MPA Molecular Psychiatry Association

2016 Poster Abstracts

Poster Abstract 1

Nava Krishnan
Cold Spring Harbor Laboratory

Targeting tyrosine phosphorylation mediated signaling in neuropsychiatric disorders

Disruption of the normal patterns of phosphorylation results in aberrant regulation of signal transduction and has been implicated in the etiology of a variety of major human diseases ranging from vascular disorders and inflammatory diseases to neurological disorders and cancer. Hence the ability to modulate signaling pathways selectively holds enormous therapeutic potential. Recently we identified a novel therapeutic target in the neurodevelopmental disorder Rett Syndrome (RTT). The X-linked neurological disorder RTT, presents with autistic features and is caused primarily by mutations in a transcriptional regulator, methyl CpG-binding protein 2 (MECP2). Current treatment options for RTT are limited to alleviating some neurological symptoms; hence, more effective therapeutic strategies are needed. We identified the protein tyrosine phosphatase PTP1B as a therapeutic candidate for treatment of RTT. We demonstrated that the PTPN1 gene, which encodes PTP1B, is a target of MECP2 and that disruption of MECP2 function is associated with increased levels of PTP1B. Pharmacological inhibition of PTP1B ameliorated the effects of MECP2 disruption in mouse models of RTT, including improved survival in young male (Mecp2-/y) mice and improved behavior in female heterozygous (Mecp2-/+) mice. We demonstrated that PTP1B was a negative regulator of tyrosine phosphorylation of the tyrosine kinase TRKB, the receptor for brain-derived neurotrophic factor (BDNF). Therefore, the elevated PTP1B that accompanies disruption of MECP2 function in RTT represents a barrier to BDNF signaling. This work raises an exciting possibility that there might be other targets, which might regulate critical signaling events in RTT and other neurological disorders, understanding which could lead to new therapeutic strategies.

Poster Abstract 2

Brendon Watson
Weill Cornell Medical College and New York University

Effects of low dose ketamine on prefrontal and hippocampal microcircuit coordination

Low dose ketamine has gathered interest as a rapid-acting antidepressant with efficacy on many patient groups including those with unipolar, bipolar and treatment resistant-depression. Here we explore the neurophysiologic correlates of anti-depressant dose ketamine in rats using silicon probe recordings in the dorsal hippocampus and frontal cortical regions.
Clinical findings indicate that ketamine may work with a truly unique mechanism of action relative to other antidepressant treatments. Firstly it is known to have unique pharmacologic properties since it acts as an NMDA antagonist, an opioid receptor agonist and has cholinergic and dopaminergic effects among others. Perhaps more importantly, it is able to alleviate depressive symptoms within hours and that effect lasts for days. Understanding the mechanism of action of this uniquely effective medication has the potential to teach us a great deal about depression treatment approaches fundamentally.
We have given 10mg/kg ketamine intraperitoneally to 7 adult male Long Evans rats while recording from frontal cortex and dorsal hippocampus. We find that during the period immediately following ketamine animals are hyperlocomotive for 25-45 minutes each before reliably going to sleep. During the period of hyperactivity we see increased average spike rates among both putative excitatory and putative inhibitory neurons. This is in contrast to published findings from similar experiments using other NMDA antagonists (1). In many cases we see specific subsets of neurons that are particularly responsive to ketamine. We also see

increased gamma oscillatory power. We observe a temporary increase in the coefficient of variation of population firing rates, decreased bursting activity and an increase in excitatory-to-inhibitory ratio. However, none of these changes deviate from what would be predicted simply by the increase in locomotion by the animal, given the relationship between each of these metrics and times of movement. It is possible that persistent elevated activity in the frontal cortex lasting ten of minutes is a mechanism itself for network change due to ketamine, consistent with optogenetic stimulation results (2).

1. Homayoun H, Moghaddam B. J Neurosci. 2007 Oct 24;27(43):11496-500.

2. Fuchikami M, Thomas A, Liu R, Wohleb ES, Land BB, DiLeone RJ, Aghajanian GK, DumanRS. Proc Natl Acad Sci U S A. 2015 Jun 30;112(26):8106-11

Poster Abstract 3

Jillian Haney
UCLA Neurology

RNA-sequencing of 11 cortical regions reveals global patterns of transcriptional dysregulation in autism

Autism Spectrum Disorder (ASD) is a highly heritable and common neurodevelopmental disorder characterized by deficits in social communication and repetitive behaviors. Recent large-scale genetic studies have begun to identify genetic risk factors for ASD and indicate substantial heterogeneity and pleiotropy in the disorder. However, little is known about how these risk factors for ASD lead to the dysfunction of the neural circuits which cause the disease. The transcriptome, as measured by RNA-sequencing or gene expression microarrays, is a valuable measure of the downstream effects of these diverse risk factors on molecular pathways. Previous work has identified global transcriptomic alterations in frontal and temporal cortex in ASD, characterized by the upregulation of neuroinflammatory signaling, downregulation of synaptic activity, and attenuation of cortical patterning. Here, we expand upon this work by using RNA-sequencing to identify gene expression changes across eleven cortical brain areas from 28 subjects with ASD and 22 neurotypical controls. Strand-specific, 50 base-pair paired-end RNA-sequencing was performed on 208 brain samples using ribosomal RNA depletion to obtain an average of 70 million reads per sample for analysis. Differential gene expression results replicate previous findings of immune-glial activation and synaptic dysregulation in ASD. These global patterns are pervasive and present across all cortical regions assessed. Furthermore, in five of six cortical lobe comparisons, the control group had at least twice the ASD group's number of differentially expressed genes between lobes. These findings suggest that cortical dysfunction is widespread in ASD, provide further support for a set of convergent molecular pathways disrupted in ASD, and implicate alterations of early brain development in disease etiology.

Poster Abstract 4

Jivan Khlghatyan
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Mood-associated Gsk3/Fxr1P pathway regulated neuronal plasticity and behavior

Neuropsychiatric disorders such as bipolar disorders, depression and schizophrenia represent a major public health problem, and a heavy burden for patients and their relatives. Drugs (such as mood stabilizers) used for management of these diseases may exert part of their therapeutic action by regulating Akt/Gsk3 pathway downstream of dopamine D2 receptors. Moreover, several genetic risk factors for mental illnesses encode proteins which either comprise or converge on this pathway. However, Gsk3 has up to 200 substrates, which makes it a non specific drug target for management of specific psychiatric symptoms. Moreover, it is not clear which targets of Gsk3 regulate particular behaviors.
We identified a new target of Gsk3β, Fragile X mental retardation syndrome-related protein 1(Fxr1P), a RNA binding protein identified as a potential risk factor for mental illnesses.Fxr1P is a Gsk3β substrate that is negatively regulated following phosphorylation. Treatment with mood stabilizers lithium, lamotrigine or valproate upregulate Fxr1P to exert mood related effects in rodents. Furthermore, interaction between human functional polymorphisms in the GSK3B and FXR1 genes contributes to mood regulation.
Studies of functional interactions between signaling proteins have historically relied on cumbersome germline systems often

resulting in developmental consequences. The CRISPR/Cas9 technology makes it possible to generate somatic gene knockout in the brain of adult animals. Here we piloted this approach to mimic pharmacological interventions and investigate functional outcomes of Gsk3β/Fxr1P pathway modulation directly in the adult brain.

Poster Abstract 5

Brooke Hjelm
University of California, Irvine (UCI)

A New Method To Detect Mitochondrial Deletion Breakpoints by Next-Generation Sequencing and Postmortem Brain Analyses of Major Psychiatric Disorders

Deletions in the 16.5kb mitochondrial genome have been implicated in a number of mitochondrial disorders, many of which are phenotypically complex but often display overlapping symptoms associated with muscle and/or neurological dysfunction due to the high energy demands of these tissues. While there has been a wealth of research evaluating the 4,977bp “common deletion”, specifically demonstrating its accumulation during aging and its high levels in some subjects with mitochondrial pathology, this approach requires an a priori hypothesis about the deletion being investigated. Here, we describe a new method to detect mitochondrial deletion breakpoints by next-generation sequencing. The bioinformatics methodology we have developed uses MapSplice, an RNA-Seq splice-junction detection program intended for the discovery and quantification of both canonical and non-canonical splice sites, which we have leveraged to detect mitochondrial deletion breakpoint junctions rather than mRNA splice junctions. Our initial investigations focus on paired, postmortem brain regions from subjects with psychiatric disorders (i.e., schizophrenia, major depressive disorder (MDD), bipolar disorder, and alcohol abuse); further analysis was performed between blood and brain samples from a subset of the same subjects. Our preliminary data suggests 1) the 4,977bp “common deletion” is neither the most frequent deletion amongst our 93 samples nor the most abundant deletion in terms of normalized reads (analogous to heteroplasmy rate); 2) postmortem brain consistently contained a greater load of mitochondrial deletions than blood; 3) approximately 30% of the mitochondrial deletions reported in MitoMap were detected in our dataset, suggesting deletions previously described for major mitochondrial disorders are present at a low level in metabolically active tissues like the brain and are not exclusive to individuals with a diagnosed mitochondrial pathology; and 4), from our analysis in psychiatric disorders, the most robust instances of clonal expansion of one or several deletions occurred in MDD. This last result is particularly interesting given depression and suicidality have previously been associated with several mitochondrial deletion pathologies. Together, these results demonstrate this new method is accurate, robust and will be a useful tool for those interested in disorders of both simple and complex etiology where mitochondrial dysfunction is suspected to be involved.

Poster Abstract 6

Kathryn Ridout
Warren Alpert Medical School of Brown University

Association between Molecular Markers of Neuroendocrine Function and Cellular Metabolism with Early Life Stress and Psychopathology

Objective: Promoter methylation of the glucocorticoid receptor (GR) gene (NR3C1) is a proposed mechanism by which early stress may impact neuroendocrine function. Mitochondria are key to cellular stress responses and recent evidence shows that mitochondrial DNA copy number (mtDNAcn) is increased in adults with a history of early stress or psychopathology. No prior work has examined the role of NR3C1 methylation in this association.
Methods: Adult participants (n=395) without lifetime bipolar, obsessive-compulsive, or psychotic disorders completed diagnostic interviews and questionnaires to characterize early stress and lifetime psychiatric symptoms. Medical conditions, current substance abuse, and prescription medications other than oral contraceptives were exclusionary. Individuals were categorized according to presence or absence of early stress (ncase=213; ncontrol=182) and threshold psychiatric disorders (ncase=170; ncontrol=225). Whole blood mtDNAcn was measured using qPCR; pyrosequencing detected NR3C1 methylation. Age and telomere length were included as covariates given prior literature regarding their effects on mtDNAcn. Multiple regression and bootstrapping procedures tested NR3C1 as a mediator of effects of psychopathology/early stress on mtDNAcn.

Results: Unconditional models revealed a positive association between early stress and mtDNAcn (p=.05) and between psychopathology and mtDNAcn (p=.002). When including NR3C1 methylation with conditional testing, early stress was no longer significantly associated with mtDNAcn (p=.18); the relationship between psychopathology and mtDNAcn became less robust (p=.013). NR3C1 methylation mediated the link between early stress and mtDNAcn (95% CI: .0042 to .026) and partially mediated the link between psychopathology and mtDNAcn (95% CI: .0046 to .026).
Conclusions: This is the first study examining associations between cellular neuroendocrine function and mtDNAcn. GR signaling may be part of a mechanism by which mtDNAcn is altered with early stress and psychopathology.

Poster Abstract 7

Jens H van Dalfsen
Maastricht University

The Serotonin Transporter Polymorphism and Cortisol Stress Responsiveness: a Modulating Role for Sleep Quality

Background: Allelic variation within the Serotonin Transporter Gene-Linked Polymorphic Region (5-HTTLPR) has been associated with a differential cortisol stress responsiveness that may ultimately convey an increased risk for affective disorders. However, this finding is not consistently replicated. Sleep quality has also been found to influence cortisol stress responsiveness and may therefore relate to this equivocality. Thus, the present study investigated whether sleep quality modulates the association between 5-HTTLPR and cortisol stress responsiveness and, moreover, whether accounting for sleep quality would enhance the predictive value of 5-HTTLPR.
Methods: A sample of healthy participants homozygous for either the 5-HTTLPR short (n = 26) or the long (n = 25) allele were assessed for sleep quality and subjected to a combined psychosocial-physical experimental stress induction. Salivary cortisol samples were collected at baseline, pre-stress as well as 20 and 40 minutes after stressor onset.
Results: Sleep quality was found to interact with 5-HTTLPR on cortisol stress responsiveness; diminished sleep quality was exclusively found to enhance the cortisol stress response in the long/long genotype. Furthermore, short/short carriers displayed higher cortisol stress responses relative to long/long carriers after accounting for the influence of sleep quality.
Conclusions: Sleep quality modulates the influence of 5-HTTLPR on cortisol stress responsiveness and, hence, may change its phenotypic expression. Moreover, accounting for this influence enhances the predictive value of 5-HTTLPR and supports the general assumption that the short-allele is associated with an elevated cortisol stress response.

Poster Abstract 8

Hyejung Won
UCLA

Genome-wide chromosome conformation elucidates regulatory relationships in human brain evolution and disease

The demonstration that chromatin exhibits a complex 3 dimensional organization, whereby short and long distance physical interactions correspond to gene regulatory processes has opened a new window to understanding the functional organization of the human genome. Chromatin remodeling has also been causally implicated in several neurodevelopmental disorders, including autism and schizophrenia. However, it remains unclear whether knowledge of chromosome organization in a tissue specific manner might inform our understanding of gene regulation in brain development or disease. Here we determined the genome-wide landscape of chromosome conformation during early human cortical development by performing Hi-C analysis in the germinal and post-mitotic laminae of human fetal brain. We show how these data permit the first large-scale functional annotation of non-coding variants identified in schizophrenia GWAS, identifying novel genes and pathways, including cholinergic receptors. We functionally validate a new candidate schizophrenia locus at FOXG1 by genome editing in human neural progenitors, demonstrating non-coding variants mediate gene regulation via formation of chromatin loops. This framework illustrates the power of tissue-specific annotation of non-coding regulatory elements, as well as novel insights into the pathogenic mechanisms of neuropsychiatric disorders.

Poster Abstract 9

Samuel Ridout
Warren Alpert Medical School of Brown University

Effects of Adversity in Early Life on Telomere length: A Meta-Analysis

Background: Early adversity increases lifetime risk for psychiatric and other medical disorders. Several recent studies have examined associations between early adversity and telomere length, a marker of cellular aging, providing insight into the biologic mechanisms underlying these associations. There is variability in the reported size and significance of this association. The objective of this study was to establish the relationship between early adversity and telomere length using meta-analytic techniques and to explore factors affecting this association.
Methods: A comprehensive search of PubMed/MEDLINE, PsycINFO, and Web of Science was conducted in June 2016. Included studies: 1) Examined the effects of early life adversity (in the form of abuse, neglect, caregiver loss through abandonment, death or separation, other adverse exposures, or socioeconomic status) occurring between the prenatal period and age 18 on human telomere length; 2) provided adequate description of the assessments used to determine early adversity exposure and telomere measurement; and 3) presented sufficient data to calculate effect sizes. Independent data extractors utilized a structured data abstraction form. Analyses were performed using Comprehensive Meta-Analysis Software (V2.2.064 Biostat, Englewood, New Jersey) with the standard meta-analysis function and random effects model. Method of moments random effects meta-regression and random effects subgroup moderator analyses were performed to examine potential sources of heterogeneity.
Results: Forty-one studies (cumulative N =30,773) met inclusion criteria. The association between early adversity and telomere length was significant (Cohen's d effect size = -0.347; 95% CI, −0.456 to −0.238, $p < 0.0001$). Sensitivity analyses revealed no outlier effects. Type and timing of adversity exposure significantly impacted the association with telomere length ($p < .0001$ and $p = .0025$, respectively). Subgroup analysis and meta-regression revealed that medication use, medical or psychiatric conditions, study design, methodological factors, age and smoking significantly affected the relationship.
Conclusions: Early adversity and telomere length are negatively related. This analysis suggests that early life adversity may have long-lasting consequences contributing to disease risk and biological aging. Further work is warranted to clarify the causality and reversibility of the observed association.

Poster Abstract 10

Tracy Bale
University of Pennsylvania

Paternal stress epigenetic reprogramming of neurodevelopment via sperm miRNA

Neurodevelopmental disorders including autism and schizophrenia have been highly associated with parental factors, including lifetime stress experience. We have developed a mouse model of paternal stress in which adult male mice exposed to chronic stress prior to breeding produce offspring with hypothalamic-pituitary-adrenal (HPA) stress axis dysregulation. Paternal sperm was examined for changes in miRNA content where 9 specific miRNAs were identified as significantly increased in stressed sperm. To test the relevance and potential mRNA targets of these miRNAs, we synthesized and injected the 9 miRNAs into single cell zygotes and found that the resulting offspring recapitulated the stress phenotype found from paternal stress sires. In addition, we have now completed single cell amplification from injected zygotes using Fluidigm technology and ascertained the stored maternal mRNAs that are targets of these sperm miRNAs and thus affecting post-fertilization development that results in a reprogrammed brain that is stress hypo-responsive. Gene set enrichment analysis of the offspring paraventricular nucleus of the hypothalamus revealed global changes in transcription, including increased representation of glucocorticoid receptor-responsive gene sets and functional annotation clustering for enrichment of extracellular matrix genes, with an exceptional representation of collagens. Analyses of blood-brain barrier changes in offspring from paternally stressed mice support a deficit that correlates with HPA axis function. Overall, these results demonstrate that paternal experience across the lifespan can induce germ cell epigenetic reprogramming and impact offspring HPA stress axis regulation, and may therefore offer novel insight into factors influencing neuropsychiatric disease risk. Identification of the specific miRNAs in germ cells that are altered long-term following stress experience may point to unique biomarkers that could identify at-risk populations.

Poster Abstract 11

Se Jin Jeon
Kyung Hee University

Swertisin ameliorates prepulse inhibition deficits and cognitive impairment induced by MK-801 in mice

Swertisin, a plant-derived C-glucosylflavone, is known to have antidiabetic, anti-inflammatory and antioxidant effects. In the present study, we investigated the effects of swertisin on glutamatergic dysfunction induced by dizocilpine (MK-801), a noncompetitive N-methyl-D-aspartate receptor antagonist, in mice. In the acoustic startle response test, MK-801 (0.2 mg/kg, i.p.)-induced prepulse inhibition deficit was significantly attenuated by the administration of swertisin (30 mg/kg, p.o.). In the novel object recognition test, recognition memory impairments that were induced by MK-801 (0.2 mg/kg, i.p.) were also reversed by administration of swertisin (30 mg/kg, p.o.). In addition, swertisin normalized MK-801-induced elevation of the phosphorylation levels of Akt and GSK-3β signaling molecules in the prefrontal cortex. These results indicate that swertisin may be useful in managing the symptoms of schizophrenia, including sensorimotor gating disruption and cognitive impairment, and that these behavioral outcomes may be related to Akt-GSK-3β signaling in the prefrontal cortex.

Poster Abstract 12

Krishna Vadodaria
Salk Institute for Biological Studies

Studying serotonergic neurotransmission using human pluripotent stem cell-derived neurons in vitro

Serotonergic neurotransmission plays an important role in brain function and its dysfunction has been implicated in neuropsychiatric disorders including Major Depression. Human stem cell technology has revolutionized our capacity to generate and study human neurons in vitro. Human neuron-based disease modeling approaches have given novel insight into pathology of human neuropsychiatric disorders as well as hope for the development of in vitro assay platforms. As a first step for studying serotonergic neurotransmission in the context of neuropsychiatric disorders, recently, we have generated human serotonergic neurons in vitro from induced pluripotent stem cells (iPSCs) and fibroblasts. Using these techniques we study serotonergic neurotransmission using multiple assay platforms including SSRI- and activity- responses of human neurons in vitro.

Poster Abstract 13

Kristopher Montrose
Okinawa Institute of Science and Technology

LMTK3 deficiency causes schizophrenic-like behaviours in mice

Protein kinases play an important function in the regulation of many neuronal processes including cell proliferation, synaptic plasticity and apoptosis. The lemur tyrosine kinase (LMTK) family consists of three serine/threonine protein kinases predominantly expressed in the brain. Here I show that LMTK3 deficiency in mice leads to abnormal behavior and cognitive defects such as hyper sociability, loss of novelty recognition and impaired spatial and working memory, behavior indicative of a schizophrenia-like phenotype. This was further evidenced by the use of antipsychotic drug clozapine, which was able to reverse or reduce the severity of symptoms exhibited by LMTK3 knockout mice. MRI of LMTK3 knockout brains revealed anatomy often associated with schizophrenic patients such as enlarged ventricles and reduced hippocampal size. Biochemical analysis revealed that AMPA receptor recycling may be impaired. Trafficking of the AMPA subunit GluR1 to the cell surface after chemical stimulation was significantly reduced in cells lacking LMTK3 expression. LMTK3 was also found to co-localize with Rab11 a marker of recycling endosomes and can also directly interact with the motor protein Myosin V which is known to transport recycling vesicles containing AMPA receptors to the cell surface. Thus, our data suggests that LMTK3 may be an important factor in the pathogenesis of schizophrenia, through its involvement in the trafficking of AMPA receptors.

Poster Abstract 14

Alannah Miranda
University of California, San Diego

Using iPSC-derived cholinergic neurons in the analysis of Bipolar Disorder-associated NTRK1 variants

Neuropharmacological and neuroimaging research suggests that the bipolar depressive state may be caused by an imbalance of cholinergic activity to adrenergic activity. Previous studies in the lab suggest that a mutation in NTRK1 is associated with bipolar disorder. NTRK1 encodes for TrkA, a receptor thought to play a role in acetylcholine release and cholinergic neuron development. This poster details methods used to differentiate patient-derived iPSCs into cholinergic neurons, utilizing a monolayer method and exposure to exogenous growth factors, rather than embryoid body states and transfection methods. Characterization of cells during various stages of differentiation (iPSC, neural stem cells, neural progenitor cells and neurons) is demonstrated using a variety of methods, including acetylcholine release, RNA expression levels and immunocytochemistry staining.

Poster Abstract 15

Daniel Howrigan
Post-Doctoral Fellow

Damaging missense de novo coding mutations contribute to schizophrenia risk

Background
Recent large-scale exome sequencing studies have illuminated the risk conferred by deleterious de novo coding mutations in a number of severe neurodevelopmental disorders. In schizophrenia, however, enrichment in deleterious de novo coding mutations has so far been modest, as no individual gene yet shows strong evidence of deleterious de novo association. Despite the weaker effect size, patterns of observed de novo mutations are converging toward genes under purifying selection and within gene networks expressed in the brain. We completed exome sequencing on 1695 schizophrenia trios, the largest exome sequenced trio cohort in schizophrenia to date.

Methods
Exome sequencing data were generated using Illumina HiSeq sequencing platforms and aligned to the hg19 reference build, and all 1695 trios underwent joint variant calling using GATK version 3.4. Validation of candidate de novo signals was performed using targeted high-throughput genotyping on Illumina MiSeq and Sequenom platforms, with Sanger sequencing validation on unconfirmed calls. De novo mutations were annotated using the Variant Effect Predictor from Ensembl, dbNSFP, and CADD. Enrichment testing of de novo mutations were compared against a mutational rate model that incorporates coding length and site-specific mutation rate into expectations of de novo mutation, as well as reported de novo mutations from 2216 published control trios and unaffected siblings. We also leveraged the non-psychiatric ExAC reference dataset (~45K individuals) to filter out de novo sites variable in the general population.

Results
Combining our results with published de novo studies, we evaluated a total of 2719 affected trios diagnosed with schizophrenia. Per-trio de novo mutation rates are significantly enriched exome-wide in both protein-truncating (p=0.01) and missense (p=0.02) de novo mutations relative to controls/unaffected siblings. In particular, the enrichment in missense de novo mutations is confined to predicted damaging missense variants not seen in the ExAC database (p=7e-6). Furthermore, the increased burden of these ultra-rare damaging de novo missense mutations is localized within genes intolerant to loss-of-function mutation (p=9e-4). Among curated gene sets, we find a significant overlap with genes implicated in de novo studies of intellectual disability. Finally, we investigated the full human Gene Ontology (GO) catalogue as an unbiased source of gene set evaluation, with chromatin silencing (GO:0006342; p=3e-5) in protein-truncating de novos and RNA splicing (GO:0008380; p=8e-5) in missense de novos as our top GO categories.

Discussion
Probands diagnosed with schizophrenia exhibit an increased burden not just in de novo protein-truncating mutations, but also in damaging missense de novo mutations, a finding concurrent with case-control reports in exome sequencing studies of schizophrenia. Such mutations are enriched for genes that show evidence of intolerance to deleterious mutation, and show overlap

with other neurodevelopmental disorders. Among the broader set of de novo mutations, gene set analysis across GO categories point towards biological processes involved in epigenetic regulation via chromatin remodeling and RNA splicing. While larger cohorts will lead to specific genes being identified as unequivocal risk factors, the increased liability toward schizophrenia due to de novo coding mutations comprises only a modest fraction of the overall genetic liability for schizophrenia.

Poster Abstract 16

Rebecca Taugher
The University of Iowa

Acid-sensing ion channel-1A dependent regulation of cerebral blood flow

Normal brain function depends on regulation of cerebral blood flow (CBF), and insufficient CBF contributes to cognitive impairment and neurological disease. Because brain pH is a strong regulator of CBF, we hypothesized that neuronal acid-sensing ion channels (ASICs) may play a key role in CBF regulation. To test this hypothesis, we manipulated ASIC1A in mice and examined the response of small pial arterioles to acidosis evoked by inhalation of 5 or 10% carbon dioxide (CO2). We found that CO2-induced vasodilation was greatly attenuated by both genetic disruption of ASIC1A or local pharmacological inhibition by psalmotoxin (an inhibitor of ASIC1A). To test the site of action we also disrupted ASIC1A specifically in neurons by generating ASIC1AloxP/loxP mice and crossing them to transgenic mice expressing Cre specifically in neurons via the synapsin I promoter. We confirmed neuron-specific loss of ASIC1A and found that these mice exhibited similar deficits in CO2-induced vasodilation, suggesting that brain neurons are a key site of ASIC1A action. In contrast to these findings, endothelium-mediated vasodilation induced by acetylcholine was not affected in ASIC1A¬¬–/– mice or by psalmotoxin. Because nitric oxide (NO) is a potent regulator of CBF and a contributor to neurovascular coupling, we next tested if ASIC1A influences NO production. We cultured brain neurons from wild-type mice and ASIC1A–/– mice and measured NO levels at baseline and following acidification of the extracellular media. We found that acidification induced NO production in wild-type neurons and that this response was attenuated by ASIC1A disruption. We also measured NO concentrations in whole brain lysates immediately after inhalation of 10% CO2 in vivo. CO2 increased relative NO levels in wild-type, but not in mice lacking ASIC1A globally or specifically in neurons. Together, these data identify ASIC1A is a key regulator of CBF and suggest that extracellular acidosis acts on neuronal ASICs, which increase intracellular Ca2+ and induce NO release likely via Ca2+-dependent nNOS, which in turn causes vasodilation in the microcirculation. We conclude that ASIC1A is critical for normal CBF regulation and may provide a novel therapeutic target for regulating CBF in psychiatric and neurological conditions in which these processes are impaired.

Poster Abstract 17

Alexander Charney
Icahn School of Medicine at Mount Sinai

A preliminary report on a multiscale investigation of the living human brain

Studies of the human brain rely on the tools of clinical observation, neuropharmacology, neuroimaging, neurophysiology, neuromodulation and molecular-cellular neuroscience. Most of these approaches require research cohorts comprised of dynamic, living individuals. An important exception, however, is molecular-cellular neuroscience, which primarily involves post-mortem or cultured human neural tissue. As a result, the human-subject neuroscience toolkit is yet to be employed in its entirety to the study of a single large cohort, rendering elusive the formation of a holistic understanding of how the brain works. Here, we provide a preliminary report on the Living Brain Project, a multiscale, data-driven investigation of the human brain wherein 500 individuals undergoing Deep Brain Stimulation will be studied using the full human-subject neuroscience toolkit. Specifically, data for each participant is derived from neuropsychological testing batteries, electronic medical records, wearable mobile health devices, multimodal neuroimaging, micro-electrode neural recordings and a suite of molecular and cellular biology assays applied to multiple tissues, including the prefrontal cortex of the brain. Data has been collected from over 100 individuals. For a small subset, pilot single-cell RNA sequencing and next-generation genome mapping experiments have been performed on brain and blood specimens. With this presentation, this new approach to human brain research will be explained in detail and its potential value explored.

Poster Abstract 18

Summer Thyme
Harvard University

Zebrafish brain activity phenotypes unify schizophrenia-associated genes

Large-scale genome-wide association studies have begun to uncover numerous candidate genes linked to schizophrenia. Yet it remains unclear how these genes function and how they contribute to the underlying molecular, cellular, developmental and behavioral processes disrupted in the disorder. Recent technological breakthroughs in zebrafish – targeted genome editing, whole-brain activity imaging, brain atlas registration, behavioral profiling – combined with the ease of studying large numbers of animals make it an ideal system for analyzing psychiatric disease genes. Combining these technologies for the first time, I have generated zebrafish mutants for over 100 schizophrenia-associated genes and am analyzing them for differences in neurological activity and morphology, as well as altered behavior. I have discovered that diverse schizophrenia-associated genes can influence activity in shared brain structures and have identified novel candidate genes in schizophrenia-associated genomic regions. The finding of shared phenotypes suggests that seemingly unrelated genes may be involved in common underlying pathways. Observed brain abnormalities in mutants also resemble known schizophrenia patient phenotypes, such as the loss of GABAergic inhibitory neurons. Understanding the molecular, cellular, developmental and behavioral processes regulated by schizophrenia-associated genes will provide the foundation to understand the causes of schizophrenia and develop new diagnostics and therapies.

Poster Abstract 19

Hyeonseok Jeong
Department of Radiology, Incheon St. Mary's Hospital, The Catholic University of Korea

Cerebellar volume deficit and its relationships with mood and cognition in firefighters

Compared to other positions in the fire department personnel, firefighters are repeatedly exposed to toxic chemicals and often suffer from mental health problems. However, neural correlates underlying these relationships are not yet known. The cerebellum may be a potential candidate area since it is particularly vulnerable to intoxication and poisoning and contributes to emotional regulation and cognitive processing, especially executive function. The present cross-sectional magnetic resonance imaging study investigated duty-specific relationships among cerebellar volume, mood, and executive function in 96 fire personnel and 96 age- and gender-matched controls. The Beck Depression Inventory, Beck Anxiety Inventory, and Wisconsin Card Sorting Test (WCST) were used to assess depressive and anxiety symptoms, and executive function, respectively. Voxel-based morphometry analysis found that volume reduction in the right cerebellar Crus I was positively correlated with only the cumulative work experience as a firefighter ($p < 0.001$), but not as a rescue or office worker. Furthermore, this area demonstrated excessive volume loss with increasing age in the fire personnel group compared with the control group. Regression analysis in the fire personnel group revealed that volume reduction was associated with increased levels of depressive symptoms and decreased performance on WCST. Our results suggest duty-related structural deficits in the cerebellum may have a negative impact on depressive symptoms and executive function in firefighters.

Poster Abstract 20

Lea Davis
Vanderbilt University Medical Center

A systematic assessment of the population genetic evidence for selection across twenty brain related phenotypes

Variation in neuropsychiatric traits is present across diverse human populations, has persisted through recorded history, and has been shown to have a genetic basis primarily accounted for by common (minor allele frequency > 5%) single nucleotide polymorphisms (SNPs). Motivated by recent observations that SNPs with high minor allele frequency (MAF) contribute disproportionately to some neuropsychiatric phenotypes (Davis et al., 2013), we tested the hypothesis that common susceptibility variants for neuropsychiatric phenotypes have experienced weak positive selection. We performed multiple analyses using

genome-wide association study summary statistics from studies of neuropsychiatric disorders, personality measures, MRI subcortical brain structure volumes, and autoimmune phenotypes to assess evidence of recent strong positive selection (i.e., hard sweeps) and polygenic selection (i.e., soft sweeps). Consistent with expectations for polygenic phenotypes, no trait displayed significant enrichment of population differentiated SNPs or strong recent positive selection. However, congruent with recent reports (Srinivasan et al., 2013) we did identify enrichment of risk alleles for schizophrenia ($p=0.004$) and neuroticism ($p<0.002$) within regions of the genome under selection since divergence from Neanderthal. Finally, we assessed each phenotype for evidence of polygenic selection using an approach that detects coordinated shifts in the MAF of many trait-associated SNPs after accounting for genetic drift (Berg and Coop, 2014). Significant evidence of polygenic adaptation was found for extraversion ($p<0.001$), schizophrenia ($p<0.001$), hippocampus volume ($p<0.001$), and putamen volume ($p<0.001$). Our results suggest that associated alleles for multiple neuropsychiatric and brain volume phenotypes have indeed experienced weak selective pressures. Importantly, however, the results of these analyses do not indicate the targets of selection. Therefore, we conducted eQTL and gene set enrichment analyses to shed additional light on the biological processes that may underlie the observed selection. Among SNPs associated with schizophrenia and putamen we found additional evidence of eQTL enrichment in brain ($p<0.001$) and immune tissues ($p<0.001$), respectively.

Poster Abstract 21

Dagmar Bruenig
Queensland University of Technology, School of Biomedical Science and Institute of Health and Biomedical Innovation, Queensland, Australia

The role of NOS1 and NOS1AP in PTSD, comorbidities and resilience

The nitric oxide pathway in the hippocampus is involved in the broader biological stress response with detrimental consequences to cells (excito-toxicity) and HPA axis feedback. Both, hippocampal atrophy and HPA axis feedback dysfunction are associated with Post-traumatic Stress disorder. Two genes, NOS1AP and NOS1 are central to the nitric oxide pathway. In a previous study we found an association of NOS1AP with increased PTSD severity and depression (Lawford et al., 2013).
In the current study we recruited age-matched Vietnam veterans including trauma-exposed cases (N = 159) and controls (N = 140). We analysed 48 SNPs of NOS1AP and 18 SNPs of NOS1 for association with PTSD. Validated clinical measures were used to assess PTSD severity (Clinician-Administered PTSD scale for DSM V (CAPS)) and other phenotypes within PTSD such as depression, anxiety and stress (DASS) and resilience (Connor-Davidson Resilience Scale (CD-RISC)). Scores from all scales were used for association with SNPs based on diagnostic status. We found significant associations for NOS1AP across all measures, and associations for NOS1 with PTSD severity, stress and resilience. Most notably, SNP rs10744891 of NOS1 showed an association of the GG genotype with PTSD severity and the combined TT-TG genotypes with resilience. Although these associations did not survive multiple correction, NOS1AP and NOS1 should be further investigated in PTSD, its comorbidities and resilience

Poster Abstract 22

Laura Perez-Cano
UCLA

Identification of multiple new ASD-risk factors by whole-genome sequencing of 2,308 familial samples

Whole-exome sequencing studies have demonstrated the importance of de novo protein-coding mutation to ASD risk in simplex families, those with one affected individual. In this study, we generated 30X whole-genome sequence data for >4,000 individuals from the family-based Autism Genetic Resource Exchange (AGRE) cohort, as part of the Hartwell Autism Research and Technology Initiative (iHART), in order to assess genetic contributions more broadly. The majority of participating families have multiple affected individuals, which provided an opportunity to elucidate the role of inherited risk loci, in addition to non-coding regions. This initial sample consisted of 491 families (2,308 individuals) containing at least two affected children (some monozygotic twins) Single nucleotide variants and indels were identified following GATK's best practices. Raw structural variant (SV) calls were generated by BreakDancer, SMuFin, GenomeSTRiP, and LUMPY. We used the Transmitted And De novo Association (TADA) test to combine evidence from rare loss-of-function (LoF) and missense variants predicted to damage the encoded protein (a probably

damaging prediction by PolyPhen-2) found in ASD participants from the current (iHART) cohort, the Simons Simplex Collection (SSC) and the Autism Sequencing Consortium (ASC) cohorts, together with small CNV deletions found in SSC probands. This analysis identifies 67 ASD-risk genes with an FDR<0.1, including 19 newly associated genes. Genes with an FDR 0.9) harboring a rare exonic SV that is transmitted to all affected children, including ASD-associated and syndromic genes such as NRXN1 and SMARCA4. In addition to inherited risk analyses, we identified de novo variants in syndromic autism genes including DDX3X, SHANK3, TSC2, and a putative somatic mutation in AFF2 in two monozygotic twins discordant for ASD. We also explored the role of noncoding variation by leveraging experimentally defined regulatory regions in human fetal brain. The iHART genome data will be housed in a cloud-computing repository and made available as a community resource. In addition to advancing our understanding of autism genetics, the iHART project represents a landmark for the study of other complex diseases and investigations using whole-genome sequence data.

Poster Abstract 23

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Multinuclear Magnetic Resonance Spectroscopy (1H and 31P) in Obsessive–Compulsive Disorder

Introduction: Obsessive Compulsive Disorder (OCD) is a public health issue as one of the most common and disabling psychiatric disorders with an estimated 40 to 60 % drug-resistance. Structural and functional imaging studies have suggested that OCD is associated with dysfunctions in the Cortico-Striato-Thalamo-Cortical network (CSTC). Considering this, several studies using proton magnetic resonance spectroscopy (1H-MRS), a metabolic imaging technique, have found some changes in neural metabolite concentrations among OCD patients, but their results remain inconsistent. The aim of our study was to describe and compare brain metabolic changes in three regions of interest (ROI): the anterior cingulate cortex (ACC), the striatum and the thalamus, between severe OCD patients and healthy control subjects, using a multinuclear MRS (1H ad 31P).
Materials and Methods: 23 OCD patients and 23 healthy controls were included and had a clinical examination. Patients were evaluated with the Y-BOCS and the MADRS. All subjects underwent an MRI examination with anatomic, 1H-MRS and 31P-MRS sequences. In 1H-MRS, levels of N-acetylaspartate (NAA), choline (Cho) and their ratios with creatine (Cr) were measured in the three ROI and compared between OCD patients and controls. As well as in 31P-MRS, levels of phosphocreatine (PCr), phosphomonoester (PME), phosphodiesters (PDE), phosphocholine (PC), glycerophosphoéthanolamine (GPE), glycerophosphocholine (GPC) were measured and compared.
Results: Significantly increased concentrations of Cho were found in OCD patients compared to controls in the ACC ($p = 0.008$), striatum ($p = 0.010$) and thalamus ($p = 0.033$). Conversely, PCr was significantly lower in OCD patients than in controls, in these same areas (respectively $p = 0{,}001$; $p = 0{,}024$; $p = 0{,}001$). Decreased of the NAA/Cho ratio was found in the ACC ($p = 0{,}008$) and the striatum ($p = 0{,}044$) in OCD patients. Finally, PME, PDE, PC, GPE and GPC were significantly higher in the striatum (respectively $p = 0{,}003$; $p < 0{,}001$; $p = 0{,}001$; $p = 0{,}049$ and $p < 0{,}001$) and thalamus (respectively $p = 0{,}005$; $p = 0{,}008$; $p = 0{,}001$; $p = 0{,}031$ and $p = 0{,}009$), in OCD patients.
Conclusion: Our results support the biochemical involvement of the CSTC network and also suggest a neural membrane alteration in OCD patients (Cho, Cho/Cr, PME, PDE, PC, GPE, GPC).

Poster Abstract 24

Carlos Cruchaga
Washington University, School of Medicine

Cerebrospinal fluid levels of amyloid beta and tau as endophenotypes reveal novel variants potentially informative for Alzheimer Disease

Background: Case-control genome-wide association studies (GWAS) have identified loci associated with risk for Alzheimer disease (AD) but they require very large sample sizes and usually identify variants with small effect sizes. GWAS of informative endophenotypes for disease have more power to identify novel variants and provide information about biological mechanisms. Cerebrospinal fluid (CSF) levels of tau, ptau181, and amyloid beta ($A\beta 42$) have been well established as endophenotypes for AD. By

analyzing data from 1,269 unrelated individuals, we previously identified risk variants for AD that were also associated with CSF levels of tau and ptau181, including a novel variant associated with AD risk.
Methods: CSF levels of tau, ptau181, and Aβ42 were collected from 3,189 unrelated individuals and linear regression was used to determine single nucleotide polymorphisms (SNPs) associated with these CSF proteins. We analyzed independent data sets to determine if associated SNPs were also associated with AD risk, age of symptom onset, or disease progression. We also performed pathway analyses to determine whether SNPs that were suggestive, but did not reach genome-wide significance, can provide information about the biology of AD.
Results: We found novel variants associated with ptau181 in OLFM4 (Chromosome 13, p=1.51×10-8) and CTDP1 (Chromosome 18, p=3.05×10-9) loci. In the analyses of CSF levels of Aβ42 we found near genome-wide significant signals in GLIS1 (Chromosome 1, p=6.41×10-8) and SERPINB1 (Chromosome 6, p=1.31×10-7) loci. We also replicated our previous findings that variants located in APOE (Chromosome 19, p=1.17×10-31), GLIS3 (Chromosome 9, p=2.63×10-8), and SNAR-I (Chromosome 3, p=2.65×10-10) loci were associated with ptau181 levels. In the previous GWAS we found a novel variant associated with AD risk, tangle pathology, and cognitive decline. Our preliminary analyses of these novel loci for ptau181 and Aβ42 levels did not indicate association with AD risk, age at symptom onset, or cognitive decline.
Conclusions: By significantly increasing the sample size for our GWAS, we were able to identify novel loci associated with CSF levels of ptau181 and near genome-wide significant associations with Aβ42 levels. We are performing additional analyses to determine potential impact of these findings in AD. We will also perform rare variant, gene-based, and additional pathway analyses.

Poster Abstract 25

Megan Puzia
University of Utah

Analysis of candidate genes in suicide decedents with personality pathology

Suicide is a serious public health problem, resulting in the loss of hundreds of thousands of lives each year and contributing to pain, sorrow, and loss in affected friends, families, and communities. There is compelling evidence that suicide aggregates in families, that genes account for some of the observed variability in suicidal behavior, and that its genetic transmission is potentially distinct from that of psychiatric disorders. In light of this understanding, researchers have channeled their efforts toward determining the molecular genetic basis for suicide. Unfortunately, molecular analyses have yielded highly inconsistent findings, and their collective interpretation does not allow definitive conclusions about the contributions of specific genes to the pathophysiology of suicide. One reason for the inconsistency of previous findings may be the heterogeneity of suicide phenotypes. The present study attempts to replicate previous genetic associations in the context of a more narrowly defined sample of suicide decedents with personality disorder (PD) diagnoses. Suicidality and PDs frequently co-occur and many outcomes associated with personality pathology (e.g., social alienation, increased risk of substance abuse, emotion dysregulation) constitute risk factors for suicide. Moreover, given that PDs, and personality traits more broadly, appear to be heritable, suicide in the context of PDs may have a substantial genetic component.

Methods: Prior to genotyping, a literature review was used to identify previously established genetic polymorphisms associated with suicide and/or PDs. This search yielded a list of 159 polymorphisms (single nucleotide polymorphisms [SNPs] and haploid variants) on 94 different genes that had previously been associated with suicidality and/or personality pathology.
Samples from 85 Utah suicide decedents with a history of a personality disorder (PD) diagnosis were included in analyses. Subjects were genotyped using the Illumina HumanExome BeadChip platform (n=24) or the Infinium PsychArray-24 Beadchip microarray (n=90). The locations sequenced on both microarrays were cross-referenced with the list of candidate genes that had previously been shown to be associated with suicidality and/or PDs. This produced a subset of 83 genes (with 1,392 total loci) for analyses in the Exome Chip sample, and 84 genes (with 4,534 loci) in the PsychArray sample. 1,349 loci were included in Exome Chip and PsychArray sequencing and were therefore analyzed in the combined sample.
Chi-square analyses were used to indicate variants that were over-represented in decedents with personality pathology compared to a population of publically available control subjects of Northern European descent. Additionally, diagnostic and demographic information from the sample was compared to a larger database of 9,688 recorded suicide cases in Utah.

Results: Compared to other Utah suicides, the sample of PD decedents was younger and more likely to be female. PD decedents also had higher rates of all analyzed psychiatric and medical diagnoses. In genetic analyses, we found variants on COL25A1, ESR1,

DDC, ABCB1, GABBR2, ANO5, and COMT to occur more frequently than expected in the current sample. Of those, variants on ANO5 and DDC survived Bonferroni corrections for multiple tests.

Discussion: We found the strongest evidence of genetic variation in our sample on the ANO5 and DDC genes. Although previous research has linked variation on ANO5 with suicide, DDC may represent the best candidate for further research. The ANO5 gene codes for the protein anoctamin-5, which is found in the skeletal and cardiac muscles and in bone cells. However, the specific function of ANO5 is not well understood. Alternatively, DDC is known to catalyze the biosynthesis of neurotransmitters dopamine and serotonin, and in tryptamine, which acts as a non-selective serotonin receptor agonist. Previously, variation in DDC it has been found to be associated with antisocial and borderline PDs. Additionally, COMT—which also contained significant variation in our sample— is also implicated in the dopaminergic synaptic pathway. More specifically, DDC and COMT, along with 34 other genes, contribute to the metabolism of tyrosine. Future studies should assess variation in other genes affecting the tyrosine metabolic pathway in other suicide and/or PD samples.

Poster Abstract 26

Samuel Clark
Columbia University

Acute amphetamine administration increases locomotion and enhances activation of D1-expressing medium spiny neurons in the mouse dorsal striatum

Amphetamine (administered as Dexedrine or Adderall) is a stimulant with therapeutic value for treating ADHD. Amphetamine also has a high potential for abuse and addiction. There is much evidence supporting the role of the nucleus accumbens (ventral striatum) in addiction-like behaviors. However, less is known about amphetamine's effect on the dorsal striatum. We used time correlated single photon counting (TCSPC) (ChiSquare Bioimaging) to optically record fluorescence changes in GCaMP6f in D1-expressing medium spiny neurons (D1MSN) in the dorsal striatum. These neurons project to the substantia nigra pars reticulata and the internal segment of the globus pallidus (termed the direct pathway). We injected AAV9 FLEX GCaMP6f virus into the dorsal striatum of mice expressing cre recombinase in D1MSNs. We then implanted imaging fibers into the same location and recorded calcium transients in mice treated with acute amphetamine or saline in their home cage. We found that amphetamine produced and enhancement of locomotion that correlated with increases in calcium transients in the D1MSNs suggesting that amphetamine may modulate the direct path in the dorsal striatum.

Poster Abstract 27

Linda Su-Feher
University of California Davis, Department of Neurobiology, Physiology and Behavior

Gene expression profiling reveals altered neurodevelopmental transcriptional networks in heterozygous Chd8 mice

The high-confidence autism risk gene CHD8, a chromatin remodeling factor, has emerged as a potential master regulator of pathways implicated in autism and neurodevelopmental disorders. Here, we report a heterozygous mouse model of Chd8, generated by CRISPR/Cas9-mediated frameshift deletion. Our Chd8+/- heterozygous mice exhibit increased brain volume, while Chd8-/- knockouts are early embryonic lethal. We performed RNA-sequencing on forebrain of heterozygous and wild-type mice across development at embryonic days (e)12.5, e14.5, e17.5, and postnatal days (P)0 and P56 in order to profile the effects of Chd8 loss on neural development. We observed widespread gene expression changes in Chd8+/- mice, including changes in autism-relevant genes such as Bcl11a, Tbr1, and Kdm5b. Down-regulated genes are most strongly enriched in RNA processing networks and regulation of gene expression. Co-expression network analysis identified networks implicated in other autism genetic studies, as well as perturbed networks that result in a shift toward increased proliferation in early brain development. Our results suggest that haploinsufficiency of Chd8 disrupts the developmental cell state and highlight the convergence of chromatin remodeling, RNA processing, and neuronal differentiation as principle gene networks potentially involved in the genetic and neurodevelopmental origins of autism spectrum disorders.

Poster Abstract 28

Jisoo Lim
Yonsei University College of Medicine

PCDH19 Epilepsy (EFMR) and the molecular characterization of PCDH19 in brain development

Epilepsy in females with mental retardation (EFMR) is an infantile epileptic disease often accompanying intellectual disabilities, which has rare inheritance patterns. Unlike the typical X-linked mode of inheritance, in which males are affected and females are unaffected carriers, EFMR only seems to affect heterozygous females and not males. EFMR is caused by mutations in the protocadherin 19 (PCDH19) gene on X-chromosome, which is a transmembrane protein predominately expressed in central nervous system (CNS). The biochemical and developmental properties of PCDH19 and its function in neurons are currently unknown, making it difficult to establish the pathophysiological mechanism of EFMR. We have used multiple approaches to characterize PCDH19 molecule which is a causative gene for EFMR. We checked the developmental profile of PCDH19 at different developmental time points in different regions of the brain. The expression level gradually increases until post-natal day 7(P7) and it dramatically decreases after this particular time point, but not their mRNA transcript levels. By treating several secretase inhibitors on both in-vitro and in-vivo models, we found that just like other membrane embedded proteins, PCDH19 also undergo secretase-dependent cleavage process and the fragmented C-terminus translocate to the nucleus. Moreover, we checked through if this secretase dependent cleavage is also time-dependent using immunostaining. The C-terminus of PCDH19 is located outside the nucleus during the early developmental period, but it is located within the nucleus as neurons mature in rat primary cultured neurons. When PCDH19 is transfected to L-cell in cell-aggregation assay, cells that express PCDH19 aggregated with each other. Moreover, PCDH19 is enriched in the exons and dendrites in the immature primary cultured neurons, promoting the synapse formation in the artificial synapse formation assay, but it gradually decreases from axons as neurons develop. Taken together, PCDH19 is a neural adhesion molecule that has homophilic interaction, and its biochemical properties are highly time-dependent, suggesting cell-cell interaction during brain development is the biological process that could be relevant to the pathophysiology of EFMR.

Poster Abstract 29

Michael McCarthy
University of California, San Diego; VA San Diego

Effects of Inositol Phosphate Manipulations on Cellular Circadian Rhythms

Bipolar Disorder (BD) is a psychiatric illness characterized by mood disturbances and abnormal circadian rhythms. Lithium restores circadian rhythms in sleep and activity, in part through its effects on PER2 expression. By inhibiting inositol monophosphatases (IMPs), lithium alters inositol metabolism. However, the precise means by which lithium acts upon the circadian clock genes like PER2 are not yet fully established. IMP inhibition by lithium extends the duration of IP3, and its conversion to higher order inositol polyphosphates. In an effort to identify new pathways for mood stabilizer development in BD, we examined whether inositol pathways engaged by lithium have effects on circadian rhythms in cells. We used mouse NIH3T3 cells transfected with Per2::luc, a circadian bioluminescent reporter to study rhythms for 5-7 days under constant conditions. We examined whether higher order metabolites of myo-inositol like inositol hexaphosphate (IP6) have effects on circadian rhythms in cells that resemble the effect of lithium. L-690330, a selective IMP1/2 inhibitor, increased rhythm amplitude and lengthened period, similar to the effects of lithium. Treatment of cells with APB, an IP3R antagonist shortened period, reversed the period lengthening effects of lithium, but had no effect on amplitude. Knockdown of inositol polyphosphate multikinase (IPMK) expression attenuated the effects of lithium on amplitude, suggesting higher-order inositol polyphosphates are involved in lithium's effects on circadian amplitude regulation. Accordingly, IP6 increased rhythm amplitude and shortened period. The IP6 effect on amplitude was attenuated by pharmacological blockade of AKT, and siRNA knockdown of GSK3B, suggesting that these molecules may be engaged by IP6 to regulate rhythms. IP6 is converted by IP6Ks into inositol pyrophosphates. Inhibition of IP6K significantly increased rhythm amplitude (by ~35%) and lengthened period. These results indicate that circadian amplitude and period are independently regulated by inositol metabolites, and contribute to lithium's action on rhythms. IP3 affects period. IP6 affects amplitude, and may involve inhibition of GSK3B through an inositol pyrophosphate mechanism as a critical step. Inositol phosphates may offer a novel means through which circadian rhythms can be regulated.

Poster Abstract 30

Nancy William
Department of Psychiatry, University of Utah

Identifying suicide genetic risk variants in a high-risk pedigree with a significant increase of female suicides

Suicide is a significant health issue, accounting for over 800,000 deaths per year globally[1]. Based on accumulated data from multiple studies, the heritability estimate of completed suicide is 45%, suggesting a strong genetic component to suicide[2], [3]. Moreover, there is a significant gender difference in suicide completion. In comparison to men, completed suicide in women is a rare event, and studies have also suggested that the genetic liability in women may be higher; completed female suicides impart 74% greater suicide risk to offspring, and female suicidal behavior shows higher heritability[4]. In our analyses, we have also found that there is a greater risk of suicide in first degree relatives of female suicide decedents than male cases. Based on these data, we hypothesized that targeting gene discovery in high-risk families with significantly more women suicides than expected, we might increase our power to detect genetic risk variants, and/or we might identify specific gene variants unique to suicide risk in women. We utilized the Utah Population Database (UPDB), a computerized genealogy database that includes medical data, demographic information, and genealogical data for over 6.5 million individuals, to identify and characterize a high-risk large extended family with a significantly increased rate of female suicide. For our genetic analyses, we utilized a software tool developed at the University of Utah called Shared Genomic Segments (SGS) to identify chromosomal regions shared among 6 suicide cases with DNA in this pedigree, using whole exome sequencing data; regions shared among these cases were hypothesized to contain variants that would contribute to suicide risk in this pedigree. We identified 5 regions on chromosomes 1,6,11, 15 and 21. Variants in these regions were identified and prioritized using the software tool Genome Mining (GEMINI) to compare variants shared among the pedigree cases to sequencing data from internal and external control populations. Rare exon variants were not identified, but frequencies were increased among pedigree cases for intronic, and upstream and downstream sequence variants of the major histocompatibility complex such as HLA-DRB1, HLA-DRB5, and HLA-DRA, and IGHV1OR21-1, BTNL2, and CD6 which are involved in T cell activation. Follow-up analyses will be performed using whole genome sequencing to verify these variants, and to extend findings to new additional suicide cases ascertained in this family.

Poster Abstract 31

Anilkumar Pillai
Augusta University

Estrogen receptor beta attenuates endoplasmic reticulum stress-induced Autism Spectrum Disorder-like behavior through an IRE/XBP-1 pathway.

Introduction: Although a number of recent studies have suggested the role of endoplasmic reticulum (ER) stress in the pathophysiology of ASD in humans, the underlying mechanism(s) is not known.

Methods: Adult mice were injected intraperitoneally (I.P.) with endoplasmic reticulum (ER) stress inducer, tunicamycin (1mg/kg) and subsequently examined in social interaction, marble burying, grooming, ultrasonic vocalization and open field tests. The role of estrogen receptor beta (ERβ) in ER stress-induced ASD-like phenotype was examined using ERB-041 (an ERβ agonist). The role of IRE-1 was examined using lentiviral injection of IRE-1 shRNA into PFC and the role of XBP1 was examined using I.P. injection of 4u8C, an inhibitor of conversion from unspliced to spliced XBP1 via IRE-1.

Results: Tunicamycin induced abnormal communication, social deficits, increased repetitive behavior, and increases in functional connectivity between medial prefrontal cortex (mPFC) and dorsal or ventral hippocampus in male mice. Reduced ERβ protein levels were found in PFC of male mice following tunicamycin treatment. ERB-041 pretreatment significantly attenuated tunicamycin-induced ASD-like behavior, hyperconnectivity and increased levels of phosphorylated IRE-1 and spliced XBP1 in mouse PFC. IRE-1 shRNA and sXBP1 inhibitor prevented the development of ASD-like phenotype.

Conclusion: Together, these results show that ER stress-induces ASD-like behavior in male mice via an IRE-1/XBP1 dependent pathway. ERbeta could be a potential therapeutic for ASD and related neurodevelopmental disorders.

Poster Abstract 32

Katrina Y. Choe
Dept of Neurology, UCLA

Cntnap2 deficiency leads to abnormal resting-state functional connectivity patterns in mice

Neuroimaging studies in individuals diagnosed with autism spectrum disorders (ASD) consistently report aberrant neural connectivity consisting of decreased long-range and increased short-range connections. Understanding the mechanistic basis for these changes warrants the use of animal models with strong construct and face validity. Mice lacking Contactin-Associated Protein-Like 2 (Cntnap2), a highly penetrant gene for ASD, display the two core behavioral phenotypes of the neurodevelopmental disorder including repetitive behavior and social deficits (Penagarikano et al., 2012). As an investigative first step, we used functional neuroimaging to study whether this validated animal model presented patterns of abnormal brain connectivity. Resting-state functional magnetic resonance imaging (rsfMRI) was performed using an ultra high field (7T) MRI scanner with a gradient-echo EPI sequence to measure BOLD contrast in dexmedetomidine-sedated wild-type (WT, n=4) and Cntnap2 knockout (KO, n=5) mice. Correlations in low-frequency (0.01-0.2Hz) BOLD signal fluctuations were computed between 26 regions-of-interest (ROIs), made up of 13 bilateral brain regions. Overall, we observed a marked reduction in the resting-state functional connectivity of KO mice. Reductions in interhemispheric connectivity were consistently observed, and those seen in the basal ganglia, as well as somatosensory, olfactory, and perirhinal cortices were statistically significant ($P<0.05$; 2-sample t-test). In agreement with these results, graph theoretical analysis revealed a strong trend of reduced mean clustering coefficient, global efficiency, and mean local efficiency in the KO, suggesting both global and local decreases in functional connectivity. These results highlight parallels between functional connectivity alterations in this mouse model of ASD and patients. We are now extending the pilot studies to validate the generalizability and reproducibility of these results. In addition, we are currently investigating whether these connectivity deficits are attenuated by oxytocin administration, previously demonstrated to rescue social deficits in these mice.

Poster Abstract 33

Abigail Clark
Columbia University

The mouse midline thalamic dopaminergic system modulates cocaine locomotor sensitization

The paraventricular nucleus of the thalamus (PVT), a midline thalamic nucleus, has recently been described as an important component of the neural circuitry underlying drug reward. In addition, work in humans, non-human primates, and rats has demonstrated dopaminergic innervation of the midline thalamus. However, little is known regarding a) the source of dopaminergic innervation to the PVT and b) the function of D2R in the PVT. Using radioactive in situ hybridization and immunohistochemistry, we first show that D2R are particularly enriched in the PVT. With in vitro electrophysiological recordings in D2R-GFP mice, we establish that tonic firing in D2R-expressing thalamic relay neurons in the PVN is inhibited by quinpirole, a D2R/D3R agonist, and increased by sulpiride, a D2R/D3R antagonist. Single synapse retrograde tracing using a pseudotyped rabies viral vector revealed that dopaminergic innervation of the midline thalamus arises from dopaminergic nuclei in both the midbrain and hypothalamus. Using anterograde viral tracing, we found that D2R-expressing midline thalamic neurons send dense projections to several regions within the amygdala and extended amygdala. Based on these findings, we next assessed the behavioral function of the midline thalamic dopaminergic system. We directly manipulated this circuitry in two directions: 1) by overexpressing D2R in the PVT and 2) by knocking down D2R in the PVT. Here we show that overexpression of D2R in the PVT decreased cocaine locomotor sensitization in mice and that downregulation of D2R in the PVT led towards a trend for increased sensitization. Our findings demonstrate for the first time the role of D2R in the PVT and add to literature suggesting that the PVT is an important component of the neural circuitry underlying drug reward.

Poster Abstract 34

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A common single nucleotide polymorphism in the AQP4 gene modulates the level of age-related hippocampal subregional atrophy among highly stressed individuals.

Both physical and psychological stress, are known to be one of the major damaging factors in the brain, especially in the hippocampus. A number of studies have repeatedly reported age-related hippocampal atrophy, of which stress was suggested as an important modulator. Particularly, the hippocampal subfields, subiculum, CA3, and dentate gyrus have been considered to be vulnerable to stress and aging.

To date, most human and animal studies of hippocampal atrophy have focused on neuronal loss. However, astrocytes, a type of non-neuronal cell, have also been suggested to play an important role in the brain dynamics involving structural and functional change. Furthermore, the astrocyte-specific water channel aquaporin-4 (AQP4) has recently received much attention as a potential major player in astrocyte physiology.

In the current study, we compared two groups cross-sectionally: 1) an experimental group consisting of firefighters who have been repeatedly and chronically exposed to physical and psychological stress, such as shift work, toxic material inhalation, heat stress, and traumatic events; and 2) an age-and sex-matched healthy control group. We identified hippocampal subregions susceptible to aging in the firefighter group compared to the control group. In addition, we examined whether genetic variations in the AQP4 gene modulates the age-accelerated atrophy.

There was no difference in the level of age-associated atrophy for both left and right overall hippocampus between groups in the mixed-effects model. On the other hand, the age-accelerated atrophy in the left subiculum was significant in the firefighter group, even after performing multiple comparisons with a Bonferroni-correction, based on the number of subregions. Interestingly, a common genetic polymorphism in the AQP4 gene, rs11659941, modulated the age-accelerated atrophy in the subiculum in the firefighter group ($p<.001$), but not in the control group. These findings suggest that the AQP4 gene might be engaged especially in the on-demand brain plasticity. The current study not only provides novel insights into the molecular basis of stress-and age-accelerated atrophy in the hippocampal subregions, but also suggests the importance of the AQP4 genetic variation in modulating brain-based alterations due to chronic stress.

Poster Abstract 35

Giulio Genovese
Stanley Center at Broad Institute

Ultra-rare protein-altering variants among 4,877 Swedish individuals with schizophrenia

Because individuals affected with schizophrenia have fewer offspring, purifying selection is expected to prevent high-risk alleles from reaching even modest allele frequencies. Similarly to rare copy number variants implicated in schizophrenia risk, variants with a large effect on schizophrenia risk are likely to be rare in populations, requiring sequencing to find them.

We generated whole exome sequencing data for 4,877 schizophrenia unrelated cases and 6,203 unrelated controls from Sweden. We define variants present uniquely in a single individual and not present in the ExAC database as ultra-rare variants (URVs). We further defined URVs as gene-disruptive and putatively protein-damaging (dURVs) those variants that are predicted to truncate or abrogate an encoded protein or that compromise protein function as assessed by 7 separate algorithms (SIFT, PolyPhen-2 HDIV, PolyPhen-2 HVAR, LRT, Mutation Taster, Mutation Assessor, and PROVEAN). We then measure the excess of synonymous URVs and dURVs in cases and controls and we measure the relative excess of dURVs across several gene sets with respect to the exome-wide excess. We further measured the excess of dURVs and more general sets of rare exome variants across each gene.

We observed, and for the first time we estimated, an excess of 0.25 (95% CI=0.17-0.32) dURVs in schizophrenia cases on a background of about 4 dURVs per individuals. We found that the excess of dURVs in schizophrenia cases largely resided in brain-expressed genes, and more specifically in genes that are expressed in neurons and genes encoding potentially synaptic proteins. We also detected this excess in previously associated protein-interaction-defined gene sets (such as PSD-95, NMDAR, ARC) but these collectively failed to explain a sizeable proportion of the exome-wide excess (collectively 4-12%). Similarly to an excess of

mutations in intellectual disability genes observed for syndromic forms of autism, we observed an enrichment in X linked intellectual disability genes (OR=1.88; 95% CI=1.34–2.64) and developmental disorder genes identified in de novo trio studies (OR=1.67; 95% CI=1.31-2.13). No individual gene surpassed exome-wide significance for association with dURVs in this analysis.

The excess of dURVs in schizophrenia cases largely resided in brain-expressed genes, and more specifically in genes that are expressed in neurons, rather than in other CNS cell types and it appears to be concentrated in a larger set of genes encoding potentially synaptic proteins. The fact that the exome data did not implicate individual genes of large effect in an unbiased exome-wide search, while documenting a very clear exome-wide enrichment of hundreds of pathogenic variants across individuals affected with schizophrenia, lends further support to the emerging impression that the high polygenicity of schizophrenia extends to rare as well as common variants.

Poster Abstract 36

Inna Gaisler-Salomon
University of Haifa

Glutamate dehydrogenase-deficient mice: a novel mouse model of schizophrenia-like phenotypes

Background: Glutamatergic abnormalities are commonly observed in schizophrenia (SZ), but all current medications for SZ symptoms target monoaminergic neurotransmitter systems, and are inefficient in treating all symptom clusters. Animal models that capture glutamatergic abnormalities in SZ and mimic its behavioral and neurochemical attributes are critical for better understanding of the neurobiology of the disorder and for the development of new pharmacological treatments. Recent findings indicate that expression of glutamate dehydrogenase (Glud1) mRNA is reduced in the hippocampus of patients with SZ, particularly in the CA1 subregion. Glud1 encodes the GDH protein, a mitochondrial enzyme that converts glutamate to alpha-ketoglutarate.
Aim: To assess whether a dose-dependent genetic alteration in CNS Glud1 expression in mice affects glutamate levels, and alters behavioral and gene expression patterns relevant to SZ, alone or with added social isolation stress in adolescence.
Methods: Male and female CND-Glud1 deficient (CND-Glud1+/- and CND-Glud1-/-) or control (CND-Glud1+/+) mice were divided into social isolation or group-housing conditions from postnatal day (PND)38 to PND60 and then tested for SZ-relevant behavior. Our behavioral battery included nesting behavior, locomotor activity and anxiety-like behavior in open field, social preference and social recognition in the three chambers paradigm, wet T-maze for reversal learning and extra-dimensional set shifting and amphetamine-induced locomotor activity (2mg/kg). Hippocampal samples from behaviorally naïve CND-Glud1-/- and control group housed mice were analyzed for glutamate content and for mRNA gene expression of excitatory (glutamatergic) and inhibitory (GABAergic) markers.
Results: CNS-Glud1-deficient mice show elevated glutamate levels in the right hippocampus. Behaviorally, CNS-Glud1-deficient mice exhibit impaired nesting behavior, baseline and amphetamine-induced hyperactivity in the open field and deficits in reversal learning and extra-dimensional set shifting. Social Isolation in adolescence induced hyperlocomotion at baseline in all mice, and triggered reversal and extra-dimensional set shifting deficits in CND-Glud1+/- mice. Expression of GAD1 mRNA was reduced in the right hippocampus of CNS-Glud1 -/- mice, and there was an overall reduction in the expression of inhibitory markers, consistent with commonly observed reductions in markers of hippocampal GABA interneurons in SZ.
Conclusion: Collectively, these studies show that Glud1 deficiency phenocopies key features of SZ in mice. Thus, Glud1 may play a critical role in SZ-related pathology and moreover, glutamate metabolism in the hippocampus may drive SZ-related pathology. These findings could lead to better understanding of SZ etiology and provide new treatment venues for its symptoms.

Poster Abstract 37

Amy Ramsey
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Adult rescue of NMDAR deficiency highlights the promise and pitfalls of neuroplasticity

Neurodevelopmental disorders like schizophrenia present challenges for treatment because diagnosis occurs decades after the causal insult to the CNS. It is not clear whether the neural circuits that mediate schizophrenia-relevant behaviours are sufficiently

plastic to overcome developmental deficits in wiring. We addressed this question by generating a new mouse line in which NMDA receptor hypofunction occurs throughout development, and can be rescued at different stages of postnatal development. In this study, we determined the extent to which schizophrenia-relevant behaviours can be normalized by restoring NMDA receptor levels in adulthood.

The NR1-KD mouse line was generated by targeted insertion of a floxed neomycin cassette into intron 17 of GluN1. Rosa26-Cre-ERT2 transgenic mice were used to achieve tamoxifen-inducible rescue at 10 weeks of age, and behavioural and biochemical testing was performed at 14 or 18 weeks of age. Using a Cre reporter line we verified complete global Cre activation with tamoxifen treatment, and no basal Cre activity in the absence of tamoxifen.

NR1-KD showed a 90% reduction in NR1 protein levels and NMDAR ratdioligand binding. Adult rescue with tamoxifen revealed brain-region-specific differences in the extent of recovery of NMDA receptor levels, with cortex and hippocampus having the greatest rates of NR1 protein and NMDA receptors.

There were also circuit-specific improvements in behaviours. Incomplete normalization of locomotor activity was observed in NR1-rescue mice, and stereotypy was not substantially improved. However, executive function, sociability, sensorimotor gating, and fear conditioning were all markedly improved, with NR1-rescue mice performing similar to WT mice. Importantly, the age at intervention or length of recovery had no effect on the extent of behavioural rescue.

We also found that NMDAR-evoked current was normalized to WT levels in cortical pyramidal neurons of NR1-rescue mice, but was only marginally improved in medium spiny neurons from the same animals. This paralleled the circuit-specific changes in behavioural improvements.

In conclusion, while some schizophrenia-relevant behaviours were highly plastic, others were less amenable to improvement. The explanation for this may lie in molecular and cellular mechanisms of plasticity that are cell-type specific. The nearly complete rescue of cognitive behaviours like executive function and sociability is remarkable, and supports the concept of profound adult neuroplasticity. Although the cognitive and negative symptoms of schizophrenia are not improved by current antipsychotics, our work suggests that these behaviours are not refractive to improvement, even in adulthood.

Poster Abstract 38

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Identifying phenotypic convergence among ASD-associated genes in Xenopus tropicalis

Autism Spectrum Disorder (ASD) is a devastating neurodevelopmental disorder of undetermined etiology. Advances in genome technology have led to the identification of 65 high-confidence ASD risk genes. Here we present a strategy to leverage CRISPR/Cas9 genome editing in Xenopus tropicalis to determine the neurodevelopmental phenotype(s) of these ASD genes. We aim to identify ‘convergent phenotypes’, which are phenotypes that occur in multiple genes of diverse cellular function, with the assumption that these phenotypes are more likely to be relevant to ASD pathology. The effects of ASD gene loss will be assayed by imaging neurons throughout embryogenesis using fluorescent reporters and by in situ RNA hybridization for neuronal cell fate specification markers. A strategy to identify transcriptional signatures of ASD gene loss using RNA-sequencing will also be presented. By combining the high-throughput capability of the CRISPR/Cas9 system, a tractable diploid vertebrate model organism, and a reliably-associated set of ASD genes, this study aims to understand the neuropathology of ASD by focusing on convergent phenotypes shared among genes.