taking lever, but in mild punishment, thereby capturing the conflict between opponent motivational states that has been suggested to characterize addictive behavior. We have investigated the neurochemical correlates and neural substrates of compulsive cocaine seeking using pharmacological manipulations of the brain.
Results: A discrete domain of the anterior dorsolateral striatum was shown to be involved specifically in punished, but not unpunished, cocaine seeking. We also identified reduced levels of 5-HT utilization across prefrontal cortical areas (as well as decreased DA utilization in the dorsal striatum) selectively in compulsive, but not non-compulsive rats, despite a very similar history of cocaine exposure. Forebrain 5-HT depletion, or systemic treatment with a 5-HT2C receptor antagonist after a short cocaine history (no rats are compulsive), resulted in increased levels of seeking under punishment. A serotonin-selective 5-HT reuptake inhibitor, citalopram, dose-dependently reduced compulsive seeking.
Conclusions: The results show that the great majority of a population of rats with an escalated, or long history of, cocaine intake are able to withhold their cocaine seeking responses — effectively achieving abstinence — when there is the intermittent risk of punishment. However, 20% of rats continued to seek cocaine compulsively. A discrete zone of the anterior dorsolateral striatum exerts a strong influence on compulsive drug seeking by mediating the influence of the threat of intermittent and unpredictable contingent punishment on cocaine seeking responses. Reductions in cortical 5-HT and striatal DA utilizatisation clearly differentiated compulsive and punishment-sensitive groups despite their common drug history. This indicates both the possible causal involvement of reduced 5-HT transmission in the compulsive cocaine seeking phenotype and the therapeutic potential of reversing this deficit and thereby the propensity to seek cocaine.
Disclosures: Nothing to Disclose.

14.3 Dynamic Changes in Phasic Dopamine Release to Drug-associated Cues Following Chronic Use and Withdrawal

Paul Phillips

University of Washington, Seattle, Washington, United States

Background: When drug-related cues are presented to drug abusers in a non-contingent manner (i.e., not dependent on their own actions), they often illicit drug craving and promote drug seeking. This type of cue exposure is considered to be one of the most robust stimuli to elicit relapse of drug use following periods of abstinence. In fact the potency of drug cues increases over periods of time without exposure to drugs, a phenomenon known as 'incubation of craving'. Prominent theories of drug addiction hypothesize that the amount of mesolimbic dopamine elicited by drug-related cues increases following chronic drug and this change accounts for hyper-responsiveness to these cues. However, there are no previous empirical reports that directly measured how phasic dopamine elicited by non-contingent drug cue presentation changes over chronic drug use and following periods of withdrawal.
Methods: Male Wistar rats underwent intravenous cocaine self-administration in one-hour or six-hour sessions followed by periods of drug withdrawal (one, seven or thirty days). Prior to and following some self-administrations (early and late training) and following withdrawal, animals were subjected to short cue (non-contingent) exposure sessions. Also following withdrawal rats underwent extinction sessions (self-administration sessions without cocaine or cue delivery) followed by cue presentation to elicit reinstatement of drug seeking, and test the degree of 'incubation of craving'. During self-administration, cue-exposure and extinction/reinstatement sessions, dopamine release was recorded in the nucleus accumbens using fast-scan voltammetry.
Results: Dopamine release to non-contingent drug cues increases following chronic drug use. This effect was more marked following six-hour cocaine self-administration sessions versus one-hour sessions, and in individuals that escalated their drug intake compared to those with stable drug use. This increase in phasic dopamine release to drug cues correlated with increased motivation to obtain the drug as assessed in behavioral sessions using progressive ratio of reinforcement. We also observed incubation of drug craving that progressed from one to seven to thirty days of withdrawal and was more pronounced following six-hour self-administration sessions compared to two-hour sessions.
Conclusions: Phasic dopamine release elicited by non-contingent drug-cue exposure increases during chronic drug use and withdrawal and enhances cue elicited drug-seeking behavior. These findings are in stark contrast to phasic dopamine transmission to response-contingent presentation of drug cues during drug taking which decrease following chronic drug use promoting escalation of drug taking. We conclude that while decreased phasic dopamine release during drug taking promotes escalation, increased phasic dopamine release contributes to craving and relapse, consistent with incentive sensitization theories.
Disclosures: Part 1: My spouse is an employee of Amgen Inc. and we own stock in that company, **Part 2:** My spouse is an employee of Amgen Inc. and we own stock in that company, **Part 3:** My spouse is an employee of Amgen Inc. and we own stock in that company, **Part 5:** My spouse is an employee of Amgen Inc. and we own stock in that company.

14.4 Functional Network Connectivity Between Rostral ACC and Insula Predicts Response to Varenicline for Tobacco Dependence (TD)

Claire Wilcox

University of New Mexico, Placitas, New Mexico, United States

Background: More than 95% of smoking cessation efforts fail. Clarifying the neural circuitry underlying relapse can

improve treatment research. Resting state functional connectivity (rsFC) and functional network connectivity (FNC) analyses provide information about coherence between brain regions and networks. Previous work has shown that the nicotine withdrawal state is associated with greater rsFC between rACC and insula (Huang 2014) or less negative rsFC between networks comprised of rACC (DMN) and insula (salience) (Lerman 2014). Insula-rACC connectivity decreases with varenicline (Sutherland 2013b). We explored whether insula-rACC FNC predicted later smoking behavior in individuals enrolled in a clinical trial of varenicline for TD.

Methods: Participants were 145 treatment-seeking cigarette smokers between the ages of 18 and 55 enrolled in a double-blind, placebo-controlled trial of varenicline, and who underwent a 6 minute resting state scan before treatment initiation. Individuals were randomized to active medication or placebo for a period of 12 wks, titrated as tolerated to 1 mg twice daily by day 8, with a target quit date of day 8. The time-line follow-back procedure was used to record tobacco use. Binary point prevalence data were not analyzed due to low numbers of quitters in the placebo group. Missing data were imputed to screen visit values. Resting state data was preprocessed using a standard pipeline. Group independent components analysis was utilized to extract individual subject time series from 2 intrinsic connectivity networks (which fell within insula and rACC) previously identified from a large sample of controls (Allen 2011). Time series were filtered and motion was regressed out. Functional network connectivity (FNC) between insula and rACC components was calculated. Two linear regressions were performed with number of cigarettes (NumCig) at screen, treatment group assignment (RxGrp), rACC-insula FNC, and an interaction term (FNC X RxGrp) as predictors. Outcome variables were NumCig smoked over the past 4 wks at 6 wk and 12 wk.

Results: There was a significant RxGrp X FNC interaction for insula-rACC FNC predicting both 6 ($p = .03$) and 12 wk ($p = .02$) NumCig. In the varenicline group, but not the placebo group, FNC predicted overall NumCig at 6 ($p = .035$, $b = -.23$) and 12 wk ($p = .05$; $b = -.21$) such that greater FNC at baseline predicted less smoking, correcting for baseline smoking. It was not a significant predictor in the placebo group (6 wk NumCig, $p = .47$, $b = .08$; 12 wk NumCig, $p = .33$, $b = .10$). When baseline smoking was removed from the model, the RxGrp X FNC interaction term was still significant at 6 wk and 12 wk ($ps = .02$), and there was a positive association between insula-rACC FNC and greater smoking at a trend level at 12 wk ($b = .22$, $p = .09$).

Conclusions: In individuals on varenicline, higher insula-rACC connectivity predicted better smoking outcomes. Greater FNC did not significantly predict outcomes when varenicline and placebo participants were combined, although there was a positive association between FNC and smoking at week 12, at a trend level. Consistent with data that excessive insula-rACC rsFC occurs during withdrawal, and evidence that varenicline decreases withdrawal, our data indicate that rsFC or FNC between rACC and insula may be an important treatment target and predictor of response to varenicline.

Disclosures: Nothing to Disclose.

Mini Panel
15. Sharing is Caring: An Overview of the Data Sharing Landscape

15.1 Data Sharing at NIMH

Bruce Cuthbert

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Background: Data sharing has become an issue for several reasons. There are broad public concerns about the reproducibility of both clinical and preclinical research. Congress worries about redundancy of research investments. NIH wants to improve the efficiency of research, noting the value of secondary data analyses. Data sharing is also one aspect of increasing transparency of the entire research process.

Methods: Beginning with the Autism Centers of Excellence, NIMH required the sharing of individual level clinical data into the National Database for Autism Research (NDAR). A similar deposition and sharing policy was deployed for genomic data. Last year, NIMH extended this policy to all clinical trials, requiring individual level data to be deposited to the National Database for Clinical Trials (NDCT). Descriptive/raw data are expected to be submitted to NDCT on a semi-annual basis (beginning six months after the award budget period has begun). Analyzed data are expected to be submitted prior to publication/public dissemination (whether the findings are positive or negative). Data access and sharing of de-identified data are worked out on an individual basis but the goal is release to qualified investigators at the time of publication.

Results: The NIMH Limited Access Datasets project, including data from 23 large NIMH-supported clinical trials, recently sent out its 300th dataset. The datasets are referred to as "limited access" because, for the protection of the human study participants from whom the data were obtained, only qualified researchers may obtain access to the datasets, and only upon the approval of a Data Use Certification (DUC), which stipulates specific terms and conditions under which the data may be used, including terms for data security and confidentiality, and acknowledgement of the original data submitters in publications. The datasets have provided the raw material for at least 160 published scientific papers, an example of how data sharing provides an avenue for multiplying the return on investment and benefit from clinical research.

Conclusions: Standardization, integration, and sharing are the principles underlying a new culture of clinical research. The transition to this new culture will not be easy for everyone, but transparency is ultimately essential for public trust and data sharing is a critical aspect of transparency. Note that data sharing introduces its own set of problems: issues of privacy, access, and data provenance will need to be addressed in this new world of open science.

Disclosures: Nothing to Disclose.

15.2 Perspectives on Responsible Clinical Trial Data Sharing

Timothy Coetzee

National Multiple Sclerosis Society, New York, New York, United States

Background: The Institute of Medicine (IOM) recently released a report entitled "Sharing Clinical Trial Data: Maximizing Benefits, Minimizing Risk" (http://www.iom.edu/Reports/2015/Sharing-Clinical-Trial-Data.aspx). The report, which was sponsored by a diverse group of stakeholders including government funding agencies, regulators, foundations, and pharmaceutical and medical device manufacturers, was produced by an expert committee with the goal of fostering emergence of a culture of clinical trial data sharing that will increase scientific knowledge and ultimately improve therapies for patients.
Methods: The committee recognized that there are several stages in the clinical trial cycle at which data can be shared and made four main recommendations for responsible sharing of clinical trial data.
Results: Stakeholders in clinical trials should foster a culture in which data sharing is the expected norm.
Sponsors and investigators should share the various types of clinical trial data at appropriate times in the clinical trial life cycle: at trial registration, 12-18 months after study completion, no later than 6 months after publication, 30 days after regulatory approval, or 18 months after abandonment.
Holders of clinical trial data should employ publicly available data use agreements that reduce risks, enhance secondary analysis, and protect public health. The public should be involved in reviewing data requests.
Stakeholders should work together to address key challenges and foster a culture toward a vision of data sharing.
Conclusions: Clinical trial data sharing has many potential benefits to the scientific community and to patients. However, risks and concerns remain, and the infrastructure and culture to support data sharing are currently only in the infancy stages. Nevertheless, finding solutions for disease demands increased data sharing. To change the culture, data sharing must be rewarded, required, and enforced. Protections of all interested parties must be in place, and user-friendly infrastructure to support data deposition must be developed and standardized. The challenge is significant, but worthy of our collective effort.
Disclosures: Nothing to Disclose.

15.3 Current Practices for Sharing Data: The Landscape in the Private Sector

Lisa Gold

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Background: According to Wikipedia "Data sharing is the practice of making data used for scholarly research available to other investigators. Replication has a long history in science. Many funding agencies, institutions, and publication venues have policies regarding data sharing because transparency and openness are considered by many to be part of the scientific method." Shifts in the research ecosystem including big data opportunities, electronic health records and data transparency policies are converging to inspire new thinking and action around the sharing of research data to drive novel research queries and innovation. In July 2013 PhRMA joined with the EFPIA in adopting Principles for Responsible Clinical Trial Data Sharing, reflecting the biopharmaceutical sector's strong support for responsible data sharing that recognizes the importance of protecting patient privacy, respects the integrity of national regulatory systems, and maintains incentives for continued investment in biopharmaceutical research. This initiative further encourages all medical researchers to promote medical and scientific advancement by adopting 5 commitments related to enhanced data sharing with Researchers, public access to clinical Study Information, sharing results with patients who participate in clinical trials, certifying procedures for sharing clinical trial Information and reaffirming commitments to publish clinical trial results. Recent focus in the regulatory arena is exemplified by the European Medicines Agency new standards for clinical trial data transparency. In January, the Institute of Medicine issued a global report concluding that a multi-stakeholder effort is needed to develop a culture, infrastructure, and policies that will foster responsible sharing—now and in the future.
Methods: In response to this changing landscape pharmaceutical companies have committed to principles on data sharing and invested in supportive policies and systems. Merck has had a data sharing policy since 2008 that has generated at least 40 manuscripts and 8 presentations to date. A revised policy in 2014 introduced a website, guidelines for submission of research proposals for data and a charter for governance of an external review board. Multi-company initiatives are helping to drive unified approaches. A GSK launched website has evolved into a multi-sponsor site involving 13 companies, where researchers request access to anonymized patient level data and supporting documents from clinical studies to conduct further research. Other stakeholder groups such as the Harvard Multi Regional Clinical Trials Center include a focus on return of results, providing clinical trial participants with plain language summaries of the trial results and information about the outcome of the study.
Non-competitive consortia are harnessing the power of collaboration across research sectors. The Innovative Medicines Initiative (IMI) does this by facilitating collaboration between key players involved in healthcare research, including universities, pharmaceutical and other industries, patient organizations, and medicines regulators. An integrated basic and clinical approach within IMI is the NEWMEDS program, Novel Methods leading to New Medications in Depression and Schizophrenia. This international consortium of scientists comprises one of the largest ever academic-industry collaboration projects.
Results: The NEWMEDS collaborative has assembled a dataset of individual patient level information from randomized placebo-controlled trials of second-generation antipsychotics by 5 pharmaceutical companies. Examination of patient and trial-design-related determinants of outcome has revealed new insights into optimal trial

duration and patient attributes that will result in reducing patient exposure to placebo and experimental treatments in future trials. Additional examples of such high impact results from data sharing activities will be presented.
Conclusions: For all its promise, data-sharing entails significant risks, burdens and challenges that must be addressed in order to fulfill its potential. These include infrastructure, patient privacy, sponsor responsibility and respect for the data. Despite these concerns, there are now enough results with positive impact to provide a strong rationale for continuing. Momentum continues to build and these efforts have the potential to transform research and improve health by speeding up the development of, and patient access to, innovative medicines.
Disclosures: Part 1: Full time employee of Merck & Co, **Part 2:** Full time employee of Merck and Co, **Part 3:** Full time employee of Merck & Co, **Part 5** Merck & Co.

Mini Panel
16. Brain-wide 'Glymphatic' Pathway: Visualization and Function

16.1 Evidence for the Impairment of Glymphatic Pathway Function in the Aging Brain

Jeffrey Iliff

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Background: Alzheimer's disease (AD), like other neurodegenerative conditions characterized by the mis-aggregation of different proteins, is primarily a disease of the aging brain. Yet the changes in the aging brain that render it vulnerable to protein mis-aggregation and neurodegeneration remain unknown. The glymphatic system is a brain-wide paravascular pathway along which CSF from the subarachnoid space recirculates through the brain parenchyma, clearing interstitial solutes such as amyloid β (Aβ) and tau, from the brain interstitium. Paravascular CSF recirculation and Aβ clearance are dependent on the astroglial water channel, which is expressed along perivascular endfeet that ensheath the cerebral vasculature.
Methods: Glymphatic pathway function was evaluated in the young by ex vivo whole slice fluorescence microscopy and radiolabeled Aβ clearance assay. Perivascular aquaporin-4 (AQP4) localization was evaluated by immunofluorescence. AQP4 localization was assessed in the post-mortem human frontal cortex by immunofluorescence.
Results: In the aging mouse cortex, perivascular localization of AQP4 was impaired, and this AQP4 mis-localization was associated with slowed glymphatic CSF recirculation and Aβ clearance in the aging brain. In α-syntrophin knockout mice, which express normal AQP4 levels, but lack perivascular AQP4 polarization, CSF recirculation was similarly impaired, suggesting that loss of perivascular AQP4 localization is sufficient to impair glymphatic pathway function. In studies carried out in human autopsy tissue, perivascular AQP4 localization in the frontal cortex declined with increasing age. Strikingly, among a cohort of so-called 'super-agers' that survived cognitively intact beyond 85 years of age, perivascular AQP4 localization was maintained at levels observed in the young cortex. Impairment of perivascular AQP4 localization was significantly associated with worsening Aβ plaque burden, more advanced Braak stage, and worsening cognitive decline.
Conclusions: These findings demonstrate that glymphatic pathway function is impaired in the aging rodent brain, and suggest that age-related impairment of glymphatic pathway function may be one factor making the aging human brain vulnerable to Aβ aggregation and the development of AD.
Disclosures: Nothing to Disclose.

16.2 Metabolic Aspects of Cerebral Capillary Water Efflux

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Background: The cerebral capillary endothelium provides extensive surface area for water exchange between the plasma and brain parenchyma. Cerebral capillary endothelial cells are in intimate contact with astroglial and pericytes which are thought to be important in control of local blood flow and ultimately drive gylmphatic flow. Combinations of neurons, glia, and microvessels have been termed "neurogliovascular units," because of their fundamental symbiotic metabolic and energetic interactions.
Methods: A 7T whole-body MRI instrument, with quadrature transmission and 24-channel phased-array receive head RF coils, was used. Dynamic contrast measurements employed a single-slice inversion recovery (IR) turboflash technique, sampling magnetization eight post-inversion times across a 0.05 mmol/kg gadoteridol injection. Data were processed using a pharmacokinetic model that incorporated two-site exchange formalism.
Results: As an example, areas of the brain damaged by multiple sclerosis (MS) disease activity have impaired energy metabolism leading to abnormal capillary mean water lifetimes; in chronic lesions we find a nearly 2-fold decrease in trans-capillary water flux and normal appearing brain tissue (NABT) shows ~20% decrease; consistent with positron emission tomography estimates of reduced cerebral metabolism in MS.
Conclusions: Results from our work suggest that tight metabolic coupling between neurons, glia, and endothelia, the "neurogliovascular unit", produces a phenomenon that allows assessment of metabolic activity using dynamic contrast MRI techniques. Water flux across the capillary endothelium is driven by the local tissue homeostatic sodium-potassium ATPase (NKA) turnover which in turn is responsive to metabolic activity behind the blood-brain barrier. The slower the metabolic activity, the slower the NKA turnover and accompanying water flux. The capillary blood water efflux is a sensitive measure of this metabolic activity, and can be mapped with dynamic contrast enhanced MRI techniques. Regional cerebral metabolic activities vary significantly across the sleep-wake cycle which is expected to result in regional differences in capillary water efflux and may be an important component of glymphatic system function.
Disclosures: Nothing to Disclose.

16.3 Brain-Wide Glymphatic Transport in the Unconscious State: Influence of Body Position

Helene Benveniste

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Background: We recently discovered a brain-wide waste removal pathway which shares many features with the classical lymphatic drainage system in other body organs. We named this system the 'glymphatic' pathway because it is dependent on aquaporin 4 water channels expressed on perivascular astrocytic endfeet. Importantly, our studies also show that soluble amyloid-beta and tau proteins are cleared from the brain along this newly discovered glymphatic pathway. Transport through this pathway is controlled by the brain's arousal level because during sleep or anesthesia the brain's interstitial space volume expands (compared to wakefulness) resulting in faster waste removal. Humans, as well as animals, exhibit different body postures during sleep, which may also affect waste removal. The objective of this study was to evaluate the influence of body position on CSF-ISF exchange rates in the unconscious state.
Methods: Dynamic contrast enhanced MRI and kinetic modeling was used to quantify CSF-ISF exchange rates in anesthetized rodents' brains in supine, prone or lateral positions. To validate the MRI data and to specifically assess the effect of body posture on clearance of amyloid beta we used a mouse model in combination fluorescence microscopy and radioactive tracers, respectively.
Results: Glymphatic transport in the anesthetized, unconscious rat and mouse was most efficient in the lateral position when compared to supine or prone positions. In the prone position, where the rat's head was in the most upright position (mimicking posture during the awake state), transport was characterized by 'retention' of the tracer, slower clearance and more CSF efflux along larger caliber cervical vessels. The optical imaging and radiotracer studies confirmed that glymphatic transport and amyloid-beta clearance was superior in the lateral and supine positions.
Conclusions: In rats, the MRI analysis showed that the reduced uptake of Gd-DTPA in the brain of PRONE rats was paralleled by increased efflux of CSF along the cervical vasculature. Imaging of fluorescently tagged CSF tracers in mice revealed that the PRONE position also was linked to reduced CSF influx in brain, while the influx of fluorescent tracers into spinal cord was increased. We suspect that the head position affect the activity of the glymphatic system similarly in rat and mice, but the difference in approach (MRI vs optical imaging) did not allow a formal species comparison. However, both sets of observations suggest that the lateral position during sleep has a clear advantage with regard to glymphatic removal of beta amyloid and other metabolic waste products of neural activity. While this is speculative and awaits testing in human subjects, other clinical studies have shown that beta amyloid content in CSF is lower in sleep than wakefulness, consistent with increased clearance. We propose that the most popular sleep posture (lateral) has evolved to optimize waste removal during sleep and that posture must be considered in diagnostic imaging procedures developed in the future to assess CSF-ISF transport in humans.
Disclosures: Nothing to Disclose.

Study Group

17. rt-fMRI Neurofeedback: Are We There Yet?

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Over a decade ago, studies that human subjects are able to modulate fMRI signals from specific brain regions in real-time (real-time fMRI neurofeedback / modulation, or rt-fMRI). rt-fMRI has been used not only for experimental research, but has been proposed as a means for non-invasively treating brain dysfunctions by having patients can learn to control activity in brain regions that mediate specific symptomology. Neuromodulation using rt-fMRI has been demonstrated recently in several contexts. For example, some studies have shown that subjects can modulate behavioral and emotional responses to pain and emotionally-valenced photographs, motor response cues, lexical stimuli and reward-related stimuli by selectively manipulating fMRI BOLD signal in anterior cingulate cortex, amygdala, insula, precentral gyrus, and Broca's area and ventral striatum.
However, rt-fMRI remains controversial as a number of issues remain unresolved. Such issues include, individual difference in people's ability to modulate rt-fMRI signals, optimal subject instructions, relation of regional brain activation to specific symptoms, and whether the proper control conditions involve rt-fMRI originating irrelevant brain circuitry, non-contingent feedback, or signals from another subject. In particular, the question of the proper control condition raises difficult ethical and policy issues that could affect the course of future research and potential clinical applications.
In 2008 NIDA issued a Request for Applications entitled "Facilitating Self-Control of Substance Abuse Related Brain Activity Through Real-Time Monitoring of fMRI Signals". In this study group, the investigators supported by this RFA will discuss the advances and challenges encountered during their studies, with an emphasis on implementation, ethical and policy issues relevant to having fMRI neurofeedback progress to being a robust research procedure and a potential treatment modality.
Disclosures: Nothing to Disclose.

Panel

18. Signals from the 4th Dimension: How the Extracellular Matrix Regulates Synaptic Plasticity and Neuropsychiatric Disease

18.1 Mechanisms Through which Extracellular Proteolysis Shapes Neuronal Structure and Function

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Background: Synaptic structural and functional plasticity underlies many forms of enduring behavioral experience,

including learning and memory, and is critical for proper formation of circuits during early postnatal development. The molecular mechanisms that drive coordinated remodeling of both synaptic form and function have been enigmatic, but there is an emerging recognition that matrix metalloproteinases (MMPs) are critically involved in the rapid remodeling of the synaptic microenvironment associated with synaptic, cellular and behavioral plasticity, both in adulthood and during development. MMPs are a large family of extracellularly-acting, mostly secreted proteases whose targets include extracellular matrix (ECM), adhesion proteins and other cell-surface molecules that are important for maintaining and modifying synaptic architecture. The focus of this talk is on the mechanistic underpinnings of these varied roles of MMPs and their canonical targets in shaping circuit structure and function.
Methods: Gain- and loss-of-function approaches (i.e. mouse genetics, exogenous inhibitors and recombinant-active MMPs) are applied to both acute hippocampal slices and adult mice and rats *in vivo* in combination with synaptic electrophysiology, live-cell imaging, novel and standard localization methods and behavioral assays in order to understand MMP control of synaptic morphology, circuit connectivity and brain function.
Results: MMP-9 is rapidly upregulated and becomes proteolytically active in hippocampus by stimuli that induce LTP as well as by a single-trial, inhibitory-avoidance learning experience. Such regulation of MMP-9 is specific MMP-9 is not regulated during LTD, nor is the closely related MMP-2 regulated by experimentally- or behaviorally-induced plasticity. Once proteolytically active, MMP-9 signals though integrin receptors both to potentiate synapses and concomitantly to enlarge the spine-heads in which they sit. These processes require dynamic actin and modification of certain actin-binding proteins. When MMP function is abrogated, stable functional and structural synaptic plasticity as well as hippocampal-dependent memory are significantly impaired. Developmentally, the effects of manipulating a canonical target of extracellular proteolysis on establishment of functional cortical circuitry was investigated in mouse somatosensory barrel cortex. Sema7A, an atypical member of the semaphorin family of guidance cues, is normally cleaved and deposited at high levels within barrel centers during early postnatal development when thalamocortical axons grow in and establish the characteristic isomorphic map of the contralateral whisker pad. We found that ablation of Sema7A disrupts barrel cytoarchitecture, reduces the polarized orientation of spiny cell dendrites, and impairs thalamocortical evoked synaptic responses.
Conclusions: Together these data provide compelling support for the idea that MMPs and other extracellularly-acting proteases function in normal synaptic physiology, coordinating both synaptic structural and functional plasticity appropriate for establishing circuit structure/function developmentally as well as enabling memory later in life.
Disclosures: Nothing to Disclose.

18.2 Role of Perineuronal Nets in Cocaine-Induced Plasticity

Barbara Sorg

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Background: Specialized aggregations of extracellular matrix called perineuronal nets (PNNs) appear during juvenile stages of development and surround primarily fast-spiking, parvalbumin-containing GABAergic interneurons in the central nervous system. Several studies have shown that removal of PNNs re-establishes juvenile-like states of plasticity. However, only a single study has examined a role for PNNs in plasticity induced by drugs of abuse. Here we determined 1) whether PNN removal in the medial prefrontal cortex (mPFC) prevented cocaine-induced plasticity; and 2) whether cocaine altered PNN intensity in the mPFC.
Methods: In the first experiment, male Sprague-Dawley rats were trained for cocaine-induced conditioned place preference (CPP). PNNs were removed by the enzyme chondroitinase-ABC (Ch-ABC) prior to training for CPP, prior to extinction of CPP, or prior to cocaine memory reactivation. In the second experiment, PNNs were removed by Ch-ABC prior to training for cocaine self-administration. In the third experiment, cocaine was given non-contingently for 1 or 5 days and PNN staining intensity was analyzed 2 hr later.
Results: Removal of PNNs within the prelimbic region of the medial prefrontal cortex (mPFC) impaired both the acquisition/consolidation and reconsolidation of cocaine-associated memories in the CPP task. Preliminary studies also indicate that PNN removal impaired the acquisition of cocaine self-administration. Consistent with reduced cocaine-induced plasticity when PNNs were removed, 1 day of cocaine decreased PNN intensity while 5 days of repeated cocaine treatment increased the intensity of PNNs, and this intensity was positively correlated with increased cocaine-induced behavioral sensitization.
Conclusions: Our studies indicate that PNNs within the mPFC impair cocaine-induced plasticity. Consistent with our findings, decreased PNN intensity is generally associated with enhanced plasticity and learning while increased PNN intensity is associated with diminished plasticity and establishment of strong memories. Our studies have implications for understanding how these extracellular matrix structures may be manipulated during the formation and maintenance of cocaine-associated memories to reduce relapse.
Disclosures: Nothing to Disclose.

18.3 Matrix Metalloproteinases and Cell Surface-Associated Substrates

Katherine Conant

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Background: Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that were named for their ability

to remodel extracellular matrix. A subset of MMPs may be released from brain-derived cells including neurons and glia. Release is increased in a neuronal activity dependent manner and plays a role in specific types of learning and memory. Because MMP activity is concentrated at the cell surface, the processing of specific cell surface molecules could be critical to MMP-dependent effects on neurotransmission. Work is focused on the processing of synaptic cell adhesion molecules (CAMs) and G protein coupled receptors.

Methods: We utilize striatal slices from mice that express the calcium indicator GCaMP3 specifically in D2 dopamine receptor-bearing neurons. Slices are pretreated with vehicle, dopamine, or the D1 receptor agonist SKF81297 and subsequent NMDA stimulated calcium flux is recorded and analyzed.

In complementary studies, we measure proliferation and neuronal differentiation of progenitor cells derived from the hippocampal dentate gyrus of adult mice that over express a glial-derived MMP.

Results: Dopamine and the D1 receptor agonist SKF81297 enhance NMDA-stimulated calcium flux in striatopallidal neurons. Potentiation is diminished by pretreatment with a broad-spectrum antagonist of MMP activity or by an inhibitor of integrin-dependent signaling, and may thus involve the generation of soluble CAM fragments that contain integrin-binding domains.

In complementary studies, increased expression of MMP-1 enhances proliferation and neuronal differentiation of progenitor cells from the adult hippocampal dentate gyrus. Both effects are reduced by an inhibitor of protease activated receptor-1, a G protein coupled receptor that is engaged following cleavage in its N-terminal domain.

Conclusions: Findings suggest that processing of specific cell surface molecules may contribute to MMP-associated plasticity. Specific findings may be relevant to addiction, as well as to anti-depressant associated neurogenesis.

Disclosures: Nothing to Disclose.

18.4 Nitric Oxide Signaling in the Accumbens Core Drives Relapse to Cocaine Seeking

Alexander Smith

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Background: Matrix metalloproteinases (MMPs) are pro-plasticity enzymes that degrade the extracellular matrix to promote synaptic growth and reorganization. Both MMP-2 and MMP-9 are required for cue-induced reinstatement of cocaine seeking and the associated transient synaptic potentiation of corticostriatal synapses. Following extinction of cocaine self-administration (SA) there is a constitutive upregulation of MMP-2 in the nucleus accumbens core (NAcore), and cue-induced reinstatement causes a transient induction of MMP-9 activity. However, it is unknown how either of these two enzymatic inductions occurs. One mechanism by which MMPs are activated is S-nitrosylation by nitric oxide (NO). NO is produced in the NAcore by neuronal nitric oxide synthase (nNOS) inside a subpopulation of interneurons that is approximately 1% of neurons in the striatum. We hypothesized that cocaine SA induces nNOS activity that in turn increases activity of MMP-2/9, and that stimulation of NO production in the NAcore would stimulate MMP activity, synaptic potentiation, and reinstatement of cocaine seeking.

Methods: Male NOS1-Cre transgenic mice were used to selectively chemogenetically target nNOS-expressing interneurons in the NAcore with a Gq-DREADD virus (AAV2-hSyn-DIO-HM3Dq). In the first experiment, we tested the ability of stimulation of these cells to stimulate MMP activity. To do this, mice were implanted with intra-NAcore guide cannula, and either CNO or vehicle was microinjected 15 minutes prior to measuring MMP activity via *in vivo* zymography. A second experiment examined the dependence of MMP activity on nNOS activity, using either a cocktail of CNO and the nNOS inhibitor NPLA, or CNO and vehicle prior to zymography. In order to determine whether nNOS activity was able to potentiate synapses on medium spiny neurons (MSNs), patch-clamp electrophysiology examined AMPA/NMDA ratios (A/N). Animals received systemic CNO or vehicle prior to being sacrificed for electrophysiological recording. In the final experiment, mice were trained in cocaine SA with conditioning light and tone cues for 10 days, and then extinguished for 10 days, then either CNO or vehicle was injected i.p. 15 minutes before either an extinction or cue-induced reinstatement session.

Results: We show that stimulating nNOS-expressing interneurons via Gq-DREADD increased MMP activity, and this effect was completely abolished by pre-treatment with the nNOS inhibitor NPLA. Furthermore, Gq-stimulation of nNOS-expressing interneurons was able to potentiate A/N on MSNs, the major cell type that constitutes 95% of the accumbens. Finally, stimulation of these interneurons was not only able to potentiate cue-induced reinstatement, but also drove reinstatement in the absence of cues.

Conclusions: We conclude that nNOS activity is not only necessary, but also sufficient to drive drug seeking behavior and the associated pathophysiology. Although nNOS-expressing interneurons constitute only approximately 1% of the NAcore, activation of these cells stimulated MMP activity globally, potentiated synapses on MSNs, and induced drug-seeking behavior. Thus, this small cell population may represent a 'master-switch' by which cocaine seeking is initiated, and may be an important pharmacotherapeutic target for the treatment of relapsing behavioral disorders.

Disclosures: Nothing to Disclose.

Panel
19. Neuroimaging, Addiction and Big Data: Opportunities and Challenges

19.1 Prediction of Substance Misuse Initiation (Alcohol, Nicotine and Cannabis): Insights from the Imagen Project

Robert Whelan

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Background: It has been demonstrated repeatedly that early substance use is a strong risk factor for adult substance

dependence and, therefore, identifying predictors of substance use in adolescence would be undeniably advantageous. Longitudinal population neuroscience studies, though logistically challenging, offer a promising approach to detecting the predictors of substance misuse as they potentially enable the causes and effects of substance misuse to be separated.
Methods: Brain data from a large number of regions of interest, encompassing the entire brain, were extracted for the following: structural gray matter (corrected for total gray matter volume), successful and unsuccessful inhibitory responses from the Stop Signal Task, reward anticipation and outcomes from the Monetary Incentive Delay Task, and responses to angry faces in a test of emotional reactivity. For all analyses (alcohol, nicotine & cannabis), participants were non-users at the time of data acquisition (age 14 years-old). At age 16, 150 non-drinkers were compared with 121 binge drinkers, 916 non-smokers were compared with 178 regular smokers, and 1216 non-cannabis users were compared to 173 cannabis users with a median of 20 lifetime uses of cannabis. A machine-learning approach was employed, utilizing forward feature selection and regularized regression via the Elastic Net. Total gray matter volume (GMV), total gray: white matter ratio (GWR), scanner location (8 sites), pubertal development status, sex, and handedness were also included as features. Results are reported with respect to performance on novel test data, using 10-fold cross validation.
Results: For all analyses, brain data were moderate predictors of future substance misuse, with area under the curve of the receiver operating characteristic (AROC) ranging from .6 (nicotine) to .63 (alcohol) to .64 (cannabis). Nicotine was primarily characterized by differences in GMV and GMR rather than by specific regional differences; alcohol by increased activity for future binge drinkers in the pre- and post-central gyri and smaller gray matter volume in the ventromedial prefrontal cortex; and cannabis by structural and functional differences (all tasks) in the temporal lobes, with smaller volumes and less activity for future cannabis users.
Conclusions: The use of a prospective, longitudinal design, in combination with a machine-learning approach to interrogate a high-dimensional data set has demonstrated that it is possible to predict, to a certain extent, future substance misuse using structural and functional brain data. Notably, no participants had engaged in substance misuse at the time of data collection. In general, these findings, such as the differences such as those found temporal lobe for future cannabis users, are consistent with previous research in this area. However, other results, such as a lack of differences in the orbitofrontal cortex, are discrepant with previous studies.
Disclosures: Nothing to Disclose.

19.2 A Selective Drug and Alcohol Prevention Programme that Targets Neurocognitive Correlates of Sensation Seeking: Focus on Reward Sensitivity

Patricia Conrod

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Background: Sensation seeking (SS) has been identified as a risk factor for substance misuse, yet very few clinical interventions have been developed to target SS in treatment or prevention. Our research demonstrates that SS is associated with a motivational sensitivity to reward which mediates the relationship between SS and early onset alcohol misuse (Castellanos-Ryan et al., 2011). We also developed a brief intervention designed to help youth better manage their SS (Conrod et al., 2006). Multiple, large prevention trials investigating the impact of this intervention approach (Conrod et al., 2010; 2013) provide unique opportunities to conduct research on personality-specific trajectories of substance misuse risk within an experimental design. With the recognition that SS is also a specific risk factor for cannabis misuse, we turn to the data in our previous and ongoing prevention trials to 1) examine if interventions targeting SS and reward sensitivity are specifically effective in reducing cannabis use; and 2) test whether reward sensitivity longitudinally mediates the relationship between SS and cannabis use, as previously shown for alcohol misuse (Castellanos-Ryan et al., 2011; 2014).
Methods: Secondary analysis of the cluster-randomised Adventure Trial (Conrod et al., 2013) examined the impact of SS interventions on cannabis use and frequency of use within a subset of SS high school students. 2) The Coventure Trial is a large (n = 3641) cluster-randomised trial of a school-based drug and alcohol prevention programme targeting 4 personality risk factors, of which one is SS. Neurocognitive factors such as response inhibition, reward sensitivity, are assessed at baseline and annually for 4 years following the brief intervention. Analyses focused on the 435 year 7 students who scored one standard deviation above the school mean on the Substance Use Risk Profile Scale - SS subscale (SURPS-SS; Woicik, et al., 2009). Reward sensitivity was measured using a passive-avoidance learning go-nogo task (Castellanos-Ryan et al., 2011), in which we contrast task performance on conditions involving reward and punishment-avoidance as incentives.
Results: Results indicate that 1) Subgroup analyses (both logistic and two-part models) reveal that the SS intervention delayed the onset of cannabis use among SS students (OR: .25, ß = -0.833, SE = 0.342, p = .015) in the Adventure Trial. 2) Mediation analyses suggest that reward sensitivity only partially explains risk for cannabis use within this personality group.
Conclusions: Targeting SS in school-based drug and alcohol interventions appears to be a promising approach to preventing risk for substance misuse in this high risk group. However, more research is needed to understand how reward sensitivity is implicated in SS and enhancement drug use motives. We suggest that the relationship between SS and addiction vulnerability is explained through a u-shaped relationship with arousal, whereby both under-arousal and over-arousal are motivational triggers for substance misuse in SS youth.
Disclosures: Nothing to Disclose.

19.3 ENIGMA Addiction Working Group: Initial Findings

Scott Mackey

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Background: An unbiased search across the whole genome for unknown associations between phenotypic traits of

interest and single nucleotide polymorphisms (SNP) typically involves testing the significance of hundreds of thousands to millions of SNP-trait associations and requires a strict multiple comparisons correction threshold, conventionally $p \leqslant 5x10\text{-}8$, to avoid reporting spurious findings. Since the sample size of a typical neuroimaging study lacks sufficient statistical power to explore unknown genomic associations with brain phenotypes, several international genetic imaging consortia, such as the ENIGMA project, have been organized in recent years to pool data across sites. This presentation will describe the formation and initial results of a large international working group that has leveraged the structure of the ENIGMA project, a template for multisite genetic imaging consortia, to study the genetic and neurobiological basis of addiction.

Methods: The Addiction working group used the genetic neuroimaging protocols developed by the Enhancing Neuroimaging Genetics through Meta-Analysis (ENIGMA) project (http://enigma.ini.usc.edu/) to harmonize data across multiple sites. The working group consists of 22 labs on 4 continents and possesses case/control and cohort datasets that collectively contain multimodal neuroimaging (e.g. task-related and resting state functional MRI, structural MRI, DTI) and genomic data on over 10,000 subjects. The first analysis examined brain volume correlates of addiction in case/controls datasets. All data were processed with freesurfer to ensure comparability of neuroimaging results across sites and the quality of data was assessed by standardized imaging protocols (http://enigma.ini.usc.edu/protocols/imaging-protocols/).

Results: Brain volumetric data relating to the use of five substances (i.e. alcohol, nicotine, cocaine, methamphetamine and cannabis) were combined to identify neural substrates of core addiction processes. This included brain scans from 3012 individuals. Total intracranial volume as well as right and left volumes of the thalamus, caudate, putamen, ventral pallidum, hippocampus, amygdala, and nucleus accumbens were compared between individuals with heavy or dependent patterns of substance use and those with little or no exposure. While the volumes of all structures were smaller in the heavy users than in the controls, after controlling for site, age, sex, and intracranial volume, two structures were significantly smaller bilaterally in heavy users: the putamen and nucleus accumbens, $p < 0.01$.

Conclusions: An initial analysis found lower grey matter volume in two subcortical brain regions was associated with heavy use or dependence on one of several addictive substances. The nucleus accumbens encodes reward value. Less grey matter volume in this brain region may alter the perception of the rewarding properties of drugs and alcohol. The putamen is involved in habitual instrumental behaviors that are not affected by devaluation of the expected outcome and which may be related to persistent drug taking despite negative consequences in addiction. The high dimensionality of follow-up genetic imaging analyses performed by the working group will be reduced by limiting the search for associations to a small set of brain regions.

Disclosures: Nothing to Disclose.

19.4 Bayesian Neural Adjustment of Inhibitory Control Predicts Emergence of Problem Stimulant Use

Martin Paulus

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Background: Bayesian ideal observer models quantify individuals' context- and experience-dependent beliefs and expectations about their environment, which provides a powerful approach (a) to link basic behavioral mechanisms to neural processing and (b) to generate clinical predictors for patient populations. Here, we focus on (b) and determine whether individual differences in the neural representation of the need to stop in an inhibitory task can predict the development of problem use (i.e., abuse or dependence) in individuals experimenting with stimulants.

Methods: One-hundred fifty-seven non-dependent occasional stimulant users (OSU), aged 18-24, completed a stop-signal task while undergoing functional magnetic resonance imaging. These individuals were prospectively followed for three years and evaluated for stimulant use and abuse/dependence symptoms. At follow-up, thirty-eight OSU met criteria for a stimulant use disorder (problem stimulant users/PSU), while fifty had discontinued use (desisted stimulant users/DSU).

Results: We found that those individuals who showed greater neural responses associated with Bayesian prediction errors, i.e. the difference between actual and expected need to stop on a given trial, in right mPFC/ACC, caudate, anterior insula, and thalamus were more likely to be diagnosed with problem use three years later. Importantly, these computationally based neural predictors outperformed clinical measures and non-model based neural variables in predicting clinical status.

Conclusions: In conclusion, young adults who show exaggerated brain processing underlying whether to "stop" or to "go" are more likely to develop stimulant abuse.

Disclosures: Nothing to Disclose.

Panel
20. Schizophrenia as a "Dysplasticity" Disorder

20.1 Experience-Dependent Dysregulation of Plasticity in the Aging and Younger "Noisy" Brain

Etienne de Villers-Sidani

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Background: Brain maturation is associated with a radical shift in the rules of regulation of neuroplasticity. During early sensitive periods of development, mere exposure to passive sensory stimuli can drive enduring changes in the structure and function of cortical circuits. In the adult mature brain, however, several cortical mechanisms powerfully restrict similar plasticity to the context of behavior. This tight control of plasticity in adults stabilizes the brain's

response to experience (i.e., to learning) and ensures the reliability of established sensori-motor representations. Cortical GABAergic tone and several brain neuromodulators such as acetylcholine play a central role in this regulation of adult plasticity.
Recent basic science evidence acquired in the sensory system demonstrates that several of these regulators of plasticity become significantly down-regulated with natural aging. The emerging data suggest that similar processes are likely to be operating in neuropsychiatric conditions associated with de-correlated or "noisy" brain activity, such as schizophrenia. In the animal experiments presented here, we investigate the consequences of a reduction in plasticity regulation on auditory learning and processing. We also investigate how chronic "noise" in brain circuits leads to altered plasticity mechanisms and destabilization of auditory processing.
Methods: Experiments were conducted in the auditory cortical fields and associated brain areas of young and older (3-30 months old) Brown Norway rats ($n = 56$) using a combination of dense intra-cortical electrophysiology, custom signal analyses, behavior and immunohistochemistry. Rats from different groups were exposed to synthetic noise stimuli with varying degree of structure for periods of a few days to a few weeks and presented at an intensity sufficient to mask ambient auditory patterns but not sufficient to cause hearing loss. The impact of these exposures on indexes of plasticity in the auditory cortex and its overall functional and structural organization was then assessed.
Results: We find that the chronic cortical disinhibition and cortical desynchronization associated with normal aging causes a short and long-term instability of sensory representations in the auditory cortex, which in turn causes slower and more rapidly decaying learning. We also find that desynchronizing the activity of auditory cortical neurons for several days in younger rats is sufficient to induce a similar state of dysregulated plasticity, abnormal sensory processing, and poor learning.
Conclusions: Our data demonstrate that a chronic desynchronization of cortical activity– whether due to endogenous factors (aging) or through exogenous abnormal sensory inputs (auditory noise exposure)– was sufficient to deregulate plasticity in the auditory cortex. Such dysplasticity resulted in negative downstream impacts on local and distributed auditory processing circuitry and learning. Our findings suggest that similar dysplastic changes induced by developmental pathophysiology that results in de-synchronized or "noisy" cortical activity may be implicated in schizophrenia.
Disclosures: Nothing to Disclose.

20.2 Dysplasticity, Metaplasticity and Schizophrenia: Implications for Risk, Illness Progression, and Novel Preventive Interventions

Matcheri Keshavan

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Background: The brain maintains plasticity throughout life in response to learning and to injury, though in varying degrees at the different epochs of age. This remarkable ability of the brain is orchestrated by the inherent networking properties of neurons, synapses and glia, as dynamically modified through neurotransmitter systems such as glutamate, GABA and neurotrophic factors. The extent to which the brain can remodel itself in response to learning events and exogenous exposures is thus determined by genetic, epigenetic and environmental influences. It is increasingly recognized that these plastic changes can be adaptive–resulting in greater levels of neural efficiency and/or increasingly fine-tuned and appropriate behavioral outputs– or can result in maladaptive cascades secondary to inherent genetic constraints, neurodevelopmental anomalies, behaviors, and environmental inputs. It is highly plausible that such maladaptive cascades underlie many of the neurobehavioral features of psychiatric illness, but such a model has only rarely been explored in schizophrenia.
Methods: We will systematically review current evidence supporting a developmental model of aberrant neuroplasticity and metaplasticity (the plasticity of synaptic plasticity) associated with schizophrenia, as well as the risk for developing the illness. We will present examples from the recent literature and our unpublished structural and functional imaging data and sleep EEG data in genetic high risk subjects and in first-episode schizophrenia.
Results: Several lines of recent evidence point to diminished neuroplasticity in widespread brain regions in schizophrenia. These include reductions in dendritic and glial density, altered function of glutamatergic, GABAergic and neurotrophic function, and *in vivo* evidence of diminished LTP and LTD-like plasticity. We will present our findings in genetic high risk and first-episode subjects that demonstrate brain structural and functional alterations, altered BDNF levels, and reduced sleep spindles as additional examples of developmental abnormalities in normal neuroplastic mechanisms. Such abnormalities may account for the core deficit symptoms of schizophrenia, while positive symptoms might result from excessive or maladaptive neuroplasticity associated with aberrant reorganization in prefrontal-limbic circuits.
Conclusions: The dysplasticity model, in conjunction with the notion of sensitive periods as they relate to the premorbid and onset periods of psychosis, allow for a parsimonious explanation of how risk states may evolve through aberrant plastic reorganization of neural circuits. Genetic, epigenetic, behavioral, and environmental factors undoubtedly influence the nature, extent, timing and persistence of such abnormalities.
Preventive and therapeutic interventions with medications, neuromodulation, and behavioral/psychosocial treatments may be directed both at reversing aberrant circuitry as well as harnessing compensatory neuroplasticity in more adaptive channels. An increased understanding of brain mechanisms underlying dysplasticity in schizophrenia may thus suggest new ways of detecting preclinical disease, better biomarkers to guide treatment selection, and novel therapeutic targets.
Disclosures: Nothing to Disclose.

20.3 Developmental Trajectory of Brain Bioenergetics and Oxidative Stress Measures in Schizophrenia: Implications for "Dysplasticity"

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Background: The brain requires a large amount of energy to support healthy neural functioning and plasticity operations. Much of this energy appears to support neurotransmission, with a 1:1 stoichiometry observed between glutamate release into the synaptic cleft and glucose oxidation for ATP generation. Several lines of evidence indicate that the generation and utilization of high energy phosphate (HEP) molecules and related processes, together termed bioenergetics, is abnormal in schizophrenia– suggesting a fundamental alteration in metabolic processes supporting neuronal functioning. These processes can be probed using noninvasive 31P magnetic resonance spectroscopy (MRS) techniques *in vivo*. Recent developments in these techniques now make it possible to examine enzyme reaction rates as well as oxidative stress markers in patient groups; however, little work has been done examining how these measures evolve from early to chronic phases of schizophrenia.
Methods: All studies were carried out at the 4 Tesla Varian MRI scanner at McLean Hospital using a custom-built surface coil tuned to the 31P frequency. We collected data from a 4x5x5cm voxel in the prefrontal cortex. T1-weighted images were also collected for grey and white matter segmentation.
In the 31P MRS scan procedures, we use outer volume suppression for localization, and magnetization transfer (MT) approach for metabolite quantification and enzyme reaction rate calculation. MT relies on the fact that the creatine kinase (CK) enzyme reversibly transfers HEP moieties from ATP to creatine to generate ADP and phosphocreatine (PCr). When the signal from ATP in the 31P MRS spectrum is suppressed using a radiofrequency pulse, this leads to reduction of the PCr signal over time (due to exchange of HEP between the two). The rate of loss of the PCr signal is the rate of the CK enzyme reaction rate. pH can also be calculated from 31P MRS data based on the distance between the PCr and inorganic phosphate resonances. Measures of oxidative stress derived from 31P MRS data have also been developed.
Results: In 22 chronic schizophrenia patients, we found a 22% reduction ($p = 0.001$) in the CK reaction rate as compared with 22 matched healthy controls. pH was also significantly reduced in the patient cohort. In 11 first episode schizophrenia patients, the CK reaction rate was reduced at a similar magnitude, but pH was less acidic and was not significantly different from a group of 8 age-matched healthy controls. We also found evidence of elevated oxidative stress in chronic patients, with even greater oxidative stress observed in first episode patients.
Conclusions: Both chronic and first episode schizophrenia patients show evidence for bioenergetic compromise in 31P MRS studies *in vivo*. First episode patients show some abnormalities similar in magnitude to that seen in chronic patients (CK reaction rate), while some abnormalities have not yet emerged (pH). Finally, abnormalities in oxidative stress measures are even more pronounced in first episode patients than in chronic patients.
This pattern indicates that there are multiple active pathophysiologic processes in the early phases of schizophrenia with a nonlinear progression. We do not see evidence of monotonic emergence of abnormalities that get more severe over time; rather a period of increased oxidative stress emerges and then weakens while reduced bioenergetics are constant in earlier and later phases of illness. Recent basic science work shows that oxidative stress in fast-spiking inhibitory interneurons induces abnormal plasticity and destabilization of cortical networks. The increased oxidative stress measures we observe in first-episode schizophrenia is consistent with the model that early psychosis represents a pathologic "sensitive period" characterized by dysplasticity.
Disclosures: Nothing to Disclose.

20.4 Abnormal Cortical Activation Patterns in People with Schizophrenia may Represent Compensatory Plasticity

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Background: Basic science research unequivocally shows that, after brain injury, extensive neuroplastic compensation and re-organization occurs throughout neural circuits, particularly when the injury occurs early in development. Concurrently, the individual animal's behaviors and experiences also impact these same neural circuits, such that there is a reverberating interaction between degenerative and regenerative cascades that ultimately determines neural reorganization and functional outcome. A well-known example is the cortical plasticity that results from compensatory reliance on the "good" forelimb in rats given unilateral cortical infarcts– compensatory plasticity that further reduces the potential for gains in the injured hemisphere.
Thus, the interaction between injury-induced plasticity and plasticity induced by experience and "learning" can have both behaviorally beneficial and detrimental effects. Although increasing attention is now being given to these processes in schizophrenia, we still know very little about cross-modality and other compensatory appropriation of cortical resources in this illness and what it might imply for plasticity-based treatment approaches. At the same time, our field has abundant evidence of aberrant neural network activation patterns and dysconnectivity across broad brain regions in individuals with the illness.
Methods: We examined abnormal activation patterns in cortical sectors during an auditory working memory task and a reward anticipation task, and we investigated their possible compensatory role in support of behavior. These two processes are known to be aberrant in individuals with schizophrenia, who typically show impaired auditory working memory task performance, as well as abnormal reward representation in experimental and real-world settings. During numerous functional imaging studies of

these processes, patients also show evidence of abnormal neural activity patterns and abnormal connectivity.
MEG was performed on 36 schizophrenia and 15 healthy subjects during a speech-sound reproduction task that taxes auditory working memory for human speech stimuli. fMRI was performed on 37 schizophrenia and 20 healthy subjects during the Monetary Incentive Delay (MID) task, with a focus on the reward anticipation period after the presentation of a monetary cue. Measures of task performance were obtained, along with standard symptom ratings, cognitive and functional capacity measures, and self-ratings of anticipatory and consummatory pleasure.
Results: During auditory working memory as assessed via MEG, schizophrenia subjects failed to show the normal correlation between posterior planum temporale (PTp) high gamma power (HGP) and working memory task performance observed in controls. Instead, they activated the visual word form area (VWFA) during both stimulus encoding and response preparation, and VWFA HGP correlated with the severity of hallucinations. In patients with severe hallucinations, VWFA activity correlated with performance accuracy on the auditory working memory task, as well as an independent neuropsychological measure of verbal working memory (Letter-Number Sequencing).
During reward anticipation as assessed via fMRI, schizophrenia subjects showed hypoactivity in ventral striatum as compared to healthy controls; they also failed to show the normal correlation between striatal activation and self-ratings of consummatory pleasure in everyday life observed in controls. Instead, they activated the right inferior parietal lobule (IPL), and right IPL activity correlated with self-ratings of consummatory pleasure and a measure of functional capacity (UPSA-B).
Conclusions: Individuals with schizophrenia may harness compensatory plasticity in cortical sectors to support basic cognitive operations. During the auditory working memory task, cross-modality compensation is observed, with the ventral visual word processing network apparently "co-opted" to support auditory working memory in patients with high hallucinations. During the reward anticipation task, compensation for ventral striatal hypoactivation appears to be subserved by the right IPL, a node in the fronto-parietal network important for "own-body" perception.
These compensatory activation patterns show a significant association with cognitive, subjective, and functional capacity measures. It is likely that they arise as the result of interactions between schizophrenia-induced "dysplasticity" and experience-dependent re-organization of distributed neural circuits. There is also no doubt that these interactions are complex, influenced by development, and affected by endogenous and exogenous factors. A better understanding of these interactions is needed to understand how to optimize treatments that focus on brain remodeling and functional outcome. For example, it is entirely unknown whether strategies should be developed that make explicit use of compensatory mechanisms, or conversely, that mitigate or disallow them. Indeed, a fundamental question will be to determine the developmental time course of this process and to develop interventions to preempt the negative consequences of dysplasticity before maladaptive neural circuit remodeling has progressed beyond a point of "no return."
Disclosures: Part 1: Site PI on an SBIR grant to Positscience Inc.; scientific advisory board to Forum Pharmaceuticals; consultant to Takeda Pharmaceuticals, **Part 4:** Site PI on an SBIR grant to PositScience, Inc.

Panel
21. Inflammation-Induced Modulation of Motivation: Impact on Neurotransmitters and Neurocircuits

21.1 Preclinical Characterization of Inflammation-Induced Motivational Deficits

Elisabeth Vichaya

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Background: Inflammation induces sickness that can transition to depression in response to activation of the kynurenine metabolism pathway. Dimensional analysis of inflammation-induced depression indicates a possible predominance of neurovegetative over psychological and affective symptoms. However, this possibility has not yet been tested. Reduced sucrose preference and motivation for food rewards are observed in animal models of inflammation. Although these results are usually interpreted to suggest that inflammation induces anhedonia, the exact dimension of anhedonia that is associated with inflammation (i.e., the liking or the wanting) is still uncertain. In the context of the Research Domain Criteria initiative, our current studies aim at specifying the basic neurobehavioral units that are induced by inflammation and the possible role of neurotoxic kynurenine metabolites.
Methods: In our basic paradigm, mice treated with lipopolysaccharide (LPS) experience an episode of sickness that peaks within a few hours followed by depression-like behavior (reduced sucrose preference and increased immobility in forced swim and tail suspension tests) concomitant with activation of the tryptophan metabolizing enzyme indoleamine 2,3 dioxygenase (IDO). Motivational deficits were characterized during the late stage of the LPS response by measuring behavioral and physiological arousal to conditioned cues associated with food delivery to food restricted mice receiving food during their subjective night. The amount of effort mice were willing to invest to gain a food reward was assessed in a concurrent performance task in which mice had the choice between a high effort high reward (10 nose pokes for 1 chocolate pellet) and a low effort low reward (1 nose poke for 1 grain pellet) response modality. Spontaneous preference for chocolate over grain was assessed at the completion of the task.
Results: Food anticipatory activity and pituitary-adrenal activation, as measured by plasma levels of corticosterone, were suppressed following treatment with LPS. This suppression of food anticipatory activity was associated with reduced expression of Per1 and Per2 clock genes in the liver. LPS treated mice still displayed a preference for chocolate over grain pellets. In the concurrent choice task, mice showed a global decrease in performance, but this

decrease affected the low effort low reward response modality more than the high effort high reward. Ongoing work is evaluating whether activation of IDO mediates the effects of LPS.
Conclusions: The reduced behavioral and physiological arousal evoked by expectation of food delivery indicates a decreased motivation in LPS-treated mice. Further, these results indicate that inflammation-induced motivational deficits are not caused by decreased sensitivity to reward since the spontaneous preference for chocolate versus grain pellets is not altered. Reduced performance in the concurrent performance task confirms that the main effect of LPS is to attenuate incentive motivation.
Disclosures: Nothing to Disclose.

21.2 A Neuro-Computational Account of how Inflammation Diminishes Sensitivity to Reward and Simultaneously Promotes Avoidance Behavior

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Background: Inflammation rapidly impairs mood and cognition and, when severe, can appear indistinguishable from major depression. These sickness responses are characterized by an acute reorientation of motivational state; pleasurable activities are avoided and sensitivity to negative stimuli is enhanced. However it remains unclear how these rapid shifts in behavior are mediated in the brain.
Methods: Here, we combined computational modeling of choice behavior, experimentally-induced inflammation and functional brain imaging (fMRI) to describe these mechanisms. Using a double-blind, randomized crossover study design, 24 healthy volunteers completed a probabilistic instrumental learning task on two separate occasions, once three hours after typhoid vaccination and once three hours after saline (placebo) injection. Participants learned to select high probability reward (win £1) and avoid high probability punishment (lose £1) stimuli. An action-value learning algorithm was fit to the observed behavior, then used within fMRI analyses to identify neural coding of prediction error signals driving motivational learning.
Results: Inflammation acutely biased behavior; reducing sensitivity to reward yet heightening sensitivity to punishment through distinct actions on neural representations of reward and punishment prediction errors within ventral striatum and anterior insula. Consequently, choice options leading to potential rewards were less behaviorally attractive, and those leading to punishments more aversive.
Conclusions: Our findings demonstrate the neural mediation of a rapid, state-dependent reorientation of reward versus punishment sensitivity during inflammation. This mechanism may aid the adaptive reallocation of metabolic resources during acute sickness, but might also account for maladaptive, motivational changes that underpin the association between chronic inflammation and depression.
Disclosures: Nothing to Disclose.

21.3 Inflammatory Responses to Stress are Associated with Altered Prediction Error Signaling During Reinforcement Learning

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Background: Psychiatric symptoms related to impaired reinforcement, anhedonia and low-motivation are common across many disorders. In both humans and animals, such symptoms have been shown to reflect alterations in a dopamine-rich corticostriatal network. Importantly, these studies have also suggested that exposure to stress may precipitate functional changes in this circuitry, thereby creating a plausible link between stress exposure and subsequent disorder onset. To date, however, the factors underlying the deleterious effects of stress are unknown. One candidate mechanism is inflammation; inflammatory signaling is both increased following stress exposure and has been found to disrupt dopamine signaling pathways. Here, we examine changes in interleukin-6 (IL-6) expression following acute stress, and its association with reinforcement learning signals in the human nucleus accumbens.
Methods: Data from 75 healthy female participants were collected in a dual-session experimental paradigm. During session 1, plasma levels of Il-6 were collected at three time points before and after participants were exposed to a cold-pressor stress challenge. In session 2, participants returned to complete a task-based BOLD fMRI scanning session. The task involved instrumental conditioning of abstract stimuli that were probabilistically associated with either monetary wins or losses. A standard Q-learning model was fit to behavioral choice data, and model-based prediction-errors were regressed against neural activity using spm8. Participants were also exposed to a social-stressor during the scan session, and completed runs of the reinforcement learning task before, during and after the social stress manipulation. An anatomical mask of the nucleus accumbens drawn from the Harvard-Oxford probabilistic atlas was used to examine associations between activity in this region during prediction-error feedback, and inflammatory responses to stress.
Results: In a sub-set of participants with available IL-6 data at the time of submission ($n = 21$), we observed a significant increase in plasma IL-6 levels following administration of the cold-pressor task during session 1 ($F(2,19) = 6.56$, $p = 0.007$). For prediction error modeling during session 2, we observed an overall main effect of prediction-error signaling in nucleus accumbens ($p < 0.05$, whole-brain cluster-corrected), though this effect was not modulated by stress. Importantly, however, PE signaling in this area was inversely associated with percent increase of stress-induced Il-6 levels ($r = -0.54$, $p < 0.05$).
Conclusions: These results suggest a critical link between inflammatory responses to stress and reinforcement learning signals in the human nucleus accumbens. Importantly, they extend prior empirical and theoretical work positing that increased risk for depression following stress may partly result from the effects of stress-induced immunoreactivity on basal-ganglia function and associated reinforcement learning behavior.
Disclosures: Nothing to Disclose.

21.4 Inflammation-Related Decreases in Dopamine and Effects on Corticostriatal Reward Circuitry: Evidence from Humans and Non-Human Primates

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Background: Administration of inflammatory cytokines or cytokine inducers to humans and laboratory animals leads to depressive symptoms, and especially anhedonia, which is believed to involve cytokine effects on mesolimbic dopamine. Neuroimaging studies in humans have shown that cytokines decrease neural activation of the ventral striatum to hedonic reward. Moreover, our non-human primate (NHP) work has revealed inflammatory cytokine-induced decreases in striatal dopamine release in a back-translational model of cytokine-induced depression. Herein, we present data from both humans and NHPs indicating that inflammatory cytokines affect synthesis and subsequent release of dopamine to affect reward circuitry in association with symptoms of reduced motivation and anhedonia.
Methods: A NHP model of cytokine-induced depressive behavior was used to examine inflammatory cytokine effects on striatal dopamine release and potential for its reversal by the dopamine precursor, levodopa (L-DOPA), administered via reverse *in vivo* microdialysis in monkeys chronically administered the cytokine interferon (IFN)-alpha. Effort-based sucrose consumption from a puzzle feeder was also assessed. Plasma and cerebrospinal fluid (CSF) biomarkers of decreased dopamine synthesis were also examined in patients administered IFN-alpha for chronic hepatitis C virus. Finally, patients with major depression exhibiting a range of inflammation from high to low, as measured by plasma C-reactive protein (CRP), underwent resting-state fMRI to assess relationships between functional connectivity within reward-related brain regions and symptoms of anhedonia and psychomotor retardation.
Results: Cytokine-induced decreases in striatal dopamine release were correlated with reduced effort-based sucrose consumption, and reversed by administration of L-DOPA, in NHPs administered chronic IFN-alpha. Patients receiving IFN-alpha exhibited decreased ventral striatal activation to hedonic reward, and deceased biomarkers relevant to dopamine synthesis in the periphery that correlated with decreased CSF dopamine and dopamine metabolites, all of which were associated with depressive symptoms including reduced motivation. In patients with major depression, increased plasma CRP was associated with decreased functional connectivity between both ventral and dorsal striatum and the ventromedial prefrontal cortex, which correlated with symptoms of anhedonia and psychomotor slowing. Moreover, preliminary data suggest that a single dose of L-DOPA can reverse inflammation-related disruptions in reward-related corticostriatal connectivity in patients with high CRP.
Conclusions: These data in humans and NHPs indicate that inflammation-related decreases in dopamine synthesis and release have functional consequences on reward circuitry in depression that are associated with fundamental alterations in motivation and motor function. This work supports development of novel therapeutic strategies to increase dopamine availability in depressed patients with increased inflammation and anhedonia, thus personalizing care.
Disclosures: Nothing to Disclose.

Panel
22. Molecular Mechanisms Underlying Psychopathology and Treatments in OCD

22.1 Role of SLITRK5 and PTPRD in BDNF-Dependent Synapse Remodeling

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Background: Slit- and NTRK-like family (Slitrks) are transmembrane proteins that localize to and function at central nervous system synapses where they mediate synapse formation through trans-synaptic interactions of their ectodomains with a presynaptic binding partner, protein tyrosine phosphatase, receptor type, D (PTPRD), a recently identified candidate risk gene for obsessive compulsive disorder (OCD). Recent studies in a genetic knock-out mouse model have also provided compelling links between Slitrk5 to OCD. Slitrk5-null mice displayed repetitive and excessive self-grooming behaviors. Treatment with chronic fluoxetine, a selective serotonin reuptake inhibitor (SSRI), alleviated the excessive grooming behavior. These mice also showed selective overactivation of the orbitofrontal cortex. In this context, overactivation of orbitofrontal-subcortical circuits has been observed in functional imaging studies of human subjects with OCD. Thus, the Slitrk5-null mouse recapitulates important aspects of the human disease. However, the molecular mechanisms underlying Slitrk5 function are not known. We hypothesized that, based on structural similarities that Slitrk5, interacts with the neurotrophin system, in particular, with the BDNF receptor, TrkB, to mediate biological responses in key cortico-striatal circuitry.
Methods: Super-resolution structured illumination microscopy (SIM) was utilized to assess localization of Slitrk5 with its interacting synaptic proteins (PTPRD, TrkB receptors) in cultured striatal neurons, as well as its impact on synapse formation.
Results: Our cell biological studies in cultured striatal neurons demonstrate that Slitrk5, a postsynaptic plasma membrane protein containing extracellular LRR domains, interacts under basal conditions with the presynaptic adhesion molecule PTPRD in trans, but in the presence of BDNF, shifts to a cis-interaction with TrkB receptor that mediates its postendocytic recycling, leading to functional resensitization of neurotrophic signaling. Our current studies elucidate a new aspect of Slitrk5 function in which Slitrk5 has a cis interaction with activated TrkB receptors on the surface of postsynaptic sites via extracellular interactions involving their respective LRR domains. Intriguingly, the cis interactions of Slitrk5 with TrkB receptors compete with trans interactions with its presynaptic partner, PTPRD, and the competition was modulated by BDNF stimulation.

Conclusions: In summary, the present studies identify an unanticipated role for Slitrk5, a cell surface transmembrane protein in mediating a balance between functioning as a synapse adhesion molecule by interacting with PTPRD, as well as a facilitator of trophic responses by interacting with TrkB receptors. By engaging with both presynaptic PTPRD and postsynaptic TrkB receptors, Slitrk5, plays a key role in striatal function that may underlie the OCD-like behaviors observed in the Slitrk5-null mice. In addition, these findings provide a potential molecular framework to understand for how PTPRD may be implicated in OCD.
Disclosures: Nothing to Disclose.

22.2 Rare Functional Mutations in SLITRK5 are Associated with OCD

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Background: Obsessive compulsive disorder (OCD) affects approximately 2% of the population, and is a leading cause of morbidity worldwide. Despite having a substantial heritable component, few specific genetic risk factors for OCD have been identified. Genome-wide association studies (GWAS) of OCD have found associations to common polymorphisms at near genome-wide significance (e.g., PTPRD) but the majority of the genetic risk for OCD remains unidentified. Knockout mice lacking Sapap3 and Slitrk5 display OCD-like phenotypes, including altered cortico-striatal circuitry and pathologic grooming behavior that is responsive to serotonin transporter targeting drugs. Rare missense variants in human SAPAP3 are statistically overrepresented in OCD cases versus controls, implicating them in risk for OCD. We screened the complete protein-coding sequence of SLITRK5 in OCD subjects to identify rare mutations that may contribute to OCD.
Methods: We re-sequenced the complete protein sequence of SLITRK5 in three hundred and seventy seven individuals with DSM-IV OCD. Rare, non-synonymous mutations (RNMs) (prevalence < 0.01) identified in the OCD sample were compared with data from the 1000 Genomes database. Putative functional effects of OCD and control RNMs were assessed in silico. To test for functional effects of SLITRK5 RNM's mutant proteins were introduced into COS7 cells and co-cultured with primary hippocampal neurons. We analyzed all OCD mutations and a subset of pseudo-matched mutations from the 1000 Genomes database sample for synaptogenic activity of hippocampal neurons onto Slitrk5-expressing COS7 cells.
Results: We identified four RNMs in the 377 OCD participants (0.011). There were 15 RNMs in the 1000 Genomes database (0.014). All of the OCD mutations were singletons, while 7 of the 15 control mutations were singletons. There was no association between OCD with either the number of SLITRK5 RNM's or the prevalence of chromosomes containing those mutations. There were no differences in the bioinformatically predicted effects of RNM's using Combined Annotation Dependent Depletion (CADD). However, all Slitrk5 alleles containing OCD-associated mutations significantly impaired synapse formation relative to wild type Slitrk5 when expressed in COS7 cells whereas none of the pseudo-matched controls did (Fisher's exact $P < 0.03$. Impaired synaptogenesis for one OCD mutation was explained by impaired surface expression and the other three displayed impaired interaction with PTP-δ, a highly associated gene in a recent OCD GWAS.
Conclusions: RNM's in SLITRK5 that impair synaptogenesis are associated with OCD. These results implicate SLITRK5 in the genetic risk for OCD and highlight the importance of biological characterization to identify genotype-phenotype relationships. They also add the growing role of corticostriatal synaptic function in the pathophysiology of OCD.
Disclosures: Nothing to Disclose.

22.3 From Good Habit to Bad: Corticostriatal Synaptic and Circuit Mechanisms in Habit and Compulsion

Nicole Calakos

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Background: Compulsivity drives maladaptive behavioral responses in many contexts, ranging from the prototypical, Obsessive Compulsive Disorder (OCD), to addiction, autism-related behaviors, and eating disorders. Although compulsive behavior has been widely hypothesized to derive from habit learning mechanisms, direct testing of how the synaptic and circuit mechanisms that give rise to these behaviors relate is largely lacking. Here we examine the relationship between corticostriatal plasticity and behavior in habit and compulsion using mouse models.
Methods: Corticostriatal plasticity was examined at the local circuit level by imaging action potential firing of striatal projection neurons (SPN) in response to defined cortical stimuli delivered in the acute brain slice preparation. Such a measurement integrates the effects of synaptic, intrinsic and homeostatic plasticity throughout the local microcircuitry and reveals the net effect on striatal output. SPN firing was imaged using two-photon, calcium imaging of genetically defined SPNs. The two types of projection neurons, direct and indirect pathway-projecting SPNs, were simultaneously evaluated to enable measurement of the balance of activity between these two mutually antagonistic pathways. Habitual behavior was induced through training in a lever-press paradigm (Dickinson et al., Quar. J. Exp. Psych., 1983). Compulsive behavior was modeled by SAPAP3 knockout (KO) mice which display persistent self-injurious grooming and anxiety-like behaviors that respond to chronic fluoxetine treatment or gene rescue in striatum (Welch et al., Nature 2007).
Results: We found that mice with persistent OCD-like behaviors and those with habitual responding share increases in striatal output excitability and pathway imbalances that favor activity in the action-promoting "direct" pathway. However, the specific features of striatal excitability affected differ between the two behavioral states. Additionally, we show that the efficacy of a novel drug treatment in normalizing circuit defects in the SAPAP3 KO model corresponds with behavioral efficacy against the OCD-like behaviors.
Conclusions: These findings reveal both common and distinguishing features of striatal circuitry in habit and

compulsion. The behaviorally predictive hypotheses generated by analysis of plasticity at the level of pathway-defined striatal output can be used to direct future mechanistic investigations as well as assist in the development of novel drug and circuit therapies.
Disclosures: Nothing to Disclose.

22.4 Pilot Trial of a Brief Course of Exposure-Based CBT in Extending IV Ketamine's Effects in OCD

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Background: A single subanesthetic intravenous (IV) dose of ketamine leads to rapid anti-obsessional effects in OCD patients with near-constant intrusive obsessions, but these effects usually do not persist. We tested whether a brief course of exposure-based cognitive behavioral therapy (CBT) could extend ketamine's effects in a 2 week pilot open trial and if this effect was maintained (without additional treatment) 2 weeks later. Our rationale was: 1) in rodents, ketamine is reported to enhance plasticity and extinction learning (and this effect is maintained for at least 14 days), and 2) enhanced extinction learning may facilitate CBT gains, as reported in prior trials combined trials of CBT with agents that facilitate extinction learning. CBT was abbreviated (i.e. 10 one-hour exposure sessions) but delivered during the putative time interval when ketamine facilitates extinction learning.
Methods: Ten unmedicated OCD outpatients (aged 18-55) with near-constant intrusive obsessions (>8 hours/day) were recruited (3/2014-3/2015) and provided informed consent. Participants met DSM-IV/5 criteria for OCD with at least moderate symptoms (YBOCS score $\geqslant 16$). Exclusion criteria included severe depression or current CBT. In an open-label design, participants received a single 40-minute IV infusion of ketamine (0.5 mg/kg), followed by 10 one-hour exposure sessions delivered over two weeks. The CBT treatment was planned in a 90-minute session prior to the ketamine infusion. At baseline, during, and up to 230 minutes post-infusion, patients rated their obsessional severity using the OCD-VAS. We focused on obsessions because the patients were supine and connected to stationary monitoring equipment during the infusion. At baseline and weekly for four weeks post-ketamine, an independent evaluator, blind to study design, evaluated patients using the Y-BOCS. Treatment response was defined a priori as $\geqslant 35\%$ Y-BOCS reduction at week 2. Y-BOCS outcomes were analyzed using mixed-effects regression to model symptoms as a function of time.
Results: Of 10 patients who started ketamine, nine completed the infusion. Eight reported a rapid reduction in obsessive severity as measured by the OCD-VAS, which persisted up to 230 minutes post-infusion in seven. Eight completed the 10 hours of exposure and the two week follow-up and were included in the Y-BOCS analyses. From baseline to 4-weeks post-infusion, OCD severity as measured by the YBOCS was significantly decreased over time ($F = 14.36$, $df = 4,28$, $p < .0001$). Compared to baseline, the mean estimated Y-BOCS score was significantly lower at week 2 (difference = -10.75 points, $SE = 1.44$, $p < .0001$) and at week 4 (difference = -6.88, $SE = 2.61$, $p = 0.01$); there was a trend-level increase between week 2 and 4 (difference = 3.63, $SE = 1.97$, $p = 0.07$). At the end of CBT (week 2) response rate was 63%.
Conclusions: These results corroborate prior findings that ketamine can rapidly relieve symptoms in unmedicated OCD patients and suggest that a brief course of CBT can extend these effects for at least two weeks in 63% of OCD patients with constant intrusive thoughts.
Disclosures: Nothing to Disclose.

Panel
23. Research Paradigms and Non-Pharmacological Interventions aimed to Prevent the Onset and Progression of Bipolar Disorder in Children

23.1 White Matter Structure of Major White Matter Tracts in Youth Offspring of Bipolar and Non-Bipolar Patents

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Background: Bipolar Disorder (BD) is a highly heritable mental illness, placing offspring of parents with BD at highest risk for this disorder. (Craney and Geller 2003; Faraone, Glatt et al. 2003; McGuffin, Rijsdijk et al. 2003; Rende, Birmaher et al. 2007; Birmaher, Axelson et al. 2009) Yet, offspring of parents with other psychiatric disorders, such as depression or ADHD are at higher risk to develop BD. Whether or not they develop the illness, these children can present ongoing emotional and/or behavioral dysregulation, associated with functional impairment.(Carlson and Weintraub 1993; Birmaher, Axelson et al. 2006; Luby and Navsaria 2010) In addition, developmental differences in the presentation of the disorder in youth (e.g., symptoms of inattention, irritability, impulsivity, etc.) can often be misinterpreted as the onset of other psychiatric conditions (e.g., ADHD, ODD), and lead to inappropriate or less efficient treatment. The identification of early neuroimaging markers of risk for BD may help shed light on the pathophysiologic mechanisms of BD, regardless of whether vulnerable children will go on to develop full syndromal BD. Yet, a limited number of studies have examined white matter structure in offspring of BD.(Frazier, Breeze et al. 2007; Versace, Ladouceur et al. 2010; Roybal, Barnea-Goraly et al. 2015)
Methods: Global probabilistic tractography of 10 major white matter tracts was performed in eighty-seven youth including age- and gender-matched offspring of BD parents (OBP: $N = 27$; mean[SD] age = 13.6[2.2]; $M/F = 14/13$), offspring of non-BD (i.e., depression, anxiety or ADHD/ODD) parents (OCP: $N = 24$; mean[SD] age = 13.9[2.5]; $M/F = 15/9$), and offspring of healthy parents (OHP: $N = 36$; mean[SD] age = 13.2[2.4]; $M/F = 21/15$). A multivariate multiple regression model was used to test putative age by genetic risk (OBP, OCP, OHC) interaction upon fractional anisotropy (FA) of 9 tracts (1. forceps major, 2. forceps minor, 3. anterior thalamic radiation, 4. cingulum, 5.

angular bundle, 6. inferior and 7. superior longitudinal fasciculus, 8. arcuate and 9. uncinate fasciculus). In this tract of interest approach, the corticospinal tract was examined as a control tract. At scan time, 13 youth had a diagnosis of emotional dysregulation disorder (6 OBP and 7 OCP) and 7 youth had a diagnosis of behavioral dysregulation disorder (2 OBP and 5 OCP).
Results: After controlling for diagnosis (offspring with emotional dysregulation disorder, offspring with behavioral dysregulation disorder, healthy offspring) and gender, there was an age by genetic risk interaction across all white matter tracts (Pillai's = .4; $F[20,144] = 1.8$; $p = 0.033$). Further analyses revealed that this interaction was significant in the forceps major ($F[20,144] = 10.2$; $p < 0.001$), in the angular bundle of the cingulum ($F[2,86] = 4.6$; $p = 0.013$), and in the inferior longitudinal fasciculus ($F[2,86] = 3.0$; $p = 0.056$).
Conclusions: Our findings suggest that the at-risk youth (both, OCP and OBP) show lower FA when compared to OHP in the forceps major (OCP, $p < 0.001$), in the angular bundle of the cingulum (OCP to a greater extent than OBP; $p = 0.006$ and $p = 0.020$) and in the inferior longitudinal fasciculus (OBP; $p = 0.021$), as previously shown11. However, while there is a decrease of FA (inverted U shape) in the OHP as they get older, such an effect is not present in the at-risk offspring of non-BD parents. Interestingly in the at-risk offspring of BD parents, FA values are lower in the angular bundle of the cingulum and in the inferior longitudinal fasciculus in younger offspring, but normalize as they get older. A steeper decline of FA might be present later in OBP. Further studies in adult OBP are needed.
Disclosures: Nothing to Disclose.

23.2 Neurofunctional Characteristics of Risk and Resilience in Youth Offspring of Bipolar Parents

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Background: Bipolar Disorder (BD) in parents is a significant source of stress for children and families. We previously demonstrated that youth offspring of bipolar parents have dysfunctional prefrontal-subcortical connectivity that correlates with chaotic family environments even before children experience frank mood symptoms (Singh et al., 2014). It is not known whether aberrant functional connectivity is associated with daily stress exposure that predisposes youth to developing mood disorders (Ellenbogen et al., 2006) or if youth are demonstrating adaptive neural functioning amidst family chaos. We used a combination of endocrine (salivary assessment of levels of diurnal cortisol production), resting state and task based functional magnetic resonance imaging (fMRI) assessments to compare healthy offspring living with parents with BD (high-risk) and healthy comparison youth without any personal or family history of psychopathology (low-risk). We hypothesized that high-risk youth would differ from low-risk youth in functional disruptions in prefrontal and subcortical regions involved in emotion regulation. We further predicted that a subset of vulnerable youth with elevated diurnal cortisol production would demonstrate disconnectivity between the prefrontal cortex and subcortical regions.
Methods: 8–17 year old healthy children of parents with bipolar I or II disorder ("high-risk," $n = 25$) and age comparable control children of parents without any psychopathology were recruited ("low-risk," $n = 25$). High-risk and low-risk youth were scanned using a 3 Tesla Signa MRI system with a standard whole head coil at rest and during an emotion face processing task (Garrett et al., 2012). We used hypothesis-driven region-of-interest (ROI)-based intrinsic connectivity to analyze the fMRI data, investigating connectivity in the following ROIs selected a priori and created from the Harvard-Oxford atlas: left and right amygdala and the left and right ventrolateral prefrontal cortex (VLPFC). We conducted partial correlations between connectivity estimates and cumulative neurofunctional, endocrine, and family chaos measures within each group. We then conducted Fisher's r-to-z transformations to determine whether the high- and low-risk groups differed significantly with respect to these within-group correlations.
Results: High-risk youth had greater levels of family chaos than low-risk youth. High-risk youth had greater resting state functional connectivity in the VLPFC ($t(48) = 6.01$, $p < .001$) and greater total diurnal cortisol production than did the low-risk group ($t(48) = 5.73$, $p < .001$). The high-risk group had greater resting state functional connectivity than did the low-risk group between the left VLPFC and left superior parietal lobule ($t(48) = 1.69$, $p = 0.048$) and greater functional connectivity between the VLPFC and amygdala while viewing happy versus neutral facial expressions ($t(48) = 2.41$, $p = 0.039$). Connectivity estimates between the VLPFC and amygdala within the high-risk group both at rest and during face emotion processing were negatively correlated with level of family chaos in high-risk ($r = -.707$, $p = 0.005$) and total diurnal cortisol production ($r = -.586$, $p = 0.008$) but not low-risk youth ($r = 0.079$, $p = 0.788$; z;s = 2.90-3.15, p's < 0.001).
Conclusions: Our results provide new evidence that in the context of high levels of family chaos and elevated total diurnal cortisol production, healthy offspring of parents with BD have increased VLPFC connectivity compared to healthy offspring with no family history of psychopathology, This increase in prefrontal connectivity may represent an early marker of resilience from developing mood disorders that can only be verified by longitudinal follow-up. Improved methods for identifying children with certain neural vulnerabilities may inform preventive and early intervention strategies prior to the onset of fully syndromal BD.
Disclosures: Nothing to Disclose.

23.3 Neural Correlates of Symptom Improvement Following Family Focused Therapy for Youth at High-Risk for Bipolar Disorder

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Background: Family focused therapy (FFT) is a well-tolerated nonpharmacological intervention that has demon-

strated efficacy in reducing depressive symptoms, anxiety, and stress and in improving function and diagnostic outcome in adults and teens with bipolar disorder (BD). A modified version of FFT for youth at high-risk for BD (FFT-HR) was shown to be effective in reducing depressive symptoms and functioning in these youth, all with a first-degree relative with BD. Understanding task-independent neural mediators of clinical improvement by FFT may suggest mechanisms underlying risk for BD and targets for treatments. This study investigates changes in intrinsic functional connectivity during rest associated with FTT –HR.

Methods: 25 youth (ages 9-18 years) with a current non-bipolar I or II mood disorder and a biological first- or second-degree relative with bipolar I disorder were recruited from an academic Bipolar Disorders Program and from the community. Subjects received either FFT ($n = 13$) or enhanced care (EC) ($n = 12$), a control condition consisting of 3-6 general and crisis intervention sessions. FFT consisted of 12 weekly hourly sessions, attended by youth, parents, and siblings. The goals of treatment included educating the family about mood disorders, managing mood symptoms, preventing adverse mood episodes, enhancing communication, and solving problems. At baseline and endpoint after treatment, subjects were scanned using a 3 Tesla GE Signa MRI system with a standard whole head coil at rest for 7 minutes. Participants were instructed to rest quietly with their eyes closed but not sleep. An implicit facial emotion task was also administered, consisting of fearful, calm, and neutral faces. Mood and anxiety symptom ratings were obtained at baseline and endpoint.

Results: Both treatment groups exhibited similar significant baseline-endpoint reductions in depression and anxiety severity scores. Baseline-endpoint intrinsic connectivity between the posterior cingulate gyrus and the bilateral medial prefrontal cortex increased after FFT ($p = .001$ height and $k = 20$ extent). Baseline-endpoint intrinsic connectivity between the precuneus and the subgenual anterior cingulate cortex decreased following FFT ($p = .005$ height and $k = 20$ extent). Increased posterior cingulate connectivity after FFT was inversely correlated with a decrease in depressive symptoms from baseline to endpoint. Furthermore, during a fearful versus calm face contrast task, greater right DLPFC activation increase from baseline to follow-up was correlated with greater decrease in depressive symptoms.

Conclusions: Reductions in depressive symptoms in bipolar offspring following FFT are associated with changes in intrinsic functional connectivity between posterior subregions of the default mode network and regions critical for emotion regulation. Furthermore, prefrontal activation increase appears to be correlated with symptom improvement, which could reflect greater prefrontal control over limbic areas in order to regulate mood. These changes in connectivity and activation may represent desired neural change following interventions to prevent the development of BD. Longitudinal assessment of these patients through the risk period of BD development is necessary to extrapolate these results toward the concept of prevention.

Disclosures: Part 1: Consultant for Sunovion, Actavis, Merck, GSK, **Part 4:** GSK, Merck.

23.4 Neurofunctional Changes Associated with Mindfulness-Based Cognitive Therapy in Youth at Risk for Bipolar Disorder

Melissa DelBello

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Background: Child and adolescent offspring of parents with bipolar disorder have a greater risk for developing anxiety disorders and emotional dysregulation than those without bipolar parents. Antidepressants, which are typically used to treat these symptoms, may increase the risk for developing mania. Therefore, studies evaluating non-pharmacological interventions for anxiety and mood dysregulation in youth with a bipolar parent are needed. Preliminary studies from our group and others suggest that mindfulness-based cognitive therapy (MBCT) is effective for reducing symptoms of anxiety and mood dysregulation. The aim of this study was to evaluate the neurofunctional effects of mindfulness-based cognitive therapy (MBCT) in youth at risk for bipolar disorder. We hypothesized that MBCT will reduce symptoms of anxiety and mood dysregulation and that reduction in these symptoms will be associated with changes in amygdala, insula, anterior cingulate, and ventral prefrontal cortical function.

Methods: Ten youth (mean age: 13 ± 2 years) with an anxiety disorder and a bipolar parent completed an fMRI session while performing a continuous performance task with emotional and neutral distractors (CPT-END) and a facial affect task of viewing fearful faces prior to and following 12 weeks of group MBCT. In a separate cohort, 10 youth (mean age: 14.0 $+1$ years) with significant mood dysregulation and a bipolar parent completed an fMRI session while performing the same facial affect tas prior to and following 12 weeks of group MBCT. Anxiety and mood dysregulation were measured using rating scales prior to and during the group MBCT.

Results: MBCT was associated with improvement in anxiety and mood dysregulation in both cohorts of at-risk youth. MBCT was associated with increases in activation of the bilateral insula, lentiform nucleus, and thalamus as well as the left anterior cingulate while viewing emotional stimuli during the CPT-END, and decreases in anxiety were correlated with change in activation in the bilateral insula and anterior cingulate bilaterally. In the facial-affect driven analysis (fear vs. neutral) of amygdala activity, MBCT was associated with a significant decrease in right amygdala activity ($p = 0.01$) in the anxious youth. In contrast, during the facial affect task (fear vs. neutral) mood dysregulated at-risk youth exhibited increases in right amygdala ($p = 0.04$), right and left anterior cingulate ($p < 0.04$), and right insula ($p < 0.03$) activity following MBCT.

Conclusions: MBCT treatment in anxious and mood dysregulated youth with a familial history of bipolar disorder reduces anxiety and mood symptoms and is associated with increased activation of brain structures that subserve interoception and the processing of internal stimuli, functions presumably improved by this treatment. However, the impact of MBCT on amygdala function during fear processing is distinct in anxious vs. mood dysregulated youth, suggesting that the underlying alterations in amygdala function may be distinct in each of these cohorts

and the primary neural impact of MBCT may be on modulatory (prefrontal and insular) regions rather than directly on the amygdala.
Disclosures: Part 1: Pfizer, Lundbeck, Sunovion, Otsuka, Supernus, Forest, Actavis, **Part 4:** Eli Lilly, Otsuka, GlaxoSmithKline, Merck, Martek, Novartis, Lundbeck, Shire, Purdue, Amylin, Sunovion, Pfizer.

Mini Panel
24. Social (Cognitive) Functioning in Schizophrenia: Course, Mechanisms, and Treatment

24.1 The 20-Year Longitudinal Trajectories of Social Functioning in Psychotic Disorders

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Background: Social deficits are a core feature of schizophrenia and common in other psychotic disorders. Nevertheless, only a few studies have systematically investigated the course of social impairment in psychotic disorders, yielding mixed findings. We will present new data on the differential longitudinal trajectories of social functioning in patients diagnosed with psychotic major depressive disorder, psychotic bipolar disorder and schizophrenia.
Methods: We used data from the Suffolk County Mental Health Project (New York, US), which is an epidemiological cohort of first admission psychotic patients (n = 485) followed over a 20-year period and Never Psychotic comparison subjects (n = 262). Social functioning was assessed at 6 months, 2, 4, 10 and 20 years after first admission. Latent Class Growth curve modeling was applied to establish latent trajectories of social functioning across diagnoses. Regression analyses were used to examine how these latent trajectories were associated with premorbid functioning scores in childhood, early- and late adolescence. Regression analyses were also used to examine whether the latent classes were related to other clinical outcome measures.
Results: Four latent classes with stable life-course trajectories of 'Preserved', 'Mildly Impaired', 'Severely Impaired', and 'Profoundly Impaired' social functioning were identified. Differences in social functioning among the groups were already evident in childhood. Specifically, those with the lowest social functioning during childhood also showed the lowest social functioning after illness onset. In addition, they had significantly more cognitive deficits and positive and negative symptoms over the course of 20 years. Overall, social functioning was poorest in schizophrenia, best in psychotic bipolar disorder and intermediate in psychotic major depressive participants. Social functioning at 20-year follow-up was still significantly worse in those with a psychotic illness (all $P < .001$) compared to Never Psychotic individuals.
Conclusions: The current results demonstrate four stable functional trajectories that differ quantitatively in severity across psychotic disorders. However, social functioning also varied widely within disorders, which suggests a need for continuous representation of social outcomes. Our findings indicate that a large group of individuals admitted to hospital with psychotic disorders have lifelong, stable social impairment. These findings highlight the importance of a detailed assessment of social functioning and emphasize the need for targeted, transdiagnostic treatment interventions aiming to preserve and improve social functioning.
Disclosures: Nothing to Disclose.

24.2 Levels of Empathy in Schizophrenia

Michael Green

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Background: The social cognitive neuroscience of empathy is a growing topic in schizophrenia research. Current models define empathy as the ability to understand and share the feelings of others. Empathy includes: affective empathy, which refers to relatively automatic processes that trigger a shared emotion response, and cognitive empathy which refers to reflective processes. We will review 3 studies of affective and cognitive empathy that compared patients with schizophrenia (SZ) and healthy controls (HC) on: 1) self-reported empathy, 2) fMRI of pain empathy, and 3) empathic accuracy.
Methods: For self-reported empathy, we compared 145 SZ, and 45 HC on The Questionnaire of Cognitive and Affective Empathy (QCAE) that assesses cognitive and affective components of empathy. Pain empathy, a type of affective empathy, typically involves examination of neural responses during first-hand experience of pain, and when watching stimuli depicting other people in pain. In this fMRI study, 21 SZ and 21 HC watched videos of people described as medical patients who were in pain and made judgments about these people. Empathic accuracy refers to the ability to accurately infer emotional states of another person, and involves both affective and cognitive empathy. Participants were asked to judge changes in emotional states of another person shown in a video clip while he/she describes an autobiographical event. In this psychometric study, 82 SZ and 59 HC were assessed on empathic accuracy at baseline, and SZ retested at 4-weeks.
Results: For self-reported empathy, factor analyses provided consistent support for a two-factor solution (affective versus cognitive) in SZ. Patients showed significantly lower cognitive ($p < .01$) but higher affective empathy ($p < .05$) than controls. Cognitive empathy showed significant correlations with negative symptoms and functional outcome. For pain empathy, both groups showed similar overall levels of anterior cingulate cortex and anterior insula activation while observing others in pain. In one experimental condition the HC showed more activation when they imagined themselves versus others in pain, whereas patients showed the opposite pattern. In the psychometric study, empathic accuracy discriminated between patients and HC (effect size = .79), showed adequate test-retest reliability (.72), had no practice effect, and was well-tolerated. The task had significant relationships with functional capacity.
Conclusions: Across studies, we found clear support for differences in SZ and HC on self-reported cognitive empathy and empathic accuracy. These measures showed

correlations with functionally meaningful external variables. In contrast, findings of affective empathy and pain empathy did not show consistent impairment. Patients self-reported normal emotional responses to close personal contacts and elevated emotional response to others in their social environment. This findings across studies suggest that affective empathy might be relatively preserved and a social cognitive strength in schizophrenia.
Disclosures: Part 1: I have consulted for AbbVie, DSP, Forum, Mnemosyne (scientific board) and Takeda, **Part 4:** I have received grants from Forum and Amgen.

24.3 Oxytocin for Schizophrenia: A Randomized Controlled Trial

Mark Weiser

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Background: Both human and animal studies have found that the neuropeptide oxytocin (OXT) is involved in regulating affiliative behaviors, including sexual behavior, mother–infant and adult–adult pair-bond formation. Social dysfunction is among the most disruptive outcomes of schizophrenia. Intranasal OXT administration has been reported to have pro-social effects in patients with autism spectrum disorders and with schizophrenia. The aim of this study was to examine the effectiveness of intranasal administration of OXT alone, and of OXT combined with social skills training in the treatment of social dysfunction in patients with schizophrenia.
Methods: Using a 2X2 design, we conducted a randomized, double blind, placebo-controlled, 3 week trial testing the effect of intranasal OXT (24IU X3/d) or placebo in combination with social skills training or supportive psychotherapy. Subjects were 51 patients with schizophrenia or schizoaffective disorder with significant impairment of their social abilities, stabilized on anti-psychotics. The primary outcome measure was a structured assessment of social interaction, done by video-taping interviews with subjects, and then having raters blinded to treatment status assessing the quality of the social interactions, specifically focusing on gaze to experimenter's face, vocalization (patient's vocal output, positive/negative tone, and fluent speech), affect, body tone, movements, and other non-verbal signals. Secondary outcome measures included PANSS and the PENN Emotion recognition tasks.
Results: Of the 51 patients randomized, 48 completed the study (23 in the oxytocin and 25 in the placebo group). Analysis of the primary outcome measure did not show significant effects of oxytocin in any of the variables rated (all p values > 0.05). However, the following variables did show non-significant improvement in patients receiving drug compared to placebo during the social interaction: positive affect, effect size (ES) $= 0.20$, negative affect ES $= 0.53$, fluency of conversation ES $= 0.35$, decreased constriction ES $= 0.378$ and decreased tension ES $= 0.44$. No improvement was found in the PENN-CNP emotion battery tasks, nor in the PANSS scales (all p values > 0.05).
Conclusions: Although changes were not statistically significant, oxytocin improved affect, reduced tension and improved fluency of social interactions at effect sizes of mild-moderate amplitude, and it is conceivable that larger sample sizes will yield statistically significant findings. These variables might reflect subtleties of social interaction not reflected in PANSS scores. The results of the published studies of add-on oxytocin vs placebo for schizophrenia are heterogeneous, with both positive and negative findings. We conclude that before further studies are performed, individual patient meta-analysis of all add-on oxytocin studies in schizophrenia should be performed.
Disclosures: Nothing to Disclose.

Mini Panel
25. Neuropsychiatric Disorders in Isolated Populations

25.1 Clustering of Mendelian Disease Loci and Bipolar Disorder Risk Alleles in a Large Multigenerational Old Order Amish Pedigree

Maja Bucan

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Background: Bipolar affective disorder (BP) is a common, highly heritable psychiatric disorder characterized by periods of depression and mania. The Old Order Amish are a genetic isolate of European ancestry currently residing in several states in North America, with a concentration in Pennsylvania and Ohio. Bipolar disorder type I (BPI) and bipolar disorder type II (BPII) in the Amish occur with similar prevalence, pattern of symptoms, clinical course and response to mood-stabilizing medicines as observed in the general North American population. Alcohol and drug abuse, which often complicate psychiatric diagnoses, are rare among the Amish. Their lifestyle provides a remarkably uniform environment in which behavioral changes can be readily and longitudinally ascertained.
Methods: To identify the genetic basis of bipolar disorder in the Amish, our research returned to the expanded multi-generational Old Order Amish pedigree available as the Amish Study of Major Affective Disorders at the Coriell Institute for Medical Research and applied contemporary genomic and statistical methodology by integrating genotype and whole-genome sequence data. Using dense SNP genotype data, we characterized copy number variants (CNVs) in 388 members of an Old Order Amish Pedigree with bipolar disorder. We identified CNV regions arising from common ancestral mutations by utilizing the pedigree information.
Results: Our findings reveal multiple linkage regions that each harbor a considerable number of sequence and CNV variants, supporting initially reported locus heterogeneity. Dissection of exonic and intronic variants that reside in these linkage peaks has identified credible candidate genes that will be further examined in large-scale population- and family-based studies (Bipolar Sequencing Consortium). We identified 541 inherited CNV regions, of which 268 are rare in a control population of European origin but present in a large number of Amish individuals. We report a trend towards a higher burden of CNVs in known Mendelian disease loci in bipolar individuals (BPI and BPII, $p = 0.06$).

Also, we identified a set of CNVs found at higher frequencies in BP individuals, including a set of deletions (encompassing PWRN2 on 15q11.2 and PARK2 on 6q26) and duplications (ANKMY1 on 2q37.3, TMEM-AS1 on 10q11.2, SALL3 and NFATC1 on 18q23) detected in an overlapping Anabaptist (Amish and Mennonite) population (see ABSTRACT by McMahon).
Conclusions: Our results suggest that both SNPs and CNVs contribute to the phenotypic presentation of mood disorders and co-morbid medical conditions in this extended pedigree. These results reinforce the hypothesis of a complex genetic architecture underlying BP disorder, and suggest that the role of CNVs should be investigated in BP and co-morbid conditions.
Disclosures: Nothing to Disclose.

25.2 Heritable White Matter Diffusion Endophenotypes and Traits in Population Isolate

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Background: Diffusion weighted imaging methods have been proposed to non-invasively decipher cerebral microstructure by examining the behavior of diffusion signals. We calculated heritability for diffusion weighted imaging parameters for cerebral white and gray matter to study the genetic contribution to the diffusion signals. Our approach is to identify diffusion weighted imaging models with the highest heritability using a family study design, here in a population isolate with large pedigree and favorable environmental homogeneity. The Old Order Amish are Caucasians with large family structure that enable heritability analyses with modest sample size.
Methods: Using a large pedigree from an Old Order Amish population isolate, we compared the heritability among three representative diffusion weighted imaging methods targeting corpus callosum white matter and cingulate gyrus gray matter: diffusion tensor imaging (DTI), the permeability-diffusivity (PD) model, and the neurite orientation dispersion and density imaging (NODDI) model. The models represent mono-, bi-, and tri-compartmental modeling of diffusion signal, respectively. We compared the heritability of these three models in 137 members (59M/78F, average age $= 51.2 \pm 15.1$; range = 18-80 years) of Amish families from Lancaster County, PA. The participants were from a total of 17 nuclear families although they were connected into a single large pedigree within 8 generations.
Results: We replicated the high heritability (h2) of the DTI-based fractional anisotropy ($h2 = 0.67$) and radial diffusivity ($h2 = 0.72$) in white matter. High heritability in both white matter and gray matter tissues were observed for the permeability-diffusivity index from the PD model ($h2 = 0.64$ and 0.84), and the neurite density from the NODDI model ($h2 = 0.70$ and 0.55), respectively. Orientation dispersion index from NODDI model was only significantly heritable in gray matter ($h2 = 0.68$). Phenotypic correlation analyses further suggest convergence vs. divergence across these diffusion measures with tissue specificity.
Conclusions: Diffusion weighted imaging parameters measured from multi-compartmental models were heritable in both white matter and gray matter with robust heritability values. Our results posit diffusion weighted imaging parameters as important phenotypes for gene search; and genes thus identified may lead to better understand on causes of mental and neurological disorders that have been extensively associated with abnormal diffusion imaging findings. Overall, the study took advantage of a large, multigenerational family design with known pedigree structure that permits genetic dissections and comparisons of the diffusion weighted imaging models with good statistical power using only modest sample size. The relative environmental homogeneity in the cohort further increased the confidence on the calculated heritability. The strong additive genetic load thus identified, particularly regardless of whether measured in white matter or gray matter, supports the use of advanced diffusion weighted imaging models to derive diffusion-based endophenotypes for genetic studies on the cerebral microstructure under normal and neuropsychiatric conditions.
Disclosures: I (or my spouse/partner) do have relevant financial interests to disclose.
Part 1 Dr. Hong has been a Consultant/Advisor to Pfizer
Part 4 Dr. Hong has received research grants from Mitsubishi Pharma, Pfizer, and Your Energy Systems, LLC.

25.3 Genome Sequencing of Anabaptist Patients with Bipolar Disorder Reveals Enrichment of Rare, Functional Variants Within Genes Involved in GTPase Signaling

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Background: Bipolar disorder is a highly heritable but genetically complex disorder afflicting about 1% of the population. Genome-wide association studies have identified a number of genetic markers, but each confers only a small increased risk of bipolar disorder. Exome sequencing studies in founder populations, such as the Anabaptists, may facilitate the identification of higher risk alleles, owing to decreased genetic heterogeneity and enrichment of otherwise rare alleles.
Methods: We have ascertained over 150 individuals with bipolar disorder within Anabaptist (Amish or Mennonite) communities in North America. All individuals were directly interviewed with the Diagnostic Interview for Genetic Studies and diagnosed by a Best Estimate Final Diagnosis procedure. Blood samples were collected from cases and all available parents. Exome sequencing was completed on an Illumina platform, followed by extensive quality control using GATK Best Practices. Here we report initial results of annotation, filtering, and gene set enrichment analyses from 57 cases with bipolar disorder and their 30 available parents.
Results: After filtering out common variants with a minor allele frequency $>1\%$ in any of several reference data sets, 706 functional variants (defined as non-synonymous, splicing, frame-shift, stop gain/loss by ENSEMBL) in 642 genes were found to be shared by 2 or more cases. Among the non-synonymous variants, 2 were found in ANK3 and one in SYNE1 - both genes have been implicated by previous genome

wide association studies of bipolar disorder. We also found 21 non-synonymous variants and two frame-shift deletions that mapped to genes within copy number variants known to be associated with schizophrenia, autism, epilepsy, or bipolar disorder. Gene set enrichment analysis indicated that genes involved with GTPase signaling were enriched for rare functional variants shared by cases (Fold enrichment = 2.49, FDR = 0.0015). In order to replicate these results, we applied the same filtering strategy to an independent, whole genome-sequencing data set drawn from an overlapping Anabaptist population (Georgi et al 2014). We identified 804 rare functional variants in 771 genes that were shared by 2 or more cases in that sample. The same 40 variants were shared by cases in both samples. In addition, 88 genes carried different variants shared cases in each sample. Enrichment of rare functional variants was again found among genes involved in GTPase signaling (Fold enrichment = 2.13, FDR = 0.019), replicating the initial results in an independent sample.
Conclusions: These results suggest that a number of different variants in many genes contribute to bipolar disorder even within an isolated population. Despite this genetic heterogeneity, we observed substantial overlap at the variant, gene, and gene-set levels between two independent samples ascertained from within the same broad isolated population. If confirmed in larger samples, these findings could point to novel drug targets for bipolar disorder.
Disclosures: Nothing to Disclose.

Study Group
26. Training Aspects of International Research Collaborations: Experiences from Multinational Initiatives in Biological Psychiatry Between the USA, Europe, Asia, and Africa

Thomas Schulze*, Raquel Gur, Akira Sawa, Frank Schneider, George Koob, Tobias Halene, Chao Chen, Chunyu Liu, Susan Weiss, Bruce Cuthbert, Triptish Bhatia, Ibtihal Ibrahim, Vishwajit Nimgaonkar, Ruben Gur, Isabella Schneider, Yoichiro Takayanagi

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International collaborative efforts are a hallmark of 21st research in biological psychiatry. They have provided samples sizes needed for statistically robust results and have propelled the harmonization of research tools and analytical strategies. Moreover, they bring together both experienced and early career researchers from around the globe to share their knowledge and innovative ideas.
They also foster the exchange of doctoral students or post-doctoral fellows for extended research stays. While the North American research community has benefited from high numbers of international early career investigators for many decades already, recent multinational collaborative endeavors are catalyzing and accelerating these developments even further. On the other hand, one can also witness a more balanced flow of young researchers between North America and the rest of the world, whereas until a few years the scientific migration was typically unidirectional, i.e. from Europe or Asia to the United States or Canada. This is in part also explained by increasingly better funding opportunities outside North America.
Funding bodies across the globe have adapted to these developments, allowing consortia to apply for funds specifically geared towards early career investigators planning on an extended research stay abroad. Highly structured programs have been set up to make the training aspect the key element in international collaborations. The International Research Training Group "Brain-behavior relationship of emotion and social cognition in schizophrenia and autism" between the University of Aachen (and affiliates) in Germany and the University of Pennsylvania in the USA, funded by the Deutsche Forschungsgemeinschaft perfectly illustrates this development. The Tri National Training Program in Psychiatric Genetics between the University of Pittsburgh and institutions in India and Egypt, funded by NIH's Fogarty International Center is another example.
This study group will bring together PIs and early career investigators from such and other training programs. Participants hail from the USA, Germany, Japan, China, India, and Egypt. The early career researchers participating in this panel have diverse educational backgrounds and are at different stages in their career. The PIs and (former) trainees can offer a unique first-hand perspective of the challenges and opportunities of multinational research training programs. They will discuss the impact on their respective labs, personal career development, funding opportunities. The audience will hear about typical pitfalls, administrative snags and creative and pragmatic ways around. Leaders and policy makers of the relevant NIH institutes, i.e. NIMH, NIDA, and NIAAA, will join the discussion and present their positions on the future development of multinational training programs for research in neuroscience and psychiatry.
With formal presentations limited to an absolute necessary minimum, the panel will be interactive, engaging the audience in sharing their own experiences. At the end of the session, the audience will have a first overview of international training programs in neuropsychopharmacological research and a basic knowledge of how to devise such programs at their respective institutions. By design, the panel is quite diverse, including male and female early career scientists at various stages, PIs, and NIH institute directors.
Disclosures: Nothing to Disclose.

Wednesday, December 9, 2015

Panel
27. Complimentary and Integrative Treatment for Mood and Anxiety Disorders

27.1 Molecular Loci for Antidepressant Effects of n-3 Polyunsaturated Fatty Acids

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Background: Chronic antidepressant treatment increases physical coupling between the G-protein, Gαs and adenylyl

cyclase (AC), increasing cAMP signaling. Biochemical and imaging studies demonstrated this is due, at least in part, to the translocation of Gαs from lipid-rafts into non-raft membrane fractions where it is more effectively coupled to AC. While perturbation of lipid rafts translocates many species of Galpha, antidepressant treatment is specific to Gαs. This effect requires sustained drug treatment and is not seen with antipsychotics or mood stabilizers.
Methods: A monomeric variant of our previously developed GFP-Gαs fusion protein was used to generate stable, clonal cell lines (C6 glioma) containing this fluorescent Gsα. GFP- Gαs not only translocates from lipid-rafts similar to endogenous Gαs in response to antidepressant treatment, but can be used to study individual cells under physiologic conditions. To do these studies, we used Fluorescence Recovery After Photobleaching measured in a Zeiss 710Meta microscope. Cells were treated with drug (at concentrations from 50 nM to 10 μM) for 1-3 days in the presence or absence of n-3 PUFA (EPA or DHA). Cells were also treated with n-3 PUFA alone. The acylation status of Gαs was also determined by mass spectrometry.
Results: Chronic treatment with antidepressants increases fluorescence recovery time. This appears due to immobility of AC as AC/ GFP-Gαs complexes are much less mobile than GFP-Gαs alone. Other G protein alpha subunits are not altered by antidepressant treatment. Mutations to Gαs that render it cytosolic shorten recovery time, and mutations that augment membrane association due to an additional acylation site retard recovery further. n-3 PUFA treatment both translocates Gαs and facilitates antidepressants in doing so. Acylation status of Gαs is modified by sustained antidepressant treatment.
Conclusions: It is suggested that both rafts and AC anchor Gαs, and removal from the former increases the latter. It is also suggested that n-3 PUFA might modify the lipid microenvironment and might also modify Gαs directly at its acylation site. We also suggest that FRAP of GFP-Gαs provides a platform that can be used for the development of antidepressant compounds and, perhaps, a prognostic indicator for drug selection in patients.
Disclosures: Part 1: Eli Lilly, Pfizer, **Part 2:** Pax Neuroscience, **Part 4:** Eli Lilly, Lundbeck.

27.2 Depression, Inflammation, and Omega-3 Fatty Acids

David Mischoulon

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Background: Omega-3 (n-3) fatty acids have been studied as a possible treatment for major depressive disorder (MDD). While the literature is generally supportive of their efficacy and safety, studies as a whole have produced mixed or contradictory results. There is evidence that n-3 fatty acids may exert at least some of their clinical effect via anti-inflammatory mechanisms of action. This suggests that individuals with MDD who have elevations in inflammatory biomarkers may be better candidates for n-3 therapy. We sought to compare the efficacy of two n-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in a sample of patients with MDD, and determine whether inflammatory biomarkers act as moderators of clinical response.
Methods: 196 adults (53% female; age 44.7 ± 13.4 years) with DSM-IV MDD and a baseline 17-item Hamilton Depression Rating Scale (HAM-D-17) score ⩾ 15, were randomized to 8 weeks of double-blind treatment with eicosapentaenoic acid (EPA)-enriched n-3 1060 mg/day, docosahexaenoic acid (DHA)-enriched n-3 900 mg/day, or placebo. Outcomes were determined using mixed model repeated measures (MMRM) analysis for the entire sample and for "high" and "low" inflammation groups based on individual and combined biomarkers.
Results: Modified Intent-to-Treat (MITT) analysis examined 177 subjects (59.3% female, age 45.8 ± 12.5 years) with ⩾1 post-baseline visit. All 3 groups demonstrated statistically significant improvement in the HAM-D-17, Quick Inventory of Depressive Symptomatology (QIDS-SR), and Clinical Global Improvement-Severity Scale (CGI-S) ($P < 0.05$), but neither n-3 preparation separated from placebo ($P > 0.05$). Response and remission rates were in the range of 40-50% and 30% respectively, for all treatments, with no significant differences between groups. 155 subjects had baseline biomarker data (IL-1ra, IL-6, hs-CRP, leptin, adiponectin) available for analysis. For the "inflammation-based" subgroups, we determined standardized treatment effect size (ES) for change in HAM-D-17 from baseline to week 8. While overall treatment group differences were negligible (ES = -0.13 to +0.04), subjects with any "high" inflammation improved more on EPA than placebo (ES = -0.39) or DHA (ES = -0.60) and less on DHA than placebo (ES = +0.21). Furthermore, EPA-placebo separation increased with increasing numbers of markers of high inflammation. Subjects randomized to EPA with "high" IL-1ra or hs-CRP or low adiponectin ("high" inflammation) had medium ES decreases in HAM-D-17 scores versus subjects "low" on these biomarkers. Subjects with "high" hs-CRP, IL-6 or leptin were less placebo-responsive than subjects with low levels of these biomarkers (medium to large ES differences).
Conclusions: Neither EPA-enriched nor DHA-enriched n-3 was superior to placebo for the treatment of MDD in our sample as a whole. However, employing multiple markers of inflammation facilitated identification of a more homogeneous cohort of subjects with MDD responding to EPA versus placebo. These results may have implications for personalized medicine in that selection of a specific cohort of depressed patients may result in better outcomes with n-3. Studies are needed to replicate and extend these preliminary findings.
Disclosures: Part 1: Dr. Mischoulon has received royalties from Lippincott Williams & Wilkins for published book "Natural Medications for Psychiatric Disorders: Considering the Alternatives, **Part 4:** Dr. Mischoulon has received research support through grants from the Bowman Family Foundation, FisherWallace, Nordic Naturals, Methylation Sciences, Inc. (MSI), and PharmoRx Therapeutics.

27.3 Massage Therapy for GAD: Lessons Learned about Outcomes, Hormones, and Immune Function

Mark Rapaport

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Background: Epidemiology studies indicate that a significant of patients in the US would prefer to seek treatment from integrative medicine healers than from traditional medical practitioners. In fact, stress, anxiety and depression are some of the most common reasons for individuals to seek massage therapy. Yet, little is known about the efficacy of massage for the treatment of GAD nor about the potential biological under-pinnings of massage therapy.
Methods: Data from 2 NCCIH- funded pilot projects will be presented and synthesized. The first study is a 40 subject 12-session, single-masked, study comparing and contrasting Swedish Massage Therapy (SMT) vs. a light touch control condition (LT) on measures of efficacy, cortisol, oxytocin (OT), AVP, and immune parameters. The second study is a 44 subject pilot study in healthy volunteers investigating the acute and longer-term effects of SMT vs. LT on hormone levels and immune function.
Results: After 12 sessions (end of week 6), clinician-rated HAM-A scores decreased 11.67 points (SE = 1.09) for the SMT group, compared to 8.41 (SE = 1.01) points for the LT group (t = -2.19; df = 106; p = 0.030), for a standardized treatment effect size (ES) -0.69 (95% CI = -1.330 to -0.051). Differences in improvement on the HAM-A total score were accompanied by non-significant but moderate effect size differences favoring SMT on both the Psychic Anxiety and the Somatic Anxiety subscales (ES = -0.429 and -0.552, respectively). We did not see differences in immune parameters between SMT and LT. the OT and AVP data are pending.
So far, these findings are in agreement with our data from healthy controls where we found that twice-weekly SMT had much greater effect on hormone levels and less effect on immune function than weekly SMT.
Conclusions: SMT was an effective treatment for subjects with GAD, it was well-tolerated and the advantage over LT could not be explained merely by differences in expectancy and credibility. This effect was not immune-mediated and was the result of improvement in both somatic and psychic anxiety. These findings with respect to twice-weekly SMT are consistent with our published data about the effects of SMT "dosage on biological measures in healthy controls.
This work highlights the challenges and some new strategies that can be used to investigate a manual therapy in psychiatric subjects.
Disclosures: Nothing to Disclose.

27.4 Complementary and Integrative Health Approaches for Mental Health Conditions

Emmeline Edwards

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Background: The National Center for Complementary and Integrative Health (NCCIH) is the Federal Government's lead agency for scientific research on complementary and integrative health strategies.
Methods: Our research portfolios include a group of diverse medical and health care interventions, practices, products, or disciplines that are not generally considered part of conventional medicine but are increasingly incorporated into integrative medicine practices and are most often used by the general public as a complement or adjunct to conventional medical care. There has been increasing interest from the psychiatry community in understanding the efficacy, safety and patterns of use of complementary and integrative health approaches for mental health conditions. In managing symptoms of mental health disorders, the most commonly used complementary and integrative approaches fall into the categories of mind and body approaches (massage, meditation, yoga) and natural products (St John Wort, omega-3 fatty acids, chamomile).
Results: N/A
Conclusions: This presentation will highlight pre-clinical and clinical work with a strong emphasis on basic mechanism-oriented research that has the potential to yield mechanistic insights, and to identify biological signals of physiological effects.
Disclosures: Nothing to Disclose.

Panel
28. Synaptic Addiction: New Insights into the Cellular Mechanisms of Drug Action and Substance Use Disorders

28.1 From Optogenetics to rTMS: A Clinical Trials on Cocaine Craving

Antonello Bonci

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Background: Human studies have shown that dysfunction of subregions of the prefrontal cortex (PFC) is strongly correlated with compulsive drug use. Importantly, recent optogenetic studies in rodents demonstrated that compulsive cocaine seeking strongly reduces prelimbic cortex activity, while optogenetic stimulation of this brain area significantly inhibits compulsive cocaine seeking, providing both a causal link between cocaine abuse and hypoactivity of the PFC, and a strong rationale for using brain stimulation techniques to reduce cocaine consumption.
Methods: 32 cocaine-addicted patients were enrolled in a 29-day outpatient study (Phase 1) and randomly assigned to either the experimental group receiving rTMS on the left Dorsolateral PFC (DLPFC), or to a control group treated with pharmacological agents (dopaminergic medications and benzodiazepines). Phase 1 was followed by a 63-day follow-up (Phase 2), during which completers in the control group were offered to switch to rTMS treatment and completers in the experimental group continued receiving rTMS.
Results: In the rTMS group, no serious or unexpected adverse events were observed. During Phase 1, there was a significantly higher number of cocaine-free urine drug tests in the rTMS group compared to control. Craving for cocaine

was also significantly lower in the rTMS group compared to the controls. Out of 13 patients who completed Phase 1 in the control group, 10 patients received rTMS treatment during Phase 2 and showed significant improvement with favorable outcomes becoming comparable to those of the rTMS group.
Conclusions: The present study, albeit very preliminary, provides data supporting the safety of rTMS in cocaine-addicted patients and suggest its potential therapeutic role for rTMS-driven PFC stimulation in reducing cocaine use.
Disclosures: Nothing to Disclose.

28.2 Strengthening Accumbal Indirect Pathway Promotes Resilience to Compulsive Cocaine Use

Veronica Alvarez

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

Background: Substance use disorders are highly prevalent and comorbid with all major mental illnesses including depression, posttraumatic stress disorders, bipolar disorder, and schizophrenia. Over the past decade, rapid technological advances have spurred unprecedented advancement in the synaptic, cellular, and circuit mechanisms underlying compulsive behaviors including addiction. A key goal of the session will be to present advances in the synaptic mechanisms subserving compulsive behaviors and addiction in a manner accessible to clinicians and non-specialists, and to highlight translational potential of research findings made at the synaptic level to higher levels of analysis at the circuit, behavioral, and ultimately clinical level.
Methods: Operant intravenous cocaine self-administration in mice combined with ex vivi electrophysiological analysis of glutamatergic synaptic transmission and two-photon laser scanning imaging.
Results: Dr. Alvarez will present results that identify a synaptic mechanism that confers resilience against the development of compulsive cocaine use in mice. Her group found that recruitment of indirect-pathway neurons in the NAc functions to restrain cocaine self-administration and suggests that individuals with robust indirect-pathway are resilience to developing addictive behaviors.
Conclusions: These findings suggest that the activity state of the accumbal-pallidum indirect-pathway strongly influences cocaine taking behavior and the motivation to seek cocaine and that strengthening of accumbal-pallidum indirect-pathway can serve as a natural protective mechanism towards the development of compulsive seeking.
Disclosures: Nothing to Disclose.

28.3 Neuroplasticity in Fronto-Cortical Circuits Associated with Compulsive Eating

Stephanie Borgland

Hotchkiss Brain Institute/University of Calgary, Calgary, Canada

Background: In an environment rich in easily accessible palatable foods, the ability to withhold food consumption is of key importance for maintaining a healthy body weight. The orbitofrontal cortex (OFC) encodes the current incentive value of food cues and is implicated in cognitive flexibility. Dysfunction of the OFC leads to perseverative behaviours including compulsive eating. Most studies have used lesions to assess the function of the OFC. However, these studies provide little information on the underlying cellular mechanisms as lesions destroy both the pyramidal neurons and the GABAergic interneurons that tightly control the firing and output of the pyramidal projection neurons. Furthermore, it is unknown if and how neurons in the OFC adapt to a pathological insult, such as compulsive behavior. Therefore, we used a model of compulsive feeding to determine if diet can induce neuroplasticity of the OFC. We hypothesized that extended access to a cafeteria diet can induces neuroadaptations in the OFC.
Methods: We used whole cell patch clamp in coronal OFC brain slices to measure basal excitability and synaptic transmission of lateral OFC pyramidal neurons from rats that had extended or restricted access to a cafeteria diet as well as chow-fed controls. We also tested for compulsive behavior using a conditioned suppression model where rats were presented with the cafeteria diet in the presence of footshock predicting cues.
Results: After 6-7 weeks of extended access to a cafeteria diet, obese rats did not suppress feeding in response to footshock predicting cues, in contrast to rats restricted to one hour a day of the cafeteria diet or chow-fed rats. OFC pyramidal neurons from extended access rats had greater excitability. This was accompanied by a reduction of inhibitory synaptic transmission to these neurons. Although restricted access rats binge eat their caloric intake during the 1 h exposure to the cafeteria diet, there were no significant changes of OFC pyramidal neurons from these rats compared to chow fed rats.
Conclusions: Taken together, extended access to a cafeteria diet can induce neuroplasticity of OFC that may alter the animal's ability to withhold feeding responses during aversive situations.
Disclosures: Nothing to Disclose.

28.4 Nucleus Accumbens Parvalbumin Expressing Interneurons Regulate Synaptic and Behavioral Plasticity

Brad Grueter

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Background: Maladaptive circuit function within the reward system is likely a key component to susceptibility to drug addiction. The nucleus accumbens (NAc) is a key brain region involved in mediating aspects of addiction-related behaviors and is comprised of parallel output loops (D1 and D2 dopamine receptor expressing medium spiny neurons (MSNs)) whose activity is governed by excitatory drive and by often overlooked inhibitory interneuronal microcircuitry. Parvalbumin (PV) expressing fast spiking interneurons (FSIs) are GABAergic cells that regulate the activity of networks through inhibition of local projection neurons. Activity of PV-FSIs is thought to gate excitatory drive

within the NAc to correctly process goal-directed behaviors. Endocannabinoid (eCB) signaling is an on-demand synapse-specific negative feed-back signal that regulates emotional and motivational states. A major established role of eCBs is regulation of excitatory and inhibitory synaptic transmission. ECB signaling via cannabinoid 1 receptors (CB1Rs) and transient receptor potential vanilloid 1 (TRPV1) inhibits D2 MSN excitatory synapses within the NAc. We hypothesize that NAc PV-FSIs differentially regulate D1 and D2 MSNs under physiological and pathophysiological conditions in an eCB dependent manner. The rationale is that, NAc MSN subtypes dynamically integrate inhibitory signaling from PV-FSIs via eCB signaling to selectively filter excitatory drive and thus propagate information through the reward circuit leading to behavioral outcomes.

Methods: We identified NAc core D1 or D2 MSNs in acute slices from transgenic mice and use targeted whole-cell patch-clamp recordings to investigate synaptic and membrane properties of NAc D1 and D2 MSNs. These mice consist of combinations of D1-tdTomato bacterial artificial chromosome (BAC) transgenic mice, parvalbumin (PV)-Cre mice, conditional ChR2 mice (B6;129S-Gt(ROSA)26-Sortm32(CAG-COP4*H134R/EYFP)Hze/J), and homozygous Cnr1floxed mice generated by Dr. Eric Delpire (INIA). 470 nm blue light stimulation activates ChR2 expressed on PV-FSIs and elicit light-evoked inhibitory post synaptic currents (oIPSCs) in cells being recorded from. Behavioral analysis consists of locomotor responding and conditioned place preference to psychostimulants and exocannabinoids. Real time place preference is performed by implanting fiber optics into the NAc of PV-cre/cChR2 mice.

Results: Our results show CB1R activation can inhibit IPSCs from PV-FSIs to D1 and D2 MSNs while TRPV1 activation inhibits GABAergic neurotransmission preferentially at D1 MSN synapses. LFS of PV-FSI to MSNs results in a state dependent depression of synaptic transmission. Long term depression of IPSCs (iLTD) from PV-FSI to D1 but not D2 MSN synapses is expressed at hyperpolarized membrane state. Conversely, while at depolarized state, iLTD is elicited at PV-FSI to D2 but not D1 MSN synapses. Preliminary studies suggest this iLTD is CB1R dependent. Further work analyzing excitatory/inhibitory (E/I) synaptic function at synapses onto D1 and D2 MSNs suggests the E/I balance is sensitive to CB1R expression on PV-FSIs. This E/I balance is also sensitive to prior cocaine experience. Conditional knockout of CB1Rs in PV-FSIs results in have a shift in CB1R agonist dependent learning and attenuated cocaine-induced learning. Finally, behavioral analysis suggests that this LFS stimulation pattern investigated in the slice elicits conditioned place aversion in PV-ChR2 mice.

Conclusions: PV-FSIs, although limited in number, make vast inhibitory connections to MSNs thereby greatly influencing NAc output. Thus, PV-FSI synaptic activity is a putative mediator of NAc circuit adaptations by influencing NAc synaptic and membrane properties. Changes in NAc circuit dynamics likely underlie addiction behaviors. Indeed, we find that manipulation of these PV-FSIs in the NAc and CB1R signaling on PV-FSIs bi-directionally modulate drug-related behaviors.

Disclosures: Nothing to Disclose.

Panel
29. Normalizing Cognitive Impairments in Schizophrenia: New Leads from Novel Glutamatergic Manipulations

29.1 Cognitive Enhancement Through Inhibition of Kynurenic Acid Synthesis

Robert Schwarcz

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Background: The levels of the tryptophan metabolite kynurenic acid (KYNA) are elevated in the brain and cerebrospinal fluid of persons with schizophrenia (SZ) and may be causally involved in pathophysiology. This view is based on the ability of KYNA to antagonize the function of α7 nicotinic and NMDA receptors, both of which are critical to normal brain development and cognitive processes, and are impaired in schizophrenia. In experimental animals, even relatively moderate increases in brain KYNA result in a spectrum of biochemical and cognitive abnormalities reminiscent of SZ. Interventions leading to a reduction of KYNA synthesis in the brain therefore provide a novel approach to achieve pro-cognitive effects. We tested this hypothesis using a new, systemically active inhibitor of the key KYNA-synthesizing enzyme kynurenine aminotransferase II (KAT II).

Methods: Using biochemical and behavioral outcome measures, we tested the effects of the specific KAT II inhibitor BFF-816 (Wu et al., Schiz. Bull., 2014) in adult rats. Microdialysis was performed in awake animals, and extracellular levels of KYNA (all regions), glutamate (dorsal hippocampus, medial prefrontal cortex) and dopamine (striatum) were determined by established HPLC methods. The Morris water maze was used to study spatial navigation and reference memory in normal animals. BFF-816 or vehicle was administered 90 min prior to the first trial of behavioral testing on each day. Finally, using an established passive avoidance paradigm, we tested the acute effects of KAT II inhibition in adult rats that show deficits in contextual memory following chronic prenatal KYNA elevation (Pocivavsek et al., Psychopharmacology, 2014). In all experiments, BFF-816 was given orally at 30 mg/kg.

Results: Compared to respective baseline levels, BFF-816 consistently reduced extracellular KYNA and increased extracellular glutamate and dopamine levels in the brain areas studied. Maximal effects (~25% decrease in KYNA and ~60% elevation in glutamate and dopamine, respectively) were seen between 90 and 150 min after BFF-816, and the levels of all analytes returned to control values after approximately 4 h. No tolerance was seen when animals were treated daily for five consecutive days. Behaviorally, daily injections of BFF-816 significantly decreased escape latency in the Morris water maze, indicating improved performance in spatial and contextual memory in normal animals. In developmentally challenged adult rats, BFF-816 restored the memory deficit when administered prior to training on Day 1, but not when given prior to testing avoidance latency on Day 2 of the contextual memory task.

Conclusions: Systemically applied BFF-816 is an excellent tool for studying the neurobiology of KYNA and, in particular, for investigating the mechanisms linking KAT II inhibition to changes in glutamatergic and dopaminergic function in brain physiology and pathology.
Disclosures: Part 1: Vistagen, **Part 2:** Vistagen, **Part 4:** Mitsubishi-Tanabe, Lundbeck.

29.2 Calcium-Permeable Ampa Receptor and Asc-1 Transporter Regulate the Cortical Extracellular D-Serine Concentration: Potential Targets for Development of Novel Pharmacotherapy for NMDA Receptor Dysfunction in Schizophrenia

Toru Nishikawa

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Background: In mammalian brains, D-serine has been shown to act as an endogenous co-agonist for NMDA type glutamate receptors (NMDARs) that are presumed to be hypofunctional in schizophrenia (SZ). This pivotal role of D-serine indicates that the control systems of the extracellular D-serine in the brain could be potential targets for analysis of the pathophysiology of and creation of new treatment for SZ. However, the molecular and cellular mechanisms underlying the D-serine signaling are largely unknown. To gain a cue to clarify this important issue, we have studied the effects of agents acting at AMPA type glutamate receptors (AMPARs) and Asc-1 neutral amino acid transporter on the extracellular D-serine concentrations in the medial frontal cortex (mFC).
Methods: The cortical extracellular concentrations of D-serine and other amino acids were measured in freely moving rats by an *in vivo* microdialysis technique in combination with high-performance liquid chromatography with fluorometric detection, because the *in vivo* conditions are needed to avoid the influence of anesthesia and to maintain integration of neuron-neuron and/or neuron-glia interaction, in which D-serine has been found to participate.
Results: The intra-mFC infusion of (S)-AMPA, an active enantiomer at the AMPAR, via the dialysis probing causes a significant reduction in the extracellular contents of D-serine in the cortical portion in a concentration-dependent, an AMPA/kainate receptor antagonist NBQX- and a calcium permeable AMPA receptor antagonist 1-naphthyl acetyl spermine-reversible manner. The reducing effects of (S)-AMPA are augmented by co-infusion of cyclothiazide that prevents AMPA receptor desensitization. Moreover, attenuation of glial activity by toxin fluorocitrate, but not cessation of nerve impulse traffic by tetrodotoxin blocked the AMPAR-D-serine interaction. The local infusion of a system A and Asc-1 amino acid transporter inhibitor, S-methyl-L-cysteine, into the mFC produces an increase in the cortical dialysate contents of D-serine in a concentration-related manner.
Conclusions: Our data support the views that a calcium permeable AMPAR subtype may exert a phasic inhibitory regulation of the extracellular D-serine release in the mammalian frontal cortex and that the liberated D-serine in the cortical glutamate synapse may be taken up into certain cells, at least in part, via the Asc-1 neutral amino acid transporter. Therefore, development of pharmacotherapy enhancing the extracellular D-serine levels by blockade of either of the two molecules could facilitate NMDAR functions and improve current treatment-resistant cognitive deficits and negative symptoms of SZ that are related to reduced NMDAR activity. It is also possible that dysfunction of these molecules might be involved in the pathophysiology of SZ.
Disclosures: Part 1: The authors declare no conflict of interest related to the subject of this presentation. Dr. Nishikawa was recently compensated for his lectures by Astllas, MSD, Eli Lilly, GSK and Otsuka pharmaceutical industries and for his consultancy by Mochida Pharmaceutical Co., Ltd. He also received grants for scientific research, but not for clinical drug assessment, from Tanabe-Mitsubishi, MSD, Pfizer, Astllas, Otsuka and Shionogi directly or indirectly through a foundation, **Part 4:** As shown in a part of the above "Disclosure Part 1", Dr. Toru Nishikawa received grants for scientific research, but not for clinical drug assessment, from Tanabe-Mitsubishi, MSD, Pfizer, Astllas, Otsuka and Shionogi pharmaceutical industries directly or indirectly through a foundation.

29.3 Endogenous D-Serine Maintains the Level of NMDA Receptor Activation Required for LTP Induction at Hippocampal Synapses

Vadim Bolshakov

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Background: Activation of NMDA receptors (NMDAR) at central synapses leads to the intraneuronal calcium influx required for the induction of synaptic and developmental plasticity. In addition to binding glutamate, NMDAR activation requires occupation of the glycine binding site on the NR1 subunit by endogenous co-agonists, glycine or D-serine. The NMDAR glycine site is not saturated at central synapses *in vivo*, and, therefore NMDAR function could be regulated through changes in the glycine site occupancy. Genetic, pharmacologic and post-mortem studies have implicated hypofunction of NMDARs in the pathophysiology of schizophrenia. Focusing on the analysis of mice with the expression of serine racemase (SR) conditionally or constitutively suppressed to inhibit D-serine synthesis, we explored whether the hypofunction of NMDARs due to the lack of D-serine may be translated into the functional deficits in the hippocampus, a brain region severely affected in schizophrenic patients.
Methods: Electrophysiological recordings were performed in slices from SR-/- mice and their control littermates. Thin hippocampal slices were cut with a vibratome and transferred to an incubation chamber filled with ACSF. Whole-cell patch-clamp recordings were obtained from visualized (with DIC/infrared optics) neurons. Extracellular fEPSPs were recorded with glass pipettes filled with the extracellular solution.
Results: We found that long term potentiation (LTP) at the Schaffer collateral-CA1 neuron synapses was markedly reduced in slices from mice in which SR was specifically inactivated in neurons (but not in astrocytes). The observed

effect on LTP was associated with significant reductions in the amplitude of NMDAR-mediated synaptic currents in CA1 neurons in these mice. Similarly, the amplitude of NMDAR-mediated synaptic currents was diminished in the dentate gyrus (DG) of SR-/- mice. The hypofunction of NMDARs, detected in SR-/- mice did not affect basal synaptic transmission but resulted in reduced LTP at the medial perforant pathway to DG synapses in the hippocampus. Chronic D-serine treatment normalized the electrophysiological deficits observed in SR-/- mice. Notably, the metabotropic glutamate receptor 5 (mGluR5) positive allosteric modulator, VU0409551, enhanced NMDAR responses and rescued LTP in hippocampal slices obtained from SR-/- mice. The alterations in LTP and its pharmacologic rescue paralleled performance in a trace conditioning memory task.
Conclusions: Our findings indicate that D-serine is required for optimal activation of postsynaptic NMDA receptors and induction of long-term synaptic plasticity in the hippocampus needed for certain cognitive functions of the brain.
Disclosures: Nothing to Disclose.

29.4 Oxidative Stress in Interaction with Nmda Receptor Hypofunction as a Core Mechanism in Schizophrenia Pathophysiology: Spatial-temporal Development and Potential Protection with Antioxidants

Kim Do

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Background: Excitatory-inhibitory (E/I) balance, as set by parvalbumin interneurons (PVI) GABAergic circuitry, is critical for high-frequency neuronal synchrony, sensory gating and cognition. PVI alterations constitute a hallmark in schizophrenia (SZ). Their fast-spiking activity implies enhanced oxidative metabolism and generation of reactive oxygen species (ROS), in tight link with inflammatory processes. If the redox regulation is deficient, as described in patients, it will lead to oxidative stress (OX) in PVI. In addition, most developmental environmental insults such as infection or psycho-social trauma induce ROS production. NMDAR hypofunction also leads to PVI impairment through OX (Hardingham): Synaptic NMDAR activation boosts intrinsic antioxidant defenses, through direct transcriptional control of glutathione (GSH), promoting its synthesis, recycling and utilization. Inversely, NMDAR hypoactivity during development leads to deleterious loss of this control. We studied the regional distribution and the developmental timing of OX induced PVI impairment and the convergence of various SZ models on OX.
Methods: Spatiotemporal distribution of OX was assessed by 8-oxo-DG and PVI integrity by PV and perineuronal net (PNN) immunohistochemistry (Cabungcal &al, 2014). Various SZ models have been investigated: One model concerns directly the redox dysregulation (gclm-/-, knock-out of the key GSH synthesizing enzyme), while the others do not: ventral hippocampal neonatal lesion (NVHL) rats, D-serine-racemase knock-out (SR-/-) mice, LgDel 22q11 deletion syndrome mice and methylazoxy-methanol acetate (MAM) rats.
Results: During development of gclm-/-, the spatio-temporal sequence of OX and PVI deficit show that they appear first in thalamic reticular nucleus (from D10 on), then in amygdala, ventral hippocampus, globus pallidus and the latest in anterior cingulate cortex. This suggests a staging of OX sensitivity and of its consequences for PVIs in various telencephalic regions, potentially affecting their respective functions.
Interestingly, various models studied also converge on OX. The NVHL that does not entail direct manipulation of redox pathways, presents OX and PVI impairment. Treatment with antioxidants such as N-acetyl-cysteine (NAC) or Ebselen prevents both, as well as their physiological and behavioral deficits, suggesting that they are secondary to OX. These reversal effects of NAC are also present when applied during adolescence. The OX induced impairment of PVI is also observed in other SZ models including SR-/-, LgDel22q11 and MAM models.
Conclusions: Combined with evidence of OX and PVI impairment both in patients and in other genetic models (DISC1, PROD, G72, NRG), our results suggest that vulnerability to OX of PVI/PNN appears as a final common pathway for various genetic and environmental risk factors and places them at the core of SZ pathophysiology. This opens perspectives for early prevention with antioxidants/redox modulators.
Disclosures: Nothing to Disclose.

Panel
30. The Research Domain Criteria (RDoC) Initiative: New Data Across Psychiatric Conditions and Age Groups

30.1 A Multi-modal Assessment of Positive Valence Systems Across Unipolar and Bipolar Depression

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Background: Current diagnostic criteria often fail to differentiate bipolar disorder from unipolar depression when a person presents in a depressed state. Consequently, for someone with bipolar disorder the average length of time from symptom onset to correct diagnosis is over ten years. This indicates an urgent need to move beyond our current diagnostic system and identify neurobiological markers that better predict bipolarity. The current study aims to address this need by identifying biomarkers that predict (hypo)mania in a transdiagnostic sample of individuals seeking treatment for mood disorders, focusing on the key domain of Reward Learning within the RDoC Positive Valence Systems matrix.
Methods: We evaluated predictors of (hypo)mania across three units of analysis that assess distinct yet complementary aspects of reward processing. First, we examined the acquisition of a behavioral response bias during a probabilistic reward task (PRT). Second, we measured the amplitude of the feedback-related positivity (FRP) during the PRT. This is an event-related potential component

elicited by positive prediction errors, thought to arise from dopaminergic burst-firing within the anterior cingulate cortex (ACC) and striatum during reward learning. Third, we measured levels of glutamate (Glu) within the ACC using magnetic resonance spectroscopy. Glu is an excitatory neurotransmitter that has been associated with motivation for rewards. To date, 25 mood disorder patients, and 9 psychiatrically healthy subjects have been recruited.
Results: Across the entire sample, levels of ACC Glu positively correlated with severity of (hypo)mania ($r = .68$, $p = .046$). Although more severe (hypo)mania was associated with greater depressive illness severity, the correlation between Glu and (hypo)mania remained significant after controlling for depressive symptoms. This indicates that elevated Glu was specifically associated with (hypo)-mania rather than illness severity. In contrast, reduced FRP to rewards was associated with more severe anhedonia ($r = -.53$, $p = .02$).
Conclusions: Findings indicate that increased ACC Glu is associated with an increased risk for (hypo)mania. The results also corroborate prior evidence of an association between blunted FRP (putatively indexing reduced dopaminergic firing during reward learning) and anhedonia. The potential for these markers to improve diagnosis of depressive disorders will be discussed.
Disclosures: Part 1: Consulting/honorarium from Pfizer, Otsuka.

30.2 The Neuroimmunology of Anhedonia and Related Positive Valence System Deficits in Adolescents

Vilma Gabbay

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Background: Adolescence represents a critical developmental stage, during which many psychiatric disorders first emerge. This phenomenon has been attributed, in part, to maturational disturbances within the neuronal reward circuitry. Clinically, such alterations can manifest as anhedonia, the reduce capacity to experience pleasure, a core symptom of major depression and a salient feature across psychiatric conditions. Utilizing a dimensional investigative approach, we have examined the role of peripheral inflammatory processes and brain chemicals in anhedonia among depressed adolescents as well as across psychiatric conditions. Further, to delineate the specific deficits of reward processes associated with peripheral inflammation and neurometabolites abnormalities, we have developed the reward flanker task to examine brain function during reward expectancy and attainment.
Methods: Subjects are adolescents ages 12-18 of Tanner $\geqslant$ 4, all medically healthy. All are diagnosed by the K-SADS-PL. Anhedonia, fatigue, sleep, and anxiety are assessed quantitatively. Immune assessment: Blood samples are collected between 8-9AM after an overnight fast ($\geqslant$ 12h), before and after the Trier Social Stress Test. PBMC are being exposed for 24 to LPS to examine response to biological stresses. Kynurenine pathway metabolite, cytokines, and composition of innate and adaptive immune system are examined. Within an hour after the blood draw participants have an imaging session including: a) 1H MRS assessing striatal and cortical glutathione (antioxidant), ACC GABA, N-acetylaspartate (neuronal/mitochondrial marker) and total choline (lipid peroxidation); b) FMRI includes resting state and the Reward Flanker Task (RFT), a combination of the incentive flanker and monetary incentive tasks, that examines brain function during reward expectancy (motivation) and immediate attainment.
Results: Adolescents with moderate to severe MDD as well as with diverse psychiatric symptoms exhibited a full range of anhedonia severity. We have reported positive relationships between inflammatory neurotoxins and anhedonia severity. We also documented that decreased ACC GABA levels in depressed adolescents were driven by the anhedonic subgroup and reported a negative relationship between anhedonia severity and ACC GABA levels in the whole sample. Using striatal based intrinsic functional connectivity, we identified specific network associated with anhedonia severity while controlled for other depressive symptomatology. Using the RFT, anhedonia severity was associated with specific brain regions during reward anticipation and attainment.
Conclusions: Peripheral inflammatory processes and neurochemical alterations implicated across psychiatric conditions may play a role in the neurobiology of anhedonia and be associated with specific deficits of reward processes. Such investigative approach can facilitate the early identification of alterations within specific neurocircuits.
Disclosures: Nothing to Disclose.

30.3 Do the Neural Mechanisms Mediating Irritability Differ Across Diagnoses?

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Background: The RDoC framework makes an implicit assumption that the neural mechanisms mediating a psychological trait or symptom (e.g., irritability) do not vary across DSM-5 diagnoses. We tested this assumption in youth with disruptive mood dysregulation disorder (DMDD) and bipolar disorder (BP). Severe, chronic irritability is the hallmark of DMDD, and is also common in pediatric bipolar disorder (BP) during euthymia; however, the two phenotypes differ in that only those with BP have distinct manic episodes. Both BP and DMDD show deficits in face emotion labeling. In this study we used fMRI and a face emotion labeling paradigm to test whether the neural mechanisms mediating irritability differ between BP and DMDD.
Methods: During fMRI, 71 youths (24 DMDD, 25 BD, 22 HV) performed an event-related face emotion labeling task with happy, fearful, and angry faces of varying intensity. In all subjects, trait irritability was characterized dimensionally on the Affective Reactivity Index (ARI). We tested, not only main effects of diagnosis (BP, DMDD, HV) and ARI on neural activity, but also diagnosis x ARI interactions in a whole-brain corrected analysis.
Results: ARI scores did not differ between DMDD and BD, and there were no behavioral differences among groups in

the scanner. We found a trait x diagnosis interaction in the amygdala, where irritability correlated with neural activity for all emotions in DMDD, but only for fearful faces in BD. Moreover, higher irritability was associated with greater amygdala activity in response to subtle fearful faces in BD, but less amygdala activity in DMDD. Other temporal, parietal, and occipital regions showed positive correlations between irritability and BOLD response to subtle negative emotion faces in DMDD, but not BD.
Conclusions: Although irritability severity did not differ between DMDD and BD, the neural mechanisms mediating irritability did differ significantly between the two patient groups. These data challenge the RDoC assumption that, across diagnoses, neural mechanisms mediating a specific trait are necessarily the same. Clearly, this assumption needs to be tested for other traits and across other diagnoses. Additionally, the current findings add to existing longitudinal, familial, and neuroimaging data suggesting that DMDD (characterized by chronic irritability, without manic episodes) and BP (characterized by episodic mania with or without chronic irritability between episodes) are distinct phenotypes.
Disclosures: Nothing to Disclose.

30.4 A Common Functional Topography Across the Major Psychiatric Disorders

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Background: Epidemiological, phenomenological, family and molecular genetic studies suggest that psychiatric disorders have overlapping aetiology and pathophysiology. Meta-analyses of the brain magnetic resonance imaging (MRI) literature have identified brain structural alterations shared across multiple disorders. The aims of the present study were (1) to identify a 'disorder-general' functional map of schizophrenia (SZ), bipolar disorder (BD), major depressive disorder (MDD), obsessive compulsive disorder (OCD), and anxiety disorders (ADs) (2) quantify the effect of functional MRI (fMRI) task paradigm, as classified by Research Domain Criteria (RDoC), on the pattern of neural engagement observed across disorders.
Methods: Following extensive search of databases available through the National Center for Biotechnology Information up to December 2013, we extracted brain coordinates from 541 task-related fMRI case-control studies in SZ, BD, MDD, OCD and ADs comprising observations from 25,626 participants. We used quantitative meta-analytic and meta-regression techniques to identify the common neural functional architecture implicated in all five disorders and define the contribution RDoC constructs while controlling for other important variables (neuroimaging parameters of the primary studies, age, sex, medication status).
Results: We identified a 'disorder-general' meta-analytic map of shared transdiagnostic functional alterations (regardless of direction) in three clusters encompassing bilaterally the amygdala, hippocampus, thalamus, striatum, insula perigenual anterior cingulate cortex, posterior cingulate cortex, ventromedial prefrontal and the entire lateral prefrontal cortex. Within these regions, patients were more likely to show increased activation in limbic and medial temporal regions and decreased activation in the thalamus and the lateral prefrontal cortex. The effect of RDoC domains was significant for subcortical regions (amygdala, hippocampus, putamen, nucleus accumbens) but not in cortical regions with the exception of the medial prefrontal cortex and frontal operculum.
Conclusions: These results provide evidence in support of a common functional topography across multiple psychiatric disorders. A model assuming disorder-specific pathogenesis would have resulted in minimal or no transdiagnostic overlap in functional architecture. Instead, the disorder-general map identified suggests that some brain regions are relatively more vulnerable and thus likely to be affected by a range of pathogenetic mechanisms.
Disclosures: Nothing to Disclose.

Panel
31. Caffeine Interactions with Dopamine in Adolescence: An Unappreciated Risk for Obesity and Addiction?

31.1 Addiction Vulnerability Characteristics Following Adolescent Caffeine Consumption

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Background: Caffeine is the most commonly used psychoactive substance worldwide, and consumption by children and adolescents has risen dramatically in recent years. Previous studies have found that caffeine intake in adults is positively correlated with substance use disorders, increased illicit drug use and increases in anxiety. We have recently demonstrated that adolescent caffeine consumption enhances acute cocaine sensitivity in adulthood suggesting that adolescent exposure may render individuals vulnerable to the development of addiction. These experiments extend our previous work by exploring the effects of adolescent caffeine consumption on cocaine intake and vulnerability traits that are associated with the development of drug addiction.
Methods: Sprague-Dawley rats consumed 0.3 g/L of caffeine for 14-28 days during the adolescent period (postnatal days 28-55). Age-matched control rats continued to receive water. Caffeine and water consumption were monitored throughout the procedure, and animals consumed approximately 30 mg/kg/day without producing significant changes in body weight gain during this developmental period. Following caffeine consumption, the caffeine solution was replaced with water for the remainder of the experiment. Behavioral testing and tissue collection occurred during adulthood (postnatal days 62-82). To evaluate how caffeine exposure influences the reinforcing properties of cocaine, rats were trained to self-administer cocaine and tested on fixed-ratio and progressive ratio schedules. We also evaluated how adolescent caffeine consumption influences incentive salience to reward-related cues in a Pavlovian learning procedure, which has been shown to correlate with a greater propensity to self-administer drugs. We also assessed how adolescent caffeine consumption alters

impulsivity using a delayed-discounting procedure. Finally, we determined how adolescent caffeine exposure alters dopamine-related protein expression and dopamine levels in the prefrontal cortex using immunoblotting and *in vivo* microdialysis, respectively.
Results: Adolescent caffeine consumption increased the acquisition of cocaine self-administration on a fixed-ratio 1 schedule and increased performance on a progressive ratio schedule of reinforcement. Adolescent caffeine consumption also increased measures of incentive salience for reward-related cues and impulsivity. Consumption of caffeine during adolescence also enhanced cocaine-induced extracellular dopamine in the nucleus accumbens and prefrontal cortex. We also observed altered protein expression of a variety of dopamine-related proteins in the nucleus accumbens and prefrontal cortex that may relate to the behavioral changes.
Conclusions: Together these findings suggest that caffeine consumption during adolescence produces changes in the mesocorticolimbic dopamine system that persist into adulthood. These neurobiological changes are thought to contribute to the behavioral changes resulting from adolescent caffeine consumption that associate with increased addiction vulnerability and enhanced cocaine intake.
Disclosures: Nothing to Disclose.

31.2 Changing Classical Pharmacology by Exploring the Allosteric Mechanisms of Caffeine Within the Adenosine A2A-Dopamine D2 Receptor Heterotetramer

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Background: Heteromerization of the adenosine A2A receptor (A2AR) with dopamine D2 receptor (D2R) takes place in a specific population of striatal neuron, the striatopallidal neuron. Previous studies suggested that the psychostimulant effects of caffeine depend largely on its ability to target the A2AR-D2R heteromer, by blocking an allosteric interaction by which adenosine decreases the affinity and intrinsic efficacy of dopamine. But in a recent human PET study using the D2R antagonist [11C]raclopride, we found that caffeine increases D2R availability in the dorsal and ventral striatum. Since the agonist-agonist interaction within the A2AR-D2R heteromer would predict the opposite effect we revisited all possible allosteric interactions between orthostheric agonists and antagonists within the heteromer with extensive experiments in transfected cells and human and sheep striatal tissue.
Methods: Allosteric effects of caffeine and other selective A2AR antagonists and the A2AR agonist CGS 21680, alone and in combination, were studied with radioligand binding experiments with [3H]raclopride or the D2R agonist [3H]quinpirole in transfected cells and sheep and human striatal tissue. Parallel MAPK signaling experiments were performed in transfected cells and sheep striatum. Bimolecular Sensor Complementation (BiSC) was used to determine the ability of synthetic peptides with amino acid sequences of transmembrane domains of A2AR and D2R (TM peptides) to disrupt A2AR-D2R heteromers in transfected cells. BRET with double BiSC was used to determine the possible tetrameric structure of the A2AR-D2R heteromer. Proximity Ligation Assay (PLA) was used to visualize A2AR-D2R heteromers in sheep striatal tissue. Patch clamp in rat striatal D2R agonist-responsive neurons and locomotor activity recording in rats were used to establish functional correlates of the allosteric effects of A2AR ligands demonstrated *in vitro* and *in situ*.
Results: Not only CGS 21680, but also caffeine and selective A2AR antagonists decreased the affinity and intrinsic efficacy of D2R agonist and the affinity of D2R antagonist. These allosteric modulations disappeared upon A2AR agonist and antagonist co-administration. This could be explained by a model that considers A2AR-D2R heteromers as heterotetramers, with A2AR and D2R homodimers, which was supported by experiments with BRET with double BiSC. TM peptides corresponding to the amino acid sequences of TM5, but not TM7, of both A2AR and D2R, disrupted A2AR-D2R heteromerization in transfected cells (BiSC) and in sheep striatal tissue (PLA). Importantly, the same disruptive peptides disrupted the ability of caffeine to modulate [3H]raclopride binding in sheep striatal tissue. As predicted by the model, high concentrations of A2AR antagonists behaved as A2AR agonists and decreased D2R function in the brain (patch-clamp and locomotor activity experiments).
Conclusions: The heterotetrameric model assumes that occupancy of the A2AR homodimer with either an agonist or an antagonist, but not with both, conduces the same allosteric modulation to the D2R homodimer. The model explains the effects of caffeine on locomotor activity under doses typically consumed and the PET imaging results with [11C]raclopride.
Disclosures: Nothing to Disclose.

31.3 Adenosine Regulation of Dopamine Release and Behavior in Adolescent Rats

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Background: Caffeine is the most widely consumed psychoactive drug in the world. Caffeine consumption by children and adolescents is significant: 75% of children and adolescents consume caffeine daily, in various forms including soda, energy drinks, tea and coffee-based beverages. While caffeine itself exerts modest reinforcing/rewarding effect, like nicotine it enhances the effects of other reinforcers, including the sugar present in the caffeine-containing beverages that young people consume. The purpose of this study was to test the hypothesis that caffeine has exaggerated effects in adolescents, perhaps due to presynaptic effects on DA release that decline with age.
Methods: Male Sprague Dawley rats (postnatal day 28, 42 or 65) from Charles River laboratories (Raleigh NC) were used in all experiments. In the first experiment, all rats received caffeine, 25 mg/kg ip, and locomotor activity was assessed in an automated device. In the next set of experiments, PN 28 or PN 65 animals were habituated to the locomotor box for 60 minutes, then lights were turned off suddenly, and

locomotor activation assessed for 15 minutes. Some animals were pretreated 30 minutes before lights off with saline, quinpirole (0.1 mg/kg ip) or the adenosine A1 agonist N6-cyclopentyladenosine (CPA, 0.3 mg/kg) to test the ability of presynaptic inhibition of DA release to suppress locomotion. Finally, PN 28 or PN 65 animals were anesthetized with urethane, and phasic dopamine release events monitored by fast-scan cyclic voltammetry at carbon fiber electrodes after saline, caffeine (25 mg/kg), caffeine followed by cocaine (15 mg/kg), the D2 antagonist raclopride (2 mg/kg) or raclopride + cocaine. Frequency, amplitude, and duration of phasic release events were quantitated. Statistics on all results were analyzed by 3 way ANOVA (age x time x treatment) using NCSS. All experiments were approved by the Duke University IACUC.
Results: Caffeine elicited an age-related increase in spontaneous locomotion, with greater locomotion observed in adolescent than adult animals. Sudden darkness triggered a surge of locomotion that was suppressed slightly by a presynaptically-active dose of quinpirole and substantially by CPA in adults. Comparison of quinpirole and CPA effects on adolescents and adults showed that CPA was more effective than quinpirole in adults, but the opposite was the case in adolescents: quinpirole was more effective than CPA. In adults, raclopride enhanced spontaneous DA release events and exaggerated the changes after cocaine, while caffeine did not affect DA release. In contrast, caffeine but not raclopride enhanced baseline and cocaine-induced DA release events in adolescents.
Conclusions: These data show that the adenosine antagonist caffeine exerts age-specific effects on dopaminergically-mediated behaviors and on dopamine release in rats. These findings as consistent with the interpretation that adenosine acting via presynaptic heteroreceptors tonically inhibits DA release and suppresses behavior in adolescents more than in adults, while presynaptic D2 autoreceptors assume greater control over DA release as animals become adult. These developmental differences in adenosine regulation of DA function suggest that caffeine might act at multiple sites to enhance the effects of reinforcers like dietary sugar more in children and adolescents than adults.
Disclosures: Nothing to Disclose.

31.4 Motivational Effects of Caffeine in Adult and Adolescent Rats

Matthew Palmatier

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Background: Caffeine is the most widely used psychoactive drug in the world. Caffeine is commonly self-administered in salient vehicles such as coffee and energy drinks and initiation of caffeine use usually occurs during adolescence. Despite the widespread human consumption of caffeinated beverages, no previously published research has demonstrated reliable and repeatable caffeine self-administration in non-human subjects. We have shown previously that caffeine, like nicotine, increases the reinforcing effects of non-drug gustatory stimuli (e.g., sucrose). We hypothesized that robust, reliable, and repeatable increases in operant behavior would be observed in adolescent and adult rats if caffeine were self-administered in conjunction with a reinforcing non-drug stimulus (e.g., saccharin).
Methods: Adult and adolescent rats were used for these experiments. Adult rats began experiments between post-natal days 60 and 90 (P60-90). Adolescent rats began experiments on P32. In Experiment 1, adult and adolescent rats were initially shaped to press a nose-response key for an oral sucrose reward (20% w/v, 0.1 ml/reinforcer) under a progressive ratio (PR) reinforcement schedule. After shaping, all rats were pretreated with caffeine (12 mg/kg) 15 min before testing sessions. In Experiment 2 adult rats were shaped to press a lever for oral saccharin (0.2% w/v) under the PR schedule. Following shaping, all rats were instrumented for intravenous (IV) self-administration and randomly assigned to one of three groups (SACC, CAFF, or CAFF + SACC). IV caffeine infusions and oral saccharin were earned by the CAFF + SACC group in subsequent sessions. There was no change in the operant contingency for the SACC group. IV caffeine infusions (0.5 mg/kg/infusion) replaced saccharin as the reinforcer in the CAFF group. In Experiment 3, adult rats were allowed to respond under the PR schedule for a complex vehicle containing decaffeinated coffee (0.5% w/v) and saccharin (0.2% w/v), oral caffeine (0.5-5 mg/ml; 0.1 ml/reinforcer) was added in a subset of these rats.
Results: In Experiment 1 caffeine significantly increased responding in both adult and adolescent rats (main effect of Drug, $p < 0.05$). Caffeine maximally increased responding in adult rats on the first day of treatment, but responding in adolescent rats increased more gradually (Drug x Age interaction, $p < 0.05$). In Experiment 2, the CAFF + SACC groups responded significantly more than both control groups across 5 days of testing ($p < 0.05$), confirming that caffeine self-administration is reliable and repeatable when it is administered in conjunction with a gustatory reinforcer. In Experiment 3 caffeine dose-dependently increased the motivation to obtain the coffee/saccharin vehicle ($p < 0.05$), with peak concentrations between 1.5 and 2.5 mg/ml.
Conclusions: Caffeine potently enhances the motivation to obtain gustatory non-drug stimuli and this effect may involve different neurobiological processes (e.g., sensitization) when initial exposure to caffeine occurs in adolescence. Inclusion of non-drug stimuli increases the reliability of self-administration in non-human subjects and suggests that the 'reinforcing' effects of caffeine in humans may be difficult to separate from the salient vehicles in which the drug is consumed.
Disclosures: Nothing to Disclose.

Panel
32. Mining a Genomic Hotspot for Psychosis: Mechanistic Insights from 22q11.2 Microdeletions

32.1 From Genes to Cognition in a Mouse Model of the 22q11.2 Microdeletion

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Background: The 22q11.2 microdeletion results in a syndrome of partially-penetrant phenotypes including

psychosis and cognitive deficits. To explore the neurobiological mechanisms underlying cognitive deficits, we study Df(16)A+/- mice, which carry a deletion of the syntenic region in the mouse genome. We have previously shown that these mice have deficits in hippocampal-prefrontal synchrony that correlate with deficits in spatial working memory. Here we set out to determine the cellular and circuit bases of these deficits using a combination of molecular and circuit-based approaches.
Methods: We have used a combination of multi-site awake, behaving neural recordings, single gene knockout mice, optogenetic terminal inhibition, and pharmacological and viral rescue to examine the neurobiological consequences of the microdeletion that may underlie spatial working memory deficits. We recorded both local field potential and multiple single unit activity from the hippocampus (HPC) and prefrontal cortex (PFC) of Df(16)+/- mice, as well as mice hemizygous for Dgcr8 and Zdhhc8, two genes within the microdeletion region in both humans and mice. We used viral overexpression and pharmacological manipulations to attempt to rescue these phenotypes, and optogenetic inhibition of HPC terminals in the PFC to mimic the phenotype.
Results: We demonstrate that hemizygous deletion of Dgcr8 or Zdhhc8, as well optogenetic inhibition of HPC terminals in the PFC, impair HPC-PFC synchrony and disrupt working memory performance. Moreover, pharmacological rescue of a downstream consequence of Zdhhc8 deficiency, and viral genetic rescue of Dgcr8, reverse the physiological and behavioral phenotypes in the single gene models.
Conclusions: These results, coupled with earlier findings demonstrating axonal and synaptic deficits in Df(16)A+/-, Dgcr8 and Zdhhc8 mice, permit us to construct a model by which deficiency of these two genes results in altered anatomical and functional connectivity within the HPC-PFC circuit, resulting in impaired spatial working memory performance. This model incorporates effects of these genes at the level of cellular, circuit and system function that explains the behavioral phenotype seen in mice. Furthermore, we demonstrate successful rescue of the behavioral phenotype by intervening in this pathway, suggesting the possibility that this understanding may lead to novel treatments.
Disclosures: Nothing to Disclose.

32.2 Thalamic MicroRNA Controls Antipsychotic Sensitivity of Thalamocortical Projections in the Auditory Cortex of Mouse Models of 22q11 Deletion Syndrome

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Background: Auditory hallucinations and other positive symptoms of schizophrenia (SCZ) typically appear during adolescence or early adulthood, and in most patients, these symptoms are alleviated by antipsychotics that inhibit dopamine receptors D2 (DRD2s). The mechanisms of SCZ symptom onset and the underlying neuronal circuits, however, remain unknown. A leading risk factor for the development of SCZ is 22q11 deletion syndrome (22q11DS), which is caused by hemizygous deletion of multiple genes in the q arm of chromosome 22. In mouse models of 22q11DS, thalamocortical (TC) projections to the auditory cortex (ACx) have emerged as candidates for mediating positive symptoms because they have disrupted synaptic transmission and aberrant sensitivity to antipsychotics. Deletion of a microRNA (miRNA)-processing gene Dgcr8, leads to increased expression of Drd2 in the auditory thalamus, abnormal sensitivity of TC projections to antipsychotics, reduced TC transmission, and deficits in acoustic-startle response, which is characteristic of patients with SCZ. The miRNA(s) that mediates this mechanism in the auditory thalamus is unknown.
Methods: To identify the culprit miRNA, we performed miRNA microarray analysis in the auditory thalamus and verified those results by using quantitative RT-PCR (qPCR). To test synaptic transmission at TC projections, we used single-cell electrophysiological recordings in TC slices containing portions of the auditory thalamus and ACx. To visualize activity of neurons in the auditory cortex, we used *in vivo* 2-photon calcium imaging. To overexpress miRNAs, we used adeno-associated viruses (AAVs) encoding miRNAs, and to knock them down, we used miRNA sponges. We also generated mutant mice lacking the miRNAs of interest. To test behavioral changes, we measured acoustic-startle response and prepulse inhibition (PPI) of startle response in mice.
Results: We identified 5 miRNAs that target Drd2 in the thalamus and are depleted 22q11DS mice and Dgcr8+/– mice. Of the 5 miRNAs, only miR-338 is enriched in the thalamus. Overexpression of only this miRNA rescued TC disruption and abnormal sensitivity to antipsychotics in 22q11DS mice. Knocking down or deleting miR-338 was sufficient to elevate Drd2 levels in the thalamus and render TC connections sensitive to antipsychotics in wild-type mice. Similar to Dgcr8+/– mice and the mouse models of 22q11DS, miR-338+/– mice were deficient in the acoustic startle response and PPI and have abnormal neuronal activity in the auditory cortex.
Conclusions: These data suggest that depletion of miR-338 is a crucial mediator of the Dgcr8–miRNA–Drd2 pathogenic disruption of TC pathways in the ACx and thus mediates the positive symptoms of 22q11DS-associated SCZ.
Disclosures: Nothing to Disclose.

32.3 Cortico-Thalamic Circuits and Psychosis Risk in 22q11.2 Deletion Carriers

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Background: Copy number variants (CNVs) that are highly penetrant for developmental neuropsychiatric disorders - like the 22q11.2 deletion - offer incredible translational potential, as the same genetic defect observed in human patients can be modeled in animals and in culture. Here we present novel data from a prospective study of a large cohort of youth with 22q11.2 deletions (22q11DS), in which we investigated a candidate neural system implicated in schizophrenia (i.e., functional connectivity within thalamocortical circuits) in relation to gene expression profiles and the development of psychotic symptoms over time.

Methods: We acquired structured clinical interviews, high-resolution T1-weighted structural magnetic resonance imaging (MRI) scans and resting-state functional MRI scans in 65 youth with 22q11.2 deletions and 60 demographically matched typically developing controls. A seed-based approach was used to assess resting thalamo-cortical connectivity. We conducted whole-genome transcriptional profiling and used systems biology methods (Weighted Gene Coexpression Network Analysis; WGCNA) to identify networks of co-expressed genes associated with these neuroanatomic traits at baseline. To test for significance of over-representation of brain-expressed genes within identified modules, we conducted hypergeometric probability tests. Gene ontology (GO) annotation was performed using DAVID (http://david.abcc.ncifcrf.gov/). A subset of the sample was followed longitudinally for one year.
Results: Resting state functional connectivity (FC) analyses revealed that 22q11DS patients showed thalamic hyper-connectivity with auditory cortex, but under-connectivity with striatal and cerebellar regions, consistent with patterns recently observed in idiopathic schizophrenia and in clinical high risk youth who subsequently developed overt psychosis. Findings also parallel those observed in a 22q11DS mouse model. Further, longitudinal data indicated that changes in thalamo-cortical connectivity over time predicted the development of prodromal psychotic symptoms in 22q11DS patients. We identified a particular gene module (Orange module) in which up-regulation of gene expression was associated with increased psychotic symptom severity ($p = .009$), increased thalamo-cortical dysconnectivity ($p = .01$), as well as reduced surface area in the left temporal pole ($p = .04$) in 22q11DS. GO analyses revealed that genes in the Orange module were primarily related to immunological processes. Hypergeometric tests revealed: 1) significant enrichment of brain-expressed genes in this module, and 2) significant overlap with genes implicated in idiopathic psychosis. This module was not associated with age, gender, antipsychotic use, scanner location, or batch.
Conclusions: Disruption of thalamo-cortical circuits is a neural phenotype characteristic of idiopathic psychosis which also predicts symptom development in 22q11DS. Further, dysregulation of peripheral gene expression in 22q11DS is significantly related to thalamo-cortical connectivity, temporal lobe structure, and to psychotic symptomatology. While 22q11DS may account for only a small proportion of risk for psychosis overall, dysregulated genes within this locus may converge on biological pathways and neural systems relevant to broader psychosis susceptibility.
Disclosures: Nothing to Disclose.

32.4 Using Patient-Derived Neurons to Gain Novel Insights into the Neuronal Basis of 22q11 Deletion Syndrome

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Background: Development of disease-modifying drugs for psychiatric disorders such as autism and schizophrenia has been difficult despite sustained efforts over the last few decades. This is partly due to the complex pathophysiology and clinical heterogeneity of these disorders, as well as restricted access to neuronal cells from patients. The advent of induced pluripotent stem cells (iPSCs) provides an opportunity to generate and directly study neurons from patients with psychiatric disease. The highly penetrant 22q11.2 deletion syndrome provides a unique opportunity to mitigate the challenges raised by the high degree of genetic heterogeneity underlying complex neuropsychiatric disorders and to enable the study of cellular neuronal phenotypes in these patients.
Methods: We generated induced pluripotent stem cells (iPSC) from a cohort of subjects carrying a 3 megabase 22q11.2 deletion and from unaffected healthy subjects, and differentiated these cells *in vitro* into cortical neural progenitors cells (NPCs) and cortical excitatory neurons.
Results: Using transcriptional profiling at multiple *in vitro* developmental stages, as well as live imaging and electrophysiological methods, we identified a series of robust cellular phenotypes in patient-derived neurons when compared to neurons derived from healthy controls.
Conclusions: These results provide novel insights into the underlying cellular defects that lead to psychiatric diseases in 22q11DS and open new therapeutic possibilities.
Disclosures: Nothing to Disclose.

Mini Panel
33. Prenatal Maternal Environment, Immune Mechanisms, and Neurodevelopment Relevant to Psychiatric Disorders and Preventive Mechanisms

33.1 Maternal High Fat Diet Alters Long-Term Metabolic, Monoamine, Neuroimmune, and Behavioral Outcomes in Mouse Offspring: A Role for Placental Inflammation?

Staci Bilbo

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Background: Maternal obesity during pregnancy and lactation can "program" offspring long-term for increased obesity themselves, along with increased vulnerability to neuropsychiatric disorders. Emerging evidence suggests that this programming by maternal diet is propagated via inflammatory mechanisms, which impact both placental and fetal brain development.
Methods: To explore the mechanisms by which inflammatory responses within the placenta impact fetal brain development and thus long-term behavioral and biochemical outcomes in offspring, we placed female mice on either a low-fat diet (LFD) or high-fat diet (HFD) for 6 weeks prior to breeding, and throughout gestation and lactation. Placenta, fetal brain, and newborn brain were assessed for monoamine and neuroimmune endpoints, along with adult offspring immune, metabolic, brain, and behavioral outcomes.
Results: PCR array analysis of 84 immune genes in mid-gestation placentas revealed that HFD altered the expression of multiple inflammatory genes in a sex-specific manner, and HPLC revealed disrupted placental serotonin synthesis

in males—a critical function for embryonic brain development. One week after birth, qPCR analysis of offspring brains revealed that HFD altered expression of both serotonergic and immune genes, as in the placenta. Despite placement on a LFD at weaning, adult male and female offspring of HFD dams had decreased insulin sensitivity and visceral fat, respectively, along with changes in leptin receptor expression within the hypothalamus and hippocampus. Adult male and female HFD offspring also exhibited increased microglial activation within the hippocampus, whereas only HFD males showed increased inflammatory IL-1β responses in serum and brain, along with evidence of inflammatory monocyte infiltration into the hypothalamus, assessed using flow cytometry. Finally, male and female HFD offspring exhibited increased anxiety-like and depressive-like behavior in adulthood, in conjunction with increased serotonin turnover in prefrontal cortex. Moreover, the male, but not female, offspring of this group were hyperactive and had altered dopaminergic metabolism, suggesting that the long-term effects of maternal nutrition are also sex-specific.
Conclusions: Perinatal diet can profoundly modulate placental and fetal monoamine and immune responses, as well as program brain development and behavior of offspring in a sex-specific manner.
Disclosures: Nothing to Disclose.

33.2 Interaction Between Serotonin Transporter Genotype and Prenatal Stress on Neurodevelopment with Implications for Autism Spectrum Disorder

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Background: Stress exposure during gestation is implicated in several neuropsychiatric conditions, including autism spectrum disorder (ASD). Previous research showed that prenatal stress increases risk for ASD with peak exposure during the end of the second and the beginning of the third trimester. However, exposures to prenatal stress do not always result in ASD, suggesting that other factors may interact with environmental stressors and increase ASD risk. Also, previous research in a mouse model revealed that a maternal heterozygous knockout of the serotonin transporter gene, known to affect stress reactivity, combined with maternal chronic variable stress late in pregnancy, resulted in altered social behavior in the offspring. This gene is well known to impact stress reactivity in clinical populations. The present study examined a maternal genetic variation in the promoter region of the serotonin transporter gene (5-HTTLPR) affecting stress tolerance and its interaction with the effect of environmental stressors on risk for ASD. Furthermore, as GABAergic changes are frequently observed in ASD, we wished to examine the effects of prenatal stress combined with this genetic variation on GABAergic migration in the rodent model. Finally, stress and the serotonergic system have significant effects on immunity, which will be of particular interest in this setting, as a range of atypical immune markers are observed in ASD patients and in mothers of patients with ASD.
Methods: Two independent cohorts of mothers of ASD children recruited by the University of Missouri and Queen's University were surveyed regarding the prenatal environment and genotyping on 5-HTTLPR was performed to explore this relationship. For the rodent model, a 2x2 design was implemented (Heterozygous serotonin transporter KO vs. wild type dams, exposed to restraint stress vs no stress during late pregnancy), and brains of the offspring were collected at E13.5, E15.5, and E18.5, and immunofluorescent calbindin labeling was performed.
Results: In both clinical samples, mothers of children with ASD carrying the stress susceptible short allele variant of 5-HTTLPR experienced a greater number of stressors and greater stress severity when compared to mothers carrying the long allele variant. The temporal peak of stressors during gestation in these mothers was consistent with previous findings. Additionally, increased exposure to prenatal stress was not reported in the pregnancies of typically developing siblings from the same mothers, regardless of maternal genotype, suggesting against the possibility that the short allele might increase the recall of stress during pregnancy. In the mouse study, at each time point tested, the offspring of the heterozygous, maternally stressed mice had significantly delayed GABAergic migration as compared to all other groups. Ongoing work is examining immune mediators of these effects.
Conclusions: The present study provides further evidence of a specific maternal polymorphism that may affect the risk for ASD with exposure to prenatal stress, and animal model studies suggest that it may occur by affecting GABAergic migration. Current work will explore potential epigenetic and immune factors that may mediate this effect.
Disclosures: Nothing to Disclose.

33.3 Inflammatory Mediators of Prenatal Stress Effects on Neurodevelopment

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Background: Prenatal stress (PS) is a risk factor for altered cognitive and emotional development in children and adolescence. In animal models, prenatal stress persistently changes behavior and the brain. The mechanisms by which stress during embryonic development can induce long-term effects may shed light on preventive strategies for childhood psychiatric disease. Our lab has shown that prenatal restraint stress influences the development of GABAergic cells in offspring, with unclear maternal factors influencing the embryonic brain. Multiple different mediators in maternal stress physiology have been implicated in prenatal stress. Here, we investigated which mechanisms involved in prenatal stress influence the brain development and behavioral outcomes seen in offspring.
Methods: We examined embryonic brain following prenatal restraint stress and repetitive maternal prenatal exposure to corticosterone, interleukin-6 (IL-6), and interleukin-1beta (IL-1beta). We assessed cellular and molecular markers of

GABAergic cell embryonic development. To test involvement of inflammatory mechanisms, Iba1+, embyronic microglia morphology was assessed. Blockade of the pro-inflammatory mediator, IL-6, was tested with repetitive maternal exposure to neutralizing IL-6antibody and examination of offspring brain development and adult anxiety-like behavior.

Results: The distribution of inhibitory neuron progenitors was initially restricted after one day of exposure by prenatal stress, corticosterone and both cytokines; however, this effect persisted after 2 days only in prenatally-stressed and IL-6-exposed offspring. Corticosterone-exposed embryos showed other distinct differences from prenatal stress in the development of GABAergic progenitors: GABAergic progenitor proliferation was reduced early, and expression of transcription factors involved in inhibitory neuron migration (dlx2, nkx2.1) was increased not decreased. Cytokine-exposed embryos showed some similar effects on GABAergic progenitor gene expression as found with prenatal stress.

Inflammatory mediation of the effects of prenatal stress was further tested by evaluation of Iba1+ microglia in the embryonic cortical plate. Prenatal stress exposure resulted in a higher density of Iba1+ cells that appeared to be actively phagocytosing apoptotic cells. This was also found in IL-6 exposed embryonic brain.

Blockade of IL-6 signaling by anti-IL-6 antibody injection during prenatal stress normalized the morphology of Iba1+ cells in embryonic cortical plate. However, migration deficits of GABAergic progenitors with prenatal stress were not normalized. Similarly, behavioral changes in adult animals after prenatal stress were not rescued by anti-IL-6 antibody.

Conclusions: In sum, exposure to glucocorticoids did not replicate the effect of prenatal stress on GABAergic cells, but cytokine exposure showed very similar trajectories in the development of these precursors and of microglia. Blockade of IL-6 in the maternal stress response did not rescue effects of prenatal stress on GABAergic progenitor development or behavior. This work demonstrates that inflammatory systems, including microglia and cytokines, which have an increasingly recognized role in brain development, are among the mechanisms by which prenatal stress may influence childhood functioning.

Disclosures: Part 1: Research Fellowship from APIRE/Wyeth (2010-2015), **Part 4:** Research Fellowship from APIRE/Wyeth (2010-2015).

Mini Panel
34. Harnessing Sex-Differences as Biological Clues in Neurodevelopmental Psychiatry

34.1 The Female Protective Effect in Autism Spectrum Disorder

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Background: The 4:1 male to female sex bias is one of the most consistent and striking observations in autism spectrum disorder (ASD). One explanation for this sex bias is the existence of a female protective effect (FPE), in which a greater burden of ASD risk factors are required for a diagnosis of ASD in females than in males. Direct observation of de novo ASD risk factors in genomic analysis supports this hypothesis, however indirect assessment of sibling ASD recurrence risk in epidemiological studies finds little supporting evidence for the FPE. Should the FPE exist, understanding the nature and mechanism of this protection may hold great potential for therapeutic strategies.

Methods: To explain the discordant genomic and epidemiological evidence for the FPE we developed a simulation of ASD risk in families to estimate the power to detect a difference in the burden of de novo mutations vs. sibling recurrence risk. Should the FPE exist, it must act through sexually dimorphic processes, including gene expression. We therefore compared gene expression data from male and female brain samples in the BrainSpan dataset ranging from mid-fetal to adult developmental stages.

Results: Under a quantitative model of ASD risk, in which 50% of ASD risk in the population comes from unique environmental exposure, 47% comes from common inherited genetic variants and 3% comes from rare de novo mutations (Gaugler et al., Nature Genetics 2014), we estimated the power to detect the FPE. Considering de novo mutations we achieve 80% power at about 500 ASD families, consistent with genomic literature. Furthermore, by combining the exome and CNV data for over 5,500 ASD cases we show that the increased burden of ASD risk factors is observed consistently to a similar extent as predicted by the simulation. In contrast, the power to detect a significant difference in sibling recurrence rate in 10,000 ASD remains below 30%. The FPE hypothesis is therefore consistent with the epidemiologic literature too.

To explore the nature of the FPE, we assessed the differential gene expression using RNA-Seq from over 1,200 region and time specific samples from males and females. We find similar developmental trajectories of gene expression in both sexes, with the exception of genes specific to microglia that appear earlier in males. Overall we find about 200 coding and non-coding transcripts with robust sexually dimorphic gene expression (FDR $\leqslant 0.01$) and a further 1,000 sexually dimorphic genes with weaker evidence (FDR $\leqslant 0.1$). Of note, these transcripts are not enriched for known ASD risk genes, suggesting that the protective effect occurs downstream of specific genetic risk factors.

Conclusions: The FPE is the leading hypothesis of the underlying mechanism of ASD sex bias, with strong supporting evidence from genomic studies. The existing large-scale epidemiological analyses remain under-powered to corroborate this finding. There is little evidence for differing rates of neurodevelopment between the sexes at the level of gene expression, with the possible exception of microglia. Finally, the ASD risk genes discovered to date do not appear to be sexually dimorphic in their expression in the human brain.

Disclosures: Nothing to Disclose.

34.2 Sex Differences in the Infant Brain: Mechanistic Considerations and Relevance to Neurodevelopmental Disorders

Rebecca Knickmeyer

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Background: Many early-onset neurodevelopmental disorders (NDDs) show a striking male-bias, including autism spectrum disorders (ASD), attention-deficit hyperactivity disorder (ADHD), early onset persistent antisocial behavior, and early-onset schizophrenia. These patterns are likely related to sex differences in typical brain development, and ASD in particular has been described by some researchers as an 'extreme male brain'. However, there is currently a major gap in research relevant to this hypothesis: data on sex differences in brain development during infancy and toddlerhood, the period of time most relevant to NDDs, are extremely limited. In addition, the mechanisms underlying sex differences in human brain development remain poorly understood, though gonadal steroid and sex chromosome effects are strong candidates for a causal role.
Methods: My research group addresses this critical research gap by integrating pediatric neuroimaging with genomics and analytical chemistry. We are characterizing sex differences in brain development in an extraordinary cohort of over 800 typically developing neonates. We test whether variation in salivary testosterone, anthropometric proxies of prenatal testosterone, and genes involved in sex steroid synthesis, transport, signaling, and metabolism, predict individual variation in sexually dimorphic outcomes. In addition, we study whether brain development is altered in infants with Turner syndrome (TS), a well-defined genetic disorder resulting from the partial or complete loss of one of the sex chromosomes.
Results: Although global brain volumes are larger in male neonates, females have relatively larger gray matter volumes around the temporal-parietal junction, a key region in the sense of agency, reorienting attention to salient stimuli, and higher level social processes. This may explain why female infants show better social interactive capacities than male infants and are less likely to be diagnosed with ASD. Studies of infants with TS suggest some of these effects are mediated by the sex chromosomes. In contrast, androgen exposure and sensitivity do not appear to be primary determinants of sexual dimorphism at this age. Sex and X-chromosome loss exert minimal effects on diffusion tensor imaging parameters.
Conclusions: Sex effects on brain development show tremendous spatio-temporal complexity. It is likely that sexual dimorphism of the brain reflects the dynamic interplay of multiple mechanisms both biological (prenatal hormone production, neonatal hormone production, pubertal hormone production, direct sex chromosome effects) and experiential (e.g. parental expectations and interactive behavior, exposure to physical hazards, culturally influenced lifestyle differences). Ultimately, a better understanding of the pathways leading to sexually dimorphic brain development and the emergence of psychiatric illness will improve diagnosis and open up possibilities for sex tailored interventions and therapeutics aimed at normalizing adverse developmental trajectories.
Disclosures: Part 1: I am co-investigator on a grant supported by Pfizer, **Part 4:** I am co-investigator on a grant supported by Pfizer. I do not receive any salary support.

34.3 Studying the where and how of Sexually Dimorphic Brain Development Through Cross-Species Neuroimaging and Genomics

Armin Raznahan

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Background: Epidemiological studies clearly establish that male sex robustly increases risk for several common early onset neurodevelopmental disorders (NDDs) including Autism Spectrum Disorder (ASD), and Attention Deficit Hyperactivity Disorder (ADHD). Studying the basis for this sex-bias could potentially advance the prevention, early detection and treatment of multiple disorders. Consequently, our laboratory uses several complementary research approaches to (i) localize brain systems that show sexually dimorphic brain development in health, (ii) test the hypothesis that sex differences in X- and Y-chromosome dosage contribute to sexually-dimorphic brain development and risk for NDD.
Methods: We map normative sex-differences in brain development at high spatio-temporal resolution using a large, longitudinal structural neuroimaging study of human brain development which spans ages 3-35 years, and includes ~1200 MRI scans from 700 individuals. We assess sex-chromosome dosage effects on brain anatomy and NDD-related phenotypes through a translational research program that integrates neuroimaging and transcriptomic methodologies in humans and mice with a range of sex chromosome complements (e.g XX, XY, XXX, XXY, XYY karyotypes).
Results: Global brain volumes are larger in males than females throughout typical development, but this well-replicated observation is underpinned by highly localized neuroanatomical sex-differences that shift their spatial distribution over development. Several such "hotspots" of sexually dimorphic brain development in health also show structural and functional abnormalities in independent clinical samples of youth with sex-biased NDDs.
Our analyses of sex chromosome aneuploidy suggest they male-female differences in sex-liked gene dosage may shape normative sex differences in overall brain size. However, X- and Y-chromosome dosage changes exert convergent effects on local brain anatomy in humans, which preferentially strike regions linked to normative and NDD-related differences in social behavior. We also identify sex-chromosome dosage effects on anatomy of social brain systems in mice, which often encompass foci of normative sexual dimorphism.
To identify candidate genomic mediators of these sex and sex-chromosome dosage effects on brain development we (i) clarify transcriptomic consequences of varying X and Y chromosome dosage in humans, and (ii) specify the gene-

expression signatures that distinguish murine brain regions with contrasting anatomical sensitivity to gonadal profile and sex chromosome dosage.
Conclusions: By specifying spatiotemporal patterns of brain development that differ between males and females in health, our findings prioritize candidate brain systems that might mediate sex-differences in the incidence and/or resilience to NDD risk factors. Our complementary imaging and genomic studies of sex chromosome dosage effects help to (i) mechanistically dissect biological contributions to sexually dimorphic brain development and NDD risk, and (ii) identify molecular pathways that might mediate these contributions.
Disclosures: Nothing to Disclose.

Study Group

35. The Sunshine Act: Implications for Neuropsychiatric Researchers and Neuropsychiatric Research - An ACNP Liaison Committee-Sponsored Study Group

Daniel Javitt*, Paul Appelbaum, William Carpenter, Daniel Carlat, Charles Beasley, Maria Oquendo, Laura Roberts

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The Physician Payments Sunshine Act requires manufacturers of drugs, medical devices and biologicals that participate in U.S. federal health care programs to report certain payments and items of value given to physicians and teaching hospitals. As part of this program, manufacturers now are required to submit annual data on payment and other transfers of value that they make to covered recipients. Information disclosed by manufacturers is then maintained on a public website. Physicians may review and dispute data prior to public release, and need to monitor published data for accuracy.
The Sunshine Act was implemented in response to concerns regarding potential undisclosed influence of manufacturers on medical practice and research. This study group has two aims. First, it will provide ACNP members and other meeting participants with information regarding specific provisions of the Sunshine Act and their implications for academic physicians who collaborate across the academia/pharma divide. Among the issues to be considered are the utility of the information disclosed under the Sunshine Act for regulators, patients, and others, and the Act's impact on academia/pharma interactions. In addition, the session will facilitate more general discussion of optimal patterns of academia/pharma interactions. The discussion will occur at a general and policy level only, with no discussion of individual cases or circumstances.
Disclosures: Part 1: Omeros; SKBP; Otsuka; Sunovion; Lundbeck; Forum/Envivo; Takeda; Glytech, Inc., **Part 2:** Glytech, Inc., **Part 4:** Roche.

Panel

36. Sex Hormones, the Medial Prefrontal Cortex and Their Role on Eating Disorder Behavior in Basic Science and Human Brain Imaging Studies

36.1 An Individual Differences Animal Model of Binge Eating Reveals Sex Differences in Binge Eating-proneness and Enhanced Activation of the Neural Reward Circuit by Palatable Food in Binge Eating-Prone Rats

Cheryl Sisk

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Background: Eating disorders emerge during puberty and disproportionately affect girls. Most theories of sex differences in eating disorders focus on sociocultural factors, however recent evidence indicates that biological factors contribute to sex differences as well. The most prevalent forms of eating disorders are characterized by binge eating. The neurobiological underpinnings of binge eating in either sex are poorly understood, but recent research points to dysregulation of food reward circuits as one component of binge eating. The purpose of this research is to examine between- and within-sex differences in binge eating using an established laboratory animal model of binge eating proneness.
Methods: Male and female rats were given intermittent access (24 hr period, 3x/wk) to palatable food (PF; Betty Crocker Frosting) for 2-3 wk, with ad lib access to standard lab chow and water. PF consumption was measured 4 and 24 hr after introduction of PF on feeding test days; chow consumption and body weights were measured daily. Binge eating prone (BEP) and binge eating resistant (BER) rats were identified on the basis of the pattern of PF consumption across the feeding tests. Rats met criteria for BEP if they ate in the highest tertile of PF intake on at least half of the feeding tests, and never ate in the lowest tertile on any feeding test. Rats met criteria for BER if they ate in the lowest tertile of PF intake on at least half of the feeding tests, and never ate in the highest tertile on any feeding test. Study 1: a sample of 30 female and 30 male adult rats was studied to determine whether there are sex differences in the proportion of rats that meet criteria for BEP or BER. Study 2: BEP and BER rats were identified in a sample of 40 adult female rats. One week after completion of the feeding tests, rats were given a PF 90 min prior to being anesthetized and perfused. Brains were removed and processed for immunohistochemical identification of fos, a nuclear transcription factor used as an index of neuronal activation. The number of fos expressing cells was microscopically quantified in subregions of the prefrontal cortex (PFC) and nucleus accumbens (NA), two primary components of the mesocorticolimbic reward circuit.
Results: Study 1: Overall, female rats consumed higher quantities of PF than male rats. Within the sample of female rats, 47% met criteria for BEP and 3% met criteria for BER. Within the sample of male rats, 3% met criteria for BEP and 37% met criteria for BER. Two-proportion z test revealed a significant sex difference in binge eating proneness. Study 2: BEP rats consumed more PF than BER rats on feeding test days, whereas 24 hr chow intake on feeding test days was higher in BER rats than in BEP rats. BEP and BER rats did not differ in body weight. BEP rats had higher PF-induced fos expression than BER rats within the NA core (pairwise comparison $p < 0.1$; Hedge's $g = 1.02$, large effect size), NA

shell ($p < 0.1$; $g = 0.82$, large effect size), prelimbic PFC ($p < 0.01$; $g = 2.59$, large effect size), infralimbic PFC ($p < 0.05$; $g = 1.55$, large effect size), and cingulate PFC ($p < 0.01$; $g = 1.64$, large effect size).
Conclusions: Using an individual differences animal model to identify extreme binge eating phenotypes, a greater proportion of female rats are BEP compared to males, providing evidence for a sex difference in binge eating that is biologically, rather than socioculturally based. Furthermore, the BEP phenotype is associated with enhanced responsiveness to PF within the neural reward circuit, suggesting that heightened food reward may underlie binge eating proneness.
Disclosures: Nothing to Disclose.

36.2 Disruption of ESRRA-HDAC4 Activity in Prefrontal Cortex Induces Eating Disorder-Related Behaviors in Mice

Michael Lutter

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Background: Eating disorders (EDs), such as anorexia nervosa and bulimia nervosa, are characterized by marked disturbances in eating patterns, social interactions, and behavioral compulsivity. While the neurobiological basis of EDs is incompletely understood, recent research has highlighted a potential role for frontostriatal circuits in the development of ED-related behaviors. We used a family-based approach to identify rare missense mutations in the estrogen-related receptor alpha (ESRRA) and histone deacetylase 4 (HDAC4) genes that are associated with the development of eating disorders. Here we use mice as a model system to dissect the functional role of ESRRA and HDAC4 activity in the medial prefrontal cortex (mPFC) in ED-related behavioral deficits.
Methods: Knock-in mice were generated with the A778T mutation in the Hdac4 gene (corresponding to the human A786T mutation). Floxed-Esrra mice were obtained and used to conditionally deleted Esrra expression from mPFC neurons. Mice expressing Cre-recombinase under control of the Esrra promoter were generated to allow for optogenetic studies.
Results: Loss of Esrra in the mPFC disrupts food intake in female mice. HDAC4A778T knock-in mice display hyperphagia in response to high-fat diet in female, but not male mice. Furthermore, AAV-mediated overexpression of HDAC4A778T, but not wild-type HDAC4 or control GFP, in mPFC recapitulates high-fat diet hyperphagia. Stimulation of mPFC-Esrra neurons by channel rhodopsin 2 induces consumption of high-fat diet in female, but not male mice.
Conclusions: Our results demonstrate that the mPFC-Esrra neurons modulate intake of high-fat diet. Furthermore, genetic manipulation of the Esrra-Hdac4 pathway, which was originally identified in human patients with EDs, in the prefrontal cortex of mice causes a bi-directional modulation in the consumption of high-fat diet (both hypophagia and hyperphagia) and that these effects are primarily observed in female mice. These findings offer new insights in the cellular and molecular basis of ED-related behaviors.
Disclosures: Nothing to Disclose.

36.3 Medial Prefrontal Cortex is Integral to Altered Social and Self Perception in Anorexia Nervosa

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Background: Anorexia nervosa (AN) is a complex psychiatric illness that includes a failure to maintain one's body weight in concert with cognitive distortions related to body size and shape. Both psychological and neural differences in social and self-perception have previously been demonstrated in AN. Here, we evaluated the function of medial prefrontal cortex (MPFC) in both currently ill as well as patients with long-term weight recovery following AN, to assess its relevance in the pathogenesis of AN.
Methods: Three groups of adult subjects, healthy comparison women (HC, $n = 19$), women currently with anorexia nervosa (AN-C, $n = 22$), and women in long-term weight recovery following anorexia nervosa (AN-WR, $n = 18$, 2 years with BMI > 19.0) performed a social self-appraisal task during 3t magnetic resonance imaging (MRI). The task involved considering social adjectives presented in three different conditions: Self ("I believe I am nice"), Friend ("I believe my friend is selfish"), and Reflected ("My friend believes I am polite"). Neural activations were analyzed using ANOVAs comparing whole-brain activations in contrasts of the three conditions with the statistical criterion set to thresholding at an initial voxel p unc $<$ 0.005 and cluster p FWE corr < 0.05.
Results: Differences in activation of the MPFC (232 vox, $Z = 4.34$, MNI -8, 48, 0) were found in the Self – Friend contrast, a comparison designed to isolate self-relevant neural processing. Both patient groups had elevated mPFC activity in contrast to the healthy subjects (mPFC beta values, HC -1.3, AN-C -0.3, AN-WR 1.23; $p < 0.001$). Furthermore, in the Reflected – Self contrast, a comparison designed to evaluate social cognitive neural processes, differences in activation of the bilateral inferior frontal gyri and anterior insula (RIFG, 79 voxels, $Z = 4.29$, MNI $x = 36$, $y = 32$, $z = 8$; LIFG, 51 vox, $Z = 3.54$, MNI -44, -16, 8), caudate (41 vox, $Z = 4.12$, MNI $x = -16$, $y = 12$, $z = 12$), and cingulate (78 voxels, $Z = 4.07$, MNI $x = 4$, $y = 32$, $z = 32$) were observed. Severity of the eating disorder (Eating Attitudes Test) amongst the patient groups correlated with less self-related activation of the MPFC ($r = 0.64$, $p < 0.003$) and R Insula (MNI $x = 36$, $y = 28$, $z = 16$, $r = 0.64$, $p < 0.0001$).
Conclusions: All the regional differences identified were in frontostriatal circuits typically engaged during reward tasks, suggesting that biological differences related to motivation may underlie altered social and self-perception in AN. Importantly, the AN-WR group showed the largest differences in MPFC compared to the HC group, suggesting that differences in social and self-perception do not resolve even after long-term weight-recovery. In sum, AN may be associated with a biological predisposition to engage the MPFC for self-evaluation, connecting environmental pressures related to appearance with psychological symptoms related to impaired self-knowledge and social perceptions.
Disclosures: Nothing to Disclose.

36.4 Brain Structure and Function Implicate the Medial Prefrontal Cortex in Anorexia Nervosa Pathophysiology

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Background: Anorexia nervosa (AN) is a severe psychiatric disorder with high mortality that typically starts in adolescence and is characterized by a complex interaction of biological, psychological and social factors. The predominantly female gender of affected individuals suggests that gonadal hormones play an important role. Here we present data from two studies that suggest that the medial prefrontal cortex may have an important role in AN and maybe in eating disorders in general.
Methods: In study 1, we compared adolescents with AN ($n = 19$, mean age 16 ± 2 years) with age matched healthy controls ($n = 16$, age M 15 ± 3 years) on brain response during a dopamine associated reward-conditioning task that tests brain response to unexpected receipt or omission of monetary reward. Adolescents with AN performed the task twice, before weight gain and 5 weeks later after weight normalization. Controls performed the task also twice, 5 weeks apart. In study 2, we compared brain white matter connectivity (estimation of fiber connections) in healthy young adults ($n = 26$, age M 23 ± 5 years) with individuals with AN ($n = 26$, age M 24 ± 3 years) or bulimia nervosa (BN, $n = 26$, age M 25 ± 4 years). Diffusion weighed imaging was acquired for tractography of white matter connection between taste-reward circuitry relevant brain regions. In both studies AN subjects were at low gonadal hormone state as evidenced by lack of menstrual cycle. Comorbid conditions and medication use were controlled for in the analyses.
Results: Study 1: Adolescents with AN showed greater brain response to unexpected win-omission compared to controls in bilateral ventral striatum and medial prefrontal cortex ($p < 0.05$, FEW corrected). On follow up after weight restoration, right ventral striatal activation had normalized, while medial prefrontal cortex activation had not. Study 2: White matter tractography indicated reduced fiber path connectivity between the ventro-medial prefrontal cortex and the hypothalamus in both AN and BN compared to controls ($p < 0.01$, Bonferroni corrected).
Conclusions: The results of those studies indicate that the medial prefrontal cortex may have an important role in the pathophysiology of anorexia nervosa and maybe in eating disorders in general. Study 1 suggests that the heightened functional response in adolescent anorexia nervosa recovers less with weight restoration in the medial prefrontal cortex compared to the ventral striatum and could be a risk factor for early relapse. Study 2 indicates that lower white matter connectivity between medial prefrontal cortex and hypothalamus could be a common finding across eating disorders and interfere with normal feeding stimulation. The medial prefrontal cortex, which receives sensory input and is strongly connected to limbic areas, is important in decision-making, fear processing and social cue processing. Disruptions in this area due to trait alterations or hormonal effects such as low estrogen during starvation could lead to disturbances in eating regulation, social interaction and emotion regulation. Importantly, the medial prefrontal cortex contributes to reward valuation and the connection to the hypothalamus may be particularly important for taste-cue dependent feeding stimulation (Holland & Petrovich, 2005). Weak fiber connectivity in this pathway in eating disorder could interfere with normal taste conditioning and impair normal food intake.
Disclosures: Nothing to Disclose.

Panel
37. A Fresh Perspective on Neuregulin in Schizophrenia

37.1 Schizophrenia-Relevant, Endophenotypes in Transgenic Mouse Models of NRG1/ErbB4 Hyperstimulation

Markus Schwab

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Background: The neuregulin 1 (NRG1) gene encodes a family of growth and differentiation factors with an epidermal growth factor (EGF)-like signaling domain that serve as ligands for receptor tyrosine kinases of the ErbB family. ErbB4 is the most prominent neuronal NRG1 receptor in the brain. NRG1/ErbB4 signaling regulates multiple aspects of nervous system development and synaptic plasticity in the mature brain. Variants of the human NRG1 and ErbB4 genes are possible risk factors for schizophrenia. Increased NRG1 expression and ErbB4 hyperphosphorylation were found in postmortem brains of schizophrenia patients, suggesting a working hypothesis according to which chronic NRG1/ErbB4 hyperstimulation represents a pathomechanisms in schizophrenia.
Methods: To test our working hypothesis, we examined transgenic mice with pan-neuronal' NRG1 type III overexpression. In addition, to study NRG1/ErbB4 hyperstimulation in a more specific *in vivo* model, we have generated a ‚conditional' transgenic mouse line, which permits Cre recombinase-mediated NRG1 type III overexpression in the brain. These transgenic mice are currently being studied using biochemical, histological, and behavioral approaches.
Results: To address our working hypothesis, we first validated that chronic NRG1 type III overexpression in the brain causes permanent ErbB4 hyperphosphorylation. NRG1/ErbB4 hyperstimulation in ‚pan-neuronal' transgenic mice was associated with synaptic dysfunctions, altered dendritic spine growth, ventricular enlargement, and deficits in sensorimotor gating. Cortex-restricted NRG1 type III overexpression was not associated with ventricular enlargement and sensorimotor gating deficits, but caused hyperactivity. Overexpressed NRG1 type III was present in synaptosomal fractions and pilot studies suggest a recruitment of the LIM kinase1/cofilin signaling pathway by hyperstimulated NRG1/ErbB4 signaling.
Conclusions: NRG1 type III transgenic mouse lines model chronic ErbB4 hyperstimulation in the brain. The spectrum of ‚endophenotypes' in ‚pan-neuronal' transgenic mice suggests that human NRG1 risk haplotypes exert a gain-

of-function effect. Findings in ‚cortex-restricted' transgenic mice indicate brain area-specific NRG1 functions, including a role of NRG1 type III signaling in subcortical networks. Recruitment of LIM kinase1/cofilin by hyperstimulated NRG1/ErbB4 signaling provides a potential mechanism for altered dendritic spine growth. These studies could provide novel targets for future treatment strategies of schizophrenia.
Disclosures: Nothing to Disclose.

37.2 Evaluating the Validity of a Novel Transgenic Mouse Model for Neuregulin 1 Type III for Schizophrenia

Tim Karl

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Background: Neuregulin 1 (NRG1) is a well-characterized risk gene for schizophrenia (SZ). Elevations in NRG1 protein and transcripts have been found in SZ with a recent study showing that the transcript for the NRG1 type III isoform (NRG1-III) is overexpressed in the forebrain of SZ patients that carry a risk haplotype for NRG1. In light of this, a mouse overexpressing Nrg1-III specifically in the forebrain was created in order to assess how Nrg1-III overexpression might cause or contribute to SZ-related deficits thereby considering construct and face validity of this novel mouse model.
Methods: Adult Nrg1-III transgenic and wild type-like control littermates of both sexes were characterized comprehensively for behaviours relevant to SZ including social and cognitive domains. Once behavioural testing was completed, brains were collected to analyse mRNA expression levels of Nrg1 type III as well as for three housekeeper genes in the prefrontal cortex (PFC) using qPCR.
Results: Nrg1-III transgenic mice were healthy and showed normal sensory abilities and neurological reflexes. Nrg1-III overexpression resulted in impaired learning of a fear-eliciting context and reduced social interaction times with a novel mouse. Furthermore, transgenic mice were characterised by deficient prepulse inhibition, one of the hallmarks of SZ mouse models. Importantly, these mice also displayed an increase in normalized Nrg1-III mRNA expression in the PFC.
Conclusions: These findings confirm that the Nrg1-III transgenic mouse has a robust overexpression of Nrg1-III mRNA in forebrain (similar to that found in the disease state of a subset of SZ patients) and that this overexpression causes behavioural deficits, which are highly relevant to SZ. These data will be discussed in relation to recently published findings on Nrg1-III knockout mice and compared to the phenotype of one of the best characterised genetic mouse models for Nrg1, the transmembrane domain Nrg1 mutant mouse. In conclusion, our results provide evidence that an overexpression of Nrg1-III may contribute to the symptomatology of SZ and that a newly developed mouse model for Nrg1-III overexpression possesses both face as well as construct validity for SZ research.
Disclosures: Nothing to Disclose.

37.3 Structural Brain Morphometry and NRG1 Gene Variants in First-episode Nonaffective Psychosis: Cross-sectional and Longitudinal Analyses

Benedicto Crespo-Facorro

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Background: Structural brain abnormalities are already present at early phases of psychosis and might be the consequence of neurodevelopmental deviance. Nonetheless, brain anomalies progression is still under debate and no clear profile of progression can be identified. The study of certain genetic susceptibility factors with neurodevelopmental implications, such as neuregulin 1 (NRG1), can be key tools to understand brain morphology anomalies in schizophrenia. We examined in first-episode schizophrenia subjects whether variations in NRG1 polymorphisms influence brain volumes at illness onset or volume changes during a 3-year follow-up.
Methods: Ninety-five minimally medicated patients experiencing their first episode of schizophrenia underwent genotyping of three SNPs within the NRG1 gene and structural brain magnetic resonance imaging. A comparison of volumes of lobar GM, lateral ventricles, and cortical CSF was made between the groups according to their genotype after controlling for total intracranial volume. In addition, 3-year follow-up magnetic resonance imaging (MRI) study on 59 minimally medicated patients who were experiencing FEP and 14 healthy control individuals underwent genotyping and structural brain MRI at baseline and at 1- and 3-year follow-up. A comparison of brain volumes, GM, WM, LV, cortical cerebrospinal fluid, and thalamus and caudate was made between the groups according to their genotype. Three NRG1 polymorphisms have been studied: SNP8NRG243177, SNP8NRG221533 and SNP8NRG221132. The possible interactive effects of NRG1 and DISC1 on brain volumes have also been investigated.
Results: The cross-sectional study reveals that the SNP8NRG243177 risk T allele was significantly associated, in an allele copy number-dependent fashion, with increased lateral ventricle volume. Genotype explained 7% of the variance of lateral ventricle volume at illness onset. Strikingly, those patients with the "at risk" allelic combinations in NRG1 and DISC1 had LV volumes which were 48% greater than those with none of the allelic combinations. In the longitudinal study, patients with the SNP8NRG6221533 risk C allele showed increased LV volume across time. C allele carriers had significantly less WM compared with subjects homozygous for the T allele after the follow-up. No significant changes according to the genotypes were found in healthy individuals.
Conclusions: Genetic variations of the NRG1 gene can contribute to the enlargement of the lateral ventricles described in early phases of schizophrenia and also can contribute to brain abnormalities described in early phases of schizophrenia and progressive changes during the initial years of the illness. Additive effect of NRG1 and DISC1 genes on lateral ventricle enlargement has been observed.
Disclosures: Nothing to Disclose.

37.4 Neurobiological Consequences of Neuregulin-1 Loci Associated with Psychosis Onset

Chad Bousman

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Background: Recent evidence suggests the schizophrenia associated Icelandic haplotype (HapICE) region may harbor single nucleotide polymorphisms (SNPs) that could assist in differentiating high-risk individuals who will or will not transition to psychosis. Two such SNPs (rs4281084 and rs12155594) were recently shown to independently predict psychosis transition in a large ultra-high risk cohort in Australia and for every additional rs4281084-A allele and/or rs12155594-T allele (allelic load range 0 – 4) the relative risk of psychosis onset increased 1.56 (95% CI = 1.20 – 2.04) (Bousman et al., Translational Psychiatry, 2013, 3, e251). However, the neurobiological consequences and mechanism by which these two newly associated polymorphisms might confer risk for transition to psychosis is not clear. The aim of this study was to utilize bioinformatic tools as well as human post-mortem brain and neuroimaging data from individuals with and without a psychotic disorder to elucidate what effect, if any, these SNPs have on NRG1 function, regulation, gene expression, and brain structure.
Methods: To examine the neurobiological consequences of the candidate SNPs and their combined allelic load a three-pronged approach was employed. First, each SNP was analyzed in silico to predict effects on transcription and splicing factor expression and publicly available GWAS data was used to identify epistatic effects with SNPs within genes that interact with NRG1. Second, three independent human postmortem brain cohorts from Sydney, Australia (37 cases, 37 controls), Melbourne, Australia (50 cases, 18 controls), and the UK Brain Expression Consortium (134 controls) were used to determine the presence of putative cis-regulatory effects on NRG1 gene expression. Third, structural magnetic resonance imaging and diffusion tensor imaging data from 333 (156 controls, 177 cases) individuals were used to identify associations with human brain structure.
Results: In silico analysis predicted variation at the rs4281084 locus would be associated with both transcription factor (glucocorticoid receptor) and splicing factor (SC35) expression. Epistasis analyses showed both rs4281084 and rs12155594 had multiple nominally significant interactions with SNPs in ERBB4, NRG1's main receptor. In healthy human postmortem brain, an increase in the number of rs4281084-A allele and rs12155594-T allele was associated with a decrease in pan-NRGI expression, particularly in the occipital cortex. Whereas, an increase in the combined allelic load of rs4281084 and rs12155594 was associated with an increase in right and left lateral ventricle volume as well as lower fractional anisotropy in a middle frontal cluster.
Conclusions: These results represent the first attempt to functionally characterize the impact of two recently identified NRG1 loci associated with psychosis onset. They build on a growing body of research supporting the functional importance of genetic variation within the HapICE region of the NRG1 gene. In concordance with previous studies our findings suggest that the functional effects conveyed by sequence variation within NRG1 are likely not driven by one SNP but rather, a diverse accumulation of nucleotide changes particularly in up-stream regions capable of regulating gene expression.
Disclosures: Nothing to Disclose.

Panel
38. Real-Life Proxies of Social Context in Affective Problems Across the Lifespan: Evidence from Human and Rodent Studies

38.1 Reward and Social Circuitry: A Link Between Adolescents' Depression and Real-Life Social Experiences?

Erika Forbes

University of Pittsburgh, Pittsburgh, Pennsylvania, United States

Background: Peer social reward becomes increasingly salient during adolescence, when reward circuitry continues to develop and reward-driven behavior increases. This vulnerable developmental point is also the time of emergence of depression, a form of psychopathology postulated to involve disrupted social functioning, reward response, and positive affect. Personally relevant reward stimuli might be particularly effective at eliciting individual differences in reward circuitry that are relevant both to depression and to affective experiences in social contexts. We investigated whether a personally relevant neuroimaging task–watching videos of a best friend from a recent conversation about a shared experience–would reveal mechanisms of adolescents' depressive symptoms and their real-world social experiences. Furthermore, because depression involves altered circadian mood patterns, with particularly low positive affect early in the day, we tested whether regions sensitive to peer social reward and depression would also predict differences in mood and social behavior at different times of day.
Methods: Community adolescents (N = 36, age 14-18, 11 male, 73% European American) varying in depressive symptom severity completed functional magnetic resonance imaging (fMRI) in Siemens Tim TRIO 3T scanner using an ecologically valid social reward paradigm. The paradigm involved viewing video clips of the participant's same-sex best friend or an unfamiliar same-sex adolescent (stranger) expressing positive and neutral affect, with best-friend video clips selected from a lab interaction between the participant and best friend. At 20 time points across two 5-day periods, participants completed experience sampling of mood and behavior during daily life. Preprocessing of fMRI data and analyses were conducted in SPM8.
Results: Participants with higher depressive symptoms exhibited less response to best-friend positive affect vs. stranger positive affect in a set of regions implicated in reward and social processing. Regions negatively associated with depressive symptoms included the precuneus, temporoparietal junction (TPJ), and ventromedial prefrontal cortex (PFC). Conjunction analyses indicated that a region of the vmPFC that responded to peer social reward was related to both higher depressive symptoms and lower emotional

closeness to companions in real life. Additionally, conjunction analyses with morning vs. evening real-life data indicated circadian modulation of depression-brain-behavior associations in 2 regions: the dorsomedial PFC, which was related to lower levels of morning positive affect, and the TPJ, which was related to greater time with peers in the evening.
Conclusions: Common associations of neural response to reward with depressive symptoms and social experience suggest a mechanism for disrupted social functioning in depression. Findings with time of day indicate possible circadian modulation of this mechanism.
Disclosures: Nothing to Disclose.

38.2 Long-Term Neurobiological Consequences of Physical Versus Emotional Stress During Adolescence in Male Mice

Carlos Bolanos-Guzman

Florida State University, Tallahassee, Florida, United States

Background: Individuals with a history of childhood maltreatment commonly suffer from a variety of adult-onset psychiatric disorders, including major depressive disorder and post-traumatic stress disorder. While much has been learned from models of early-life stress, current paradigms emphasize physical stressors, while models of emotional stress focus on parental neglect and social isolation. Thus, it is critical to develop animal models that allow for independent assessment of the neurobiological consequences of emotional stress (ES). Here we introduce a novel social stressor that is insulated from the effects of physical stress.
Methods: In this study, adolescent male C57/BL6J mice were forced to witness the social defeat of another mouse. The home cage of a male CD-1 retired breeder mouse was divided by a Plexiglas divider into two adjacent compartments, and an adolescent male (postnatal day 35) mouse was then introduced into the compartment territorialized by the CD-1 where it was repeatedly attacked (PS) and demonstrated escape-like behaviors, vocalizations, and submissive posturing, while a second mouse witnessed (ES) this interaction from the adjacent compartment.
Results: Acute and chronic ES and PS exposure increased serum corticosterone, and these mice also lost more weight than controls. ES and PS exposure increased anxiety- and depression-like behaviors: ES and PS exposure induced social avoidance, while decreasing exploratory behavior in the elevated plus-maze and open field, along with increased novelty-induced hypophagia and depression-like behavior in the forced swim test. Molecular analyses yielded reduced expression of the scaffolding protein Shank3 within the ventral tegmental area (VTA), a brain region implicated in responses to stress. Overexpressing shank3 within the VTA in adulthood was sufficient to restore control levels of social interaction in both ES- and PS-exposed mice.
Conclusions: Together, these data indicate that witnessing traumatic stress early in life stress is a potent stressor capable of inducing life-long biological and behavioral dysregulation, and changes in shank3 expression within the VTA mediate these effects.
Disclosures: Nothing to Disclose.

38.3 Endogenous Opioid Release and BOLD Activation During Romantic Rejection and Acceptance: Implications for Impaired Social Functioning in Major Depressive Disorder

David Hsu

Stony Brook University, Stony Brook, New York, United States

Background: Seeking out romantic relationships is a significant part of adolescent and adult life. However this process often involves rejection by desired partners, which can strongly elicit negative moods. In animal models, the endogenous opioid peptides acting at μ-opioid receptors (MORs) have been shown to be critical for both behavioral recovery during social distress and promoting social motivation during social reward. In humans it is not known if the MOR system plays a similar role. In this talk I will present studies using both positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) to support the hypothesis that abnormal MOR functioning and neural activity contribute to poor social functioning in major depressive disorder (MDD).
Methods: PET STUDIES: Subjects were 18 HCs (13 women; 32 ± 12 years) and 17 medication-free adult patients with current MDD (13 women; 30 ± 10 years). Prior to PET scanning, subjects rated online profiles of preferred-sex individuals with whom they would most like to form a close relationship. A few days later they were given feedback that they were not liked (rejection) or liked (acceptance) by their highest-rated profiles during PET with intravenous administration of the selective MOR radiotracer [11C]carfentanil. MOR activation (i.e., increased endogenous opioid release) was measured as acute reductions in receptor availability during rejection or acceptance compared to baseline blocks, which did not contain feedback. FMRI STUDIES: Subjects were 19 female HCs (31 ± 12 years) and 18 medication-free female patients with current MDD (30 ± 11 years). An identical profile-rating procedure was used as described above. During fMRI scanning, subjects experienced blocks of rejection, acceptance, and neutral trials.
Results: PET STUDIES: Both HCs and MDDs showed sustained negative affect in response to rejection, although only HCs showed broad MOR activation, which was found in the nucleus accumbens, amygdala, midline thalamus, and periaqueductal gray. In contrast, MDDs showed MOR deactivation in the amygdala and persistent negative affect after rejection trials had ended. During acceptance, HCs but not MDDs showed a positive correlation with MOR activation in the nucleus accumbens (a structure involved in reward and motivation) and an increased desire for social interaction. FMRI STUDIES: Preliminary analyses show that during rejection, MDDs have greater BOLD activation in the right anterior insula compared to HCs, indicating that rejection may be more salient and/or unpleasant in MDDs.
Conclusions: In HCs, rejection and acceptance activated the endogenous opioid system, potentially dampening negative emotions and increasing social motivation, respectively. In

MDD, altered opioid and neural function may be a mechanism for slower/incomplete recovery from rejection and poorly sustained engagement during positive social interactions. Together, these alterations may contribute to difficulties in seeking and maintaining relationships.
Disclosures: Nothing to Disclose.

38.4 How does the Brain Understand the Death of a Loved One? Neural Correlates of Complicated Grief in Older Adults

Mary-Frances O'Connor

The University of Arizona, Tucson, Arizona, United States

Background: Complicated Grief (CG) is marked by a persistent and intrusive grief lasting beyond the expected period of adaptation. Hypothesized neural mechanisms distinguishing CG from Noncomplicated Grief (NCG) include deficits in updating reward-related processes related to attachment behavior, and emotion regulation deficits.
Methods: In Study 1, 23 bereaved women (11 CG, 12 NCG) participated in an event-related functional magnetic resonance imaging (fMRI) scan, during grief elicitation with photos of their deceased loved one and a stranger. In Study 2, 28 older adults (CG = 8, NCG = 9 and Nonbereaved, married controls = 11) completed the emotional-counting Stroop task with self-relevant grief and neutral words.
Results: Analyses of Study 1 revealed that whereas both CG and NCG participants showed pain-related neural activity in response to photos of the deceased (compared to a stranger), only those with CG showed reward-related activity in the nucleus accumbens (NA). This NA cluster was positively correlated with self-reported yearning, but not with time since death, participant age, or positive/negative affect. For Study 2, behavioral Stroop data showed that the CG group had slower reaction times to grief-related words compared to NCG and Nonbereaved groups. FMRI studies investigating the neural networks associated with Stroop performance consistently implicate the rostral anterior cingulate cortex (rACC). Those with CG showed an absence of rACC recruitment. Activity in the medial prefrontal cortex was significantly elevated in the NCG group compared to Nonbereaved controls, consistent with this as an emotion regulation region.
Conclusions: For those with CG, reminders of the deceased may still activate neural reward activity, which may interfere with adapting to the reality of the loss. In addition, those with CG show a relative inability to recruit the regions necessary for successful grief-related emotion regulation when processing reminders of their loss. In addition to learning about distinct regions of activation during expected grief, neuroimaging of CG provides insights into the neuroanatomical correlates of behaviors seen in this disorder.
Disclosures: Nothing to Disclose.

Panel
39. Advances from Three Hallmark Genetic Consortia on Endophenotypes in Schizophrenia to Four Collaborations Operating at the Exciting Frontiers of Genomic Science

39.1 De Novo Mutations in Schizophrenia Map to Prefrontal Cortical Network

Jon McClellan

University of Washington, Seattle, Washington, United States

Background: Schizophrenia is characterized by extreme genetic heterogeneity. Rare damaging mutations, many of which are de novo, or arose in recent generations, appear to play an important role in the illness. Given that most affected persons may have different genetic causal events, strategies are needed to identify shared pathways or neurobiological processes that, when disrupted, lead to schizophrenia.
Methods: Using quads and trios from COGS and PAARTNERS, we previously found that persons with sporadic schizophrenia are more likely to harbor damaging de novo mutations that disrupt genes in developing fetal brain, as compared to healthy siblings (Gulsuner et al., 2013). We have constructed expanded gene networks, using seed genes harboring damaging de novo events in affected persons, to identify genes that operate in related neurobiological processes predicted to be relevant to schizophrenia.
Results: Genes in the expanded network cluster into modules with distinct patterns of brain expression, based on RNAseq data, and operate in pathways important to early brain development. The next step is to determine whether the disruption of different genes, and their related neurobiological functions, predicts patterns of endophenotypic profiles in affected persons and families.
Conclusions: Each candidate gene implicates mechanisms and pathways potentially important to disease. Well-characterized family-based cohorts can be exploited to define genomic and neurodevelopmental aspects of schizophrenia, and to guide the next generation of intervention research.
Disclosures: Nothing to Disclose.

39.2 Associations of Gene Expression with Schizophrenia and Related Neurocognitive Endophenotypes

Laura Almasy

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Background: There is strong evidence for a genetic contribution to risk of schizophrenia. It is likely that some variants contributing to genetic risk are regulatory and act through alterations in the amount, timing or location of gene expression rather than through changing protein structure. We are using RNA expression levels as a means of scanning the genome for potential schizophrenia risk loci by assessing correlations between gene expression and established neurocognitive risk factors for schizophrenia in the Consortium on the Genetics of Schizophrenia (COGS), Multiplex Multigenerational Investigation of Schizophrenia (MGI), and Project among African Americans to Explore Risk for Schizophrenia (PAARTNERS) studies.

Methods: RNA transcription levels were assayed using Illumina HT-12 Expression BeadChips in 4,369 lymphoblastoid cell lines from COGS, MGI, and PAARTNERS. Variance component methods implemented in SOLAR were used to assess correlations between gene expression and seven measures of cognition, assessed using the PENN computerized neurocognitive battery. These include measures of abstraction and mental flexibility, emotion processing, face memory, sensorimotor dexterity, spatial memory, spatial processing, and verbal memory. Age, sex, self-reported ethnicity (African-American versus European-American), and study were included as covariates.
Results: Measures of gene expression are available for 1,029 individuals with schizophrenia, 2,729 unaffected family members, and 611 healthy community controls. Analyses of the first half of these data showed 828 probes associated with risk of schizophrenia and 55 associated with neurocognitive endophenotypes at $p < 5x10\text{-}5$. Permutation analysis comparing these results to 1,000 sets of randomly selected probes, indicated overrepresentation of genes associated with a variety of KEGG pathways, including calcium signaling, MAPK signaling, circadian rhythm, peroxisome proliferator-activated receptor signaling, and tyrosine metabolism. However, in these analyses many genes showed differing mean mRNA levels between African- and European-Americans and between the COGS, MGI and PAARTNERS samples, even after controlling for ethnicity.
Conclusions: Correlations of mRNA levels with schizophrenia and neurocognitive endophenotypes show promise as a means of scanning for loci harboring potential non-coding, regulatory variants affecting schizophrenia risk. However, evidence for differences in gene expression by ethnicity and study raise concern. To address these issues, analyses in the full sample are being run separately by study and using principal components-derived covariates to control for ethnicity and for other unmeasured, systematic sources of variance.
Disclosures: Nothing to Disclose.

39.3 Linking Clinical Outcome and Genotype to Schizophrenia Predisposition using Stem Cells

Kristen Brennand

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

Background: Schizophrenia (SZ) is a debilitating neurological disorder. Though postmortem studies have revealed reduced neuron size and spine density in SZ brain tissue, the molecular mechanisms underlying the disease state remain unclear.
Methods: We directly reprogrammed fibroblasts from SZ patients into human induced pluripotent stem cells (hiPSCs) and subsequently differentiated these disorder-specific hiPSCs into neural progenitor cells (NPCs) and neurons. We and others have found that SZ hiPSC NPCs show evidence of aberrant migration, increased oxidative stress, perturbed responses to environmental stressors; while SZ hiPSC neurons exhibit diminished neuronal connectivity, decreased neurite number, reduced synaptic maturation and reduced synaptic activity. Now, we wish to test if rare mutations, identified in COGS, PGC and/or NIH genetic studies, are causal contributors to SZ.
Results: First, we are investigating the link between genotype and gene expression. From two related individuals with large (289kb) heterozygous deletions in CNTNAP2 and discordant clinical outcomes, we generated hiPSC neural cells, observing exon-specific changes in CNTNAP2 expression in both carriers as well as allele-biased expression in CNTNAP2 that was consistent with both clinical outcome and neural migration *in vitro*. Second, we intend to explore the relationship between genotype and neuronal function by restoring defined mutations in SZ hiPSC neurons and recapitulating them in controls, in order to assess whether they are necessary and sufficient for disease across a range of genetic backgrounds.
Conclusions: Taken together, we believe these studies will inform the relationship between genotype, neuronal phenotype and clinical outcome.
Disclosures: Nothing to Disclose.

39.4 Epigenetics of Schizophrenia

Andrew Feinberg

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

Background: Our group, working with COGS, PAARTNERS, and MGI, have been investigating epigenetics changes in schizophrenia in a large case-control study, as well as genetic and epigenetic mediators of neurocognitive epigenetic transmission.
Methods: We have used a combination of assays, including CHARM, Illumina 450K, and integration of epigenetic data with GWAS to search for differences in a large case control series, as well as, with Laura Almasy, familial transmission of neurocognitive traits linked to epigenetic modification.
In the case control set, we measured the methylation levels of 456,513 CpG autosomal loci using the Infinium HumanMethylation450 BeadChip assay in independent case-control discovery and replication sets of blood and brain tissue. We implemented linear regression analysis for disease outcome, adjusting for age, gender, race, smoking, batch and cell heterogeneity. The discovery sample set included samples of 689 SZ cases and 645 controls. Replication samples were from the Genomic Psychiatry Cohort, frequency matched with respect to smoking status, age, and sex (Janet Sobell collaborating).
Results: We identified SZ-associated methylation differences which were replicated in controls, and included genes likely to play a role in SZ either by known genetic association or neurodevelopmental phenotype. We also noted that at least some previous epigenetic studies are affected by cell type confounding, and by confounding with smoking, which we went to great lengths to avoid. However, it remains to be determined whether the changes we observe occur before or after disease onset.
Conclusions: Purely genetic analysis of schizophrenia can miss disease associations that become apparent through a combined approach.
Disclosures: Nothing to Disclose.

Panel
40. The Role of Neuroinflammation in Depression: Pet Imaging and Clinical Implications

40.1 Positron Emission Tomographic Imaging of Translocator Protein (TSPO) as a Biomarker of Neuroinflammation in Alzheimer's Disease

Robert Innis

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

Background: In addition to reviewing the use PET imaging of translocator protein (TSPO) as a biomarker of neuroinflammation, Dr. Innis will provide novel results on TSPO imaging in Alzheimer's disease. His laboratory found that this marker of neuroinflammation is increased in patients with AD compared to subjects with mild cognitive impairment (MCI, a precursor syndrome) and to control subjects (Kreisl et al., Brain, 136: 2228, 2013). Furthermore and unlike amyloid, the amount of inflammation, indirectly measured as TSPO binding, was correlated with the severity of cognitive impairment. The results of this between-group comparison have been confirmed in a larger groups of subjects (unpublished) and, more importantly, have been extended with repeated PET scans in patients over a two to three year follow-up period (unique data to be presented in this talk).
Methods: Eleven patients with either AD or mild cognitive impairment (Mini Mental State Exam score $= 21.6 \pm 5.2$) and 8 cognitively-normal age-matched controls underwent 11C-PBR28 PET at baseline and after median follow up of 2.7 years. Patients were amyloid-positive and controls were amyloid-negative on 11C-PIB PET. Dynamic PET images were corrected for partial volume effects. Relative 11C-PBR28 binding was calculated 60-90 min post-injection using cerebellar gray matter as a pseudo-reference region.
Results: For patients, 11C-PBR28 binding in target regions was 2.5 – 17% greater at follow up than baseline, with greatest increase in entorhinal cortex. For controls, regional binding at follow up was within $\pm 5\%$ of baseline values. Corrected for baseline values, patients had greater 11C-PBR28 binding at follow up than controls in superior and inferior parietal lobule, occipital cortex, superior and middle temporal cortex, and entorhinal cortex ($P < 0.03$). Change in Clinical Dementia Rating scale score correlated with change in 11C-PBR28 binding in prefrontal cortex, inferior parietal lobule, precuneus, and superior and middle temporal cortex ($r > 0.66$, $P < 0.023$).
Conclusions: TSPO binding increases with progression of AD but not in healthy aging.
Furthermore, the increased TSPO binding was significantly correlated with the increase of cognitive impairment during the follow-up period. Overall, these results suggest that neuroinflammation is a marker of the conversion from MCI to AD and that neuroinflammation correlates with disease severity both between groups and within subjects during progression of the disease. Of relevance to this panel, this study in AD shows that TSPO imaging can visualize and quantify neuroinflammation in a disorder, whose pathology is known to include significant neuroinflammation at the time of death. Thus, TSPO imaging can now be used as a validated tool to explore the role on neuroinflammation in Major Depression Disorder, whose neuropathology if far less clear than that in AD.
Disclosures: Part 1: Research collaboration and financial support from Eli Lilly, **Part 4:** Research collaboration and financial support from Eli Lilly.

40.2 New Evidence that Microglial Activation, an Important Component of Neuroinflammation, Is found Throughout Grey Matter Regions in the Brain During Major Depressive Episodes

Jeffrey Meyer

Center for Addiction and Mental Health, Toronto, Canada

Background: The neuroinflammatory model of major depressive disorder (MDD) is supported by the several main findings including commonality of sickness behaviors with symptoms of major depressive episodes (MDE), the association of elevated peripheral inflammatory markers with MDD, and high rates of MDE in neuroinflammatory illnesses. However, a key limitation has been the lack of brain inflammation studies in reasonably large samples of MDE secondary to MDD. Recent advances in positron emission tomography (PET) enable measurement of TSPO VT, an index of translocator protein levels which elevate when microglia are activated. The aim of the study was to determine whether TSPO VT, is elevated in the prefrontal cortex (PFC), anterior cingulate cortex (ACC) and insula in MDE secondary to MDD.
Methods: 20 subjects with MDE secondary to MDD and 20 healthy controls, underwent an [18F]FEPPA PET scan. TSPO VT was measured in the PFC, ACC, and insula. MDE subjects were medication-free for at least 6 weeks. All participants were otherwise healthy, and non-smoking.
Results: In MDE, TSPO VT was significantly elevated in the PFC, ACC, and insula (average 30%, multivariate analysis of variance, $F(3,35) = 4.73$, $P < 0.001$). A similar increase was observed in other brain regions. In MDE, greater TSPO VT in the ACC and insula correlated with greater depression severity and lower body mass index (BMI), respectively (ACC: $r = 0.628$, $P = 0.005$; insula: $r = -0.605$, $P = 0.006$).
Conclusions: This finding provides strong evidence for brain inflammation, and more specifically, microglial activation, in MDE, implying that novel therapeutics which either modulate or reduce microglial activation may be promising for MDE. The correlation between higher ACC TSPO VT and the severity of MDE is consistent with the perspective that neuroinflammation in specific regions may contribute to sickness behaviors that overlap with symptoms of MDE.
Disclosures: Part 1: Drs. Meyer has received operating grant funds for other studies from Janssen, Eli-Lilly, GlaxoSmithKline, Bristol Myers Squibb, Lundbeck, and SK Life Sciences in the past 5 years. Dr. Meyer has consulted to several of these companies, as well as Takeda, Sepracor, Trius, Mylan, and Teva, **Part 2** It is possible that the total amount from Trius or Teva could have reached $10000, **Part 4:** Janssen, **Part 5:** not applicable. I have several patents

(submitted/completed) related to use of MAO-A markers and inflammatory markers as biomarkers in mood disorders as well as one for a dietary supplement to prevent mood disorders. These have potential to generate income in the future.

40.3 Using PET Imaging of Translocator Protein (TSPO) to Investigate the Link Between Inflammation and Depression

Erica Richards

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

Background: Neuroinflammation may be a predisposing factor for major depressive disorder (MDD). Translocator protein 18 kDa (TSPO) is a highly expressed protein in glial cells of the brain and, therefore, a potential biomarker of neuroinflammation. TSPO can be accurately quantified using positron emission tomography (PET) and [11C]PBR28, a TSPO tracer developed in our laboratory. During this panel, in an earlier presentation, Dr. Jeffrey Meyer will report findings from his group showing increased TSPO binding in multiple brain regions of unmedicated MDD patients currently experiencing a major depressive episode. Our current study has three aims. The first aim is to replicate the findings presented by the Meyer group. The second aim is to investigate antidepressant effects on TSPO binding in patients with MDD. The third aim is to determine the relationship of peripheral and central inflammatory markers to TSPO binding.
Methods: Unmedicated MDD (n = 13), medicated MDD (n = 10) and healthy control (n = 12) subjects underwent PET imaging using [11C]PBR28. We measured total distribution volume (VT, proportional to Bmax/Kd) using arterial input function and corrected for TSPO genotype. Based on previous post-mortem findings, we chose the subgenual prefrontal cortex and anterior cingulum as regions of interest and compared VT values obtained in medicated and unmedicated MDD subjects and healthy controls. We also obtained peripheral blood samples and cerebrospinal fluid, for later analysis, to investigate the relationship between peripheral and central inflammatory markers and TSPO binding.
Results: The interim results of this ongoing study show no significant differences in TSPO binding in depressed patients compared to healthy subjects in any of the predetermined brain regions. In the anterior cingulate, VT was 12.5% higher in unmedicated MDD patients compared to healthy controls ($p = 0.25$, Cohen's $d = 0.49$) and 10.3 % higher in medicated patients compared to healthy controls ($p = 0.45$, Cohen's $d = 0.31$). In the subgenual cortex, VT was 11.1% higher in both the unmedicated ($p = 0.30$, Cohen's $d = 0.43$) and medicated patients ($p = 0.44$, Cohen's $d = 0.32$) compared to healthy controls. TSPO binding did not correlate to peripheral blood C-reactive protein levels.
Conclusions: With about 50% recruitment completed for this study, we have not replicated the previous findings of Meyer's group showing increased TSPO binding in brain regions of depressed patients compared to healthy controls. However, based on the Cohen's d effect sizes reported, there is a moderate effect showing increased TSPO binding in the anterior cingulate and subgenual cortex of unmedicated patients. The moderate effect sizes noted indicate that increasing the sample size may result in significant differences, specifically with increased TSPO binding in unmedicated depressed patients. Effect size decreases when medicated patients are compared to healthy controls. For future analysis, there may be some utility in polling the data of these two similar studies to look for greater significance and to subgroup the patient populations given the heterogeneous nature of major depressive disorder. These findings are important because they may help further elucidate pathways involved in the development of MDD as well as identify potential novel treatments and pharmacological targets.
Disclosures: Nothing to Disclose.

40.4 Efficacy of the Anti-Inflammatory Agents Minocycline and Aspirin in Bipolar Depression

Wayne Drevets

Janssen Pharmaceuticals of Johnson & Johnson, Inc., Titusville, New Jersey, United States

Background: The literature suggests a subgroup of individuals with mood disorders manifests elevated release of pro-inflammatory cytokines in the peripheral blood and cerebrospinal fluid (CSF), and activation of microglia in the brain. The latter finding has been informed by post mortem assessment of microglial activation in the cingulate cortex of suicide victims with mood disorders, and appears consistent with the results of *in vivo* PET-TSPO binding in some, but not all studies, of major depressive disorder. Studies in rodents show that suppressing microglial activity using minocycline or drugs targeting microglia-based receptors reduce depression-like behaviors in chronic stress models. To examine whether anti-inflammatory treatments improve depressive symptoms in bipolar disorder (BD) we evaluated the efficacy of minocycline, which selectively inhibits the microglia polarization to a pro-inflammatory state, and aspirin, at a dose expected to relatively selectively inhibit cyclooxygenase 1 (COX-1). Both minocycline and COX-1 inhibition exert neuroprotective effects in preclinical models. The rationale and study design appears in Savitz et al (BMJ Open 2012; 2:e000643; Clinical Trials.gov: NCT01429272. LI-Jonathan Savitz; PI-Sheldon Preskorn). The study completes enrollment in summer, 2015, and final results will be presented.
Methods: Outpatients 18 to 65 years of age, who met DSM-IV-TR criteria for BD (types I, II, or NOS) in a current depressive episode were recruited to participate in a randomized, double-blind, placebo-controlled, parallel-group, clinical trial following a 2×2 design. The total enrollment target was $n = 120$. The study was conducted at three sites: Laureate Institute for Brain Research, Tulsa, OK, Kansas University School of Medicine, Wichita, KS and University of Oklahoma School of Community Medicine, Tulsa, OK. As adjuncts to existing treatment, subjects were randomized to receive one of four treatment combinations: placebo-minocycline plus placebo-aspirin, active-minocy-

cline plus placebo-aspirin, placebo-minocycline plus active-aspirin or active-minocycline plus active-aspirin. The dose of minocycline and aspirin is 100 mg twice daily and 81 mg twice daily, respectively. Antidepressant response was assessed by changes in the MADRS score between baseline and the end of the 6-week trial. As secondary outcome measures, the anti-inflammatory effects of minocycline and aspirin was tested by measuring pre-treatment and post-treatment serum levels of C reactive protein and inflammatory cytokines.
Results: Using an adaptive trial design during this study, a blinded interim analysis was conducted once 60 individuals had been randomized to determine whether any of the cells were separating from the others. Two cells appeared to be separating. A power calculation was performed based on the mean difference between cells in relationship to the variance. That calculation determined that 30 subjects in each of the two cells would produce power $>$ 80% for alpha $=$ 0.05. An unblinded investigator not involved in the study conduct then evaluated the two cells to determine whether a reduction to just these two cells going forward was reasonable from a scientific perspective. That decision allowed for a reduction of the total enrollment goal from $n = 120$ (i.e., 30 subjects in 4 cells) to $n = 100$ (30 subjects in each of the two critical cells plus 40 individuals already enrolled in the two intermediate cells). The last patient in is anticipated in July, 2015.
Conclusions: The final study results will be presented and will address the therapeutic potential of anti-inflammatory agents in bipolar depression. Relationships between the clinical outcome measures and the pre- and post-treatment serum biomarkers will be assessed to explore whether patients manifesting evidence of a pro-inflammatory process may particularly benefit from anti-inflammatory treatment.
Disclosures: Part 1: Johnson & Johnson, Inc., Use Patent filed "Composition and Method for Treating Bipolar Disorder", Use Patent awarded, "Scopolamine in the Treatment of Depression" (no financial proceeds to date), **Part 2:** Johnson & Johnson, Inc., Equity and Salary, **Part 3:** Johnson & Johnson, Inc., **Part 5:** Johnson & Johnson, Inc.

Panel
41. Fear Generalization: Neurobiological and Behavioral Mechanisms Across the Pre-clinical and Clinical Spectrum

41.1 Fear Learning Circuitry is Biased Toward Generalization of Fear Associations in Posttraumatic Stress Disorder

Rajendra Morey

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Background: Fear conditioning is an established model for investigating posttraumatic stress disorder (PTSD). However, symptom triggers may only vaguely resemble the initial traumatic event, differing on a variety of sensory and affective dimensions. We extended the fear-conditioning model to assess generalization of the conditioned-fear response on fear processing neurocircuitry in PTSD.
Methods: Military veterans ($n = 67$) consisting of PTSD ($n = 32$) and trauma-exposed comparison ($n = 35$) groups underwent functional MRI during fear-conditioning to a low fear-expressing face while a neutral face was explicitly unreinforced. Baseline responses before, and generalization responses after, fear conditioning used stimuli that varied along a neutral-to-fearful continuum.
Results: Compared to trauma-exposed controls, PTSD patients exhibited more severe post-study memory distortion of the fear-conditioned stimulus toward the stimulus expressing the highest fear intensity. PTSD patients exhibited biased neural activation toward high-intensity stimuli in fusiform gyrus ($p < .02$), insula ($p < .001$), primary visual cortex ($p < .05$), locus coeruleus ($p < .04$), thalamus ($p < .01$), and at the trend-level effect in inferior frontal gyrus ($p = .07$). All regions except fusiform were moderated by childhood trauma. Amygdala-calcarine ($p = .01$) and amygdala-thalamus ($p = .06$) functional connectivity selectively increased in PTSD patients for high-intensity stimuli during generalization. In contrast, amygdala-vmPFC ($p = .04$) connectivity selectively increased in trauma-exposed controls compared to PTSD patients for low-intensity stimuli during generalization, representing a safety-learning response.
Conclusions: Fear generalization in PTSD is biased toward stimuli with higher emotional intensity than the original conditioned-fear stimulus. The functional brain differences provide a putative neurobiological model for fear generalization where PTSD symptoms are triggered by threat cues that merely resemble the index trauma.
Disclosures: Nothing to Disclose.

41.2 Erring on the Side of Caution: One Cell at a Time

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Background: The early stages of fear memory formation involve strengthening of sensory afferents from the thalamus to the amygdala. As useful as this simple behavioral model has been in studying basic cellular mechanisms of associative learning, it does not capture some of the essential features of learning in the "real world". What makes learning a challenging problem is that it requires that animals generalize appropriately from experience. There are costs associated with both too little and too much generalization: If an animal under-generalizes it may overlook future signs of danger, whereas if it overgeneralizes it may fail to explore and thereby miss opportunities for feeding, mating, etc.
Methods: I will present recent findings obtained using a combination of *in vivo* unit recordings and optogenetics in awake behaving rodents, on the cellular mechanisms in the amygdala mediating generalization during fear learning.
Results: We identified distinct neuronal populations in the lateral amygdala (LA) of rats that signaled generalized versus cue-specific associations and determined how their distributions switched during fear generalization. Notably,

the same LA neurons that were cue-specific before the behavioral shift to generalized fear lost their specificity afterwards, thereby tilting the balance of activity toward a greater proportion of generalizing neurons (Fig. 1). Neuronal activity in the LA, but not the auditory cortex, was necessary for fear generalization. Furthermore, targeted activation of cAMP/PKA signaling in the LA increased neuronal excitability of LA neurons and led to generalized fear.
Conclusions: These results provide a cellular basis in the amygdala for the alteration of emotional states from normal to pathological fear.
Disclosures: Nothing to Disclose.

41.3 Aversive Learning and Generalization Predict Sub-Clinical Anxiety Symptoms Six Months Later

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Background: The identification of premorbid markers of risk for psychopathology is one of the most important challenges for present-day psychiatric research. There is a pressing need for efficient, low-cost, low-effort screening tools in order to deliver targeted prevention and cure. This study focuses on deviant generalization as a key behavioral vulnerability factor, and uses simple behavioral tasks to assess the level of generalization. The first experiment examines the predictive value of aversive learning and generalization for the development of sub-clinical anxiety. The second and third experiment focus on the diagnostic value of non-aversive learning and generalization in panic disorder and autism, respectively.
Methods: The behavioral task involved two rings of different size, with one ring (CS+) consistently followed by an aversive picture (Experiment 1) or a neutral picture (Experiments 2 and 3), whereas the other ring not (CS-). Learning was tracked by asking participants during each ring to rate the probability that the (aversive) picture would follow. Subsequent generalization testing comprised repeated presentations of rings of varying sizes. In Experiment 1, 375 college students completed the task, while levels of anxiety were assessed by questionnaires (DASS and STAI) at that moment and after a six-month follow-up. In Experiment 2, learning and generalization were compared between 22 panic disorder patients, 27 other anxiety patients, and 29 healthy controls. In Experiment 3, learning and generalization were compared between 18 high-functioning autistic patients and 19 matched controls.
Results: Experiment 1 showed that both discrimination learning and generalization added significantly to the explained variance in anxiety symptomatology at 6 months follow-up. Experiment 2 showed weaker discrimination learning in panic disorder patients, due to heightened expectancies during the non-associated control stimulus (CS-). Experiment 3 showed weaker discrimination learning in high-functioning autistic patients, due to lowered expectancies during the associated stimulus (CS+) as well as heightened expectancies during the non-associated stimulus (CS-).
Conclusions: Simple behavioral tasks that probe discrimination learning and generalization can be used as efficient, low-cost, low-effort screening tools for the prediction and diagnosis of various forms of psychopathology. The behavioral and neural processes underlying explicit expectancy learning have relevance for the study of psychopathology and should be studied in more detail.
Disclosures: Nothing to Disclose.

41.4 The Effect of Generalized Fear Learning on Episodic Memory

Joseph Dunsmoor

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Background: Pavlovian fear conditioning has been used successfully to investigate fear learning in humans and nonhuman animals for stimuli in the environment that signal aversive events. Beyond understanding how fear is acquired and expressed, fear learning can also provide insights into other memory systems involved in remembering episodic details of a fearful event. Little research has been conducted in the effects of fear conditioning on episodic memory for neutral details associated with aversive experiences because most experimental protocols in humans utilize only one or two conditioned stimuli repeatedly paired with the aversive event.
Methods: Here, we used a novel trial-unique form of fear conditioning in which 119 healthy subjects learned to fear an entire category of objects (either animals or tools). This procedure enabled us to test memory for items from a conceptual category viewed prior, during, and after Pavlovian fear conditioning of semantic categories of stimuli.
Results: We demonstrated that memory for pictures from a category paired with an electric shock (animals or tools) was greater than for an unpaired control category (tools or animals, respectively). At 24 hour ($N = 30$) and 6 hour ($N = 30$) retrieval tests, enhanced episodic memory was observed for items from the feared category viewed before fear conditioning, suggesting a retroactive enhancement in episodic memory for items related to a future threat. The latter finding is in accord with recent animal studies on "behavioral tagging" in which weak learning is enhanced through subsequent activation that engages common neural pathways minutes to hours later.
Conclusions: These results provide new evidence for a generalized tagging process during memory encoding, whereby seemingly inconsequential information can be retroactively credited as relevant, and therefore selectively remembered, if conceptually similar information acquires salience in the future.
Disclosures: Nothing to Disclose.

Panel
42. Probing the Perinatal Expression of Risk for Mental Disorder: Basic Molecular, Neurobiological, Neuroimaging and Clinical Intervention Studies in Pregnancy and Fetal Development

42.1 Neurexin 1 (NRXN1) Gene Expression Across the Normal Human Lifespan: Implications for Normal and Abnormal Neurodevelopment

Amanda Law

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Background: Genetic studies have identified neurexin-1 (NRXN1; 2p16.3) as a risk gene for several neurodevelopmental disorders, including autism spectrum disorder, schizophrenia, developmental delay and mental retardation, implicating a pleotropic role for NRXN1 in human cortical development. NRXN1, a presynaptic cell adhesion molecule and receptor that plays critical roles in synaptogenesis, encodes two major isoforms, NRXN1-α and NRXN1-β. To gain insight into NRXN1's involvement in human cortical development we used quantitative real time PCR to examine the expression trajectories of NRXN1, and its predominant isoforms NRXN1-α and NRXN1-β in prefrontal cortex (PFC) from fetal stages to aging and in patients with schizophrenia or bipolar disorder.
Methods: Postmortem human brains from the Clinical Brain Disorders Branch were obtained at autopsy from the Washington, D.C. and Northern Virginia medical examiners' offices (protocol 90-M-0142 approved by the NIMH/NIH IRB). Additional postmortem fetal, infant, child, and adolescent brain tissue samples were provided by the National Institute of Child Health. Dorsal lateral prefrontal cortex (DLPFC) was available from 245 normal individuals (0-85 years), 110 patients with schizophrenia and 34 patients with bipolar disorder. Fetal PFC was derived from 39 individuals (14-39 weeks). NRXN1 and its predominant splice isoforms NRXN1-α and NRXN1-β, were measured using Taqman Gene Expression Assays by real time RT-PCR using an ABI Prism 7900 sequence detection system with 384-well format (Applied Biosystems) and quantified via the standard curve method.
Results: Linear Regression revealed that in the human fetal brain, NRXN1-α ($P = 0.01$) and NRXN1-β ($P = 1.38E-5$) increased significantly with gestational age. Highly significant effects of age on NRXN1-α ($\beta = -0.57$; $P = 2.1E-18$) and NRXN1-β ($\beta = -0.49$; $P = 6.28E-15$) expression were also observed during postnatal development, whereby NRXN1 expression was highest at birth until 3 years of age, after which expression declined and remained steady throughout childhood, adolescence and aging. Expression levels of NRXN1-α were significantly elevated in patients with bipolar disorder compared to age matched healthy controls ($p = 0.01$). Conversely, expression levels of NRXN1-β were significantly higher in the DLPFC of patients with schizophrenia compared to age matched controls ($p = 0.01$).
Conclusions: Our data provide novel insight into NRXN1 splice isoform expression profiles during normal human neocortical development and demonstrate that expression is highest during critical periods of plasticity in pre- and early postnatal development, consistent with the association of NRXN1 with a broad spectrum of neurodevelopmental disorders. Our data also demonstrate abnormal patterns of NRXN1 expression in psychiatric disorders, suggestive of an immature molecular phenotype. These data will be discussed together and in the context of emerging animal studies and the roles of NRXN1 in early pre-and perinatal brain development.
Disclosures: Part 1: Dr. Law has served as a paid consultant for Astra Zeneca Pharmaceuticals.

42.2 Genetic Neuropathology in Human Brain Development and Schizophrenia

Joel Kleinman

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Background: Recent advances in genome-wide association studies have led to the identification of 108 regions of genetic variation, single nucleotide polymorphisms (SNPs), associated with increased risk for schizophrenia ($p < 1.0e-8$) (PGC2, Nature 511, 2014). Although these genetic variants each increase risk to a relatively small degree, understanding their mechanisms has the potential to have a large impact with regard to diagnosis and treatment. The molecular biology mechanisms by which genetic variation increases risk for schizophrenia involve expression of specific alternative transcripts thought to be critical for early brain development. Many of these transcripts may be brain and/or primate specific and several are preferentially expressed in fetal human brain.
Methods: We have generated qRT-PCR and RNA sequencing data on over 604 -701 postmortem prefrontal cortical (PFC) specimens that have been genotyped for over 650,000 SNPs (Illumina Bead chips). We have used this data to look for mechanisms involving genetic variants that increase risk for schizophrenia from the PGC2 data including ZNF804A and the locus at 10q24.32 as well as other genes of interest including CHRNA7 and CHRFAM7A. Comparisons in expression of these genes have been made across the human lifespan in normals ranging in age from week 14 in the fetus to 80 years of age ($n = 313$) as well as between patients and controls (schizophrenia ($n = 169$), bipolar disorder ($n = 57$), major depression ($n = 133$) and normal controls ($n = 198$)). N's are for the ZNF804A study (Tao R et al, JAMA Psychiatry 71, 2014). Comparable sample sizes were used for CHRNA7/CHRFAM7A and the 10 q24.32 locus study).
Results: ZNF804: The risk allele is associated with decreased expression of a novel truncated transcript in ZNF8O4A specifically in PFC of fetal human brain ($p < .05$). This transcript is preferentially expressed in fetal human PFC relative to postnatal specimens. Moreover, this same transcript is underexpressed in PFC of patients with schizophrenia and overexpressed in patients with affective disorders ($p = 1.8e-19$).
CHRNA7/ CHRFAM7A: CHRFAM7A is preferentially expressed in fetal PFC while CHRNA7 is relatively unchanged across the lifespan. CHRFAM7A is expressed more in PFC of all patient groups relative to normals ($p = 1.3e-7$), while CHRNA7 is decreased in PFC of patients with schizophrenia ($p = 2.8e-5$) and increased in major depression ($p = 1.0e-17$). Gene expression for transcripts of these genes are not associated with any known risk alleles.

10q24.32 locus: rs7085104 is associated with increased expression of a novel truncated transcript in AS3MT ($n = 604$, $p = 1.99e-30$). Although the transcript is expressed in fetal PFC it is not preferentially expressed in fetal human brain. The expression of this transcript is increased in PFC of patients with schizophrenia relative to controls ($p = 7.2e-4$).
Conclusions: Postmortem human brain is critical for elucidating the mechanisms by which genetic variation increases risk for schizophrenia. In so far as the transcripts may be brain and primate specific and are developmentally regulated, fetal human brain may also be important for fully understanding the genetic neuropathology of schizophrenia. Genetic variations that increase risk for schizophrenia in ZNF804A and AS3MTare associated with expression in normal PFC that is in the same direction as that seen between patients with schizophrenia and controls. The differences in expression in CHRNA7 and CHRFAM7A in PFC remain to be determined.
Disclosures: Nothing to Disclose.

42.3 Altered Amygdala Functional Connectivity in Neonates at Risk for Schizophrenia or Bipolar Disorder

John Gilmore

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Background: Emotional processing deficits are common in schizophrenia and appear to be related to abnormal functional connectivity of the amygdala with the prefrontal cortex and other regions. Amygdala dysconnectivity has been observed in adolescents at risk for psychosis, indicating that functional networks associated with emotional processing and the amygdala may predate and potentially contribute to the onset of psychosis.
Methods: Neonates of mothers with schizophrenia (SCZ), bipolar disorder (BD), and Mood Disorder not otherwise specified (MD-NOS) underwent resting state functional magnetic resonance imaging (rsfMRI) shortly after birth. Neonates were scanned unsedated in a natural sleep. Twenty SCZ-risk, 26 BD-risk, 23 MD-NOS-risk neonates, and 20 matched control subjects (CONTROL) were scanned. Seed-based functional connectivity analyses of the amygdala were conducted. Nine brain areas that show gray matter deficits in adult schizophrenia patients were also studied to test the specificity of amygdala-based findings and explore if additional functional connections are altered as well.
Results: The left amygdala demonstrated hyper-connectivity with the right dorsal lateral prefrontal cortex while the right amygdala demonstrated common hyper-connectivity with the thalamus for both SCZ-risk and BD-risk groups. The exploration analysis revealed that two of nine brain areas, including the thalamus and ventral anterior cingulate cortex, demonstrated additional schizophrenia-specific functional connectivity alterations. The levels of thalamic connectivity detected predicted cognitive development scores at 1 year of age.
Conclusions: This is the first study of functional connectivity in neonates at risk for schizophrenia and suggests that functional connectivity abnormalities of the amygdala associated with schizophrenia and bipolar illness arise during prenatal brain development. We found both common and disorder-specific abnormal profiles of functional connectivity in neonates at genetic risk for schizophrenia, bipolar illness, and mood disorder NOS. It may be possible to develop imaging-based biomarkers for the early identification of risks for later psychiatric illness.
Disclosures: Nothing to Disclose.

42.4 Human Perinatal Choline Supplementation Decreases Preschool Parent-Reported Attentional and Social Withdrawal Symptoms via an alpha7 Nicotinic Cholingergic Receptor Mediated Effect on Infant Developmental of Sensory Gating

Randal Ross

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Background: Most neuropsychiatric illnesses—including ADHD, anxiety disorders, autism, bipolar, and schizophrenia—are neurodevelopmental disorders, where onset illness is the end result of brain developments changes which begin prenatally. Thus, one potential window for primary prevention is the perinatal period. In animal models, prenatal stimulation of the alpha7 nicotinic cholinergic receptor with dietary choline supplementation leads to improved development of sensory gating, improved memory, and decreased anxiety. Previously published work has supported perinatal choline supplementation's positive impact on human infant sensory gating development at 1 month of age. This report is a follow-up of these same children to 30 months of age.
Methods: Randomized controlled trial of perinatal choline supplementation in 100 healthy mothers (phosphatidylcholine 6300 mg QD) and infants (phosphatidylcholine 700 mg QD). Outcomes of P50 sensory gating (1 months of age) and parent-reported behavior utilizing the Child Behavior Checklist (CBCL; 40 months of age). All infants were genotyped for a schizophrenia-associated SNP in CHRNA7, rs3087454.
Results: Infant sensory gating predicted the 40-month CBCL total problems score. Homozygosity for a schizophrenia risk allele in either CHRNA7 is associated with delayed development of infant cerebral inhibition and increased CBCL total problems at 40 months of age. Both genetic effects are reversed by perinatal choline supplementation, with a particular benefit of perinatal choline supplementation on 40-month-old attention and social withdrawal.
Conclusions: Prenatal choline supplementation compensates for genetic vulnerability's impact on the infant development of sensory gating and the 3-year-old development of behavior. The effect of choline is moderated by CHRNA7 genotype supporting perinatal stimulation of the alpha7 nicotinic receptor as the mechanism of action. Universal prevention strategies have a role in preventing major mental illnesses.
Disclosures: Nothing to Disclose.

Study Group
43. Neurocircuit-based Interventions in Addictions: When and How?

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This Study Group will discuss opportunities and challenges in developing neurocircuit-based interventions for the treatment of addictive disorders. The group will draw from experience of the use of deep brain stimulation (DBS) and other interventions for neuropsychiatric disorders, as well as basic science experience in modulating addictive behaviors in model organisms.
The development and growing application of neurocircuit interventions for the treatment of addictions has been stimulated by a confluence of clinical epidemiologic facts and relevant neuroscience observations. High rates of relapse, disability and mortality suggest that more effective interventions are needed. Preclinical studies as well as human neuroimaging studies have demonstrated that addictions are mediated and maintained by circuits rather than a single brain region or neurotransmitter system. Addictions are systems-level disorders affecting integrated cortical, subcortical, and limbic neural circuits, and maintained by allostatic alterations in expression of their related neurotransmitter and molecular mediators.
Within this framework, successful treatments for addiction should be capable of modulating limbic and executive control circuits implicated in craving and relapse.
Multiple neuromodulation techniques are FDA-approved for clinical and investigational use in humans. Three of the most promising include deep brain stimulation (DBS), repetitive transcranial magnetic stimulation (rTMS), and brain lesioning by focused ultrasound.
Beyond direct electrical stimulation, encouraging preclinical studies suggest that a number of focused chemogenic approaches may be possible, including optogenetic stimulation, DREADDS, and genetic transfection (e.g., with dopamine receptor genes).
Each is potentially feasible, but safety data have to be developed in each instance, requiring a substantial institutional commitment, in addition to confirming efficacy in preclinical models.
Therefore, a question equally as contentious as when, is how. Potentially, many methods may be used against a multiplicity of targets. A unifying theme across different methodologies and neural targets could include some commonalities of assessment and enrollment, including assessment of neuropsychological changes associated with addiction and neuroimaging of brain connectivity and responses.
This study group will address, or debate: 1) Which regions, neurocircuits or molecules are the most appropriate target for neurocircuit-based intervention in addictions? (2) Where treatment can be calibrated, for example stimulation parameters, how should this be done, and most importantly, standardized across studies? (3) Who should be eligible? (4) What are the clinical outcomes providing the most realistic and consistent measures of efficacy (5) Can we design human neurocircuit intervention studies so that they can back to model organisms to unravel mechanisms of addiction and understand how the human interventions are working, or why they are not?
This study group will bring together investigators with experience in neurocircuit-based interventions to discuss the challenges, strategies, and solutions applied to date. A major goal will be to identify common elements of concern and, through audience interaction, raise awareness of the obstacles and potential future approaches.
Disclosures: Nothing to Disclose.

Thursday, December 10, 2015

Panel
44. The Role of Impulsivity vs. Impulse Control on the Developmental Trajectories of SUD - New Insights from Neuroimaging Research

44.1 A Longitudinal fMRI Study of Reward and Inhibitory Control in Youth at Risk for Substance Use Disorder

Mary Heitzeg

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Background: Evidence suggests that the relatively early maturation of subcortical incentive-responding systems compared with prefrontal inhibitory systems may bias adolescents toward seeking out reward. This bias is proposed to underlie the impulsive, risky decision-making and related escalation of substance use that occurs during adolescence. The longitudinal work reported here aims to investigate inhibitory control and reward system functioning as both prospective predictors of early problem substance use and, in a separate sample, as markers of substance use disorder (SUD) in young adults.
Methods: The Michigan Longitudinal Study (MLS) is an ongoing prospective study of youth at high-risk for SUD based on parent history. Longitudinal fMRI is being conducted in two cohorts recruited from the MLS. The child cohort had a baseline scan prior to substance use initiation ($n = 125$; average age $= 10$). The young adult cohort had a baseline scan during the age where normative peaks in SUD are observed (age 18-21; $n = 160$; average age $= 20$). Scans were collected during inhibitory control (go/no-go) and reward (monetary incentive delay; MID) tasks. Participants completed annual questionnaires regarding substance use and behavior problems as part of the MLS assessment. For the child cohort, prospective predictors of the initiation of problem substance use by age 13-16 were investigated using baseline scans. For the young adult cohort, differences between participants with and without SUD were investigated.
Results: Activation to successful inhibition did not predict initiation of problem substance use in the child sample

whereas blunted activation of left middle frontal gyrus (LMFG) during failed inhibition was a significant predictor of later problem substance use. Blunted LMFG activation was also observed in the young adults with an SUD compared with controls. In both cohorts, less LMFG activation was associated with increased externalizing behavior problems. During the MID, nucleus accumbens (NAcc) activation to reward anticipation did not predict problem substance use involvement in the child sample; however, activation in those who went on to problem use was increased compared with controls after problem substance use initiation. Higher NAcc activation was also observed in older adolescents with an SUD compared with controls. NAcc activation was not associated with externalizing behavior problems in either cohort. In the older cohort, NAcc activation was positively correlated with past year drinking volume.
Conclusions: Blunted activation during performance errors in childhood may underlie problems with adapting behavior appropriately, leading to undercontrolled behavior generally and problem alcohol and drug use specifically. The replication in an older sample with SUD suggests it may be a persistent, stable marker of risk for SUD identifiable in childhood. In contrast, hyper-activation of the reward system in childhood is not an early predictor of the initiation of problem substance use. Ongoing analyses of the longitudinal data are investigating whether heightened activation of the reward system is a later-emerging risk factor or a result of substance use.
Disclosures: Nothing to Disclose.

44.2 Neural Activation to Response Inhibition Predicts Subsequent Substance Use Initiation and Escalation in Adolescence

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Background: Response inhibition and cognitive control have been recently appreciated as risk factors for substance use and related disorders. Our early work suggested that poor scores on cognitive tests of attention predicted greater substance use and dependence symptoms 8 years later, even in youth without ADHD, conduct disorder, or learning disorders (Tapert et al, 2002). Here, we sought neurocognitive and neuroimaging markers of response inhibition and cognitive control systems during early adolescence in predicting substance use in mid- to late- adolescence.
Methods: We recruited 295 substance-naïve 12-14 year-olds from community schools, and followed them into early adulthood. At each time point, participants were given a comprehensive neuroimaging session and neuropsychological test battery, plus quarterly interviews on substance use. Brain activation to response inhibition (i.e., a go/no-go task) and performance on a test of inhibiting prepotent responses (i.e., a Stroop task) was assessed prior to the onset of substance use. Annual follow-up interviews assessed substance use and mental health symptoms.
Results: Poorer baseline performance on tests of cognitive inhibition interference predicted higher follow-up peak drinks on an occasion, more days of drinking, and more marijuana use days ($p < .001$) by ages 17 to 18, above and beyond covariates. Prior to the onset of substance use, youth who later transitioned into heavy drinking showed significantly less activation during response inhibition than those who went on to remain non to minimal users throughout adolescence, in right inferior frontal gyrus, left dorsal and medial frontal areas, bilateral motor cortex, cingulate gyrus, left putamen, bilateral middle temporal gyri, and bilateral inferior parietal lobules (corrected $p < .01$/cluster $\geqslant 32$ contiguous voxels). In mid-adolescent substance users, less ventromedial prefrontal activation during response inhibition predicted increased substance use and dependence symptoms at follow-up ($p < 05$). These variables will be examined as predicts of substance use outcomes in a single model.
Conclusions: Blunted activation during response inhibition and poorer performance on tasks of inhibition may be linked to behavioral undercontrol and propensity to substance use and use disorder. Ongoing analyses will incorporate additional neuroimaging markers to examine the degree to which substance use can be predicted prior to the onset of substance use.
Disclosures: Nothing to Disclose.

44.3 A Longitudinal Investigation of Reward and Control Brain Systems as Predictors of Adolescent Drug Use

Hugh Garavan

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Background: Individual differences in neurodevelopment during adolescence may explain why some youth are more inclined to use and abuse alcohol and other drugs. The IMAGEN study, a multi-site, longitudinal study of 2,000 European adolescents, was designed to investigate this possibility. Extensive assessments at age 14 included genetics, structural and functional imaging and numerous phenotypic measures (e.g., personality, cognition, IQ, mental health, drug use, family histories of drug use). The fMRI tasks included an assessment of inhibitory control (the STOP task) and reward processing (the Monetary Incentive Delay task). Subjects were assessed two years later on their drug use and mental health. The longitudinal design allows us to search for baseline predictors of drug use. Moreover, the large sample size allows us (a) to identify youth who were drug-naïve at baseline enabling us to rule out confounding influences of exposure and (b) allows us to quantify the generalizability of our findings using cross-validation techniques.
Methods: The STOP task requires motor response inhibition yielding activation associated with successful inhibitions as well as failures to inhibit. The MID task assesses both the anticipation and the receipt of rewards. These brain measures were included with numerous phenotypic measures to develop regression models predicting future use and assessed using ten-fold cross-validation. We report the models from two separate analyses that predict future binge drinkers ($n = 121$ 16 year olds with just 1-2 lifetime

drinks at baseline) and future cannabis users (n = 170 16 year olds who were cannabis naïve at baseline). Future users were compared to subjects who did not increase their alcohol use (Binge Drinking analysis) or remained cannabis-naïve (Cannabis analysis).
Results: We were able to predict future drug use with relatively high accuracy (Binge Drinking: area under the ROC = .75; Cannabis use: area under the ROC = .80). The best predictors of future binge drinking and future cannabis use were personality factors, a family history of drug use, and a history of stressful life events. Brain structure (total and regional grey matter volumes) and brain activation associated with both inhibitory control and reward processing were shown to predict future drug use. The best predictors included medial prefrontal and premotor areas (Binge Drinking) and parietal cortex and premotor cortex (Cannabis).
Conclusions: The analyses reveal that individual differences at age 14 in brain function associated with reward and control predicted binge drinking and cannabis use two years later. Although the cannabis group analyses are ongoing, the initial sets of results suggest that of the two brain systems, individual differences in control systems are better predictors than individual differences in reward systems. This conclusion would be consistent with the critical role that prefrontal maturation is thought to play in adolescence in regulating impulsive and risky behaviors.
Disclosures: Nothing to Disclose.

44.4 Impulsivity and Reward Processing in Drug Naïve Youth at Risk for Substance Use Disorders

Iliyan Ivanov

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Background: Extensive evidence has shown that impulsivity in childhood is associated with elevated risk for the development of substance use disorders (SUD). Impulsivity, however, is not a unitary contract and does interact with other behavioral traits (e.g. reward sensitivity) that also contribute to the development of SUDs. These interactions are purportedly linked to the activity of distinct brain systems. This presentation will report on studies investigating reward processing and impulsivity in typically developing youth and youth at various clinical risks for SUD (i.e. childhood disruptive behavior disorders and familial SUD) before exposure to abusable substances.
Methods: We examined the interaction between reward processing and impulsivity in 2 cohorts: 1) 47 drug naïve pre-adolescents aged 8-13 divided in a) low risk group (LR, N = 14) - participants with ADHD only; b) high risk group (HR, N = 18) - participants with ADHD and familial SUD, and c) controls (N = 15). All participants performed a novel Anticipation, Conflict, Reward (ACR) imaging task; 2) 472 drug-naïve youth aged 13-14 selected from a larger cohort of typically developing youth (e.g. IMAGEN study). These youth were further characterized by measures of impulsivity (IMP) and sensation seeking (SS) in 4 groups: High IMP/High SS (N = 205), High IMP/Low SS (N = 59), Low IMP/High SS (N = 149) and Low IMP/Low SS (N = 60). All subjects performed the Monetary Incentive Delay (MID) imaging task.
Results: Higher activation in the brain reward system (e.g. insula, OFC, VS) during reward outcomes of the ACR task was documented in the HR group vs. LR and control groups whereas controls showed higher activation in the behavioral control system (inferior frontal gyrus, caudate, and anterior cingulate gyrus) compared to both LR and HR groups during the ACR flanker trials.
For the typically developing sample ANOVA analyses showed significant main effect for IMP and significant IMP by SS interaction in the left VS during the cue component of the MID so that High IMP/Low SS group showed the lowest activation vs. the other 3 groups. Further analyses will examine if differences in brain activation at baseline may be linked to drug experimentation at age 16.
Conclusions: These preliminary results suggest that the activation in the brain reward system varies among drug-naive youth in relation to impulsivity, sensation seeking as well as clinical risk factors for SUD (e.g. childhood ADHD and familial SUD). Possible clinical implications are related to identification of biological predictors of drug experimentation/use in typically developing youth and selecting optimal pharmacological treatments for children with ADHD with and without familial predisposition for SUD.
Disclosures: Part 1: DSMB member for Lundbeck- honoraria.

Panel
45. Ontogeny of Autism: Identification of very Early Signs of Autism Spectrum Disorder in Humans and Mice

45.1 Onset of ASD and Behavioral Signs in the First Year of Life

Sally Ozonoff

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Background: Autism Spectrum Disorder (ASD) is diagnosed at a mean age of 4 years in the United States, but studies demonstrate that behavioral signs of ASD can be detected reliably by the first birthday. Some of the earliest indicators of ASD documented in infants who are later diagnosed with the disorder include reduced interest in faces, increased interest in objects and atypical looking patterns at objects, reduced vocalizations and babbling, and limited shared affect.
Methods: Participants were 25 infant siblings of children with ASD, all of whom were later diagnosed with ASD themselves, and 25 gender-matched younger siblings of children with typical development. A prospective longitudinal design was used and participants were evaluated at 6, 12, 18, 24, and 36 months of age. Infants were video-recorded during an object-based interaction with an examiner. Frequencies of gaze to faces, social smiles, and directed vocalizations were coded by researchers unaware of risk or outcome group.

Results: A Generalized Estimating Equations approach was used to analyze developmental trajectories for the dependent variables. The frequency of gaze to faces, shared smiles, and vocalizations to others were highly comparable between groups at 6 months of age, but significantly declining trajectories over time were apparent in the group later diagnosed with ASD, while the trajectories of the control group significantly increased with age. Group differences were significant by 12 months of age and widened over time.
Conclusions: This work demonstrates that atypical signs of development, particularly in the realms of social engagement and vocalization frequency, are present prior to the first birthday in infants later diagnosed with ASD. These findings may lead to the development of more sensitive methods for diagnosis in infancy, which may in turn permit the application of preventive therapies and earliest intervention to lessen the symptoms of ASD or even prevent onset of the full syndrome.
Disclosures: Nothing to Disclose.

45.2 Vocal Development in Infants and Children with ASD

D. Kimbrough Oller

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Background: Kanner himself posited vocalization anomalies in autism. Yet descriptions of ASD speech often seem contradictory—pitch is said to be too high and on other occasions too low, with pitch too variable or too monotonous. At Univ. of Memphis, and in collaboration with other universities and the LENA Research Foundation (LRF), we have developed descriptions of vocalizations of infants/children with ASD designed to clarify the diverse vocal patterns in ASD as well as providing a basis, especially an automatic basis, for differentiating children with ASD from typically developing (TD) or language delayed (LD) infants and children.
Methods: We use home movies, laboratory audio-video, and all-day audio recordings with a battery-powered, wearable recorder. The materials come from our own laboratories, and from collaborations with individuals at other universities and with the LRF, which maintains a database of 100,000 hours of all-day recordings. In many instances we observe recordings and code the vocalizations within an infraphonological framework. In other cases we use automated tools, some from the LRF, some developed by us or our collaborators.
Results: Young ASD children showed high proportions of vocalizations with non-normal phonation. Some were high in pitch and some low. Variability of phonation then was the key to differentiation. The finding also suggested that vocalizations of the ASD children were less mature than those of TD children, because younger TD children also show high variability in phonation. A subsequent study with over 230 children at 8-48 months included over 20,000 hours of recording conducted in homes, and over 3 million automatically identified child utterances. Automated analysis showed that 12 acoustic parameters could differentiate ASD from TD with 86% accuracy, and could predict age in the TD group accounting for $>50\%$ of variance. Principal components analysis revealed that the primary factors predicting age and group classification were related to well-formed syllabification: production of syllables within a duration range found in mature speech, phonation as occurs in speech, well-timed, rapid transitions between consonant-like elements and vowel-like elements, and speech-like spectral entropy within syllables. We then confirmed that ASD children show late onset of well-formed syllables in a study of home movies with 9-12 month olds later diagnosed with ASD. In most recent results we are attempting to develop a workable clinical assessment based on the automated procedures, an approach that focuses on prediction of language development rather than age. Results suggest much better performance within TD, ASD and LD groups than in prior age-based modeling.
Conclusions: Research is rapidly developing new perspectives on vocalization in ASD. Based on the results, we anticipate within the next decade that diagnostic vocal markers will become practical for infants within the first year.
Disclosures: Nothing to Disclose.

45.3 Modeling Social Communication Deficits in Mouse Models of Autism

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Background: Autism Spectrum Disorder can include impaired abilities to express emotions or respond to the emotions of others. Speech provides a mechanism for expressing emotions, by both what words are spoken and by the melody or intonation of speech (prosody). Some aspects of autistic speech incorporate both features of the diagnosis: repetitive behaviors, deficits in the ability to express emotions and in communication. For instance, some children with autism repeat the use of certain sounds, syllables or words, more than typically developing children, or fail to use appropriate patterns of intonation to communicate. Prosodic features of mouse ultrasonic vocalizations (USVs), indicated by changes in frequency and amplitude, also convey social and emotional information. Dams retrieve pups that emit separation calls, females approach males emitting solicitous calls, and mice can become fearful of a cue associated with the vocalizations of a distressed conspecific. Because acoustic features of mouse USVs respond to drugs and genetic manipulations that influence reward circuits, USV analysis can be employed to examine how genes influence social motivation, affect regulation, and communication. This talk will focus primarily on a detailed spectrographic evaluation of ultrasonic vocalizations emitted during infant social isolation and dyadic interactions in inbred, outbred mice and three synapsin mutant lines.
Methods: At postnatal days 2, 4, 6, 8 and 12, spontaneous movements and ultrasonic vocalizations were recorded (3-min) in response to social separation (from mother and siblings). At three months of age, male mice were socially isolated for one hour before the test and social behaviors

and ultrasonic vocalizations were recorded (3-min-session) during an interaction between a male mouse and a female in estrous (C57BL/6J).
Results: Each mouse strain emits distinct pattern of vocalizations associated with different levels of social investigation throughout lifespan. In the same social contexts, deficits in spontaneous behaviors and ultrasonic vocalizations measured during the first two postnatal weeks of age and at adulthood were detected in SynI, SynII mutant mice. By contrast, SynIII mice did not show deficits at either infancy or adulthood.
Conclusions: Overall, motor and social communication deficits observed in SynI and Syn II mutant mice support the view that these genes are involved in the expression of autistic-like behavioral traits and identify these mutant lines as useful experimental models of ASD and epilepsy. These data support the role of vocalizations as a valuable tool for identifying alterations in several mouse models of human neurodevelopmental disorders, starting from those in which deficits in social communication are a primary core symptom e.g. autism spectrum disorders.
Disclosures: Nothing to Disclose.

45.4 Structure and Function of Neonatal Social Communication in a Genetic Mouse Model of Autism

Noboru Hiroi

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Background: Babies with incipient autism spectrum disorder (ASD) and pups of genetic mouse models of ASD exhibit atypical vocalizations. However, the precise sequence structure of and functional impacts of such atypicalities on social communication between babies and mothers have not been isolated and determined.
Methods: We used vocal call data from a genetic mouse model of ASD to test the hypothesis that call type sequences have functional impacts on maternal approach. Calls were recorded from Tbx1 heterozygous and wild-type littermates at postnatal fay (P) 8 or P12 during 5-min maternal separation. The sequence structure of vocal calls was analyzed by Shannon entropy analysis, Markov model, and Sparse Partial Least Square Discriminant Analysis (sPLS-DA). The functional impact of call sequences on maternal approach was examined by playing back wild-type, heterozygous and randomized wild-type calls to C57BL/6J mothers.
Results: Tbx1 heterozygous pups emitted significantly fewer complicated call types, compared to wild-type pups at P8; vocal calls considerably declined thereafter for wild-type pups so that the two groups were indistinguishable for any call type by P12. Wild-type pups emitted longer complicated call types than heterozygous pups at P8. Wild-type pups exhibited decreased lengths of these calls by P12 so that the two genotypes no longer differed at that time. Wild-type and heterozygous pups did not differ in the pitch or peak amplitude of vocal calls. Shannon entropy analysis showed that pups non-randomly chose call types to emit two, three and four successive calls. A sequence structure of calls exists in normal mouse pups and Tbx1 heterozygous pups have a higher degree of non-random sequence. Markov modeling determined the predominant sequences of calls of wild-type and heterozygous pups. Wild-type pups more frequently connected complicated call types than heterozygous pups. In contrast, heterozygous pups more frequently formed connections among simple call types than wild-type pups. A Sparse Partial Least Square Discriminant Analysis (sPLS-DA) showed that wild-type pup call sequences were more individually variable along the two identified components, compared to call sequences of heterozygous pups. Finally, C57BL/6J mothers stayed longer in the tube from which wild-type calls were presented compared to that from which no call was presented; heterozygous calls did not induce such a preference. When a randomized wild-type sequence was presented, mothers did not show a preference for the sound tube compared to the no-sound tube.
Conclusions: Our data suggest that an ASD risk gene has a negative impact on social communication with mothers due to atypical sequence structures of vocalizations during the neonatal period.
Disclosures: Nothing to Disclose.

Panel
46. Using Neural Connectivity Biomarkers in Major Depressive Disorder (MDD) to Identify Subtypes and Predict Treatment Response

46.1 Abnormal Resting State Functional Connectivity as a Biomarker for Major Depressive Disorder (MDD) and Clinical Subtypes

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Background: Major depressive disorder (MDD) is a chronic, serious psychiatric illness that has been linked to abnormal communication among distributed brain systems, as assessed via resting state functional connectivity (RSFC). However, differences between studies in methods and analytic approach have made it difficult to unify this research. In addition, heterogeneity in the presentation of MDD suggests the presence of clinical subtypes with distinct neurobiological profiles, which have rarely been targeted by previous RSFC studies. Unifying the evidence for abnormal RSFC in MDD through meta-analysis and extending this research to identify potential biomarkers of MDD subtypes may help to clarify the nature of depression and inform more precise delivery of treatment.
Methods: First, a coordinate-based spatial meta-analysis was conducted of functional magnetic resonance imaging (fMRI) studies comparing seed-based RSFC of 556 individuals with MDD to 518 healthy controls. Second, in a new and independent sample, voxelwise RSFC of seed regions in the amygdala and nucleus accumbens (NACC) was compared between 103 individuals with MDD and 109 healthy controls. Third, in a subset of the above sample, potential biomarkers of the anhedonic subtype of depression were investigated by testing the association between anhedonic symptoms of depression and voxelwise RSFC of amygdala or NACC within the MDD group.

Results: The meta-analysis revealed abnormal RSFC in MDD between frontoparietal and limbic brain systems. Results of empirical analyses comparing MDD with healthy controls were consistent with the meta-analysis, showing weakened RSFC between frontoparietal systems and both NACC and amygdala. Within the MDD group, increased severity of anhedonia predicted weakened RSFC between NACC and temporoparietal and insular regions, and increased RSFC between amygdala and medial prefrontal regions.
Conclusions: Meta-analytic results and new empirical evidence both suggest that MDD is characterized by abnormal RSFC among brain systems involved in processing and regulating emotion. Critically, additional patterns of abnormal RSFC were detected as a function of anhedonia between brain regions implicated in processing reward and positive emotion and those involved in processing salience; or between regions implicated in processing fear and negative emotion and those involved in self-referential processing. Together, these results suggest that anhedonia may be distinguished by particular patterns of imbalanced RSFC, and RSFC may be a useful tool for identifying biomarkers of MDD subtypes.
Disclosures: Nothing to Disclose.

46.2 Cognitive Control Performance and Salience Network to Cognitive Control Hyperconnectivity Predict Depression Relapse in Early Adulthood

Scott Langenecker

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Background: A practical gap exists in our understanding of major Depressive Disorder. While the neuroimaging, affective, and cognitive markers associated with acute state of illness are well known, and emerging work on prediction of treatment response is present, there is limited work that is directed toward understanding early course risk features using the neuroimaging milieu. There are significant advantages to determining and refining neuroimaging, performance, and psychosocial markers and features that are predictive of risk for illness recurrence. Identifying those at high risk is a way to direct limited resources toward secondary prevention that is aimed at reversing or mitigating these risk features.
Methods: This is a two-site study of young adults between ages 18 and 23 who report 1-3 previous episodes of MDD and are currently remitted from MDD. Resting state functional connectivity and functional MRI during completion of a Parametric Go/No-go test were used to predict relapse of illness between the index assessment and one year follow-up, conducted with the Longitudinal Interview Follow-Up Evaluation (LIFE). Performance on the PGNG, activation during correct rejections (fMRI), and seed-based connectivity from the left amygdala, subgenual cingulate, and dorsolateral prefrontal cortex were used to evaluate differences at the baseline measurement that might predict risk for recurrence in the subsequent year. Recurrence was defined as experiencing a MDE episode ($n = 19$) and/or reinitiaton of treatment ($n = 2$), and resilience was the absence of these ($n = 29$). 29 Healthy comparison (HC) with no emergence of illness were used for comparison/reference.
Results: There were a number of significant predictors of relapse from baseline to the naturalistic follow-up point 1 year later. Reaction Time on the PGNG (with age) predicted 26% of the variance in relapse, ($X = 9.34$, $p = .009$). Decreased activation during correct rejections in the relapse group relative to both the HC and resilient groups were present in right inferior frontal gyrus (predicted 42% of variance, $X = 14.33$, $p = .001$). Increased connectivity from the left subgenual cingulate to bilateral anterior middle frontal gyrus (MFG) and left anterior nucleus (AntNuc) of the thalamus was higher in the relapse group relative to both the resilient and HC groups ($F = 14.98$, $p = .000006$) accounting for 71% of relapse status ($X = 26.0$, $p = .00001$, LMFG Wald $= 5.16$, $p = .023$, LAntNuc Wald $= 1.78$, $p = .182$, RMFG Wald $= 3.87$, $p = .049$; 84% prediction accuracy for resilient, 80% prediction accuracy for relapse). An omnibus model with all significant predictors resulted in two predictors, activation during rejections, and left subgenual cingulate to rMFG connectivity ($X = 25.0$, $p = 0.000004$, 73% R2, 100% prediction in both groups).
Conclusions: Multimodal imaging and performance markers can enhance predictive capacity for longitudinal illness course in MDD. These techniques can aid in identifying higher risk groups and may lead to earlier and more targeted prevention trials in the area of secondary prevention. Secondary prevention strategies have the distinct advantage over primary prevention in that the risk ratio is 50% for recurrence. Identifying the high risk group can focus limited resources and can also define primary domains (cognitive control) and inter network relationships (salience network-cognitive control).
Disclosures: Nothing to Disclose.

46.3 Functional Connectivity Predictors of Response to Behavioral Activation Therapy for Depression

Gabriel Dichter

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Background: Despite the heterogeneous symptom presentation and complex etiology of Major Depressive Disorder (MDD), functional neuroimaging studies have shown with remarkable consistency that dysfunction in mesocorticolimbic brain systems are central to the disorder. Relatively less research has focused on the identification of biological markers of response to antidepressant treatment that would serve to improve the personalized delivery of empirically-supported antidepressant interventions. To this end, the purpose of the current study was to evaluate functional connectivity predictors of response to Behavioral Activation Therapy for Depression (BATD), an empirically validated psychotherapy modality designed to increase engagement with rewarding stimuli and reduce avoidance behaviors.
Methods: Thirty-eight outpatients with MDD and twenty matched controls completed functional MRI scans after which the MDD group received an average of 12 sessions of

BATD, and the average decline in BDI scores was 11, a statistically significant ($p < .0001$) and clinically meaningful response. The scan session included a resting-state scan (five minutes with eyes open) as well as task-based scans using the Monetary Incentive Delay (MID) task and a positive emotion regulation task. Heart rate and respiration were collected during scanning to regress out these sources of physiologic noise. Hierarchical linear modeling analyses were used to evaluate the predictive effects of pre-treatment functional connectivity on response to BATD measured by BDI scores collected every two weeks during treatment.
Results: Resting-state connectivity revealed that response to treatment was predicted by pre-treatment connectivity of the right insula with the right middle temporal gyrus. Task-based connectivity using Psychophysiological Interaction Analyses (PPI) revealed that response to treatment was predicted by increased endurance of nucleus accumbens connectivity with a number of cortical regions during the MID task, whereas response to treatment was predicted by increased connectivity between the anterior insula with clusters in the thalamus and left caudate during the emotion regulation task.
Conclusions: Findings highlight the critical role of meso-corticolimbic connectivity in predicting which patients with MDD respond to a given treatment modality. Future research will evaluate the capacity of these metrics to predict response to different antidepressant modalities to inform prospectively treatment decisions for individual patients with MDD. Additionally, future research will likely need to include genetic, epigenetic, or neuroendocrine markers to improve algorithms that predict patient-specific response to different antidepressant modalities.
Disclosures: Nothing to Disclose.

46.4 Differences in Resting State Functional Connectivity Between Patients with Treatment-Resistant Versus Treatment-Responsive Depression

Paul Holtzheimer

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Background: Treatment-resistant depression (TRD) is a significant public health concern. The medial prefrontal cortex (BA10) has been implicated in the neurobiology of depression and antidepressant treatment response. We hypothesized that BA10 functional connectivity would differ between treatment-responsive (RESP) and TRD patients actively taking antidepressant medications.
Methods: Resting state functional MRI (rs-fMRI) was performed in 22 TRD and 22 matched RESP patients, aged 22-70 years. All patients were taking adequate doses of at least one antidepressant medication and had no other clinically significant psychiatric or neurological comorbidities. The CONN toolbox and SPM12 were used to perform univariate, seed-to-voxel functional connectivity analyses.
Results: Compared to the RESP group, TRD patients showed decreased functional connectivity of left BA10 with premotor, dorsal anterior cingulate, and dorsal frontal cortices (FDR $\leqslant 0.05$). Secondary analyses identified additional differences in connectivity of the right dorsal anterior cingulate and right putamen between the groups.
Conclusions: In TRD patients, despite adequately dosed antidepressant treatment, BA10 functional connectivity with several regions involved in the neurobiology of depression was weaker compared to connectivity in treatment responders. This may represent an imaging biomarker of TRD or an "uncorrected" abnormality in TRD patients. Either way, this difference may indicate a potential target for further treatment development (e.g., altering BA10 FC directly via focal brain stimulation).
Disclosures: Part 1: St. Jude Medical Neuromodulation (consultant), **Part 4:** Cervel Neurotech; Otsuka Pharmaceuticals.

Panel
47. Combining Imaging Modalities in Understanding and Treating Stress-related Disorders

47.1 Disrupted Neural Synchrony During Social Interaction in Borderline Personality Disorder

Edda Bilek

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Background: Recent developments in social neuroscience provided first linked neuroimaging data sets from subjects interacting live using a two-person fMRI environment (hyperscanning), which enables synchronized data acquisition and interaction-based fMRI tasks. While previous research mostly emulated social interaction (using photographs, recorded videos or computerized partners), these new data give insight into the coupling of two brains working together. Our work examining healthy subjects during cooperation revealed a coupling of specific neural systems, indicative of the flow of information between brains, that is unique to subjects interacting live, only present during the interaction, and based on right temporo-parietal junction (rTPJ) function of both subjects.
We investigate this pair marker of neural coupling in a Borderline Personality Disorder (BPD) sample performing identical social-interactive tasks, since difficulties in social contexts, interaction, and associated emotions describe the core of BPD diagnosis.
Methods: Subjects were examined in pairs either formed from one patient with BPD and one healthy subject, or from two healthy subjects. Pairs completed a cooperative Joint Attention task during hyperscanning, which allowed for simultaneous fMRI scan and task presentation, as well as live video streaming of task partner and interaction-based paradigms.
Recently, we reported a data analysis routine fit to identify unique properties of truly interacting subjects, which is now applied to the current data set. The analysis included a group independent component analysis of fMRI data, followed by calculation of neural coupling indices as a measure cross-brain information flow within interacting pairs of subjects. True interaction is assessed by means of permutation-based tests of coupling indices against

permutated data sets from non-interacting (i.e., sequentially scanned) data pairs. Finally, group differences are assessed.
Results: Neural coupling differs significantly depending on health status, and is higher in healthy pairs than in the patient subsample (HC > BPD). Only healthy pairs exhibit increasing neural coupling through familiarity with a task partner, i.e. learn from experience. Coupling of non-interacting (sequentially scanned) individuals is the same across subsamples.
Conclusions: Neural coupling represents a neural marker that is sensitive for health status in BPD even during basal social interaction. Pairs involving BPD exhibit considerably less synchronization of relevant brain systems than healthy pairs, indicating impaired flow of social information. This is not due to generally lower brain activation in this region or missing engagement in the task.
While this study confirms prior conclusions regarding synchronization of interacting brains, and expands validation evidence to clinical subgroups, future studies need to investigate the causal status of the described indices. For example, stability and treatment factors are of high interest in BPD; similarly generalizability to other psychiatric disorders involving social impairment as well as validity in varying social-behavioral contexts need further focus in research. However, online second-person approaches such as fMRI hyperscanning are mandatory.
Disclosures: Nothing to Disclose.

47.2 Amygdala Neurofeedback Modulates Amygdala-VMPFC Connectivity in Healthy Participants and Borderline Patients

Christian Schmahl

Central Institute of Mental Health, Mannheim, Germany

Background: Neurofeedback training by functional magnetic resonance (fMRI) signals has the potential of enabling voluntary control over brain processes and treating impaired brain circuits in a directed closed-loop intervention. Providing patients with neurofeedback from the amygdala might be a way to normalize affected prefrontal-limbic brain circuits in mental disorders of emotion dysregulation, such as borderline personality disorder (BPD).
Methods: Healthy females (N = 32) were randomized in two age-matched groups with same group size and eight female BPD inpatients were recruited. Participants viewed aversive pictures while receiving fMRI. One group of healthy subjects and BPD patients received amygdala feedback, the second healthy group was provided with feedback from a control region. Healthy participants completed a single session and BPD patients participated in four sessions. Psychophysiological interaction (PPI) analysis was used to investigate functional connectivity. Subjects rated stimulus arousal after the session outside the MRI suite. Ratings on dissociation (DSS-4) and difficulties in emotion regulation (DERS) were collected from BPD patients on during each session.
Results: Voluntary amygdala down-regulation by amygdala neurofeedback was associated with increased connectivity of the right amygdala to the ventromedial prefrontal cortex (vmPFC) in healthy subjects, as evidenced by a significant group interaction. Furthermore, BPD patients were able to alter functional amygdala-vmPFC connectivity towards the healthy pattern with repeated sessions. Consistent with a transfer of emotional information, picture-associated arousal was positively correlated with increases in amygdala-vmPFC connectivity. Neurofeedback training was associated with improvements in self-assessments of dissociation and emotional awareness in patients.
Conclusions: These results show demonstrate that key neural networks of emotion regulation can be changed with amygdala neurofeedback down-regulation. This is initial evidence for a potential benefit of amygdala neurofeedback for the therapy of BPD patients. A randomized controlled trial study with BPD patients is needed.
Disclosures: Nothing to Disclose.

47.3 Real-time fMRI Emotion Regulation Training in PTSD: Altered Amygdala Activity and Connectivity as a Function of Depersonalization

Ruth Lanius

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Background: Real time fMRI (rtfMRI) neurofeedback has increasingly been used as a noninvasive tool to better understand the neurobiological correlates of psychiatric disorders, as it allows for precisely localized brain regions to be self-regulated through signal feedback. Emerging evidence points towards unique amygdala activity and resting state functional connectivity among dissociative subtype and non-dissociative PTSD patients. The objective of the present study was to examine amygdala activity an amygdala-medial prefrontal cortex connectivity during real-time fMRI emotion regulation training in PTSD patients with various levels of dissociative (depersonalization/derealization) symptomatology.
Methods: BOLD signal from the amygdala was displayed to PTSD patients (n = 10) while viewing words associated with their past traumatic experiences. Participants were asked to regulate their emotional state by decreasing their amygdala activity. Functional scans were acquired, and regions-of-interest (ROIs) processed in real-time via Turbo-Brain Voyager. Brain activity was then relayed back to the participant in the scanner as a simple thermometer during three runs. Subsequently, patients were asked to regulate their amygdala activity in the absence of neurofeedback during a transfer run. Patients' ability to self-regulate was also evaluated as a function of state dissociation, as previous evidence has suggested that amygdala activity would be attenuated and amygdala-medial prefrontal cortex connectivity would be altered in the trials in which state dissociation occurs.
Results: Within PTSD patients, we observed initial increased amygdala activation in response to the trauma-related words, when conducting offline analyses. Over trials of rtfMRI emotion regulation training, we found decreased amygdala activation, which was sustained during the transfer run. Critically, correlational analyses revealed a significant relationship between self-reported state dissociation and activity in the amygdala, within dissociative

subtype PTSD patients. Moreover, altered amygdala connectivity patterns and emotion regulation capacities as a function of dissociative symptomatology were observed.
Conclusions: This study has the potential to develop promising non-invasive treatments for different PTSD phenotypes.
Disclosures: Nothing to Disclose.

47.4 Identification of a Causal Pathway for Amygdala Control in Humans and Abnormalities in PTSD

Amit Etkin

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Background: Anatomical tracing in non-human primates has revealed largely uni-directional pathway from the ventrolateral PFC (vlPFC) to amygdala. These areas often co-activate in human neuroimaging studies of emotion regulation but, to date, no causal evidence of communication between these brain areas has been established in humans. Thus, we do not know whether and how the vlPFC and amygdala causally interact.
Methods: To determine the directional causal influence of vlPFC activation on the amygdala and to investigate possible abnormalities in this pathway in PTSD, we stimulated the right vlPFC (or a control site in the superior frontal gyrus; SFG) first in healthy participants ($N = 24$) and then in patients with PTSD ($N = 22$, DSM-IV-TR, CAPS). Stimuli were delivered as single transcranial magnetic stimulation pulses (spTMS) using MRI-based neuronavigation, concurrent with interleaved acquisition of fMRI BOLD.
Results: Among healthy participants, vlPFC stimulation resulted in deactivation (ie inhibition) of the right amygdala (FWE $p < .05$), while stimulation of the SFG did not affect amygdala activity. Similarly, vlPFC stimulation resulted in greater amygdala inhibition in healthy participants than patients with PTSD (FWE $p < .05$ for group and group x stimulation site interaction). We found no evidence that vlPFC stimulation resulted in medial prefrontal activation, suggesting that amygdala inhibition occurred through a direct vlPFC-amygdala pathway. The vlPFC and amygdala were also disconnected in patients during a resting-state scan, relative to healthy participants. Patients' failure to inhibit the amygdala correlated as well with PTSD severity, with a greater failure in the most severe patients. Finally, failure in patients of vlPFC stimulation to inhibit the amygdala correlated with their failure to do so in a reappraisal-based emotion regulation task in the absence of stimulation.
Conclusions: We provide the first evidence for a causal and inhibitory pathway for amygdala control in humans, and demonstrate its disruption in PTSD – disruption that correlates with both their clinical severity and deficits in emotion regulation. These data have implications for understanding normal mechanisms in the human brain by which amygdala activity is regulated and identify an important, and never previously examined, brain pathway for potential treatment of emotion regulatory abnormalities in PTSD.
Disclosures: Nothing to Disclose.

Panel
48. Genomes and Cells: New Models for Target Discovery and Validation

48.1 Probing Neural Phenotype in Macrocephalic Autism

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Background: Autism spectrum disorders (ASD) comprise a group of complex neurodevelopmental disorders that affect more than 1% of children in the United States. ASD is characterized by impaired social interaction and limited and repetitive interests and behavior at its core. Family history and twin studies suggest that, at least in some cases, these disorders share genetic roots (Piven et al., 1997; Ronald et al., 2006). Mounting evidence proposes that heritable and de novo genetic variation plays a significant role, but these studies also demonstrate striking genetic heterogeneity (Garber, 2007; Marchetto et al., 2010; Sanders et al., 2012). Neuropathological imaging and gene expression studies of postmortem brains from ASD patients have revealed disruption of developmental and proliferation gene networks (Chow et al., 2012; Voineagu et al., 2011). One relevant observation in ASD pathophysiology has been the occurrence of macrocephaly and altered growth trajectory with early overgrowth and later normalization in a subset of affected individuals. (Courchesne et al., 2011a; Courchesne et al., 2003; Courchesne et al., 2001; Courchesne et al., 2005; Hazlett et al., 2011; Shen et al., 2013). The major impediment to testing ASD hypotheses is the lack of relevant animal and cell models.

Methods: We reprogrammed fibroblasts to generate induced pluripotent stem cells (iPSCs), neural progenitor cells (NPCs) and neurons from 8 ASD Individuals with early brain overgrowth and 5 non-ASD gender and age-matched controls with normal brain size.

Results: ASD-derived NPCs display increased cell proliferation due to dysregulation of a β-catenin/BRN2 transcriptional cascade. ASD-derived neurons display premature differentiation, reduced synaptogenesis and altered levels of excitatory and inhibitory neurotransmitters, leading to functional defects in neuronal networks. RNA expression analysis revealed that ASD-derived NPCs displayed significant enrichment for genes involved in brain development, whereas ASD-derived neurons displayed up- and down-regulation of genes related to extracellular matrix and cilium/axoneme, consistent with the observed synaptic dysregulation.
Conclusions: This work demonstrates that, in heterogeneous conditions such as ASD, the selection of subjects based on endophenotypes improves the power to detect biologically relevant pathway disruption that may help the development of novel therapies.
Disclosures: Nothing to Disclose.

48.2 Using IPSC Models to Identify Cellular Phenotypes Associated with Autism and Schizophrenia

Ricardo Dolmetsch

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Background: Human neurons derived from Induced Pluripotent Stem Cells (IPSCs), can provide important clues about the underlying cellular and molecular basis of complex psychiatric disorders like autism and schizophrenia. IPSCs can be developed from patients with penetrant mutations that lead to syndromes that include autism and schizophrenia. Cells can also be developed from patients with less penetrant mutations that are associated with idiopathic disease though these are presently difficult to analyze. In addition, IPSCs can be engineered to express mutant alleles that are associated with disease. These tools are helping us identify cellular and molecular phenotypes associated with psychiatric disease.
Methods: We have developed automated methods of generating a neurons from iPSCs to model human neuropathologies *in vitro*. Our industrial approach is allowing us to generate cells from many patients in parallel with the quality and reliability that is required to identify new drug targets and develop new drugs. We have automated the process of making a subset of neurons and brain organoids.
Results: We have identified both biochemical and molecular phenotypes associated with monogenic forms of autism like Timothy Syndrome, Phelan-McDermid Syndrome and 22q11 deletion syndrome. We are using these phenotypes to identify new drug targets as well as to screen for new drugs and have identified some candidate molecules that may be helpful for developing future treatments. We have also embarked on a project to study gene expression and identify phenotypes associated with less penetrant disease risk alleles.
Conclusions: IPSC-derived neurons are an important new tool for identifying cellular phenotypes. They can be useful for identifying cellular phenotypes that may provide insights into the underlying basis of psychiatric diseases. We have improved many of the methods for making and analyzing iPSC derived neurons making them a more reliable method for identifying new targets and developing new drugs. However there are still many limitations associated with IPSC based drug discovery including the slow maturation of the cells, the difficulty of identifying cellular phenotypes that are important clinically and the lack of reliability of the assays.
Disclosures: Part 1: Novartis Institutes for Biomedical Research, **Part 5:** Novartis.

48.3 Ectopic Expression of Peripheral Ion Channel Genes in the Central Nervous System Underlies an Autism Spectrum Disorder

Brady Maher

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Background: Genome-wide association studies (GWAS) have identified a number of loci associated with risk for SZ and several of these risk variants are located within introns of Transcription Factor 4 (TCF4; E2-2, ITF2). In addition, autosomal dominant mutations in TCF4 result in Pitt-Hopkins Syndrome (PTHS), a rare neurodevelopmental disorder characterized by a spectrum of symptoms including hyperventilation, seizures, autistic behaviors, and intellectual disability. Currently, the molecular mechanisms and underlying pathophysiology responsible for these two disorders are not understood. Our goal is identify and validate therapeutic targets identified by modeling PTHS in a cell-autonomous rodent model.
Methods: To model PTHS we altered the in utero expression of TCF4 by transfecting neuroprogenitor cells that give rise to layer 2/3 pyramidal cells in the rat medial prefrontal cortex by in utero electroporation (IUE). We knockdown TCF4 expression using shRNA and Crispr/Cas9 constructs that target independent sequences within the TCF4 transcript or gene, respectively. Resulting physiological phenotypes were assayed using whole-cell electrophysiology. A novel molecular profiling technique that combines IUE with translating ribosome affinity purification (iTRAP) was used to identify candidate ion channels underlying cellular phenotypes. Molecular phenocopy and pharmacological rescue were used to validate candidate target genes.
Results: Knockdown of TCF4 produced a severe and consistent reduction of action potential (AP) output ($p < 0.0001$) and an increase in the resting membrane potential ($p < 0.0001$) compared to control cells. Crispr/Cas9 mutation of TCF4 resulted in a similar deficit in AP output compared to cells expressing an empty Crispr/Cas9 vector ($p < 0.0016$). The decrease in AP output was attributed to a significant increase in the afterhyperpolarization (AHP, $p < 0.0003$). Using iTRAP, ribosomes from transfected layer 2/3 neurons were affinity purified and the associated RNA was processed for expression analysis on prefabricated 384-well plates that contained primers for all known ion channels in the rat genome. Knockdown of TCF4 resulted in a significant increase (>2-fold) in the expression of KCNQ1 ($p < 0.005$) and SCN10a ($p < 0.01$) compared to control transfected cells. Application of antagonists to KCNQ1 (UCL2077 $p = 0.0002$; JNJ303 $p = 0.0084$) or SCN10a (A-803367; $p < 0.04$) significantly rescued AP output in TCF4 knockdown cells but had no effect on control cell firing. Furthermore, molecular phenocopy of reduced AP output and increased RMP was observed when recombinant SCN10a was expressed in control cells ($p < 0.0006$).
Conclusions: Our results describe a novel approach to model psychiatric risk at the cellular level. We model an autism spectrum disorder in cortical neurons and identify physiological phenotypes associated with deficits of intrinsic excitability. Using a novel molecular profiling technique (iTRAP) we identify two candidate genes that are associated with the observed phenotypes. These target genes were validated using pharmacological rescue and molecular phenocopy. We propose that ectopic expression of peripheral ion channel genes in the central nervous system may underlie cognitive deficits observed PTHS patients.
Disclosures: Nothing to Disclose.

48.4 Modeling Major Mental Disorders Using Patient-derived iPSC and Humanized Mouse Models

Hongjun Song

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Background: Psychiatric disorders, such as schizophrenia, are disabling brain disorders and development of rational therapeutics based on knowledge of the etiology and pathogenesis is critically needed. Patient-derived induced pluripotent stem cell (iPSC) has been proposed as a promising model for understanding disease mechanism and for drug discovery. However, there are major gaps in our knowledge on whether disease modeling and drug testing using iPSC-derived developing immature cell types in dish would provide predictive validity for *in vivo* pathophysiology and drug efficacy in adulthood.
Methods: We used iPSCs derived from psychiatric patients with a mutation in Disrupted-in-Schizophrenia 1 (DISC1) as an example for mechanism-based drug discovery to correct pathophysiology-relevant cellular phenotypes *in vitro*, followed with *in vivo* testing using a humanized animal model carrying the same mutation in adulthood.
Results: Our previous study using isogenic iPSC lines has revealed that mutant DISC1 causes defects in the presynaptic synaptic function and gene expression. We found that multiple phosphodiesterases (PDEs) were elevated in forebrain neurons with the DISC1 mutation, and rolipram, a PDE4 inhibitor, rescued the presynaptic deficit of mutant neurons with little effect on isogenic normal neurons. Similar to iPSC-derived developing cortical neurons *in vitro*, adult DISC1-KI mice exhibit aberrant gene expression, including elevated expression of some PDEs, in both cortex and hippocampus, as well as increased pair-pulse facilitation, indicating reduced presynaptic release probability. Furthermore, DISC1-KI mice exhibited behavioral deficits, some of which can be rescued by rolipram treatment.
Conclusions: Our study provides a proof-of-principle example for drug discovery-in-dish with predictive validity for efficacy in the animal model at the adult stage and has significant implications for application of patient-derived iPSCs for personalized medicine.
Disclosures: Nothing to Disclose.

Panel
49. Studies of Stress Identify Novel Signal Transduction and Epigenetic Antidepressant Targets

49.1 REDD1/mTORC1/S6K1 Signaling and Synapse Formation in the Pathophysiology and Treatment of Depression

Ronald Duman

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Background: Major Depressive Disorder (MDD) affects nearly one fifth of the population and is the second leading cause of disability worldwide. Although the precise molecular mechanisms underlying MDD remain largely unknown, our lab has recently demonstrated an important role for the mechanistic target of rapamycin complex 1 (mTORC1)/S6 Kinase 1 pathway in mediating stress-related behavior and rapid antidepressant responses. The mTORC1/S6K1 pathway is a critical regulator of protein synthesis, cell growth and cytoskeletal rearrangement, and inhibition of this pathway could contribute to the atrophy of neurons seen in cortical and limbic structures in depressed patients and in rodent stress models. Our recent studies have extended this work in two important areas.
Methods: We use a combination of molecular, cellular and behavioral approaches to examine the role of REDD1 and S6K1 in the regulation of mTORC1 and antidepressant synaptic and behavioral responses.
Results: First, we have identified an up-stream negative regulator of mTORC1, referred to as REDD1 (regulated in development and DNA damage responses 1) that is induced by chronic stress and is increased in postmortem dlPFC of depressed subjects. Viral expression of REDD1 in the mPFC is sufficient to cause atrophy of neurons and depressive behaviors in rodent models. Conversely, mice with a deletion of REDD1 display a resilient phenotype in that chronic stress exposure does not cause atrophy of pyramidal neurons in the mPFC or anhedonic behaviors. Second we have investigated whether direct modulation of a key downstream element of the mTORC1 pathway, S6K1 is sufficient to control depressive behavior. S6K1 is decreased in postmortem PFC of depressed subjects and we have found that chronic stress decreases the phosphorylated and active form of S6K1 in the mPFC. Importantly, we show that infusion of a constitutively active S6K1 in the mPFC produces an antidepressant response, while infusion with a kinase-inactive S6K1 form demonstrated results in pro-depressive behavior. We have also found expression of S6K1 in primary cortical cultures increases neuronal complexity. Together, these data suggest that up-regulation of REDD1 in response to stress or aberrant protein synthesis through S6K1 contributes to the pathology of MDD.
Conclusions: The results also raise the possibility of developing drugs that inhibit REDD1 or that active mTORC1-S6K1 signaling as novel therapeutic targets for the treatment of depression.
Disclosures: Nothing to Disclose.

49.2 RGS4 Plays a Key Role in the Efficacy of Classical and Fast-Acting Antidepressants

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Background: Most major depression patients undergo treatment with monoamine targeting antidepressant drugs. As 30-40% of treated patients fail to show remission, there is a great need for development of more efficacious medications. Understanding the cellular adaptations induced by chronic stress may provide important information for the development of new drug targets or adjunct medications. Recent research efforts have been directed towards the development of fast acting antidepressants (ketamine-like drugs) that target glutamatergic transmission. We recently demonstrated that the GPCR modulator RGS4 plays a key role in adaptations to stress and

antidepressant drug efficacy. RGS4 controls the function of monoamine, opiate and other GPCRs via interactions with Galpha subunits, and it is expressed in several brain regions involved in mood, motivation and cognition. Using conditional knockout models, or viral mediated gene transfer, we manipulated RGS4 expression in the Nucleus Accumbens (NAc) and in the medial prefrontal cortex (mPFC) and demonstrated that in the NAc, RGS4 acts as a positive modulator of monoamine-directed antidepressants. Interestingly, in the mPFC, RGS4 acts a negative modulator of the actions of the NMDA receptor antagonist ketamine. We also investigated the key protein interactions and signal transduction events underlying these phenotypes.
Methods: Advanced genetic mouse models for brain region specific manipulations of RGS4 activity in the NAc and mPFC have been used along with the chronic unpredictable stress paradigm. For targeted RGS4 overexpression, we generated Adenoassociated Viruses (AAV) expressing RGS4. To knockdown RGS4 in the NAc and mPFC we used a line of floxed RGS4 mice, and stereotaxically infused AAV-Cre and AAV-CamkII-Cre (or AAV-EGFP) vectors in the targeted region. qPCR and western blot analysis have been used to monitor RGS4 expression following chronic stress or drug treatment. Finally, protein interactions in the mPFC were monitored using co-immunoprecipitation assays.
Results: RGS4 in the NAc plays a potent positive modulatory role in the actions of TCAs, SSRIs and SNRIs, and promoting RGS4 activity in this brain region improves antidepressant efficacy. On the other hand, RGS4 in the mPFC acts as a negative modulator of ketamine actions. Our work also reveals that adaptations in RGS4 expression in the mPFC (but not in the NAc) modulate responses to chronic stress. Using western blot analysis and co-immunoprecipitation assays we show that RGS4 is dynamically regulated in the mPFC by chronic unpredictable stress, whereas this molecule is also playing a negative modulatory role in the antidepressant effects of ketamine by forming complexes with components of the mTOR pathway as well as with metabotropic glutamate receptors.
Conclusions: The study demonstrates that the signal transduction modulator RGS4 plays a potent role in chronic stress vulnerability and modulates the actions of monoamine and fast acting antidepressants via distinct mechanisms. Interventions in the activity of RGS4 complexes may provide a new pharmacological target for the treatment of depression.
Disclosures: Nothing to Disclose.

49.3 Cell Type-Specific Epigenetic Reprogramming of the Fosb Gene Controls Depression-Related Behaviors

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Background: Genome-wide histone posttranslational modifications have been shown to underlie the pathophysiology of stress exposure, leading to the characterization of many highly relevant genes. We have found that Fosb gene expression is repressed in the nucleus accumbens of depressed human subjects and that this repression is associated with increased histone methylation at the Fosb promoter. To test the hypothesis that increased Fosb methylation is a causal mechanism underlying depression, it is necessary to manipulate the epigenome solely at this locus. While stress represses deltaFosB protein in total NAc, recent findings have demonstrated an increase in deltaFosB expression in the Drd2-expressing medium spiny neuron subtype following chronic social defeat stress. We found that this cell-type and locus-specific epigenetic manipulation of FosB expression is sufficient to regulate depressive behaviors. The role of engineered transcription factors are a novel tool to study the function of epigenetic reprogramming at a single gene in a single brain region *in vivo*, for the study of neuropsychiatric disease and beyond.
Methods: We have targeted the Fosb gene promoter in NAc using a HSV-mediated expression engineered zinc-finger protein (ZFP) fused to the transcriptional activator, p65, which promotes histone acetylation or the transcriptional repressor, G9a, a histone methyltransferase. qRT-PCR and immunohistochemistry were used to measure Fosb expression. Quantitative chromatin immunoprecipitation (qChIP) was used to measure the chromatin modifications. To achieve cell-type specific expression, we generated Cre-depended HSV constructs into the NAc of transgenic mice expressing Cre recobinase under control of either the Drd1- or Drd2- promoter, which specify each of the two medium spiny neuron cell types. For behavioral studies, mice were subject to social defeat stress following NAc infection with the HSV-ZFP constructs.
Results: FosB-ZFP-p65 efficiently and robustly activate FosB/ΔFosB expression in NAc neurons, while FosB-ZFP-G9a represses expression. HSV-G9a deposits H3K9me2 specifically at the FosB gene *in vivo*, while FosB-ZFP-p65 activates FosB via H3K9/14 acetylation. In addition, we found epigenetic remodeling at the FosB promoter, including enrichment of histone protein 1a and depletion of H3K9me3. Repression of Fosb in total NAc by Fosb-ZFP-G9a sensitizes animals to subthreshold defeat stress, as does activation of Fosb expression by Cre-dependent Fosb-ZFP-p65 in Drd1 + NAc medium spiny neurons.
Conclusions: Using engineered transcription factors, we have identified a direct molecular mechanism for stress-mediated repression of the Fosb gene, and have efficiently manipulated behavioral responses to social defeat stress. This approach allows a functional analysis of chromatin modifications that underlie affective disorders.
Disclosures: Nothing to Disclose.

49.4 Maternal Stress Epigenetic Programming Through Maternal and Fetal Exosomes

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Background: Perturbations during gestation, including maternal stress, are associated with an increased risk for neurodevelopmental disorders. Therefore, understanding the mechanisms by which stress affects the maternal and fetal milieu is important for identifying factors involved in dysregulation of neurodevelopment. In our well-established mouse model, male offspring exposed to early prenatal stress (EPS) have altered HPA axis programming and

increased behavioral stress sensitivity, similar to endophenotypes identified in autism and schizophrenia. Previously, we established in this model that gene sets important for endo- and exosomal cellular processes were significantly down-regulated in male placentas in response to EPS, suggesting that stress was imparting a programming effect on maternal and fetal exosome signaling. Exosomes are small lipid vesicles secreted locally and into the circulation by most tissues, and through the transfer of proteins, microRNAs (miRNAs), and other signaling factors between cells and tissues, are able to communicate unique information regarding the environment. Importantly, exosomes can cross the blood-brain barrier to impact neural gene expression, and potentially alter brain development. Less is known regarding their ability to cross the maternal:fetal barrier and directly impact fetal development.
Methods: To examine the impact of EPS on exosome signaling, maternal and fetal serum and tissue samples are collected on embryonic day 18.5 from control and stressed pregnant dams. Exosomes are first isolated from the serum samples, and then protein and RNA are extracted for further proteomics and small RNA-Seq analyses. Bioinformatics analyses will determine the impact of stress on total exosome production, and exosomal protein and miRNA content. Comparisons between maternal and fetal tissues and the exosomal content will identify stress effects on exosome secretion and the target tissues involved.
Results: In these studies, we have found that maternal stress during the first week of pregnancy produced lasting and significant effects on exosome signaling, as well as intriguing sex differences in the overall exosomal production in fetal and neonate circulation. Our proteomics data suggest that stress induces long-term changes in exosome production and cargo from a variety of maternal sources, including maternal immune cells and the placenta.
Conclusions: These studies provide exciting insights into a novel mechanism by which cellular communication from maternal and fetal tissues can carry information regarding dynamic changes in the environment. Exosomal cargo, proteins and miRNAs, are especially important in this signaling, and maternal stress can impart significant and lasting changes in exosomal production that may directly be altering the course of neurodevelopment. Understanding placental and maternal serum exosomal changes with stress can be developed as a potential biomarker of perturbations during pregnancy related to neurodevelopmental disease risk.
Disclosures: Nothing to Disclose.

Panel
50. Orphan GPCRs and Psychiatric Disorders

50.1 Orphan GPCRs in Psychiatry

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Background: The GPCRs bind all the known neurotransmitters, neuropeptides and peptide hormones and are the omnipresent modulators of brain function. Many bind ligands that have not been thus far described. These are the orphan GPCRs. We will present our results on the deorphanization of the melanin-concentrating hormone (MCH) receptor. MCH is a neuropeptide that is mainly produced in the lateral hypothalamus. Its receptor is highly expressed along the mesocorticolimbic dopamine pathway. The MCH receptor is present in the circuitry in which dopamine system overactivity is thought to lead to schizophrenia. We examined whether the MCH system activity modulates the prepulse inhibition (PPI) of the startle reflex, directly or when disrupted by dopamine-related drugs, which serves as an animal model that is relevant to schizophrenia symptoms.
Methods: Startle reactivity was measured using startle chambers. One week before drug testing, animals underwent a brief baseline session to create matched treatment groups. PPI session consisted of startle, prepulse and no-stimulus trials and these were presented in a pseudorandom order. The amount of PPI was calculated as a percentage score for each acoustic prepulse trial type: % PPI = 100-{[(startle response for prepulse + pulse)/(startle response for pulse-alone)]x100}.
Results: Regarding the prepulse inhibition (PPI) of startle, we found that there is no significant effect of central MCH injection on either startle or PPI level, although there was significant main effect of prepulse intensity on PPI. Because the mixed D1/D2 agonist apomorphine is known to disrupt PPI, we then tested whether MCH could affect apomorphine-induced PPI disruption and found that MCH dose dependently increased PPI deficit upon low doses of apomorphine. In contrast, central MCH injection did not affect stereotyped behaviors. We then used an animal model of schizophrenia, apomorphine-susceptible (APO-SUS) and apomorphine-unsceptible (APO-UNSUS) rats to test whether modulating the MCH system activity affects PPI and stereotyped behaviors.
Conclusions: We found that the MCH system can modulate dopamine-related responses. In sensorimotor gating, MCH is able to increases the disruptions induced by low doses of apomorphine. Because the MCH1R is expressed at a very low level in the striatum, these data position the MCH system as a unique target for therapies directed at modulating the dopamine tone selectively in the nucleus accumbens.
Disclosures: Nothing to Disclose.

50.2 An Orphan GPCR Highly Enriched in the Medial Habenula and Lateral Septum Detects L-Tryptophan and L-Phenylalanine and May Represent a Novel Sensor that Modulates Behavior

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Background: L-Tryptophan (TRP) and L-phenylalanine (PHE) are essential amino acids required for protein synthesis as well as biogenic amine neurotransmitter synthesis. In susceptible humans and some animal models, reduced TRP and PHE intake has been shown to be depressogenic, whereas anecdotal reports of TRP and PHE loading are reported to be mood elevating. The effects of altered TRP and PHE levels on behavior has been hypothesized to be mediated by downstream conversion to serotonin or dopamine. GPR139 is a highly conserved orphan G-Protein Coupled Receptor that is

expressed in the Medial Habenula and Lateral Septum. The Medial Habenula is a relatively understudied but conserved region in the brain implicated as a key part of the limbic circuits that may mediate mood and stress.
Methods: HEK293 cells transfected with GPR139 were subjected to various orphan ligands and tissue extracts and the effects on GTPgS activity was measured. In situ hybridization of GPR139 antisense riboprobes and immunohistochemistry of anti-GPR139 antibodies were evaluated on rat and mouse brain sections. Rats implanted with EEG electrodes were injected with GPR139 agonists and monitored for behavior and sleep EEG parameters. A high throughput screen was run to identify lead agonists and antagonists and these compounds were optimized for potency and drug-like properties.
Results: Random screening of amino acids, orphan ligands, and tissue extracts revealed that, compared to untransfected cells, HEK293 cells transfected with GPR139 responded only to TRP and PHE. Neither serotonin nor dopamine nor any other known neurotransmitters had any effect on GPR139-transfected cells. Chromatographed extracts of rat brain, rat plasma, and human plasma revealed two peaks of activity which corresponded to the elution peaks of TRP and PHE. A selective small molecule agonist (JNJ-63533054) with low nM affinity and potency, when radiolabelled, bound to GPR139-transfected cells and showed specific displacement by TRP and PHE. mRNA for GPR139 was mapped to habenula and lateral septum. Anti-GPR139 antibodies detected reactivity in medial habenula and lateral septum. Animals injected with GPR139 agonists exhibited decreased locomotor activity and increased sleep.
Conclusions: Using multiple biochemical and cell-based assays we demonstrated that the two essential amino acids, L-tryptophan and L-phenylalanine activate a G protein coupled receptor GPR139 with an EC50 in the 20-200 uM range, consistent with the physiological concentrations of TRP and PHE in human blood. Our findings suggest that TRP and PHE are likely physiological ligands for GPR139 and might explain the biological and behavioral effects of these substances without relying on their conversion to biogenic amines. It is tempting to speculate that the brain has system to detect levels of these essential amino acids and that changes in the availability of these amino acids may elicit behavioral responses. GPR139 could respond to dynamic changes of TRP and PHE under physiological conditions and thus represent a potential new drug target for CNS diseases.
Disclosures: Part 1: I am an employee of a pharmaceutical company, **Part 5:** Janssen Pharmaceutical R&D. LLC.

50.3 GPR88: An Orphan GPCR for the Potential Treatment of CNS Disorders

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Background: GPR88 is an orphan G-protein coupled receptor (GPCR) of the rhodopsin family, expressed in striatum, caudate putamen, nucleus accumbens and olfactory tubercle. Its CNS expression is particularly robust in striatum, paralleling that of the dopamine D2 receptor, suggesting the receptor may play a role in regulating dopaminergic activity. GPR88 knock-out (KO) animals display increased locomotor activity in response to treatment with dopaminergic compounds, demonstrating a role for GPR88 in dopaminergic signaling. Additional studies with GPR88 knock-out mice showed increased glutamatergic excitation and reduced GABAergic inhibition in medium spiny neurons, thereby enhancing neuronal firing rates *in vivo* and resulting in hyperactivity, poor motor coordination and impaired cue-based learning. In addition, transcriptional profiling studies have revealed that GPR88 expression is altered in a number of CNS related diseases, providing additional evidence that GPR88 is an essential modulator of CNS signaling pathways related to psychiatric disease.
Methods: To develop an understanding of the *in vivo* phenotype, GPR88 knock-out mice were generated and examined in a battery of *in vivo* tests. Localization studies were carried out to determine the expression pattern of GPR88 in the CNS. Additionally, small molecule agonists of GPR88 identified from a high throughput screening campaign were characterized *in vitro*.
Results: The GPR88 KO mice were shown to potentiate the locomotor effects of the dopamine D2 agonist quinpirole. Additionally, GPR88 KO mice were shown to have impaired prepulse inhibition (PPI) as well as enhanced quinpirole sensitivity in the PPI model, suggesting that GPR88 is a negative regulator of dopaminergic (D2 receptor) signaling. GPR88 mRNA was found to be most abundantly expressed in the nervous system and prominently expressed in striatum paralleling that of Dopamine D2. Finally, a series of small molecule agonists of GPR88 were identified and shown to have excellent potency in a cAMP assay as well as in a GTP□S binding assay. The compounds were examined in GRP88 KO and wild type (WT) tissue showing the activity to be GPR88 dependent.
Conclusions: Our preclinical findings with the GPR88 KO mouse phenotype and CNS tissue distribution add further evidence to support the hypothesis that modulators of GPR88 activity may have the potential to treat CNS related diseases.
Disclosures: Nothing to Disclose.

50.4 Neural Functions of SREB - The Most Evolutionarily Conserved G-Protein Coupled Receptor Family Associated with Psychiatric Disorders

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Background: The SREB (Super-conserved Receptor Expressed in Brain) family of SREB1/GPR27, SREB2/GPR85 and SREB3/GPR173 is a unique subfamily of G protein-coupled receptor (GPCR) expressed in the CNS. SREB2 is virtually the most conserved GPCR in vertebrate evolution, i.e. the primary amino acid sequence is 100% identical between humans and rodents. Conservation rates of SREB1 (97%) and SREB3 (99%) are also very high and equivalent to glutamate and GABA receptors, suggesting the existence of undiscovered fundamental neural systems involving SREB family members. Human genetic studies have indicated a possible link between SREB2 and schizophrenia

and autism spectrum disorder. Studies using SREB2 transgenic (over-expression) and knockout (KO) mice revealed that SREB2 is implicated in determining brain size and regulating hippocampal adult neurogenesis and its related cognitive functions. Although the highest conservation rate of SREB2 indicates its critical roles in the CNS, clear-cut neural functions of SREB2 have not been revealed yet by studies of single SREB2 KO mice presumably due to compensational mechanisms involving SREB1 and SREB3.
Methods: To investigate neuronal functions of SREB family, we have generated all single SREB gene knockout mice (SREB1 KO, SREB2 KO and SREB3 KO mice) and double SREB genes knockout mice by intercrossing of single SREB KO lines. To avoid compensational mechanisms and elucidate clear-cut neural functions of SREB family, triple knockdown/knockout of all SREB genes in neurons has been attempted. We have applied RNAi approach to knockdown the third SREB gene expression in neurons established from double SREB genes KO mice.
Results: SREB2 transgenic (over-expression) mice showed 20% brain weight reduction compared to wild-type littermates (WT). Reciprocally, increased brain weight ($\sim$ 15%) was observed in SREB2 KO mice. Single SREB1 KO mice ($\sim$ 10%) and SREB3 KO mice ($\sim$ 5%) showed a significant brain weight increase compared to WT. In addition, macroscopic analysis using Nissl staining in coronal brain section of single SREB1 and SREB3 KO mice demonstrated no gross structural abnormalities in laminar formation of cerebral cortices or position of major brain nuclei, which is consistent with previous data from single SREB2 KO mice. By intercrossing of single SREB1 KO and SREB2 KO lines, SREB1/2 double KO mice have been generated and turned out to be viable. Primary cultured neurons established from SREB1/2 double KO mice were treated with SREB3 shRNA. RT-qPCR assays confirmed null mutation of SREB1 and SREB2 genes and knockdown of SREB3 gene expression in the cultured neurons.
Conclusions: Single SREB1 KO and SREB3 KO mice showed similar brain phenotypes with single SREB2 KO mice. Our results indicate that SREB family members presumably share the same downstream pathway to regulate brain development and function. Molecular analyses of neurons with triple knockdown/knockout of all SREB members will reveal clear-cut neural functions of the SREB family, which addresses two questions existing: why SREB family has been so conserved in vertebrate evolution and how SREB family is involved in psychiatric disorders.
Disclosures: Part 1: I am a full-time employee of Astellas Pharma Inc., **Part 5:** Astellas Pharma Inc.

Study Group
51. Addictions Neuroclinical Assessment: The Search Continues

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The focus of the proposed study group is on a new clinical framework for understanding addictive disorders, which we call Addictions Neuroclinical Assessment (ANA). The need for this new framework is apparent in the relatively static nosology for addictive disorders, which has clinical reliability but significant within-diagnosis heterogeneity. This heterogeneity limits our ability to understand, treat, and prevent addictive disorders, which comprise significant public health problems. ANA builds on advances in our understanding of the neurobiologic underpinnings of addictive disorders and emphasizes the assessment of three neurofunctional domains: Cognitive Control, Reward, and Negative Emotionality. Each domain may be associated with various aspects of the addiction cycle, including binge-intoxication (Reward), withdrawal (Negative Emotionality), and preoccupation-anticipation (Cognitive Control). There is significant overlap between these three functional domains and those included in the National Institute of Mental Health's major initiative to redefine the research framework for varied psychiatric disorders, the Research Domain Criteria (RDoC) initiative. RDoC includes five functional domains (Cognitive Systems, Negative Valence Systems, Positive Valence Systems, Systems for Social Processes, and Arousal and Regulatory Systems). The primary differences between ANA and RDoC are that RDoC focuses on multiple psychiatric disorders, while ANA emphasizes addictions, and that RDoC is a research tool, while ANA is clinical in nature.
Proposed assessments to be incorporated within ANA include specific magnetic resonance imaging (MRI)-based measures of each functional domain, along with MRI assessments of brain structure, volume, and connectivity. These neuroimaging measures will be supplemented by collection of blood for genomic analysis and a battery of ancillary instruments, including assessments of specific drug use patterns, psychiatric disorders, and psychosocial functioning. Thus, the neuroimaging and ancillary instruments will comprise a deep phenotyping of addicted individuals, to be analyzed in conjunction with the genomic data. This assessment package will be piloted within the National Institute on Alcohol Abuse and Alcoholism's (NIAAA) intramural research program, which includes inpatient and outpatient treatment facilities. ANA will further be expanded on and disseminated through collaboration with universities, health providers and organizations, and other research and treatment facilities. This study group would describe ANA in further detail and allow for a discussion of how best to maximize this opportunity to better understand the heterogeneity of addictive disorders, reconceptualize the nosology, and, ultimately, improve efforts at prevention and intervention.
Disclosures: Nothing to Disclose.

Panel
52. The Re-Emergence of Serotonergic Hallucinogens as Tools for Neuropsychopharmacology

52.1 Regulation of 5-HT2A Receptor-induced Behavioral Responses by mGlu2/3 and mGlu5 Receptors

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Background: Metabotropic glutamate (mGlu) receptors have been suggested to play a role in schizophrenia and depression.

Because serotonergic hallucinogens increase glutamate release and mGlu receptors modulate the response to serotonin (5-HT)2A activation, the interactions between serotonin 5-HT2A receptors and mGlu receptors may prove to be important for our understanding of these disease states, and may help to unravel the mechanisms underlying the potential therapeutic effects of hallucinogens.
Methods: One series of experiments assessed whether the head twitch response (HTR) induced by the highly selective 5-HT2A agonist 25CN-NBOH in C57BL/6J mice is modulated by acute or chronic treatment with the mGlu2/3 agonist LY379268. In the acute experiment, mice were treated with LY379268 (0.1-10 mg/kg SC) 30 min prior to administration of 25CN-NBOH. In the chronic experiment, mice were treated with vehicle or LY379268 (10 mg/kg/day SC) for 21 days, and then challenged with 25CN-NBOH after a 48-h washout period. We also tested whether deletion of the mGlu5 gene in mice alters the locomotor hyperactivity induced by the 5-HT2A agonists DOM (0.5 mg/kg IP) and TCB-2 (0.3 mg/kg IP).
Results: 25CN-NBOH induced the HTR (ED50 = 0.40 (95% CI 0.20-0.81) mg/kg) in C57BL/6J mice. The response to 25CN-NBOH (1 mg/kg SC) was significantly attenuated (52.5% reduction) by acute treatment with 10 mg/kg LY379268 ($F(3,20) = 5.00$, $p < 0.01$). When mice were treated with LY379268 for 21 days and then challenged 48-h later with 25CN-NBOH, the HTR was attenuated 26.7% relative to mice treated chronically with vehicle ($F(1,54) = 30.91$, $p < 0.001$). The locomotor hyperactivity induced by DOM (gene x drug: $F(1,28) = 12.83$, $p < 0.002$) and TCB-2 (gene x drug x time: $F(5,125) = 3.30$, $p < 0.008$) was potentiated in mGlu5 knockout mice relative to their wild-type littermates.
Conclusions: These studies demonstrate that mGlu2/3 and mGlu5 receptors modulate the behavioral responses induced by 5-HT2A activation. Additionally, these findings show that repeated activation of mGlu2/3 receptors can reduce the response to a 5-HT2A agonist. These studies provide additional support for the link between the serotonergic and glutamatergic systems. Additional studies are necessary to understand why 5-HT2A responses are altered by chronic mGlu2/3 activation and by the loss of mGlu5 signaling.
Disclosures: Part 1: L-3 Communications; Roche, **Part 4:** Roche.

52.2 Mood, Craving, and Self-efficacy in Psilocybin-Assisted Treatment of Alcoholism

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Background: Evidence suggests that classic hallucinogens have clinically relevant effects in alcohol and drug addiction. It is hypothesized that acute pharmacological and psychological effects trigger longer-term changes in psychological variables such as mood, craving, and self-efficacy, and that these changes in turn lead to persisting improvement in substance use behavior. This report explores the effects of psilocybin administration on changes in mood, alcohol craving, and self-efficacy, and the relationship of these changes to short-term drinking outcomes that were previously reported.
Methods: Ten patients with DSM-IV alcohol dependence received psilocybin 0.3 mg/kg PO in a supervised 8-hour session after 4 weeks of outpatient psychotherapy. Nine of these participants remained in the study for at least 4 additional weeks, during which no psilocybin was administered. Measures of acute medication effects included the total score from the Mystical Experience Scale, the General scale from the Altered States of Consciousness Scale (5D-ASC), and the Intensity subscale of the Hallucinogen Rating Scale. Measures of mood (the Profile of Mood States), self-efficacy (the Alcohol Abstinence Self-Efficacy Scale, Confidence subscale), craving (the Penn. Alcohol Craving Scale), and drinking were obtained at baseline and at intervals during and following treatment. Drinking outcomes were percent drinking days and percent heavy drinking days.
Results: Mean Profile of Mood States subscale scores did not change significantly in the week following psilocybin administration (week 5) relative to the week before (week 4). However, individual changes in tension, depression, vigor, and confusion were significantly ($p < .05$) correlated with one or more of the three measures of the subjective experience during the first psilocybin session ($r = .680$ to $.920$). In all cases, more favorable mood outcomes were observed in participants who had stronger subjective experiences. Changes in mood symptoms at week 5 were significantly correlated with changes in drinking during the month following the first psilocybin session (weeks 5-8) relative to the month before (weeks 1-4). Similarly, craving was not significantly decreased at week 5, but change in craving was strongly correlated ($r > .8$, $p < .01$) with two of three measures of subjective experience intensity, and this change in craving was correlated with subsequent changes drinking outcomes in weeks 5-8 ($r > .7$, $p < .05$). Significant correlations were observed between change in craving and change in depression, vigor, fatigue, and confusion. An increase in self-efficacy was also observed at week 5, and this change too was significantly correlated ($r > .7$, $p < .05$) with two of the three measures of subjective experience. However, change in self-efficacy was not significantly correlated with subsequent change in drinking, or with measures of mood and craving.
Conclusions: In this proof-of concept trial, stronger experiences with psilocybin produced more positive change in mood, craving, and self-efficacy, and changes in mood and craving were in turn predictive of short-term improvement in drinking. Controlled trials are necessary to test whether the causal mechanisms suggested here can be reproduced prospectively.
Disclosures: Part 4: Research grant for a non-pharmacologic treatment study from the Lundbeck, Foundation, through the University of Southern Denmark.

52.3 A Single Dose of Psilocybin Produces Substantial and Enduring Decreases in Anxiety and Depression in Patients with a Life-Threatening Cancer Diagnosis: A Randomized Double-blind Trial

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Background: Patients with cancer often develop chronic, clinically significant symptoms of anxiety and depression

that have a significant negative impact on the quality of their life. Existing pharmacological and psychological treatments are very limited. Several trials in the 1960s and 1970s with the classic hallucinogens LSD and DPT in cancer patients showed clinically significant improvement in ratings of depression and anxiety. These trials involved 236 cancer patients. Recently, Grob and colleagues (2011) reported a pilot study with a moderate dose of the classic hallucinogen psilocybin (about 14 mg/70 kg) showing decreases in anxiety and depression in 12 cancer patients.
Methods: The study used a randomized, double-blind, cross-over design to investigate the acute and sustained effects of a very low psilocybin dose (1 or 3 mg/70 kg) vs. a moderate-high dose (22 or 30 mg/70 kg). Instructions to participants and staff minimized expectancy effects. 51 patients with a life-threatening cancer diagnosis who had symptoms of anxiety or depression received a low or high dose of psilocybin in counterbalanced order with about 5 weeks between sessions and a final follow up at 6 months. For this preliminary analysis, results between the low ($n = 25$) and high ($n = 26$) dose groups on the first session were compared. Enduring effects were assessed at a 6 month follow-up.
Results: On session days, the high dose group showed substantially greater effects including perceptual changes, mystical-type subjective experiences, and labile mood. At the 5-week follow-up the high dose group showed significantly lower anxiety (STAI Trait Anxiety, HAM-A) and depression (BDI, HAM-D) compared to the low dose group (effect size mean and range 0.98, 0.60-1.30). The participants attributed significantly greater positive changes in attitudes about life/self, positive social effects, and positive behavior changes to the experience, and a higher percentage reported the experience to be among the 5 most personally meaningful of their lives (54% vs. 16%). Total mystical experience scores at the end of the session showed significant negative correlations with the above measures of anxiety and depression at 5 weeks. Partial correlation analysis showed this relationship remained significant after controlling for ratings of intensity of drug effect. The decreases in anxiety and depression were sustained at 6 month follow-up.
Conclusions: A single moderate-high dose of psilocybin, when administered under supportive conditions to carefully screened and prepared participants, can produce substantial and enduring decreases in anxiety and depression in patients with a life-threatening cancer diagnosis.
Disclosures: Part 1: I am a consultant to Merck and Co and Jazz Pharmaceuticals. I am on the Board of Directors of the Heffter Research Institute, **Part 4:** Heftier Research Institute has provided grant funding of some of my research.

52.4 Results: Of a Multi-Modal Neuroimaging Study of LSD and a Psilocybin for Treatment-Resistant Depression Clinical Trial

Robin Carhart-Harris

Imperial College London, London, United Kingdom

Background: Our research team have conducted a series of MRI and MEG studies with the 5HT2A receptor agonist psilocybin in comparison with MDMA, an entactogen that releases 5HT. The MRI studies of psilocybin (both ASL and fMRI) revealed unexpected reduction in brain blood flow and a decrease in BOLD signal (Carhart-Harris et al 2013 PNAS) in high-level cortical regions and the thalamus, with post-hoc analysis showing large increases in brain connectivity between, rather than within, the usual resting state networks (Petri et al J Roy Soc 2014). The cortical psilocybin MRI findings were confirmed by a later MEG study that revealed a major loss of power in all measured frequency bands (1-100Hz) after psilocybin with decreases in alpha power in the posterior cingulate cortex correlating with ego-dissolution measures (Carhart-Harris & Muthukumaraswamy et al 2013 J Neurosci). Results from the fMRI and MEG work suggested psilocybin has antidepressant properties and from these we are now conducting the first study of psilocybin in resistant depression. Data from a pilot phase will be ready for the ACNP meeting.
MDMA also decreased blood flow and BOLD signal but the effects were largely subcortical, particularly in the amygdala, and no psychedelic effects were seen (Carhart-Harris et al 2014 Biol Psych). Negative memories were attenuated and positive ones enhanced by MDMA and these effects were associated with fMRI-measured changes in brain activity (Carhart-Harris et al 2013 Int Neuropychopharm).
LSD is the prototypical hallucinogen, with much greater use than the others in psychiatric and research settings, with over 1000 papers published before it was banned in 1967. Since then, and only in the past year, there have been 3 research reports, but none using modern brain imaging methods.
Methods: Over the course of 6 hours, 20 healthy volunteers were scanned sequentially with ASL/BOLD-fMRI/ and MEG following 75 microgm LSD iv or saline placebo in a cross-over design at least 2 weeks apart. Subjective ratings of psychedelic experiences were then correlated with the imaging data. Twelve patients with resistant depression were treated with two sessions of psilocybin. Significant improvements in symptom severity were observed for up to 5 weeks post-treatment, with a far greater before and (1 week) after treatment effect size (Cohen's $d = 3.4$) than seen with currently available anti-depressant interventions.
Results: LSD decreased integrity and segregation of brain networks and this effect correlated with subjective ratings of changes in consciousness, including ego-dissolution. Increased functional connectivity between the visual cortex and high-level cortical regions correlated strongly with ratings of visual hallucinations. Patients treated with psilocybin for resistant depression have shown marked improvements in the symptom severity post-treatment.
Conclusions: The LSD data and our three prior psilocybin studies show that 5HT2A agonist hallucinogens provoke profound changes in consciousness due to decreased integrity of brain networks and a decrease in between-network segregation found in resting state measures, leading to a more chaotic or "entropic" brain state. These effects may also explain the utility of these drugs in addiction and mood disorders. The psilocybin for depression findings suggest that psilocybin is a safe and effective treatment for severe depression.
This work was led by R Carhart-Harris and supported by D Nutt, M Kaelen, L Roseman, S Muthukumaraswamy

and A Feilding as part of the Beckley-Imperial College psychedelic research program.
Disclosures: Nothing to Disclose.

Panel
53. Beta Arrestin Signaling: An Avenue to Novel Psychopharmacology

53.1 Physiological Responses to Mu Opioid Receptor Agonists Promoting Bias Toward G Protein Signaling Pathways

Laura Bohn

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Background: Mu opioid receptors (MOR), the cognate targets for endorphins, endomorphins and enkephalins, are pharmaceutical targets for pain treatment, depression disorders and addiction therapies. As a G protein-coupled receptor, MOR signals through G proteins to dampen pain responses. These receptors also recruit βarrestins, which are intracellular regulatory proteins. Studies in βarrestin2-knockout mice suggest that preventing βarrestin2 interactions with MOR may lead to better analgesics with fewer side effects.
Methods: A series of novel MOR agonists were generated and analyzed for their ability to promote G protein signaling over βarrestin2 recruitment. Mathematical application of the operational model of nonlinear regression analysis was used to rank the degree of bias for G protein signaling over βarrestin2 recruitment. Compounds with high G-preferring bias factors were analyzed in mouse for their ability to induce a wide array of behavioral responses. Pharmacokinetic evaluations were also performed.
Results: We demonstrate that antinociceptive properties of these drugs are maintained, with potencies and efficacies comparable to conventional agonists. However, we find that agonists biased for G protein signaling lead to a loss of some of the behavioral effects induced by balanced agonists, such as morphine. In further comparison to morphine, the MOR biased agonists produce less tolerance, constipation, respiratory suppression and locomotor activity. Pharmacokinetic analysis reveals that the compounds are long lasting in plasma and they are brain penetrant.
Conclusions: Biased agonists identified *in vitro* are shown to display diverse physiological responses *in vivo* when compared to morphine. While the direct demonstration of βarrestin2 activation *in vivo* is still forthcoming, the current findings support the earlier observations made in the βarrestin2-KO mice: reduction of the MOR-βarrestin2 interaction may lead to preserving antinociception with reduced side effects.
Disclosures: Part 1: A patent has been filed by TSRI on this work, **Part 4:** Funding from Eli Lilly in Company, not on the work presented here. (Funded by NIH DA033073, DA031927, DA038964).

53.2 A Unique Dual Cortico-Striatal Action of a Beta-arrestin Biased Dopamine D2 Receptor Ligand

Nikhil Urs

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Background: β-arrestin2 (βarr2) signaling at D2 receptors (D2Rs) plays an important role in antipsychotic responses, allowing development of signaling biased therapies. In preclinical studies βarr2 biased D2R ligands behave as efficacious antipsychotic compounds. The dopamine (DA) hypothesis of schizophrenia postulates hypodopaminergia in the prefrontal cortex (PFC) and hyperdopaminergia in the striatum. Current antipsychotics effectively reverse excess striatal activity, but do not fully reverse cortical deficits. Using cell-specific βarr2KO mice and β-arrestin biased ligands we address this problem here.
Methods: To achieve cell type-specific deletion of βarr2 we crossed βarr2 floxed mice to D1R, D2R or A2aR CRE mice. We then tested the ability of clinically effective antipsychotics haloperidol (HAL), clozapine (CLOZ), aripiprazole (ARI) and the β-arrestin-biased D2R ligands UNC9994A (94A) and UNC9975A (75A) to inhibit psychostimulant-induced hyperlocomotion in these neuron-specific βarr2KO mice. We employed *in vitro* GPCR signaling assays to test ARI, 94A and 75A for their antagonist/partial agonist activity at D2Rs.
Results: Deletion of βarr2 in striatal D2R + (A2aCRE) or all D2R + (D2CRE) but not D1R + (D1CRE) neurons causes β-arrestin-biased D2R ligand 94A but not 75A to lose its antipsychotic activity against amphetamine. However other antipsychotics tested (HAL, CLOZ and ARI) were still effective in all βarr2KO mouse lines. Interestingly, unlike AMPH, when tested against phencyclidine (PCP), 94A lost its antipsychotic activity only in D2R + (D2CRE) but not striatal D2R + (A2aCRE) or D1R + (D1CRE) βarr2KO mice suggesting a role for cortical βarr2 in this effect. Upon western blot analyses we observed higher expression of βarr2 and GRK2 in the PFC compared to the striatum. *In vitro* signaling assays revealed that upon over-expression of GPCR Kinase2 (GRK2) - ARI and 94A but not 75A have partial agonist activity at βarr2 recruitment at the D2R. However, with endogenous expression levels of GRK2 - ARI, 75A and 94A antagonize βarr2 recruitment to the D2R but that only ARI and 75A antagonize Gi mediated D2R signaling.
Conclusions: Using neuron-specific βarr2KO mice and the β-arrestin-biased D2R ligand 94A, we show that βarr2 antagonism in striatal D2R + neurons is sufficient for antipsychotic activity against amphetamine. However, for antipsychotic activity against phencyclidine, 94A displayed a unique regional selectivity, suggesting a role for PFC D2R/βarr2 agonism. The switch of 94A from antagonism to agonism is due to higher PFC expression of βarr2 and GRK2 compared to striatum. Therefore, unlike current antipsychotics, β-arrestin-biased D2R ligands that behave as agonists in the cortex but antagonists in the striatum may be sufficient for clinical antipsychotic efficacy, with a superior ability to correct cortical hypodopaminergia. Such a mechanism would allow for the amelioration of not only psychosis but also cognitive and negative symptoms observed in schizophrenia.
Disclosures: Nothing to Disclose.

53.3 D2 Beta Arrestin-Signaling Enhances Prefrontal Cortical Interneuron Activity

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Background: Fast spiking interneurons (FSI) in the prefrontal cortex exhibit a protracted developmental trajectory with the acquisition of their adult profile of dopamine modulation during adolescence. In juvenile animals FSI can be activated by D1 and inhibited by D2 agonist, while in adult animals D2 activation becomes excitatory. We tested the hypothesis that such excitatory effect, unlikely driven by Gi activation, is dependent on signaling through beta arrestin 2 (bARR).
Methods: We conducted whole-cell recordings in FSI from wildtype and bARR KO mice testing the effects of the unbiased agonist quinpirone, the unbiased partial agonist aripiprazole and the bARR biased ligand UNC9994A (94A).
Results: All compounds increase excitability and firing in prefrontal FSI, but 94A had a much stronger effect on FSI than the other compounds, and did not exert an excitatory effect on pyramidal neurons. The excitatory effect of 94A was abolished in bARR KO mice.
Conclusions: In conclusion, the data suggest that biased signaling could have significant impact on dopamine modulation of FSI in the prefrontal cortex, an effect that could provide cognitive improvement by bARR biased D2 agonists.
Disclosures: Part 1: Employee and stockholder at Pfizer, **Part 5:** Pfizer.

53.4 Novel Cellular Mechanisms Underlying Actions of Dopamine D2 Receptors on Prefrontal Pyramidal Neurons

Vikaas Sohal

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Background: Dopaminergic modulation of prefrontal function has been implicated in a wide variety of normal and pathological processes. Recently our laboratory described a novel action of dopamine D2 receptors (D2Rs) on a specific subtype of subcortically projecting (SC) pyramidal neurons in layer 5 of the prefrontal cortex (PFC). Specifically, when D2Rs are activated in the presence of synaptic NMDAR activation, these neurons produce prolonged afterdepolarizations (ADPs) that can drive spiking for several seconds in the absence of further input. Thus, this phenomenon may powerfully regulate top-down output from the prefrontal cortex to subcortical structures. Several key questions about this phenomenon remain. Does this phenomenon influence the excitability of SC neurons in other ways, besides producing ADPs? How exactly do NMDARs contribute? Does this phenomenon reflect canonical or non-canonical D2R signaling? And finally, how does this phenomenon contribute to normal or pathological behaviors?
Methods: We made whole cell patch clamp recordings from visually identified L5 pyramidal neurons in the medial PFC in acute brain slices from 8-10 week old mice. To activate D2Rs, we used 10-20 micromolar quinpirole. To activate synaptic NMDARs, we optogenetically stimulated callosal fibers, or bath applied 4 micromolar NMDA. To knockout the NR2B subunit, we used NR2B conditional knockout mice injected with a virus to drive expression of Cre in the mPFC. For behavioral experiments, we used TH-Cre mice to optogenetically stimulate TH-positive projections from the VTA to mPFC.
Results: First, we confirmed that interactions between D2Rs and NMDARs can modulate prefrontal SC neuron excitability in other ways, besides simply eliciting ADPs. Activating D2Rs in the presence of synaptic stimulation (to recruit NMDARs) increases the sensitivity of SC neurons to brief inputs. Next, we explored mechanistic aspects of this phenomenon. The ability of synaptic stimulation to facilitate D2R-induced increases in excitability is suppressed when hyperpolarizing current is delivered concurrent with synaptic stimulation, suggesting that it requires $Ca2+$ influx via NMDARs. Surprisingly, knocking out the NR2B subunit increases D2R-induced afterdepolarizations, suggesting that NR2A and NR2B-containing NMDARs differentially contribute to this phenomenon. Antagonists for cAMP-PKA mediated signaling consistently block the D2R-induced afterdepolarization, suggesting that it is not mediated through canonical Gi signaling pathways. Finally, we have been exploring the effects of stimulating TH-positive VTA to mPFC projections during a rule switching task. We paired phasic stimulation of these fibers with either correct or incorrect choices and found that both pairings disrupted learning of a new rule, while also suppressing perseverative behavior. Thus dopaminergic signaling in the mPFC does not simply transmit feedback about recent choices and reinforces specific behaviors, but rather can nonspecifically destabilize behavioral strategies in a way that could facilitate exploratory behavior.
Conclusions: Our results confirm that D2Rs can powerfully regulate PFC output in ways that may guide flexible behaviors. This appears to reflect non-canonical signaling through D2Rs, as well as novel D2R-NMDAR interactions.
Disclosures: Part 1: Research support from Roche.

Panel
54. Novel Molecular Targets in Cocaine Addiction

54.1 Acid Sensing Ion Channel: A New Player in Addiction-Related Behavior

John Wemmie

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Background: Synaptic physiology and structure in the nucleus accumbens (NAc) is known to be altered as a consequence of chronic exposure to drugs of abuse, and these changes are believed to be critical components in the pathology of drug addiction. A potential regulator of these changes in the NAc are acid-sensing ion channels (ASICs). In particular, acid-sensing ion channel 1A (ASIC1A) is abundant in the nucleus accumbens (NAc), and previous evidence from our laboratory has suggested that ASIC1A influences learning and memory

mechanisms dependent on other brain regions. However, the function of ASIC1A in the accumbens in regulating NAc-dependent behavior and functioning within the NAc is unknown. Therefore, we hypothesized that manipulating ASIC1A in the NAc would alter addiction-related behavior, including drug-seeking behavior, in rodents.
Methods: To address this issue, we conducted studies in both mice and rats, utilizing the strengths and capabilities of each approach to understand the role of ASIC1A in the NAc. In mice, we explored our hypothesis by: 1) examining the effects of manipulating ASIC1A in the mouse on cocaine conditioned place preference, 2) investigating the effects ASIC1A disruption on synaptic transmission and dendritic spine morphology in the NAc, and 3) determining how alterations in ASIC1A affect cocaine-evoked synaptic plasticity. Based on the results from our mouse studies, we then examined the function of ASIC1A in the NAc of rats using self-administration models. Specifically, we expressed ASIC1A in the NAc in the rat and examined cocaine self-administration, post-withdrawal cocaine-seeking behavior, and synaptic transmission.
Results: We found that disrupting ASIC1A in the mouse NAc increased cocaine-conditioned place preference and over-expressing ASIC1A in the rat NAc reduced cocaine self-administration. Investigating the underlying mechanisms, we identified a previously unknown postsynaptic current during neurotransmission that was mediated by ASIC1A and ASIC2 subunits and thus well positioned to regulate synapse structure and function. Additional studies of ASIC1A in the rat NAc further suggest significant effects of ASIC1A on cocaine craving following cocaine withdrawal, as ASIC1A overexpression in the NAc of rats produced a significant increase in cocaine-seeking behavior following a withdrawal period. In contrast, such overexpression had no effect on food-seeking behavior.
Conclusions: Together, these findings suggest that ASIC1A contributes to excitatory synaptic transmission in the NAc and, moreover, that altering ASIC1A in the NAc had profound influences on synaptic functioning and addiction-related behaviors. In particular, the evidence that manipulating ASIC1A selectively influences measures of relapse to cocaine-seeking, but not food-seeking, behavior suggests an interaction between chronic cocaine use and ASIC1A functioning. As a result, our work raises the possibility of developing therapies for drug addiction by targeting ASIC-dependent neurotransmission.
Disclosures: Nothing to Disclose.

54.2 Regulation of Protein Translation in the Nucleus Accumbens

Marina Wolf

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Background: Cue-induced cocaine craving progressively intensifies ("incubates") during withdrawal from extended access cocaine self-administration. After prolonged withdrawal, expression of incubated craving is mediated by calcium-permeable (CP)-AMPARs that accumulate in NAc synapses. Group I mGluR plasticity is also altered. Our recent results suggest that ongoing dendritic protein translation is required to maintain these adaptations. Thus, when NAc slices were prepared from "incubated rats" (rats subjected to extended-access cocaine self-administration followed by >40 days of withdrawal) and then exposed for ~ 1 h to inhibitors of protein translation, synaptic transmission was normalized (Scheyer et al., 2014). Is protein translation globally upregulated after incubation? Or, is there dysregulated translation of a few critical proteins that are required to maintain cocaine-induced plasticity? Information about regulation of protein translation in neurons comes mainly from studies of hippocampus. For example, group I mGluR stimulation increases translation, while NMDAR transmission can exert a suppressive effect. Our goals are: 1) determine if the same pathways regulate translation in the NAc and if this is altered after incubation of cocaine craving, and 2) identify specific proteins that are differently translated in the NAc of "incubated rats".
Methods: Metabolic labeling was used to tag newly synthesized proteins in freshly dissected NAc tissue from drug-naive rats or rats killed >40 days after discontinuing extended access saline or cocaine SA. Tissue was incubated with 35S-Met/Cys in the presence of vehicle (control) or antagonists of mGluR5, mGluR1 or NMDARs, and then processed for SDS-PAGE/autoradiography to quantify incorporation of radiolabel into newly synthesized proteins. This method is useful to measure overall rates of translation but not translation rates of individual proteins. Therefore, parallel studies are underway using bioorthogonal non-canonical amino acid tagging (BONCAT) combined with immunoprecipitation and mass spectrometry.
Results: The mGluR5 antagonist MTEP increased 35S-Met/Cys incorporation into newly translated proteins in the NAc of drug-naive rats and cocaine-exposed rats. The mGluR1 antagonist LY367385 had no effect in any group. The NMDAR antagonist APV increased translation in drug-naive but not cocaine-exposed rats.
Conclusions: Our results show that tonic mGluR5 signaling suppresses protein translation in the NAc of drug-naive rats, opposite to what has been found in hippocampus, and that this effect is not altered after incubation of craving. In contrast, mGluR1 blockade did not affect translation in any experimental group, despite emergence of mGluR1-LTD in the NAc after incubation. Finally, we found that NMDARs suppress translation in the NAc of drug-naive rats, as in hippocampus, but this braking influence is lost in the cocaine group. This may explain, at least in part, the abnormal translation that maintains neuroadaptations in NAc synapses of "incubated rats". Using BONCAT, we will test the hypothesis that loss of NMDAR-mediated inhibitory tone permits excessive translation of proteins that regulate AMPAR transmission.
Disclosures: Nothing to Disclose.

54.3 Cocaine-Induced Adaptations in Astrocyte-neuron Communication Mediate Cocaine Seeking

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Background: Recent studies indicate that cocaine use alters astrocytes and neuron-astrocyte communication within the

brain's reward circuitry. In particular, downregulation of astroglial glutamate transporter GLT-1 is a hallmark feature observed after extended withdrawal from multiple drugs of abuse. A growing list of compounds known to affect glial physiology and restore glutamate uptake, including N-acetylcysteine (NAC), ceftriaxone, and propentofylline, impair reinstatement of cocaine seeking. Given this, it is of great interest to determine specifically how cocaine experience affects astrocytes and contributes to maladaptive cellular dynamics that underlie relapse.
Methods: Prior to behavioral training, astrocytes in the nucleus accumbens were transduced with an AAV2/5 expressing membrane-tagged Lck-GFP under the control of an astrocyte-specific promoter. Rats then either underwent cocaine self-administration or served as yoked-saline controls. Following two weeks of extinction training, properties of fluorescent accumbens core astrocytes were measured, including surface area and volume. Slices were also immunostained for synapsin I, to allow for colocalization of synaptic puncta with GFP-positive pixels containing peripheral astrocyte processes. Astrocytes were imaged at 63x on a Zeiss confocal microscope, and analyzed using Imaris software. For behavioral studies, rats were treated with riluzole (1 or 4 mg/kg, i.p.) thirty min prior to each extinction session and reinstatement test. For electrophysiology, rats were overdosed with pentobarbital and nucleus accumbens slices were taken for whole-cell patch-clamp recording.
Results: Following cocaine self-administration and extinction, nucleus accumbens astrocytes are significantly smaller and make fewer presynaptic contacts than following saline experience. Moreover, the decrease in synaptic colocalization is reversed by administration of the glial modulator ceftriaxone during extinction training. Thus, a model is emerging in which retraction of astrocytes from neurons exacerbates decreased glutamate uptake caused by decreased GLT-1 expression, leading to glutamate spillover and stimulation of accumbens neurons which drive reinstatement. Based on this model, we should expect other compounds known to enhance GLT-1 to also impair cocaine reinstatement. In fact, we have found that in addition to NAC, ceftriaxone, and propentofylline, the GLT-1 regulator riluzole also impairs both cue- and cocaine-primed reinstatement. Interestingly, we find that riluzole also normalizes excitability of prelimbic (PL) prefrontal cortical neurons, which is increased by cocaine self-administration and extinction. Thus, we hypothesize that the mechanism of action of riluzole against cocaine seeking includes decreased PL neuron excitability paired with enhanced glutamate uptake by astrocytes in the nucleus accumbens.
Conclusions: These studies collectively demonstrate that nucleus accumbens astrocytes are retracted from synapses in the nucleus accumbens following cocaine self-administration and extinction, an effect that is ameliorated by administration of ceftriaxone during extinction. Moreover, a growing list of compounds that restore GLT-1 expression also impede cocaine seeking, including NAC, ceftriaxone, propentofylline, and riluzole. We propose that reduced astrocyte contact with neurons in the nucleus accumbens represents an important functional adaptation to chronic cocaine use and an amenable target for pharmacological intervention. Ongoing studies are designed to more fully understand the functional consequences of this phenomenon, as well as the mechanism by which GLT-1 is downregulated.
Disclosures: Nothing to Disclose.

54.4 Corticosterone Potentiates Cocaine-induced Reinstatement of Drug Seeking by Inhibiting OCT3-mediated Dopamine Clearance in the Nucleus Accumbens

Paul Gasser

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Background: Cocaine addicts report that craving responses to drug-associated stimuli are intensified during periods of stress, resulting in heightened susceptibility to relapse of drug use. These studies suggest that stress may act as a "stage-setter", inducing state-dependent changes in the sensitivity of brain reward circuits to the reinforcing properties of drugs, and enhancing the potency of drugs of abuse or drug-associated cues to induce relapse. We recently demonstrated that the stress hormone corticosterone acutely blocks dopamine clearance in the nucleus accumbens (NAc) via a dopamine transporter-independent mechanism likely mediated by organic cation transporter 3 (OCT3). We provided evidence that, through this mechanism, corticosterone potentiates the actions of low-dose cocaine on dopamine signaling and reinstatement of drug-seeking behavior in rats. We have hypothesized that decreased clearance of dopamine in the NAc, due to inhibition of OCT3 by corticosterone, enhances dopaminergic neurotransmission, resulting in increased sensitivity to cocaine, and heightened vulnerability to relapse of cocaine-seeking behavior. The present studies test this hypothesis by examining: 1) corticosterone effects on the reinstatement of drug-seeking behavior in OCT3-deficient mice; 2) the effects of corticosterone and cocaine treatment, alone and in combination, on sub-second dopamine dynamics in the nucleus accumbens; and 3) the subcellular distribution of OCT3 in the mouse brain.
Methods: 1.) We examined the effects of corticosterone and normetanephrine, two inhibitors of OCT3-mediated transport, on low-dose cocaine-induced reinstatement of conditioned place preference in wild type and OCT3 knockout mice.
2.) Using fast scan-cyclic voltammetry, we measured naturally-occurring transient dopamine release events during a baseline period, after a systemic injection of corticosterone (2 mg/kg) or vehicle, and after a subsequent systemic injection of low-dose cocaine (2.5 mg/kg).
3.) Coronal sections of aldehyde-fixed mouse brains were incubated with OCT3 antiserum and processed for avidin-biotin-peroxidase labeling. Ultrathin sections were examined with an electron microscope.
Results: 1.) OCT3-knockout mice were both: a) more sensitive to low-dose cocaine-induced reinstatement of conditioned place preference, and b) insensitive to corticosterone-induced potentiation of reinstatement.
2.) Low-dose cocaine injection did not lead to increases in time-averaged dopamine concentrations in the nucleus

accumbens unless it was preceded by corticosterone injection. Corticosterone injection alone tended to increase extracellular dopamine concentration.
3.) OCT3 immunostaining was observed on plasma membranes of dendritic spines adjacent to putative monoamine release sites, as well as in glial processes.
Conclusions: These data indicate that OCT3 represents an important post- or peri-synaptic clearance mechanism, and that inhibition of OCT3 by corticosterone potentiates the effects of low-dose cocaine on both dopamine signaling in the nucleus accumbens and drug-seeking behavior. As OCT3 is capable of transporting norepinephrine, serotonin and histamine in addition to dopamine, its presence in the brain has profound implications for the regulation of monoaminergic neurotransmission under basal and stress conditions.
Disclosures: Nothing to Disclose.

Panel
55. Visualizing Neurocircuit Dynamics in Rodent Models of Addiction and Anxiety

55.1 Neural Activity in Processing Positive and Negative Valence

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Background: Dopamine in the medial prefrontal cortex (mPFC) has been implicated in aversion, yet the circuits mediating avoidance have yet to be identified. Our goal is to understand how mPFC neuronal subpopulations encode the positive or negative valence of discrete stimuli and where this information is communicated downstream to instigate adaptive behavioral responses, such as approach or avoidance. We also seek to understand how dopamine may signal aversion in mPFC circuitry.
Methods: We use freely-moving deep-brain endoscopic calcium imaging to visualize the dynamics of mPFC neurons in behaving mice across a number of behavioral tasks. We also use *in vivo* optogenetics to manipulate circuit components and fast-scan cyclic voltammetry to measure dopamine transients related to the optogenetic manipulations.
Results: We show that an aversive stimulus causes dopamine release in the mPFC, as the increase in catecholamine content is unaffected by locus coeruleus inactivation but is blocked by ventral tegmental area inactivation. We have found that the mPFC neurons have diverse response profiles. Approximately 50% of neurons ($n = 273$) responded similarly, perhaps encoding general salience. $\sim$26% showed selective responding to only one of the stimuli. Another subset of mPFC neurons ($\sim$19%) showed opposing responses, characterized by increased activity to one stimulus and decreased activity to the other. mPFC neurons encoding positive or negative valence likely have distinct downstream targets. The periaqueductal gray (PAG) is has been linked to aversive behaviors and we have found that optogenetic activation of mPFC terminals in the PAG produces avoidance [$n = 6$ ChR2, $p = 0.01$] and anxiety-related behaviors [$p = 0.04$]. Further, stimulation of this pathway evokes defensive [$p = 0.03$] and escape [$p = 0.01$] behaviors in the marble burying assay, suggesting that stimulation of the mPFC:PAG circuit triggers active avoidance behaviors. These effects were not observed in control animals [$n = 6$ eYFP; $p > 0.05$]. The paraventricular nucleus of the thalamus (PVT) has been implicated in reward, and receives dense input from the mPFC. To selectively manipulate mPFC neurons terminating in PVT, we used an anterogradely traveling viral vector carrying ChR2 in a double inverted open reading frame in the mPFC and a retrogradely traveling viral vector carrying cre-recombinase in the PVT. In animals expressing ChR2 only in neurons originating in the mPFC and terminating in the PVT, we show that activation of the PVT-projecting mPFC neurons is positively reinforcing [$n = 3$ ChR2, $p = 0.04$]; an effect not observed in controls [$n = 3$ eYFP; $p > 0.05$].
Conclusions: Together, these data suggest that dopamine is released in the mPFC with an aversive stimulus and the mPFC controls avoidance and approach behaviors through its projections to the PAG and PVT, respectively. Our results advance us towards a circuit-level explanation for how the mPFC can exert control over valence-defined motivated behaviors.
Disclosures: Nothing to Disclose.

55.2 Visualization of a Feeding Authorization Mechanism

Christian Luscher

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Background: Feeding satisfies metabolic need but is also powerfully controlled by external stimuli, like palatability or predator threat. Nucleus accumbens shell (NAcSh) projections to the lateral hypothalamus (LH) are implicated in such feeding control, but the neurons involved and their mechanism of action remains elusive.
Methods: To address these questions, we established a paradigm in which food consumption can be monitored on a moment-to-moment basis in genetically modified mice, permitting the observation and control of identified cell types. We monitor neuroal exctivity with *in vivo* electrophysiology (tetrode recordings in freely moving animals) and by visualizing calcium transients with genetically encoded calcium indicators. We then establish links of causality with bidirectional optogenetic control of neural activity while observing the effect on feeding.
Results: We show that dopamine D1 receptor expressing NAcSh neurons (D1R-MSNs) provide the dominant source of accumbal inhibition to LH and control food consumption via LH GABA neurons. In freely feeding mice, D1R-MSN activity reduced during consumption, while their optogenetic inhibition prolonged feeding, even in the face of distracting stimuli. Conversely, activation of D1R-MSN terminals in LH was sufficient to abruptly stop ongoing consumption, even during hunger. Direct inhibition of LH GABA neurons, which receive strong accumbal inhibition, suppressed feeding, while their activation generated unrestrained consummatory behavior.

Conclusions: Our study identifies a permissive feeding circuit that overrides immediate metabolic need to enable rapid consumption control in response to changing external stimuli.
Disclosures: Nothing to Disclose.

55.3 Neural Substrates of Anxiety-Like Behavior in the Prefrontal Cortex

Ilana Witten

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Background: The medial prefrontal cortex subserves a staggering range of behaviors, and accordingly, its dysfunction is implicated in many neuropsychiatric conditions, including addiction and anxiety disorders. Subsets of prefrontal cortical neurons send descending projections to various subcortical areas, including the nucleus accumbens, the ventral tegmental area, the raphe and the amygdala. This "top-down" control is thought to play an important role in anxiety and addiction, in part because the targeted subcortical areas are also implicated in the same conditions. However, due to technological limitations and the complexity of the underlying circuitry, it has been difficult to ascertain what information is being encoded by each of these top-down projections. We believe that characterizing the top-down regulation of subcortical structures will play a crucial role in the generation of novel and valid hypotheses about the etiology and possible therapies for these conditions.
Methods: In order to image cellular resolution activity from neurons that project from the prefrontal cortex to the nucleus accumbens, a Cre-dependent gCaMP6f virus was injected into the medial prefrontal cortex of mice, and a retrograde virus expression Cre-recombinase was injected into the nucleus accumbens. Subsequently, a GRIN lens was implanted above the dorsal region of the prefrontal cortex (prelimbic cortex). Mice explored anxiety assays (elevated plus maze, open field test) while neural activity was imaged using a head-mounted microscope (Inscopix).
Results: Over 400 neurons were imaged from 6 mice. Activity of many neurons within this population encodes the mouse's location while it explored the elevated plus maze (an anxiety assay), with some neurons responding more strongly in the non-preferred, unprotected region (open arm), and other others responding more strongly in the preferred, protected location (closed arm). In addition, several analyses revealed consistent differences in the population-level encoding of the open versus the closed arms. For example, the average activity across the population of neurons was stronger in the open arms rather than the closed arms. In addition, neural activity was correlated across the closed arms within the population of neurons, while it was anti-correlated across the open arms.
Conclusions: Neurons that project from prefrontal cortex to the nucleus accumbens encode an animal's location while exploring an anxiety assay, with distinct coding schemes in the protected and unprotected locations in an environment. These data provide an important step in characterizing the role of top-down signals from the prefrontal cortex in rodent models of anxiety.
Disclosures: Nothing to Disclose.

55.4 Imaging Network Dynamics to Rewards and Predictive Stimuli in VTA and PFC Circuits

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Background: Accurate neuronal encoding and representation of reward availability is critical to survival, and this adaptive function of the brain becomes highly disruptive in addiction and other neuropsychiatric conditions. While midbrain dopamine neurons are hypothesized to encode a reward prediction error signal, it is unclear whether individual neurons in the VTA or in upstream targets such as the lateral hypothalamus (LH) or medial prefrontal cortex (mPFC) develop learned signals in response over many repeated cue-reward pairings.
Methods: To address this, we have developed *in vivo* imaging approaches to record neural activity dynamics of 100's of genetically or circuit defined neurons in awake and behaving mice. We have selectively expressed the genetically-encoded calcium indicator in select subsets of neurons in the VTA, LH, or mPFC based on their molecular phenotype or projection targets. Following expression of the calcium indicator in targeted cells, micro-endoscopic GRIN lenses or optical cannula are implanted directly above the VTA, LH, or mPFC. We then use single photon or two-photon imaging approaches to resolve neural activity dynamics in these structures in response to primary rewards (sucrose) or reward-predictive stimuli (CS+).
Results: By imaging activity in the LH, we have demonstrated that individual LH GABAergic neurons, many of which project to the VTA, encode appetitive stimuli or primary reward consumption, but rarely both. Appetitive and consummatory encoding LH neurons are largely intermixed within the LH, suggesting the encoding of these processes occurs on a cell by cell basis and may be dictated based on the project targets of individual LH GABAergic neurons, or their afferent inputs. In addition, We show that using imaging approaches, we can reliably track the activity dynamics of individual neurons throughout repeated behavioral sessions, thus demonstrating that changes in the neuronal representation of rewards or predictive cues can now be resolved over many days. The experiments investigating VTA and mPFC neuronal encoding are still ongoing, but we will present our unpublished results of these experiments in this session.
Conclusions: By imaging neuronal activity in the LH our data demonstrate that individual LH GABAergic neurons can encode select behavioral aspects critical for motivated behavior. In addition, by establishing methods to resolve neural activity from the same neurons over many repeated behavioral sessions, we demonstrate the feasibility of studying how neuronal representations of behavior are altered following learning, or throughout the progression of a maladaptive behavioral state such as addiction.
Disclosures: Nothing to Disclose.

Panel
56. Translational Neural Network Approaches for Identifying Individualized Targets for Neurostimulation in Mood Disorders and OCD

56.1 Cortical Nodes in the Ventrolateral Prefrontal and Anterior Cingulate Cortex and the Functional Segmentation of the Internal Capsule: Implications for Potential Treatment Targets for Psychiatric Disease

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Background: The dorsal anterior cingulate cortex (dACC), ventrolateral prefrontal cortex (vlPFC), along with the orbital prefrontal cortex (OFC), dorsolateral prefrontal cortex (dlPFC), and amygdala are associated with several psychiatric diseases. Non-invasive stimulation methods, including transcranial direct current stimulation (tDCS) and the invasive method, deep brain stimulation (DBS), are currently being explored as treatment options for several diseases including obsessive-compulsive disorder (OCD) and depression. In the noninvasive approaches stimulation is placed over specific cortical regions. Our hypothesis is that there exist 'critical nodes' within cortical areas that receive converging inputs from several functionally diverse areas (i.e. a combination of different emotion and cognitive control areas). These nodes are potentially an important focus for treatment targets that are designed to modulate the balance between top-down and bottom-up control. Here we show the location and composition of 2 nodes, one in the vlPFC and one in the dACC. In contrast to tDSC, DBS targets white matter, with one major target, the anterior limb of the internal capsule (ALIC). We sought to delineate where, within the ALIC, fibers from the vlPFC and dACC travel. We tested these fiber trajectories in humans using diffusion MRI (dMRI) and determined which fibers are likely to be involved at different DBS sites in DBS treated-patients.
Methods: Using animal tracing experiments, we analyzed the specific cortical and amygdala inputs that converge in the vlPFC and dACC and the location of these critical nodes. We identified a similar region in the human brain using resting state functional MRI that could be used as a tDCS target. We also determined where fibers from the vlPFC, dACC, and dlPFC traveled through the ALIC in animals, combining the analysis with previous data of OFC fiber location in the ALIC. We used this data to guide dMRI identified fiber locations in the human ALIC and the likely pathways stimulated at different DBS sites within the capsule.
Results: We found critical nodes located in specific dACC and vlPFC regions. The dACC region receives convergent inputs from the amygdala, OFC, dlPFC, vlPFC. The vlPFC node receives inputs from the amygdala, OFC, dlPFC, dACC, and pSMA. Regions adjacent to the nodes do not receive these inputs. These data guided specific seed placements for a resting state analysis in humans. The results show a functional connectivity map that was consistent with the anatomical connections. Fibers from these cortical nodes travel within specific parts of the ALIC. In particular, DBS of the central ALIC involves dACC and vlPFC fibers, but not OFC fibers.
Conclusions: Specific areas of the dACC and vlPFC receive inputs from the amygdala and OFC that converge with those from cognitive control areas (dlPFC, dACC, and vlPFC). We found that each DBS electrode captures a specific combination of fibers depending on the electrode location.
Disclosures: Nothing to Disclose.

56.2 Associations Between Distinct Patterns of Reward Circuitry Function and Impulsive Sensation Seeking Provide Novel Neural Targets for Transcranial Direct Current Stimulation as an Intervention to Reduce Risk-Taking Behaviors

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Background: Neuroimaging studies of mood disordered individuals highlight relationships among measures of function and structure in ventrolateral prefrontal (vlPFC)-striatal-amygdala neural circuitry and reward-related behaviors, e.g., risk-seeking and impulsive sensation seeking. This dimensional approach is elucidating neural mechanisms associated with dysfunctional behaviors in psychiatric disorders, and can identify key nodes in neural circuitries implicated in underlying psychopathological processes in these disorders that can serve as targets for interventions with novel neurostimulation techniques.
Methods: In a large, ongoing study of neural mechanisms underlying dysfunctional reward-related behaviors in distressed, treatment-seeking 18-25 year-olds, we have, to date, scanned over 40 such individuals during performance of different tasks, including a number-guessing reward task. Each trial of the reward task includes an expectancy period, when participants anticipate uncertain reward or loss future outcome, and the outcome phase itself. In a pilot study in healthy volunteers, cathodal (inhibitory) and sham transcranial direct current stimulation (tDCS) were implemented in a randomized, single-blind, within-subjects study design, during neuroimaging. The goal of this pilot study was to determine the extent to which this neurostimulation technique could perturb functioning within reward circuitry during performance of the above reward task.
Results: We show significant positive relationships between a measure of trait impulsive sensation seeking and activity during uncertain outcome expectancy in distributed reward circuitry, including bilateral visual cortices (cuneus; $p < 0.05$, FWE) and left vlPFC ($p < 0.005$). These data replicate our previous findings in independent studies of a significant positive relationship between fun-seeking and activity in left vlPFC across individuals with bipolar disorder and healthy volunteers, and abnormally elevated left vlPFC activity in individuals across the bipolar disorder spectrum, during uncertain reward and outcome expectancy. Pilot study findings in healthy volunteers show that cathodal (vs. sham) tDCS applied to the left vlPFC results in decreased positive functional connectivity between this

region and ventral striatum during reward task performance.
Conclusions: The vlPFC links specific stimulus and specific reward outcome representations. Our new and previous findings suggest that abnormally elevated visual cortical and left vlPFC activation to uncertain outcome expectancy may reflect heightened representation of visual stimulus-reward outcome relationships that, in turn, may be a neural mechanism for impulsive sensation seeking. Our pilot findings suggest that left vlPFC cathodal tDCS may help reduce vulnerability to risk-taking behaviors, especially in individuals with higher trait impulsive sensation seeking.
Disclosures: Part 1: I am a consultant for Roche Pharmaceuticals.

56.3 Mapping Functional Connectivity Networks in the Individual

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Background: The capacity to identify the unique functional architecture of an individual subject's brain is a critical step towards personalized medicine and understanding the neural basis of variations in human cognition and behavior. Clinical and imaging studies have demonstrated marked inter-individual variability in the organization of different functional systems of the brain, particularly in higher order association areas. Localizing specific functional circuitries in a particular subject is therefore a fundamental requirement in clinical procedures such as surgical planning or non-invasive brain stimulation therapies. However, functional imaging techniques are generally limited in accuracy and reliability at the single-subject level.
Methods: Here we developed a novel brain parcellation approach to accurately map functional organization at the individual level using resting-state fMRI. A population-based functional atlas and a map of inter-individual variability were employed to guide the iterative search for functional networks in individual subjects. This strategy allows the idiosyncratic functional organization of the individual to drive the network solution. Reliability and accuracy of the resulting functional maps were tested in several independent datasets.
Results: Functional networks mapped by this approach were highly reproducible within subjects and effectively captured the variability across subjects, including individual differences in brain lateralization. The algorithm performed well across different subject populations and data types including task fMRI data. The resulting parcellation networks were significantly more reliable than networks localized by traditional task-evoked response. This novel technology can also reliably identify the ventrolateral prefrontal (vlPFC)-striatal circuitry at the single subject level, a potential neurostimulation treatment target for OCD.
Conclusions: Functional connectivity variability has a specific topographic distribution with heteromodal association cortex being most variable therefore within-subject functional mapping is particularly important in psychiatric research. The novel functional mapping technique developed in this study can provide an individual-level functional atlas which may help the identification of personalized therapeutic targets for various diseases including OCD.
Disclosures: Nothing to Disclose.

56.4 DBS Electrode Position and Clinical Outcomes in the Context of Tractography

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Background: Deep brain stimulation (DBS) for intractable OCD, which has regulatory approvals in the U.S. and E.U., remains the subject of ongoing research. Similar to its uses in movement disorders, the method requires time-consuming programming to select the individual electrode contacts, or groups of contacts, where stimulation is associated with the best clinical responses, and the greatest tolerability, over time. Our DBS target, the VC/VS or ventral capsule-ventral striatum, spans the ventral anterior limb of the internal capsule (ALIC) and the adjacent ventral striatum (VS). Previous work by ourselves and others has provided hints of systematic relationships between activation of DBS contacts in certain locations within the target region and both therapeutic and adverse effects. In general, enhanced positive affect (and later OCD improvement) has been somewhat more likely with use of contacts at or dorsal to the anterior commissure, a landmark along the trajectory of the DBS leads. Conversely, adverse affective and autonomic effects may be more likely when more ventral contacts are stimulated. However, both kinds of effects differ notably across individual cases, even with electrode placements which appear comparable when visualized with high-resolution structural MRI. It is likely that this variability in outcomes, which adds complexity and patient burden to programming, can be explained by relationships between electrodes and specific fiber tracts connecting the ventral prefrontal cortex to thalamic and brainstem nuclei which course through the DBS target.
Methods: Our multicenter NIMH-supported controlled trial of DBS for OCD has enrolled 27 patients, the last of whom will exit the masked phase by July 2015. In contrast to earlier open-label studies of VC/VS DBS in intractable OCD, using a lead covering a relatively large territory (contacts spanned 24mm, each electrode 3mm in length), in this trial we selected a device which was more than twice as compact (10.5mm electrode span, electrodes 1.5mm long). This choice was based on prior clinical outcomes and 3D modeling of fiber tracts hypothesized to be most relevant which were based on nonhuman primate tracer experiments informed by human DTI tractography (S. Haber et al.). Among the baseline pre-surgical measures obtained in this trial is diffusion-weighted MRI. These were obtained on the same research magnet for patients from three of the nine study centers participating in the trial.
Results: Open-label data suggest that there is wide case-by-case variability in the DBS electrode contacts associated with the greatest improvements in YBOCS OCD severity.

The most optimal responses are seen with cathodal stimulation at contact 0 (most ventral) in some individuals, and with activation of contact 3 (most dorsal) in others, and in others when the middle two contacts 1 and 2 are used for chronic stimulation. The same pattern obtains for symptoms of comorbid depression and nonspecific anxiety. Localization of clinically-identified contacts will be confirmed using data from the study masked phase. The contact sites will then be placed within ventral PFC-thalamic and ventral PFC-brainstem pathways using DTI-based tractography.
Conclusions: Open-label data suggest substantial variability in locations of DBS electrodes associated with the greatest benefit and lowest burden of adverse effects, even when using a stimulating lead with less than half the anatomical extent of that in prior studies. Our hypothesis that clinical outcomes is explained by specific relationships between electrodes and fiber tracts connecting the ventral prefrontal cortex to subcortical nuclei will be tested using tractography.
Disclosures: Nothing to Disclose.

Panel
57. New Twists on Transmembrane Transporter Function in Psychiatric and Neurodegenerative Disorders

57.1 Missense Mutations in the Dopamine Transporter Gene: Commonality Between Neuropsychiatric and Neurodegenerative Diseases?

Ulrik Gether

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Background: Dopamine dysfunction is of central importance in neuropsychiatric diseases, such as schizophrenia, affective disorder, ADHD and autism, as well as in neurodegenerative parkinsonism. The presynaptic dopamine transporter (DAT) mediates reuptake of dopamine and thereby plays a key role in regulating dopamine homeostasis by terminating dopamine signaling and ensuring maintenance of reusable pools of transmitter. There is accumulating evidence that rare genetic variants in the DAT gene, including de novo mutations, can play a hitherto unknown key role in the pathophysiology of both neuropsychiatric and neurodegenerative disorders.
Methods: Patient cohorts are screened for genetic variants in the DAT gene. This includes patients with atypical movement disorder and large cohorts of patients ($>10{,}000$) with psychiatric disease (schizophrenia, bipolar disorder, ADHD and autism). Missense variants in the coding region are phenotypically characterized *in vitro* to assess alterations in uptake, pharmacology, trafficking and electrophysiological properties. Knock-in mice of selected mutations are generated to study causality and disease mechanisms.
Results: We recently identified two novel DAT coding variants in an adult male diagnosed with both neuropsychiatric disorder and early-onset neurodegenerative parkinsonism. The variants included Ile312Phe in transmembrane segment 6 and a presumed de novo mutant Asp421Asn in the second sodium site. In heterologous cells, both mutants exhibited markedly reduced dopamine uptake capacity but preserved membrane targeting, consistent with impaired catalytic activity. For Asp421Asn, substrate efflux experiments revealed a constitutive, anomalous efflux of dopamine, and electrophysiological analyses identified a cation leak that might contribute to perturbed dopaminergic neurotransmission. To assess causality and investigate disease mechanisms, knock-in mice expressing Asp421Asn and/or Ile312Phe have been generated. Importantly, our genetic screening has led to identification of yet other DAT variants. This includes a variant, located in the C-terminal PDZ binding sequence, which is also associated with neuropsychiatric disorder and early-onset neurodegenerative parkinsonism. Preliminary data suggest impaired surface targeting and an interesting dominant negative effect on the wild type transporter. Moreover, sequencing of 155 patients with severe affective disorder has revealed new variants that currently are subject to phenotypic characterization *in vitro*.
Conclusions: Our data provide strong evidence that missense mutations in DAT can cause or contribute to both neuropsychiatric diseases and movement disorders, and that the resulting disease phenotype depends on the nature of the functional perturbations caused by the mutations. Moreover, the results suggest a yet unappreciated commonality between neurodegenerative and neuropsychiatric diseases and accordingly that the study of DAT missense variants can lead to novel understanding that pertain to dopamine pathologies in general.
Disclosures: Nothing to Disclose.

57.2 An Inside Job: Endosomal Na+/H+ Exchangers in Autism and Neurological Disorders

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Background: A subset of Na+/H+ exchangers (eNHE) localize to endosomal compartments where they mediate proton leak, countering the action of proton pumps. This important safety valve precisely sets the pH of the lumen to control the trafficking and turnover of cargo critical for neurological function. Dysregulation of eNHE has been implicated in both neurodevelopmental and neurodegenerative disorders, including autism, ADHD, intellectual disability and Alzheimer disease. Rare variants in SLC9A9, the gene encoding NHE9, may underlie autism whereas significant depression in gene expression links to the APOE4 variant associated with Alzheimer and other neurodegenerative disorders. Although genetic approaches are important in candidate gene identification, functional analysis of transporter activity is essential to predict clinical outcome and for personalized therapy.
Methods: We use homology models based on evolutionary conservation to distant bacterial orthologs of known structure to predict functional consequences of rare variants in NHE9. This structure-driven assessment is followed by sequential phenotype screening in the yeast model organism and primary astrocytes to distinguish

harmless polymorphisms from disease-causing mutations. Lentiviral mediated knockdown and overexpression strategies in astrocytes and genotyped patient fibroblasts allow us to correlate changes in endosomal pH to delivery, removal and activity of cell membrane receptors and transporters that mediate specific cellular phenotypes, including glutamate uptake, growth factor signaling and amyloid beta peptide clearance.
Results: Functional assessment of autism-associated NHE9 variants revealed that changes in conserved residues led to loss of transporter phenotypes in both yeast and astrocyte model systems. Unexpectedly, substitution of a variable side chain caused functional deficiency in astrocytes but failed to show differences from wild type protein in yeast. Loss of eNHE associated proton leak in NHE9 mutants resulted in endosomal hyperacidification and reduction in uptake of the neurotransmitter glutamate, consistent with elevated levels of brain glutamate and predisposition to seizures in patients. Similarly, decreased NHE9 expression in patients with Alzheimer disease correlated with reduced clearance of the amyloid peptide Abeta and missorting of endosomal cargo, both pathological hallmarks of neurodegeneration.
Conclusions: Endosomal dysfunction is central to several neurological disorders, ranging from autism to Alzheimer disease. A mechanistic understanding of the role of Na+/H+ exchanger NHE9 could lead to potential therapeutic intervention. Several FDA-approved drugs have been reported to mildly alkalinize endosomal pH and could be repurposed to counter loss of eNHE function in the treatment of these neurological disorders. With the advent of personal genomics on the horizon, our phenotype screening approach offers a rapid, inexpensive and accurate evaluation of human variants, distinguishing disease-causing mutations from harmless polymorphisms.
Disclosures: Nothing to Disclose.

57.3 Unraveling Mechanisms Contributing to Lack of Antidepressant Efficacy in Juveniles and Adolescents: are Organic Cation Transporters to Blame?

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Background: Depression is a major public health problem for which most patients are not effectively treated. This problem is further compounded in children and adolescents where only two antidepressant drugs are currently approved for clinical use. Both belong to the selective serotonin (5-HT) reuptake inhibitor (SSRI) class of antidepressant, and act by blocking the high-affinity uptake of 5-HT from extracellular fluid via the serotonin transporter (SERT). The therapeutic utility of SSRIs is thought to be triggered by downstream events that occur in response to their ability to increase extracellular levels of 5-HT. However, in juveniles and adolescents, little is known about mechanisms contributing to 5-HT uptake. Essentially nothing is known about expression and function of SERT during this period, and nothing is known about expression and function of organic cation transporters (OCTs) and the plasma membrane monoamine transporter (PMAT), low-affinity, high-capacity transporters for 5-HT. Here we present data that PMAT is a promising new target for the treatment of pediatric depression.
Methods: All studies were carried out in male mice (C57BL/6 background). Chronoamperometry was used to measure clearance of 5-HT from extracellular fluid in hippocampus. Radioligand binding together with western blot was used to quantify expression of SERT, OCT3 and PMAT. The tail suspension test was used to assay for antidepressant-like effects of drugs.
Results: During juvenile and adolescent periods, we found that PMAT expression is increased and SERT function is decreased, relative to adults. The increase in PMAT was magnified in SERT+/- and SERT-/- mice suggesting that during adolescence PMAT upregulates to compensate for a constitutive reduction in, or loss of SERT expression/function. In contrast, in adults OCT3 expression is increased in SERT+/- and SERT-/- mice. Functionally, we found that clearance of 5-HT from extracellular fluid in hippocampus was equally efficient among adolescent SERT+/+, SERT+/- and SERT-/- mice, indicating contributions to 5-HT clearance by a mechanism(s) other than SERT, putatively PMAT. In contrast, in adult mice, the same concentration of 5-HT was cleared with lower efficiency in SERT+/- and SERT-/- mice than in SERT+/+ mice, suggesting that OCT3 is not as effective in clearing 5-HT as PMAT. Importantly, we found that D22 (an inhibitor of OCTs and PMAT) produced antidepressant-like effects in wild-type juvenile mice, whereas in adult mice, antidepressant-like effects of D22 were only apparent when SERT was pharmacologically or genetically inactivated. In adult mice the potency of D22 to produce antidepressant-like effects was reduced in OCT3 knockout mice. Studies using juvenile and adolescent PMAT and OCT3 knockout mice are ongoing.
Conclusions: Here we show that during juvenile and adolescent periods, PMAT plays a previously unsuspected role in 5-HT uptake, whereas in adults, OCT3 contributes to 5-HT clearance. The antidepressant-like activity of D22 suggests that activity of these transporters likely limits the therapeutic utility of SSRIs. Moreover, the role of PMAT and OCT3 in 5-HT clearance increases when SERT function is genetically compromised. This is of particular interest given the link between low expressing and/or functioning variants of the SERT gene and psychiatric disorders. Our findings point to PMAT and OCT3 as promising targets for the development of new antidepressants with improved therapeutic potential.
Disclosures: Nothing to Disclose.

57.4 Towards Chemical Screening of Antidepressant Efficacy via Voltammetric Characterization of *In Vivo* Serotonin Clearance

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Background: Antidepressants treat the symptoms of depression, a debilitating neurological disorder, and are

among the most widely prescribed medications. Unfortunately, most antidepressants have limited therapeutic benefits. Resources for antidepressant drug discovery are declining primarily because of the unavailability of preclinical tests that predict clinical efficacy. Behavioral tests, such as the forced swim and the tail suspension tests, in rodents are not always reliable predictors of antidepressant efficacy.

Most antidepressants influence serotonin neurotransmission either by blocking the serotonin transporter, or by targeting dopamine and norepinephrine systems to indirectly modulate serotonin neurotransmission. Here we describe using *in vivo* fast scan cyclic voltammetry (FSCV) at carbon fiber microelectrodes (CFMs) for measuring hippocampal serotonin clearance kinetics in mice treated with different antidepressants. We outline how different antidepressants create unique voltammetric serotonin profiles which correlate to clinical efficacy, finally discussing the potential of FSCV for chemical screening of antidepressant efficacy.

Methods: A single carbon fiber was aspirated into a glass capillary, pulled apart under heat and cut to 150 μM. The resulting CFM was electroplated with Nafion. Adult, male C57BL/6 mice weighing 20-25g were anesthetized with urethane. Mouse procedures were in compliance with WSU's Guide for the Care and Use of Laboratory Animals, approved by the Institutional Animal Care and Use Committee (IACUC). Stereotaxic surgery was performed to implant the CFM into the CA2 region of the hippocampus, a stimulating electrode into the medial forebrain bundle (MFB) and a reference electrode into the contralateral brain hemisphere. Electrical pulses were delivered via a linear constant current stimulus isolator.

Results: We tested the effects of a large, acute antidepressant dose on stimulated serotonin release and reuptake. Escitalopram increased serotonin release amplitude rapidly (~5 minutes) after administration. Additionally, and surprisingly, escitalopram caused an increase in the rate of serotonin reuptake: an effect which persisted for over 2 hours. This effect may be associated with transporter up-regulation and/or shift to high efficiency state. Importantly, when we tested the effects of other different antidepressants on serotonin, we found a similar increase in the rate of serotonin reuptake. Most interestingly, we found that antidepressants with a greater ability to increase serotonin reuptake (independent of class) are reported to be the most efficacious in the clinical literature. We therefore present the potential power of FSCV measurements for chemical screening of antidepressant efficacy.

Conclusions: Antidepressants continue to carry limited therapeutic benefit. Elucidating techniques to more powerfully predict clinical efficacy of novel antidepressants would greatly facilitate drug discovery. In this study, we found that *in vivo* FSCV of serotonin provides important, novel insights into antidepressant mechanisms. Furthermore, different antidepressant's effects on serotonin uptake correlate to clinical efficacy, giving rise to the potential of FSCV as a chemical screen for antidepressant efficacy.

Disclosures: Nothing to Disclose.

Mini Panel
58. Revisiting the Mu Opiate Receptor for the Treatment of Depression

58.1 Opioid Receptors and Mood

Brigitte Kieffer

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Background: The roles of opioid receptors in pain and addiction have been extensively studied, but their function in mood disorders has received less attention. Accumulating evidence from animal research reveals that the three receptors (mu, delta and kappa) exert highly distinct controls over mood-related processes, and the potential of each receptor for the treatment of major depressive disorders is being actively investigated.

Methods: In this presentation, we will first summarize data from genetic mouse models by our group and others, which have allowed positioning each opioid receptor in the control of hedonic homeostasis and mood control. Those studies have used behavioral models of anxiety and depression, as well as a recent model for emotional deficits in protracted abstinence from drugs of abuse from our laboratory, which involves serotonergic neurotransmission. We will then focus on the mu opioid receptor and present two new genetic mouse models that (i) allow deciphering the neuroanatomy of mu opioid receptor expression *in vivo* with subcellular resolution and (ii) produce conditional ablation of the mu opioid receptor gene in targeted neuron populations within reward and aversion pathways.

Results: Data indicate that mu opioid receptors mediate rewarding properties of both drugs of abuse and natural rewards, with implication for autism spectrum disorders. Delta receptors show anxiolytic and antidepressant activities, which have now translated to clinical trials, and the kappa/dynorphin system contributes to dysphoric states induced by drug abuse or chronic stress. Further, delta and kappa receptors oppositely influence susceptibility to develop depressive-like behavior and social withdrawal upon prolonged abstinence to chronic heroin. Finally, knock-in mice expressing a functional fluorescently-tagged mu opioid receptor (mcherry) show highest receptor expression in cholinergic and substance P neurons of the medial habenula, and we currently identify afferent and efferent pathways within and outside the habenular complex using tracing experiments. We also currently develop mice with a conditional deletion of mu receptors in targeted neurons of this intriguing, yet poorly investigated brain microstructure structure to test the hypothesis that this receptor regulates negative affect states at the level of the medial habenular-interpeduncular pathway.

Conclusions: Our current knowledge of opioid receptor function in mood control clearly posits delta receptor activation and kappa receptor blockade as promising therapeutic approaches to improve mood states. The role of mu receptors in mediating reward is well-established, but basic knowledge of its role in depression-related behaviors is still missing. This new area of investigation hold promises for treatment, should the addictive liability of mu opiates be circumvented.

Disclosures: Nothing to Disclose.

58.2 The Antidepressant-Like Effects of Tianeptine are Mediated by the Mu Opiate Receptor

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Background: Tianeptine is an antidepressant used in Europe for the treatment of Major Depressive Disorder and Anxiety Disorders. In preclinical studies tianeptine was shown to decrease depression and anxiety-related behaviors and to prevent stress-induced decreases in neuronal dendrites in the hippocampus. Despite earlier suggestions that tianeptine influenced the serotonergic and glutamatergic systems, its mechanism of action has remained elusive.
Methods: We have used a combination of pharmacology, cell-based assays and gene knockouts in mice to study the mechanisms underlying the physiological and behavioral effects of tianeptine.
Results: Using radioligand binding and cell-based functional assays, including bioluminescence resonance energy transfer (BRET)-based assays for G-protein activation and cAMP accumulation, we identified tianeptine as an efficacious mu-opioid receptor (MOR) agonist (Ki-Human of 383 ± 183 nM and EC50-Human of 194 ± 70 nM for G-protein activation). Tianeptine was also a full delta-opioid receptor (DOR) agonist, although with much lower potency (EC50-Human of 37.4 ± 11.2 μM for G-protein activation). In contrast, tianeptine was inactive at the kappa-opioid receptor (KOR). We have also shown that the behavioral and analgesic effects of tianeptine in a mouse model of depression are blocked by the opioid antagonist naltrexone and are absent in MOR knockout mice.
Conclusions: On the basis of these pharmacological and behavioral data, we propose that activation of MOR (or dual activation of MOR and DOR) could be responsible for the antidepressant and anxiolytic effects of tianeptine in humans. Targeting the MOR may therefore represent a novel approach in the treatment of depressed patients who do not respond to classic antidepressants.
Disclosures: Nothing to Disclose.

58.3 Evaluation of Agonist-Antagonist Opioid Modulation with ALKS-5461 in Major Depressive Disorder

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Background: An accumulating body of evidence derived from animal models, human PET imaging investigations and human autopsy studies indicate that major depressive disorder (MDD) is associated with significant dysregulation of the endogenous opioid system. The contemporary use of opioids to treat MDD and related mood disorders, however, is very limited due to their potential for abuse and addiction. In an effort to therapeutically address endogenous opioid dysregulation in MDD while avoiding the potential for abuse and addiction, we studied a combination of buprenorphine (BUP), a μ-opioid partial agonist and κ-opioid partial agonist with low intrinsic activity, and samidorphan (SAM), a potent μ-opioid antagonist.
Methods: Fixed ratios of BUP and SAM were co-formulated in a single sublingual tablet (ALKS 5461). Pupilometry and subjective pharmacodynamic effects of ALKS 5461 were evaluated in $N = 16$ non-addicted opioid experienced subjects. Safety and antidepressant effects were evaluated as adjunctive treatment in subjects with MDD and an inadequate response to SSRI or SNRI therapy in two trials; a 1-week pilot study ($N = 32$) and a follow-up 2-stage phase 2 study ($N = 142$). The clinical studies were double-blind, randomized and placebo-controlled and utilized the Montgomery–Åsberg and Hamilton Depression Rating Scales (MADRS and HAM-D17). The phase 2 MDD study employed sequential parallel comparison design (SPCD) to minimize placebo effect. Nonclinical *in vivo* microdialysis studies in Wistar rats measured regional changes in extracellular concentrations of brain neurotransmitters following subcutaneous administration of BUP and SAM individually and in combination.
Results: In the study of non-addicted, opioid experienced subjects, maximal blockade of pupilary and opioid subjective and physiologic effects was observed with a 1:1 ratio of BUP:SAM. In both the pilot and the follow-up phase 2 MDD studies, adjunctive treatment with the 1:1 ratio demonstrated statistically significant and clinically important antidepressant efficacy vs placebo (pilot study: MADRS $p = 0.032$, HAMD-17 $p = 0.054$; phase 2 study MADRS $p = 0.004$, HAMD-17 $p = 0.026$). Comparison of the individual stages of the phase 2 MDD study demonstrated that the SPCD design successfully attenuated placebo response. ALKS 5461 was generally well tolerated. The most common AEs were nausea, vomiting, and dizziness. There was no evidence of opioid withdrawal post-treatment.
Microdialysis studies in rats showed that combined BUP/SAM resulted in sustained low-level, ceiling-limited increases in extracellular dopamine, serotonin, and/or metabolites in the nucleus accumbens (NAc) shell, medial prefrontal cortex (mPFC) and other brain regions. A small reproducible increase in mPFC glutamate with delayed (2-3 hr) onset was also observed.
Conclusions: A "balanced" agonist-antagonist opioid modulation with ALKS 5461 is a novel treatment approach for patients with MDD. Microdialysis studies in rats suggest that the observed clinical response may be mediated via modulation of the release of multiple neurotransmitters in key brain regions including the NAc and mPFC. Large phase 3 studies to confirm and extend the results of the current work are ongoing.
Disclosures: Part 1: Full-time employee of Alkermes, Inc., **Part 5:** Alkermes, Inc.

Mini Panel
59. DBS and the Identification of Circuits Mediating Depression

59.1 Using DBS to Define Circuits Mediating Depression

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Background: Recently several targets have been identified as potential sites for DBS to treat treatment resistant depression

(TRD). Defining the circuits activated in successful responses following DBS may provide detailed information on the circuits that are central to depression. It is now more than 10 years since the first subcallosal cingulate DBS implant for TRD. The research has made steady progress with emerging clues as to which patients are most likely to benefit and the technique has now been refined to optimize surgical targeting using diffusion tractography with improved outcomes. As testing of DBS for TRD evolves, imaging will continue to play a crucial role, with recent work now focused on refinement and optimization of the procedure using combined structural, function and diffusion MRI data combined with real-time behavioral and electrophysiological metrics, providing a more precise method to identify the optimal target location for the individual patient. In an animal models combining new studies combining optogenetic activation of a circuit with fMRI is allowing a detailed investigation of downstream effects of stimulation. Using these methods in animal models and subjects is leading to a clear analysis of the circuit changes necessary to lead to remission of TRD. Data on the ongoing MT. Sinai DBS study involving l.habenular stimulation for treatment resistant depression will be presented with a discussion of new targeting methods.
Methods: In this presentation using animal studies involving optogenetics, fMRI, proteomics and histological staining, circuits will be defined that appear to mediate depression. Using data from patient DBS studies involving DTI, activation volume, probabilistic tractography and 7T segmentation activated tracts will be identified and correlated with clinical outcome.
Results: The discussion will formulate an approach to defining *in situ* measures from successful DBS targeting. This will include an analysis of the activation of medial frontal cortex, uncinate fasciculus and rostral and cingulate cortex as well as sub cortical nuclei such as VTA, DRN and inhibition of the l. habenula. Of interest is the examination of targets for change in one procedure, such a l. habenula inhibition or cingulate cortex stimulation, in procedures targeting another area. Patient characteristics that predict successful DBS, such as previous remission with ECT, will also be examined. These results suggest a common final pathway mediating depression through changes in cortical and monoaminergic pathways and a patient profile that better predicts response to DBS.
Conclusions: There are extensive networks involving input from an anxiety and fear circuit involving hippocampus, amygdala, BNST, mPFC and l. habenula, a circuit involving reward and the control of monoaminergic nuclei and feedback to the mPFC. A proposed methodology for identifying candidates with a high probability of positive outcomes to DBS using the above methods will be presented.
Disclosures: Part 4: Medtronic donated devices for study of Lateral Habenula DBS in TRD.

59.2 Delineating Alterations of Brain Circuitry Due to Lateral Habenula DBS in TRD

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Background: The Lateral Habenula (LHb) has received growing attention as a key player in the pathophysiology of major depression. Down-regulation of LHb activity by (unspecific electrical) deep brain stimulation has been successfully demonstrated in two patients by our group. An animal model of treatment-resistant depression (congenital Learned Helplessness, cLH) exhibits altered habenula activity as well as increased functional connectivity between, among others, the cingulate gyrus and retrosplenial cortex. This finding is homologous to the midline resting-state network, which has repeatedly been reported to be over-active in human depression.
Methods: Using the novel combination of opto-genetics and high-field functional Magnetic Resonance Imaging (og-fMRI), we sought to reduce habenula activity in cLH animals. This approach unifies a highly specific perturbation of brain circuitry while still observing whole brain connectivity changes. Three weeks after ArchT-containing adenovirus injection in cLH animals (a control group received an empty viral cassette) optic fibers were implanted at the same site ($n = 19$ in total). Laser stimulation was performed during resting-state image acquisition in a high-field 9.4 Tesla animal scanner. Additionally, resting state data (without laser stimulation) were acquired before and after stimulation. Virus expression was assessed histologically after sacrifice.
Results: In our hypothesis-driven analyses we found laser-dependent specific reductions in the connectivity between retrosplenial cortex and cingulate cortex. This difference was present both when comparing connectivity during laser stimulation versus baseline condition (i.e. before laser application), and after laser stimulation vs. baseline. The changes were significant when compared to the control group.
Conclusions: Midline resting state hyperconnectivity is a well-described depression-related intermediate endophenotype that can be studied in a translational manner. Our results further support the lateral habenula as a promising target for treatment of (especially treatment-resistant) depression.
Disclosures: Nothing to Disclose.

59.3 Refined Methods: for Subcallosal Cingulate DBS Targeting using Network-Specific Structural Connectivity Maps

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Background: The first studies of subcallosal cingulate deep brain stimulation (SCC DBS) for treatment resistant depression (TRD) were initiated in 2003 [1] with follow-up now exceeding 7-10 years [2-3]. While a 6-mo response rate of 40-60% is reported, there is variability in onset of initial effects and the rate of symptom improvement. Mechanisms mediating antidepressant effects have generally focused on regional change patterns evoked by chronic stimulation; new studies are additionally examining acute as well as chronic changes in network dynamics using electrophysiological recordings, with precise delineation of essential network 'nodes' an emerging priority [4]. Based on post-hoc analyses of the structural connectivity pattern differences in 6-mo responders and non-responders to SCC DBS using DTI-based probabilistic tractography (sc-DTI) [5], we hypothesized that pre-surgical delineation and individualized targeting of this specific combination of white matter tracts would improve response over standard methods using solely SCC anatomy and trial and error testing of individual lead contacts.

Methods: Eleven patients with TRD scheduled for SCC DBS had pre-operative MRI, including DTI. A software tool employing deterministic tractography was used to visualize the surgical target–namely the intersection of four well-defined white matter bundles converging at the SCC: bilateral uncinate fasciculus, cingulum bundle, forceps minor and cingulo-subcortical fibers. Intra-operative, blinded testing of behavioral and physiological effects with stimulation of each of the 8 contacts along the DBS lead was performed to test the specificity of the predetermined optimal site. Chronic stimulation at this 'optimal' location was initiated 1 mo post implantation with both location and stimulation parameters held stable for 6 mo (130Hz, 60usec, 6-8mA). Symptom improvement was monitored using the Hamilton Depression Rating Scale (HDRS).
Results: 11/11 patients showed maximal behavioral effects during blinded testing in the predefined optimal target location in each hemisphere. The 6-mo response rate (>50% HDRS decrease) improved from 41% (7/17 pts in the original cohort) where lead placement and contact selection was based on structural anatomy alone, to 73% (8/11) in the new group using pre-surgical sc-DTI guidance. Group-wise probabilistic sc-DTI maps of the active contacts used for chronic DBS (location verified by post-op CT scans) confirmed that the pre-defined 4- bundle template was impacted in all subjects.
Conclusions: Individualized, pre-operative DTI targeting of a 4-bundle SCC-network improves DBS outcomes over the previous approach based on local SCC anatomy. Further, 'effective' versus 'ineffective' contacts were confirmed using a combination of behavioral, autonomic and electrophysiological changes elicited during intra-operative testing. These findings have implications for refining and standardizing targeting methods for future trials of this potential intervention.
Disclosures: Part 1: Consultant, licensing of IP to St Jude Medical, Inc., **Part 2:** licensing of IP to St Jude Medical, Inc. (neuromodulation), **Part 4:** Medtronic Inc. and St Jude Medical Inc. (Donation of unapproved devices). Support: Dana Foundation, Hope for Depression Research Foundation. Devices donated by St. Jude Medical Inc.and Medtronic, Inc.
Co-authors: P Riva Posse, K Choi, O Smart, V Tiruvadi, S Garlow, A Crowell, P Holtzheimer, R Gross.
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Study Group
60. Methodological Challenges in Human Pharmacogenetic Studies in Alcohol and Drug Abuse – What has Early Experience Taught us, Where to Next?

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As knowledge about human genetics has grown, it is possible to evaluate the interaction of substances (e.g., alcohol, drugs, medications) and genetic variation on consumption and treatment. In contrast to inherited psychiatric disorders (e.g. schizophrenia, bipolar illness), substance use disorders have known etiologic agents (e.g., alcohol, cocaine, marijuana) with well-characterized biological mechanisms. Because brain tissue for human studies is generally unavailable, genetic and epigenetic variation could provide a useful entry point to understand the neurobiological effects of substances. In addition, using identified biological targets of alcohol and drugs, one can examine the effects of potential pharmacotherapeutic agents on these targets based on genetic moderators. Further, brain-imaging data, used to elucidate the pathophysiology of the disorders, when combined with pharmacogenetics could expand knowledge of the neuroanatomical and neurochemical pathways involved. These findings could enhance the translational value for prognosis and "personalized treatment".
As we move into the pharmacogenetic era, there are challenges that need to be overcome and strategies developed to enhance the efficiency, validity, and interpretation of the growing body of research. Early work has focused on alcohol and drug effects in individuals based on their genetic variation (e.g., alcohol effect differences based on allelic differences in GABRA2) and genetic moderator effects on therapeutic efficacy (e.g., the OPRM1 Asp40 allele as a predictor of response to naltrexone in alcohol dependence). Functional MRI (fMRI) studies have shown differential effects of alcohol, nicotine, and cannabinoids on brain activation, based on variation in opioid, nAChR, CNR1, and dopamine receptor genes, with some of these being studied as predictors of treatment efficacy.
Despite early successes, there are troubling non-replications of results, in part due to small or genetically heterogeneous samples, and inadequate characterization of salient endophenotypes (e.g., psychiatric diagnosis, impulsivity, exposure to stress). In addition, many apparently relevant (e.g., functional) alleles have low population prevalence, or interact with other gene variants producing epistatic effects, both of which increase the sample size necessary for adequate statistical power.
This study group will bring together investigators with experience in pharmacogenetic studies in alcohol and drug populations to discuss the challenges, strategies, and solutions applied to date. A major goal is to identify common elements of concern and, through audience interaction, raise awareness of the obstacles and potential future research approaches. For instance, in pharmacotherapeutic trials, the value of prospective genotyping and selection needs to be balanced against limitations due to low allele frequency and biased subject selection, while retrospective analyses could introduce randomization bias. The study group will consider how to balance these conflicting priorities. Another important question is which targets to examine in brain imaging/genetic studies to identify medication interaction or therapeutics and type 1 error minimized.
Brief presentations by experienced investigators with attendee discussion should enhance knowledge in this important area of pharmacogenetics of alcohol and substance abuse.
Disclosures: Part 1: I chair the ACTIVE workgroup affiliated by the ASCP which is supported by Lilly, Lundbeck. Abbvie, Pfizer, Ethylpharm. In the last year I received grant funds from Lilly and have been a paid consultant for Novartis, **Part 4:** Lilly.