Morning Poster Session
Location: Row A
Poster #1

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Yunhong Huang	Graduate	Thathiah	Department of Neurobiology

The phosphorylation barcode of GPR3 modulates Aβ generation

Alzheimer's disease (AD) is the most common type of dementia and is characterized by the insidious degeneration of brain networks involved in memory and cognition. Accumulation and aggregation of the amyloid β (Aβ) peptide in the brain are pathological hallmarks of AD. Sequential cleavage of the amyloid precursor protein (APP) by the β- and γ-secretases generates Aβ. Given that both secretases cleave numerous substrates selective modulation of APP cleavage is a major concern with targeting these two secretases for AD therapeutic development. The orphan G protein-coupled receptor 3 (GPR3) selectively regulates activity of the γ-secretase in the absence of an effect on Notch proteolysis one of the major γ-secretase substrates. Genetic deletion of Gpr3 reduces amyloid pathology and alleviates the cognitive deficits in various AD transgenic models suggesting that GPR3 is a relevant therapeutic target for AD. Mechanistic studies indicate that an interaction between GPR3 and the scaffolding protein β-arrestin 2 is involved in the modulation of Aβ generation. β-arrestin 2 belongs to a small family of multifunctional GPCR regulatory proteins which bind to activated GPCRs and play an almost universal role in facilitating traditional GPCR desensitization; however these proteins are also capable of initiating distinct signals in their own right conveying receptor subtype-specific signaling events. Here we demonstrate that the phosphorylation status of GPR3 regulates β-arrestin 2 recruitment and Aβ generation. Mutagenesis of specific serine residues in the C-terminus of GPR3 differentially regulate the interaction between GPR3 and β-arrestin 2 and subsequent Aβ generation. Genetic deletion of individual G protein–coupled receptor kinases (GRKs) which regulate GPCR phosphorylation leads to a reduction in Aβ generation suggesting that GRK-dependent phosphorylation of GPR3 is involved in the modulation of Aβ generation. Collectively these studies demonstrate that differential phosphorylation of specific serine residues in the C-terminus of GPR3 by GRKs are important for β-arrestin 2 recruitment and Aβ generation.

Morning Poster Session
Location: Row A
Poster #2

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Matthew Phillips	Graduate	Wills	Neuroscience

Enhancement of NMDA Receptor Desensitization by the Alzheimer's Disease Drug Memantine

NMDA receptors (NMDAR) play an essential role in synaptic development plasticity and neuronal survival principally through their collective effect upon magnitude and timing of Ca2+ influx. However pathological conditions can lead to overactivation of NMDARs resulting in excitotoxicity and cell death due to excess Ca2+ influx. Calcium-dependent desensitization (CDD) of NMDARs is a crucial process that under normal conditions works to prevent excess Ca2+ influx via a negative feedback mechanism initiated by increased intracellular levels of Ca2+. Although the structure of the NMDAR Ca2+-dependent desensitized state is unknown interactions of the carboxy-terminal domain (CTD) of the GluN1 subunit with allosteric regulators and CTDs of other NMDAR subunits are vital to CDD. Additionally recent work in our lab has found that the NMDAR channel blocker memantine (Mem) slows NMDAR recovery from calcium-dependent desensitized states. Here we further examine the relation between the GluN1 subunit CTD the Ca2+-dependent desensitized state and Mem using whole-cell recordings from HEK cells expressing wild-type NMDARs composed of the GluN1 and GluN2A subunits (GluN1/N2A receptors) or mutant receptors with truncated CTDs (ΔCTD). As expected GluN1ΔCTD/N2A receptors were found to exhibit reduced desensitization. Interestingly GluN1ΔCTD/N2A receptors displayed a decreased sensitivity to Mem and Mem had no effect on the time course of recovery from desensitization of GluN1ΔCTD/N2A receptors. These results support the hypothesis that Mem enhances desensitization of NMDARs through stabilization of a Ca2+-dependent desensitized state giving new insight into Mem’s mechanism of action.

Morning Poster Session
Location: Row A
Poster #3

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Yanjun Zhao	Postdoctoral	Wills	Neurobiology

Amyloid Beta Peptides Block New Synapse Assembly by Nogo Receptor Mediated Inhibition of T-Type Calcium Channels

Compelling evidence links amyloid beta peptide (ABeta) accumulation in the brains of Alzheimer's disease (AD) patients with the emergence of learning and memory deficits; yet a clear understanding of the events that drive this synaptic pathology are lacking. We present evidence that neurons exposed to amyloid beta peptides are unable to form new synapses an unappreciated feature of AD pathology. Further we demonstrate the Nogo receptor family (NgR1-3) act as amyloid beta receptors mediating an inhibition of synapse assembly and synaptic plasticity. Dual fluorescent sensor live imaging studies reveal that amyloid beta peptides activate NgRs on the dendritic shaft of neurons triggering an inhibition of calcium signaling. Finally we define T-type calcium channels as the target of ABeta-NgR signaling mediating ABeta's inhibitory effects on calcium synapse assembly and plasticity. These studies highlight deficits in new synapse assembly as a potential initiator of cognitive pathology in AD and pinpoint calcium dysregulation mediated by NgRs and T-type channels as key components.

Morning Poster Session
Location: Row A
Poster #4

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Amanda Gleixner	Postdoctoral	Donnelly	Neurobiology

Assessment of FG Nup function in C9ORF72 ALS

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease characterized by the degeneration of the motor neurons and interneurons in the brain and spinal cord. The majority of ALS occurs sporadically (sALS; 90%) with no family history. Genetic studies of patients with a family history of ALS familial ALS (fALS; 10%) have been studied to identify ALS-causing mutations within the human genome. Despite 31 known ALS-causing mutations a G4C2 repeat expansion in the first intron of the C9orf72 gene has been identified as the most common known genetic cause of both fALS and sALS comprising 30% and 8% of cases respectively (Renton et al. 2013; DeJesus-Hernandez et al. 2013). A molecular mechanism conferring neurotoxicity for the repeat expansion mutation is the generation of toxic G4C2 RNAs that sequester RNA binding proteins and the accumulation of dipeptide repeat proteins (DPRs) through the non-canonical repeat associated non-ATG translation (RANT) pathway (Donnelly et al 2013; Wen et al 2014; Ash et al 2013). Recent studies show that the expression of G4C2 or arginine-rich DPRs products of the C9orf72 repeat expansion dramatically alter the nucleocytoplasmic transport pathway (Zhang et al 2015; Freidbaum et al 2015; Jovičić A). This results in the nuclear retention of RNAs and the reduction in the rate of nuclear import of proteins that contain a classical nuclear localization sequence (NLS) and the cytoplasmic enrichment of TDP-43 (Freidbaum et al 2015; Zhang et al 2015). Moreover components of the nuclear pore complex (NPC) were identified as potent modifiers of both nuclear transport defects and neurodegeneration in C9orf72 ALS Drosophila models (Freidbaum et al 2015; Boeynaems et al 2016). The NPC functions to regulate passive transport of molecules >40kDa across the nuclear membrane. It is comprised of thirty different nucleoporins and approximately half of the nucleoporins contain intrinsically disordered phenylalanine-glycine repeat domains (FG domains). FG Nups comprise the selective barrier of the NPC and their dysfunction alters the compartmentalization of nuclear and cytoplasmic proteins. Loss of some FG nucleoporins (FG nups) have been shown to modulate degeneration in C9ORF72 ALS Drosophila models. In this work we begin to address the role of FG Nups in C9orf72 ALS pathobiology. Here we employed a genetic screen in RNA and DPR Drosophila models of C9orf72 ALS and iPSC motor neurons to determine FG Nups important for C9orf72 mediated neurodegeneration. Next we assessed how C9orf72 RNA and/or DPR products alter FG Nup biology including stability and post-translational status. Finally we determine if modulating FG Nup function and contributes to or rescues nucleocytoplasmic trafficking deficits in C9ORF72 ALS.

Morning Poster Session
Location: Row A
Poster #5

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Paige Rudich	Graduate	Lamitina	Pediatrics/Cell Biology

A C. elegans model for C9orf72-associated dipeptide toxicity

Amyotrophic lateral sclerosis (ALS) is a rapidly progressing age-related neurodegenerative disease that affects ~30 000 Americans. ALS causes degeneration of the upper and lower motor neurons leading to paralysis. Currently there are no effective treatments for ALS and only ~50% of patients survive beyond three years after diagnosis. A recently discovered expanded hexanucleotide repeat in the first intron of the C9orf72 gene is the most common known genetic cause of familial and sporadic ALS. The repeat expansion is not thought to cause disease through alteration of C9orf72 function. Rather the expanded repeat is transcribed in both sense and antisense directions to produce repeat-containing RNAs that are then translated in multiple reading frames to yield up to five distinct dipeptide repeat proteins. This unusual mode of translation is termed Repeat Associated-non-ATG (RAN) translation. It is controversial whether the expanded repeats cause ALS through RNA toxicity RAN translated dipeptide toxicity or both. Using codon-varied transgenes we created a 'dipeptide-only' model in the nematode C. elegans to better understand the mechanisms of dipeptide toxicity. The arginine rich dipeptides PR and GR were toxic in C. elegans when expressed in multiple cell types including motor neurons. This toxicity was dependent on both the length of the dipeptide as well as its subcellular localization. Genetic inhibition of the insulin pathway a conserved regulator of ageing delayed age-onset toxicity caused by PR dipeptides suggesting that physiological age rather than chronological age is a determinant of PR toxicity. Currently we are performing RNAseq and using unbiased forward and reverse genetic screens to identify modifiers or arginine-containing dipeptide toxicity. Defining these modifiers will allow us to determine potential mechanisms for dipeptide toxicity and may lead to new disease biomarkers and/or therapies.

Morning Poster Session
Location: Row A
Poster #6

Presenting Author:	Author Type:	Mentor/Lab:	Department:
William Reynolds	Undergrad	Wu	Developmental Biology

MRI Investigation of Hydrocephalus in Mutant Mouse Models with Congenital Heart Disease: Insights into the Pathogenesis of Congenital Hydrocephalus

INTRODUCTION: Neurodevelopmental disabilities are the most common and potentially most disabling long-term complication of congenital heart disease (CHD) and yet their etiology is not well understood. There are high incidents of hydrocephalus in particular external hydrocephalus with excess external cerebrospinal fluid (CSF) among CHD patients. The current therapeutic procedure for hydrocephalus the ventriculoperitoneal shunt to relieve the excessive CSF is invasive with high malfunction rate and revision surgery is often needed. Moreover despite the ventriculoperitoneal shunt many patients still suffer from life-long neurological disabilities. The causes of congenital hydrocephalus is not well understood and its pathophysiologoy is thought to entail ventricle enlargement with mechanical injury and eventual destruction of the periventricular white matter. While the etiology of congenital hydrocephalus is largely unknown this can be seen in patients with primary ciliary dyskinesia (PCD) a sinopulmonary disease due to motile cilia dysfunction. The goal of this study is to elucidate the pathophysiology of hydrocephalus in a PCD mutant mouse model to better understand the underlying cause for the poor neurological outcomes associated with hydrocephaly. METHODS: Animal model: Studies were conducted using mutant mice harboring a mutation in Dnah5 a gene encoding the outer dynein arm of motile cilia and a gene commonly associated with PCD. Dnah5 (Dynein Axonemal Heavy Chain 5) encodes an axonemal heavy chain dynein that comprise a force-generating protein with ATPase activity whereby the release of ADP is thought to produce the force-producing power stroke. The wild-type (WT) littermates were used as age-matched controls. The homozygous mutant mice and WT controls were subject to MRI evaluation. Brain MRI Analysis:\tMulti-modal magnetic resonance imaging (MRI) was performed at 7-Tesla (Bruker Avance III). In vivo T2-weighted RARE and T1-weighted anatomical images are used to quantify ventricular and gray matter development. Different brain areas such as ventricles hippocampus olfactory bulbs cerebral cortex thalamus hypothalamus caudate mid-brain cerebellum and brain stem are segmented computationally for volumetric and morphometric evaluation. Diffusion MRI followed by graphic analysis is used to evaluate brain injury white matter development and neuronal network. Motile cilia function assessment: Cilia in the tracheal respiratory epithelia as proxy for the ependymal cilia were analyzed for ciliary motion using vidoemicroscopy. Ciliary wave form and beat frequency were analyzed using the digital videos. RESULTS: In vivo MRI of the Dnah5 mutant mouse brains showed severe ventriculomegaly excessive CSF and extra-axial fluid in regions dorsal to the brain parenchyma. In several cases there were intraventricular and subdural hemorrhages and subdural collections. There was also significant cortical necrosis and overall abnormal gray matter dysplasia. It is generally believed that hydrocephalus in mutant mice in BL6 background was caused by aqueductal stenosis because BL6 mice have narrower cerebral aqueducts than other mouse strains. However despite significantly increased CSF and ventricular volume no aqueductal stenosis was observed in any of the hydrocephalus mice (n>100). Our data suggests the hydrocephalus in Dnah5 mutants may arise from motile cilia dysfunction rather then aqueductal stenosis. Our volumetric and morphometric analysis showed the hydrocephalus mutant mice displayed brain dysplasia especially in the olfactory bulb hippocampus cerebellum and

supratentorial regions. This observation is consistent with clinical presentation of hydrocephalus patients. In addition to gray matter and ventricular abnormality neural network analysis with diffusion MRI followed by graph analysis of fiber tracks showed aberrant neural networking with substantial disruptions in fiber tracks between various brain regions. Together these findings support the notion that cilia may play an important role in regulating neurogenesis and brain development. CONCLUSION: Our results suggest motile cilia plays an essential role in normal brain development with motile cilia defects causing not only ventriculomegaly but also brain dysplasia and aberrant neural network formation. These findings suggest the poor neurological outcomes associated with hydrocephalus despite intervention with ventriculoperitoneal shunt may arise from neurogenesis defects caused by the ciliary dysfunction and not simply mechanical injury from the ventriculomegaly.
ACKNOWLEDGEMENT: \tWe thank Michael Wang and Nikolai Klena for genotyping; Jennifer Hess Caleb Radomile and Jacob McLeary for animal breeding and care and Dr. Fang-Cheng Yeh for diffusion MRI analysis.

Morning Poster Session
Location: Row A
Poster #7

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Yijen Wu	Faculty	Wu	Developmental Biology and Neurology

MR SPECTROSCOPIC AND IMAGING STUDIES OF A VARIABLE RAT MODEL OF EPILEPSY

Rationale: The development of spontaneous recurrent seizures (epilepsy) is a complex process that commonly ensues after an initial cerebral insult. While it is well known that metabolic dysfunction is common during this process there is little experimental data on how variation in seizure duration in experimental status epilepticus (SE) influences metabolic injury. Using lengthy periods of status epilepticus that are highly likely to result in the development of epilepsy several groups (1 2) have reported on the metabolic injury seen during the epileptogenesis period in chemoconvulsant models of epilepsy. These changes have been characterized as neuronal and glial in nature with declines in N-acetyl aspartate (NAA) increases in myo-inositol (Ins) and glutamine. In this report we use a variant of the Hellier Dudek model with a much shorter period of status epilepticus to assess the metabolic changes. In a subset of these animals we obtain histology to assess for neuronal injury and gliosis. Method: A short variant of the Hellier Dudek (3) low dose kainate (KA) model of temporal lobe epilepsy was used. Briefly 180-200g male rats (Charles River) were injected ip with 5 mg/kg KA (n=21) until a stage 3/4/5 seizure (modified Racine scale) was elicited. Status epilepticus was defined as the time after the first motor seizure started until 45min later when 20mg/kg diazepam was administered to terminate behavioral seizures. Controls (n=10) were similarly treated with sterile saline. Rats were evaluated twice by MR after status (3days 3d) and during the latent period (3weeks 3w). Rats were video monitored 24-7 for the duration of the study. MRI: A Bruker Biospec 7T 40cm horizontal MR system was used throughout with a 72mm volume transmit coil and 2 element receive array. Rapid T2 weighted images were acquired for optimal positioning of the hippocampus. Single voxel MR spectroscopy (8ul) was acquired with TR/TE 1.7s/10ms (17min per acquisition) from the left right dentate gyrus and CA3 region. LCM analysis was performed for determination of the metabolite profiles and taken as a ratio to total creatine tCr accepting Cramer Rao bounds of <12%. A total of n=21 kainate treated rats were studied with n=7 controls. Results: A hierarchical cluster analysis performed on the 3day data from n=21 kainate treated animals (dentate gyrus voxel) segregated into two clusters denoted by KM (more injured n=6) and KL (less injured n=15). While there was no difference in kainate dosing or seizure count between them the metabolic pattern of injury was different. The KM group displayed the largest significant changes in neuronal and glial parameters; the KL group displayed milder but significant changes. At 3weeks the KL group returned to normal compared to controls while the KM group persisted with declines in NAA/tCr Glutamate/tCr increased Inositol/tCr and Glutamine/tCr. The classification was also consistent with subsequent patterns of histology at 3weeks. Table 1 shows the differences in these groups. Conclusions: While a short status period might be expected to generate a continuous distribution of metabolic injury these data show that the short Hellier Dudek model appears to generate two levels of injury. The changes seen in segregated groups persisted into 3weeks and can be interpreted based on neuronal and glial biomarkers consistent with histology results. This work is supported by NIH R21 NS83035 and R01NS090417

Morning Poster Session
Location: Row A
Poster #8

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Ashwinee Manivannan	Undergraduate	Modo	Department of Neuroscience

Evaluating tractography parameters to visualize connectivity at the mesoscale in an ex vivo human hippocampus from a patient with temporal lobe epilepsy

Understanding the biology of hippocampal atrophy due to mesial temporal lobe epilepsy is imperative to improving treatment for the condition. A key hypothesis states that an aberrant connection between the dentate gyrus and stratum moleculare is an underlying cause of the disorder. This cannot be investigated using macroscopic or microscopic techniques. Instead mesoscale diffusion tensor imaging (DTI) a type of magnetic resonance imaging that measures the direction of diffusion of water molecules may provide better insight. Using DTI diffusion tensor tractography (DTT) computes streamlines visualizations of neuronal connections. Although tractography calculation has many parameters that influence streamline connectivity parameters that afford a reliable and accurate representation of neuronal connections have not been previously investigated. Diffusion images of the left hippocampus sample of a 42-year-old man with intractable epilepsy were analyzed in DSI Studio a program used to analyze DTT. Our investigations found that a lower threshold for step size functional anisotropy and minimum length provided reliable results while a higher threshold was better for angle. Seed sample was inconclusive. Further investigation will allow for accurate and reliable visualization of extra- and intra-hippocampal connections as well as the ability to non-invasively investigate the human hippocampus for better understanding of tissue architecture.

Morning Poster Session
Location: Row A
Poster #9

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Daniela Leronni	Postdoctoral	Friedlander	Neurological Surgery

Toward a Gene Therapy for Huntington Disease

Huntington Disease HD is an autosomal dominant neurodegenerative disease due to an extended CAG repeat in the gene encoding for the protein huntingtin (htt). Melatonin has been shown to be neuroprotective in cellular models of HD and to decrease mortality in mouse models of HD. Melatonin inhibits cytochrome c release activation of the caspase cascade and cell death. HD patients show a gradual decrease of melatonin blood level and a reduction of Arylalkylamine N-acetyltransferase (AANAT) the rate-limiting enzyme in the production of melatonin from serotonin. We want to investigate if restoring of the level of melatonin produced in HD cells could provide a potential treatment for HD. To overcome the problem of continuous drug administration and to restore the melatonin synthesis in HD cells one potential powerful approach is gene therapy. HSV-based vectors have provided one method of long-term delivery of transgenes. We aim to overexpress two enzymes involved in the synthesis of melatonin (AANAT) and its precursor serotonin (aromatic l-amino acid decarboxylase AADC) in HD cells. We have created three different HSV-based vectors: one for each enzyme independently and one overexpressing both. We will then deliver our transgene(s) in wt(htt) and mut(htt) mouse stratal neurons derived cell lines in order to compare the level of melatonin and consequently the release of cytochrome c in the two cell lines. The results of these experiments will indicate 1) which step of the melatonin synthesis pathway is impaired in HD cells and 2) if restoring or even increasing levels of melatonin produced in HD cell can have a neuroprotective effect. Future experiments will attempt to apply these vectors for infection of mouse brain by direct inoculation.

Morning Poster Session
Location: Row A
Poster #10

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Mark Schurdak	Faculty	Schurdak	University of Pittsburgh Drug Discovery Institute

Application of Quantitative Systems Pharmacology to identify mechanistic probes and combinations of neuroprotective agents for Huntington's Disease

Huntington's disease (HD) is a devastating chronic neurodegenerative disorder currently afflicting 30 000 Americans with 150 000 more at risk of inheriting the disease from a parent. HD is caused by a highly penetrant autosomal dominant mutation in the HTT gene. Expansion of a series of CAG triplets at the 5'-end of this gene increases the number of tandem glutamine residues in the encoded protein (HTT) and is associated with neuronal death. Although the number of glutamine repeats negatively correlates with age of onset the mechanistic basis between glutamine expansion in HTT and neuronal death is not completely understood. Despite more than two decades of research since the discovery of the causative mutation and the development of more than 20 transgenic mouse models that recapitulate many aspects of the clinical phenotypes no effective treatment for HD neurodegeneration is available and the disease is universally fatal. It has been hypothesized that the pleiotropic effects of mutant HTT represent a major barrier to understanding mechanisms of HD progression and designing therapeutic strategies. To meet this challenge we have implemented a Quantitative Systems Pharmacology (QSP) platform. QSP is an approach to drug discovery and development that melds the fundamental principles of pharmacology and systems biology into a modular multidisciplinary broadly applicable platform enabling a quantitative network-centric understanding of the biology underlying disease progression (Stern et al. 2016). A hallmark of QSP is its iterative use of experimental and computational models that when integrated with comprehensive and unbiased multiplexed system-wide measurements and existing knowledge can identify emergent properties of diseases and corresponding drug-target interactions at multiple levels of biological complexity. Data-driven iteration through a QSP cycle is expected to improve the clinical relevant design of its component models with the prospect of increasing the robustness of target and companion biomarker selection. Accordingly it is anticipated that implementation of QSP will enable optimization of therapeutic strategies for HD and serve as a paradigm for studying other neurodegenerative diseases. Here we present the chemogenomic component of QSP to understand pathways involved in neuroprotection in HD. Chemogenomics is the systematic screening of small molecule probes with known targeted interactions to help elucidate the molecular modes of action of compounds giving rise to a specific phenotype. Using computational and experimental methods we identified a number of drugs/probes with different canonical mechanisms of action that exhibited neuroprotection as measured by a cytotoxicity experimental model. Further combinations of these drugs/probes showed enhanced protective effects relative to single probes suggesting distinct mechanisms may be involved. These data will help guide the elucidation of pathways/networks involved in HD neuroprotection.

Morning Poster Session
Location: Row A
Poster #11

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Svitlana Yablonska	Postdoctoral		Neurological Surgery

Localization of Huntingtin in mammalian mitochondria

Mitochondrial dysfunction is believed to be a crucial driver of Huntington's disease (HD) pathophysiology. HD is a hereditary progressive neurodegenerative disorder characterized by selective neuronal loss in the striatum and cortex caused by mutated huntingtin protein (mHtt). Though many mitochondrial related effects of Htt have been described the precise location and translocation of Htt in mitochondria has not been shown. We utilized fresh brain tissue of wild-type (WT) and transgenic mice expressing both full length mHtt (140CAG) and N-terminal fragment (R6/2) to purify synaptosomal and non-synaptosomal mitochondria. Utilizing treatment with protease and permeabilizing agent we localized WT and mutant full length Htt as well as fragments of mHtt inside mitochondria. By conducting the same assay on mitochondria isolated from post-surgical frozen human cortex we confirmed localization of human WT and mHtt in isolated brain mitochondria. Immunogold electron microscopy (IEM) demonstrated co-localization of mHtt fragments with synaptosomal mitochondria of R6/2 mice. The N-terminal 17-amino acids sequence (N17) of Htt plays a role in its mitochondrial targeting. We conducted structured illumination microscopy (SIM) and confirmed mitochondrial translocation of Htt's N17 fused with fluorescent protein. Therefore results of our study clearly demonstrate localization of Htt in mammalian mitochondria pointing towards its role in mitochondrial functions and involvement of mHtt in development of mitochondrial pathology.

Morning Poster Session
Location: Row A
Poster #12

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Victor Van Laar	Postdoctoral	Berman	Neurology

Evaluating mitochondrial biogenesis in a cell model of Parkinson disease via mitochondrial DNA replication in neuron cell bodies axons and dendrites

Evidence implicates dysregulation of mitochondrial homeostasis and quality control in neurodegenerative diseases such as Parkinson's disease (PD). We had previously found that exposure of primary neurons to chronic sublethal doses of rotenone a Complex I inhibitor linked to PD was associated first with increased mitochondrial density in distal neurites followed by later increases in mitochondrial density in cell bodies. The increased mitochondrial density in neurites prior to cell bodies was not accounted for by changes in mitochondrial transport or localized changes in mitochondrial degradation. This suggested the possibility that localized changes in mitochondrial biogenesis could be occurring in axons/dendrites but methodology for direct studies targeting neuroanatomical localization of mitochondrial biogenesis was lacking. We have optimized methodology to directly image localize and quantify replicating mitochondrial DNA (mtDNA) in neurons using BrdU incorporation and immunocytochemistry. We are able to visualize newly synthesized mtDNA replication within minutes in neurons in vitro. The BrdU incorporation is inhibited as expected by the addition of 2' 3'-dideoxycytidine (ddC) inhibitor of mitochondrial DNA polymerase gamma. Interestingly as neurons 'age' in culture from 7 to 21 days rates of mtDNA synthesis increase. This increase is also associated with increased expression of PGC1alpha suggesting increased mitochondrial biogenesis with senescence. To address the effects seen in our previous studies we are also evaluating the effects of chronic sublethal rotenone on localized mtDNA replication. These studies give us the ability to better elucidate mtDNA replication/mitochondrial biogenesis in neurons and under PD-relevant conditions.

Morning Poster Session
Location: Row A
Poster #13

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Meghan Bucher	Graduate	Hastings	Neuroscience

Dopaminergic degeneration following viral mediated dysregulation of dopamine: Implications for Parkinson's disease

Dopaminergic neuronal health is dependent upon the proper handling of dopamine (DA). Following synthesis in the cytosol DA is quickly packaged into acidic vesicles via the vesicular monoamine neurotransporter 2 (VMAT2). Disruptions in the packaging of DA can lead to increased cytosolic DA oxidation forming ROS and highly reactive DA quinone which can attack and covalently modify protein cysteinyl residues leading to detrimental downstream consequences. Cytosolic DA oxidation has been proposed to contribute to the neurodegenerative process in Parkinson's disease (PD). Previously it was shown that mice expressing only 5% of normal VMAT2 levels showed an age dependent loss of substantia nigra (SN) DA neurons and increased DA oxidation (Caudle et al. 2007). More recently Pifl et al. (2014) showed impaired VMAT2 function within the remaining dopaminergic terminals in PD patients suggesting that a dysregulation of DA storage can contribute to the pathogenesis of the disease. To investigate the effects of reduced VMAT2 expression in an adult animal an adeno-associated virus containing a plasmid coding for small-hairpin ribonucleic acid against VMAT2 was generated (AAV2-sh[VMAT2]). Rats were stereotactically injected unilaterally into the left SN with AAV2-sh[VMAT2]. Six weeks following the viral injection SN coronal sections were immunostained for VMAT2 tyrosine hydroxylase (TH) MAP2 and DAPI. Stereological counting of TH- and MAP2-positive neurons showed a significant loss of SN DA neurons on the AAV2-sh[VMAT2] injected side (-38.74%) compared to the contralateral non-injected SN (N=5 p<0.05). Likewise decreased density of striatal TH-immunoreactive terminals and the presence of dystrophic axons on the viral injected side also suggested neurodegeneration. In the remaining transduced TH-positive neurons VMAT2 protein levels were significantly decreased and there was an increase in insoluble alpha-synuclein. Consistent with the loss of DA neurons rats injected unilaterally into SN with AAV2-sh[VMAT2] showed significant asymmetric motor deficits as determined by the postural instability test and the cylinder test. These results show that a substantial reduction in the expression of VMAT2 within dopaminergic neurons is sufficient to cause neurotoxicity impairing proper VMAT2 functioning and DA sequestration in the maintenance of dopaminergic neuronal health.

Morning Poster Session
Location: Row A
Poster #14

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Jianming Chen	Postdoctoral	Burton	Neurology

Parkinson's disease-related protein α-synuclein modulates dynamic RedOx responses in CNS dopaminergic neurons in vivo

Parkinson's disease (PD) is characterized pathologically by death of specific neuronal groups (most prominently dopaminergic neurons of the substantia nigra) and accumulation of the presynaptic protein α-synuclein in cytoplasmic and axonal aggregates. Oxidative damage and mitochondrial dysfunction are thought to be central to the events leading to degeneration of dopaminergic neurons in PD. It is known that α-synuclein can influence both mitochondrial physiology and cellular oxidative biochemistry; however it is not known how α-synuclein modulates the production of ROS or physiologic maintenance of the RedOx potential in disease-vulnerable neurons. In order to address this question in CNS dopaminergic neurons in vivo we generated novel transgenic zebrafish expressing ratiometric reporters to allow measurement of dynamic RedOx responses in by direct intravital imaging. roGFP2-Orp1 is a sensitive detector of peroxide and is therefore a reporter of ROS generation. Grx1-roGFP2 senses changes in the oxidation status of the glutathione 2GSH/GSSG RedOx couple and is a reporter of oxidative stress. Our initial data using these novel transgenic reporters indicate that we can measure levels of ROS production and oxidative stress in vivo including dynamic responses after application of an oxidative challenge. By crossing these lines with further novel transgenic zebrafish expressing human α-synuclein we have shown that α-synuclein causes increased ROS production and disrupts RedOx homeostasis following a sub-lethal oxidative insult. The effect appears to be specific to dopaminergic neurons. These results are important since the direct measurement of these proximate pathogenic events for the first time in disease-relevant neuronal groups in vivo will allow us to exploit the genetic and chemical approaches possible in zebrafish to dissect the underlying biochemical mechanisms and to identify novel therapeutic targets.

Morning Poster Session
Location: Row B
Poster #15

Presenting Author:	Author Type:	Mentor/Lab:	Department:
April Dukes	Postdoctoral	Burton	Pittsburgh Institute for Neurodegenerative Diseases and Neurology

Role of Parkinson's disease-related protein α-synuclein in mitochondrial transport and degradation in CNS dopaminergic neurons in vivo

Parkinson's disease (PD) is characterized pathologically by neuronal loss and accumulation of α-synuclein in large protein aggregates (Lewy bodies) in surviving neurons. Extensive oxidative damage and loss of mitochondrial respiratory function has been found in PD autopsy material suggesting that mitochondrial dysfunction is a key pathogenic mechanism. Maintenance of a healthy mitochondrial population requires a delicate balance of biogenesis fission fusion transport and degradation by mitophagy. Rare Mendelian Parkinsonism phenocopies are associated with loss of function in proteins that are centrally involved in mitochondrial quality control; however little is known about the role of mitochondrial transport and degradation in sporadic PD or how these processes are influenced by α-synuclein. We previously developed a method for measuring mitochondrial transport in dopaminergic neurons in the intact CNS of live transgenic zebrafish lines expressing fluorescent reporters within their mitochondria. In this model low sublethal concentrations of a mitochondrial inhibitor previously implicated in PD pathogenesis (MPP+) caused a dramatic 3-fold increase in retrograde mitochondrial transport in dopaminergic axons (Dukes et al. Neurobiology of Disease 2016:95:238). We now aim to determine (i) whether the large increase we observed in retrograde transport following MPP+ exposure is part of a compensatory cellular response encompassing mitophagy within the cell body of irrevocably damaged presynaptic mitochondria; and (ii) how this process is influenced by α-synuclein. In order to directly address these questions in dopaminergic neurons in vivo we have generated additional novel transgenic lines including zebrafish that express an LC3-GFP fusion protein that labels autophagosomes in living cells and zebrafish that express human α-synuclein in dopaminergic neurons. By a combination of genetic crosses and high-end in vivo imaging we now have the tools to observe mitophagy in CNS dopaminergic neurons in vivo and to determine how this is modulated by mitochondrial inhibitors and α-synuclein. These unique reagents and approaches will elucidate how α-synuclein and mitochondrial quality control intersect in the pathogenesis of PD.

Morning Poster Session
Location: Row B
Poster #16

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Vladimir Ilin	Postdoctoral	Burton	Neuroglogy

Evaluation of presynaptic dopaminergic function in the intact CNS of a genetic model vertebrate

Evaluation of presynaptic dopaminergic function in the intact CNS of a genetic model vertebrate Vladimir Ilin MD Edward A. Burton MD DPhil FRCP Pittsburgh Institute for Neurodegenerative Diseases and Deaprtment of Neurology University of Pittsburgh School of Medicine Parkinson's disease (PD) is characterized by loss of substantia nigra dopaminergic neurons and formation of Lewy bodies (cytoplasmic inclusions containing aggregates of α-synuclein) in surviving cells. To determine the role of α-synuclein in PD pathogenesis we have generated zebrafish over-expressing human α-synuclein in their dopaminergic neurons or lacking endogenous zebrafish synucleins. In order to understand how these experimental manipulations affect the synaptic physiology of dopaminergic neurons prior to degeneration of terminals or cells we have developed an experimental approach to record dopaminergic synaptic potentials in live intact zebrafish larvae. The zebrafish homologue of the mammalian substantia nigra is found in the ventral diencephalon; dopaminergic neurons in this location project both to the forebrain and to the spinal cord. There are direct synaptic connections between the dopaminergic diencephalospinal tract and spinal cord motor neurons which are relatively accessible for intracellular electrophysiological recordings from intact animals. We have obtained stable whole-cell patch recordings from zebrafish spinal cord motor neurons and identified glutamatergic GABAergic and dopaminergic synaptic inputs. By application of pharmacological agents we have isolated and recorded spontaneous dopaminergic postsynaptic potentials. This approach will allow us to analyze how the function of dopaminergic terminals is altered by accumulation or loss of synucleins or by other genetic or environmental factors relevant to PD. Importantly we predict that changes in synaptic physiology will be informative for understanding upstream events in pathogenesis and for testing therapeutic interventions designed to target proximate mechanisms.

Morning Poster Session
Location: Row B
Poster #17

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Manish Verma	Postdoctoral	Chu	Pathology

Role of Mitochondrial Calcium Uniporter SIRTUIN-3 and Calcium in LRRK2 mediated neurodegeneration.

Background: Leucine-rich repeat kinase 2 (LRRK2) is a large (~280 kDa) multi-domain protein containing GTPase and kinase domains that is predominantly localized to the cytoplasm. Mutations in various domains of LRRK2 have been associated with familial Parkinsonian neurodegeneration. Impaired oxidative phosphorylation perturbations of mitochondrial dynamics and increased sensitivity to mtDNA damage have been observed in cells expressing LRRK2. LRRK2 has been implicated in the regulation of various cellular pathways including regulation of calcium (Ca2+) homeostasis. Furthermore dysregulation of Ca2+ homeostasis has been shown to induce mitophagy leading to loss of dendritic mitochondria in LRRK2 mutant expressing primary cortical neurons. These studies indicate that dysregulation of Ca2+ handling may play a central role in neurodegeneration; however the mechanism through which LRRK2 acts to modulate mitochondrial Ca2+ homeostasis remains unclear. In the current study we delineate the possible molecular pathway through which mutant LRRK2 induces mitochondrial damage. Results: Mouse primary cortical neurons expressing PD-associated LRRK2 mutants showed simultaneous increase in cytosolic and mitochondrial Ca2+ upon KCl stimulation compared to those expressing either pcDNA or LRRK2-wild type. This increase in mitochondrial Ca2+ uptake in LRRK2 mutants was due to transcriptional upregulation of one of the mitochondrial calcium uptake transporter called mitochondrial calcium uniporter (MCU). Moreover pharmacological or siRNA mediated inhibition of MCU in neurons protected against LRRK2 mutations induced dendrite retraction. Interestingly inhibition of ERK signaling pathway prevented increased expression of MCU and in turn partially rescued mutant LRRK2 mediated neurite retraction suggesting that LRRK2 may function through ERK pathway. Additionally restoration of mitochondrial Ca2+ handling by overexpression of mitochondrial sodium/Ca2+ exchanger (NCLX) or SIRTUIN-3 (SIRT3) also protected against LRRK2 mediated neurite retraction. Conclusion: Mitochondrial calcium overload has been implicated in various diseases. Mutations in LRRK2 have been shown to dysregulate Ca2+ homeostasis leading to loss of functioning mitochondria. In the present study we show that LRRK2 causes increased mitochondrial Ca2+ uptake by inducing the expression of MCU. This increase in MCU expression could potentially increase the susceptibility of neurons to mitochondrial calcium overload leading to LRRK2 associated neurotoxicity. Mutant LRRK2 (R1441C and G2019S) increased ERK1/2 phosphorylation and inhibition of ERK activity protected against LRRK2 mediated neurotoxicity by repressing MCU expression. On the other hand increasing mitochondrial Ca2+ efflux by overexpression of NCLX was also shown to be neuroprotective.

Morning Poster Session
Location: Row B
Poster #18

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Kevin Mastro	Graduate	Gittis	Neurobiology

Cell-specific pallidal intervention induces long-lasting motor recovery in dopamine depleted mice

In Parkinson's disease (PD) the external segment of the globus pallidus (GPe) is a key contributor to the induction propagation and maintenance of network dysfunction within the basal ganglia. In the classical rate model of basal ganglia dysfunction the loss of dopamine shifts the balance of the two functionally opposing pathways: motor-facilitating direct and motor-suppressing indirect. An overactive indirect pathway leads to the cardinal symptoms of PD: bradykinesia and immobility. To test the efficacy of pallidal stimulation to reduce indirect pathway activity and alleviate parkinsonian motor symptoms we used an optogenetic approach to modulate activity in the GPe in a global or cell-selective manner. Our results demonstrate that global increases or decreases in GPe activity are minimally effective at restoring movement in bilaterally dopamine-depleted mice but in contrast cell-specific stimulation strategies were highly effective. Specifically activation of Parvalbumin-positive (PV-GPe) neurons or inhibition of Lim homeobox 6-positive (Lhx6-GPe) neurons restored movement to near pre-lesion levels. Intriguingly this behavioral rescue did not cease at the end of stimulation but persisted for hours. At the end of the 4-hour experiment all mice still exhibited near pre-lesion levels of locomotion. For comparison we tested the ability of direct-pathway stimulation to rescue movement in bilaterally-depleted mice. Behavioral recovery as a result of direct pathway stimulation was neither as robust nor as persistent as cell-specific manipulations in the GPe. In future experiments we will use in vivo electrophysiology within the output nucleus of the BG to observe circuit-level alterations before during and after the optogenetic stimulation. In summary these results demonstrate that cell-specific activation of PV-GPe or inhibition of Lhx6-GPe neurons provide a long-lasting recovery in motor function and establish the existence of two functionally distinct cell populations in the GPe.

Morning Poster Session
Location: Row B
Poster #19

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Amber Van Laar	Faculty	Van Laar	Neurology

A novel progressive endogenous synucleinopathy model of Parkinson disease in rats

One of the greatest obstacles in developing effective neuroprotective therapeutics for Parkinson disease (PD) is lack of a predictive preclinical research model that replicates the human disease with fidelity. We now report a new rat model in which brief pesticide exposure causes progressive accumulation and aggregation of endogenous α-synuclein culminating in a delayed and progressive behavioral and pathological parkinsonian phenotype over a period of months. Lewis rats (6-9 months old) received baseline behavioral testing and then were treated with rotenone (i.p.) once daily for 5 days only. During treatment rats became mildly parkinsonian but there was no morbidity or mortality. All rats recovered to their behavioral baseline over the succeeding week. They remained behaviorally normal until about 3 months at which point all rats began to show mild progressive parkinsonian symptoms including postural instability and bradykinesia. From onset symptoms progressed over 3-4 months and stabilized thereafter. Pathological studies indicate that during the quiescent latent period before symptom onset nigrostriatal neurons accumulate α-synuclein which becomes progressively consolidated into inclusions by 3 months. The accumulation of α-synuclein is accompanied by progressive microglial activation - and many microglia also contain intracellular α-synuclein apparently derived from nigral neurons. By the time of symptom onset there is loss of nigrostriatal dopamine neurons which continues to progress over a period of months. By 9 months there is α-synuclein accumulation in other brain regions including in the cortex and there are legitimate Lewy bodies in some remaining nigral neurons. These results indicate that a remote environmental exposure has the potential to set in motion a pathological cascade that results after a long latent period in parkinsonism. The model has many advantages over conventional models including the fact that (i) it is a spontaneously progressive endogenous synucleinopathy and (ii) potential disease-modifying treatments can be started at symptom onset which is analogous to current clinical practice.

Morning Poster Session
Location: Row B
Poster #20

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Mary Cheng	Faculty	Cheng	Departments of Computational & Systems Biology

Human dopamine transporter: transport and modulation mechanism

Human dopamine transporter: transport and modulation mechanism Mary H Chenga E Blockb J Pinoc G Torresc A. Sorkinb and Ivet Bahara Departments of Computational & Systems Biology a cell Biology b University of Pittsburgh School of Medicine Pittsburgh PA 15261; and c Department of Pharmacology and Therapeutics University of Florida Gainesville FL 32610 Dopamine transporters (DATs) control neurotransmitter dopamine (DA) homeostasis by reuptake of excess DA assisted by sodium and chloride ions. The recent resolution of DAT structures (dDAT)1 from Drosophila permits us for the first time to directly view the sequence of events involved in DA re-uptake in human DAT (hDAT) using homology modeling and full-atomic microseconds accelerated simulations. Major observations are2: spontaneous closure of extracellular (EC) gates prompted by DA binding; stabilization of a holo-occluded intermediate; disruption of N82-N353 hydrogen bond and exposure to intracellular (IC) water triggered by Na2 dislocation; redistribution of a network of salt bridges at the IC surface in the inward-facing state; concerted tilting of IC-exposed helices to enable the release of Na+ and Cl- ions; and DA release after protonation of D79. The observed time-resolved interactions confirm the conserved dynamics of LeuT-fold family while providing insights into the mechanistic role of specific residues in hDAT.2 The model provides excellent structural basis for understanding hDAT interactions with regulatory proteins and drug molecules. Further simulations show the ability of DAT to readily translocate DA and amphetamine; whereas orphenadrine (our predicted re-purposable drug) acts as a blocker like cocaine.3 We also note the propensity of DAT C-terminal end to bind Gβ consistent with experiments. Currently the potential mechanism of DA efflux induced by either Gβ or AMPH binding is being investigated. Acknowledgement: NIH grants P30DA035778 and P41GM103712 (I.B.) R01DA014204 (to A.S.) and R01DA038598 (G.T.) References: 1.\tKH Wang A. Penmatsa E. Gouaux. Nature (2015) 521 (7552): 322-7. 2.\tMH Cheng and I Bahar (2015) Structure; 23: 2171-81 3.\tMH Cheng E Block F Hu M Cobanoglu A Sorkin and I Bahar (2015) Frontiers Neurol 6:134

Morning Poster Session
Location: Row B
Poster #21

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Cynthia Kenmuir	Faculty	Kenmuir	Neurology

Acute Stroke Thrombectomy Outcomes for Patients Transferred Directly to the Angiosuite in an Effort to Reduce Delay to Reperfusion

Introduction: Time from symptom onset until reperfusion is correlated to outcome following acute stroke intervention. Ongoing efforts focus on streamlining the time needed to adequately assess a patient during an acute stroke in order to offer endovascular therapy as quickly as possible. Methods: A retrospective chart review was conducted to evaluate outcomes for acute stroke patients treated with endovascular therapy at the University of Pittsburgh Medical Center comparing patients who were taken directly from the helipad to the angiosuite versus those who received additional assessments in the emergency room prior to endovascular reperfusion. Results: Cases were reviewed from Jan 2013 to July 2015 in order to capture all cases who were brought directly to the angiosuite upon arrival. Of 379 endovascular stroke cases 32 (8.4%) were taken directly to the angiosuite – 30 (8%) had large vessel occlusions (LVO) – 19 MCA occlusions and 11 basilar occlusions. 8 patients received IV tPA. 9 patients had tandem cervical lesions that required intervention. Mean door to puncture time was 21.1 minutes. All 30 patients were successfully revascularized (TICI2b/3 reperfusion). There was a trend towards smaller final infarct volumes in patients taken directly to the angiosuite versus patients who underwent additional assessment prior to endovascular treatment (26.4 cc vs 34.0 cc). Mean length of stay was similar between the groups (5.4 days ICU 12.8 days total). MRS at discharge was improved in patients taken directly to the angiosuite (mean 2.9 versus 3.9) but MRS at 90 days was unchanged (3.4). At 90 days 12 patients (40%) had a good outcome (MRS0-2) though 8 patients were deceased (26.7%). Conclusion: Taking patients directly to the angiosuite for endovascular reperfusion during an acute stroke can reduce the delay from symptom onset to reperfusion. In 30 patients with LVO there was a trend towards smaller infarct volumes without significant change in 90-day MRS.

Morning Poster Session
Location: Row B
Poster #22

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Lingjue Li	Graduate	Poloyac	School of Pharmacy

No-reflow phenomenon Revisted: Alteration of Cerebral Microcirculation after Cardiac Arrest in Developing Rats

Introduction: As early as 1968 the no-reflow phenomenon was described by Ames in a global ischemia rabbit model however this phenomenon has never been observed in vivo. Our previous studies in a pediatric asphyxial cardiac arrest (CA) model observed decreased cortical blood flow from 15-180 min post-CA. In order to further elucidate the mechanism underlying the cortical hypoperfusion we propose to evaluate cortical microvascular dysfunction in our pediatric CA model with the goal of enhancing the understanding of vascular dysfunction post-CA and establishing a scaffold to evaluate potential vasoactive therapeutic agents. Hypothesis: Disturbances in cortical microcirculation are present post-CA consistent with the no-reflow phenomenon. Methods: Postnatal 17 day old rats underwent tracheal intubation arterial and venous cannulation and were equipped with a cranial window for in-vivo two photon laser scanning microscopy. Asphyxial CA of 9 min was induced by cessation of mechanical ventilation after neuromuscular blockade. Rats were resuscitated with chest compressions epinephrine and sodium bicarbonate. Using in-vivo multiphoton microscopy we serially assessed cortical microcirculatory blood flow pre- and post-CA. We measured the diameter of cortical microvessels red blood cell (RBC) velocity and density pre- and post-CA. We quantified the mean transit time using intensity tracking during bolus dye injection. Data were processed in MATLAB and SPSS. Results: We assessed 44 capillary branches from 12 rats. The RBC flow was continuous at baseline. At 30 min post-CA 14 (25.4%) capillaries had no-reflow at 30 min and 8 (18.1%) capillaries have no-reflow at 60 min post-CA. For capillaries with continuous RBC flow we collected 33 capillary branches from 4 sham rats and 39 capillary branches from 8 CA rats. No significant differences were identified in RBC velocity or density post-CA compared with baseline or sham animals. Microvessel diameters were found to have high variability post-CA. The mean transit time from cortical artery to veins was increased at 30 min post-CA compared with sham rats suggesting obstruction in cortical microcirculation. Conclusions: We are first to identify the no-reflow phenomenon in-vivo in a clinically relevant pediatric asphyxial CA model. Additionally we observed important alterations of the cerebral microvascular circulation with highly variable vessel diameter and increased mean transit time. Significance: Approximately 16 000 pediatric patients suffer CA each year in the US with asphyxia as the major cause. Hypoxic ischemic encephalopathy is the limiting factor for recovery post-CA. We identified important alterations of the cortical microcirculation consisting of areas with no reflow areas of low flow and increased variability of capillary diameters. Understanding the cerebral microvascular dynamics post-CA is paramount to identifying therapeutic targets for mitigation of ischemic encephalopathy post-CA.

Research / Grant Support: NIH R01 HD075760 1S10RR028478-01 Brain 2015 Conference Young Investigator Travel Bursary

Morning Poster Session
Location: Row B
Poster #23

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Pablo Iturralde	Graduate	Torres-Oviedo	Bioengineering

Intertrial variability of EMG reveals lack of bilateral or inter-joint muscle synergies for walking in unimpaired and post-stroke patients

Activation of 'muscle synergies' has been proposed to underlie neural control of movement and cortical damage is thought to change the structure of these neural commands (Cheung et al. 2014). To test these hypotheses we identified muscle synergies in unimpaired and post-stroke subjects that were independent from task requirements. We specifically recorded 30 bilateral muscles in 16 chronic post-stroke subjects and 16 age and sex matched controls under two walking conditions imposing distinct movement demands: normal walking vs. split-belt walking in which legs move at different speeds. We identified muscle synergies by computing covariance matrices indicating correlated activity across muscles. Importantly we dissociated covariations in EMG signals due to task requirements and those due to common neural drive by analyzing 1) rectified and filtered EMG data ('full dataset') and 2) fluctuations in EMG recordings from the mean activity across strides ('intertrial dataset'). We found anatomical multijoint and bilateral muscle co-activation in the full dataset analysis but only anatomical in the intertrial dataset analysis. This was indicated by significant correlations across muscles within the same anatomical groups (22/22 signif. correlations median r2=.73) across multiple-joints (119/188 r2=.30) and legs (149/225 r2=.29) identified in the full dataset. However only anatomical correlations were observed in the intertrial dataset (22/22 r2=.36) while the others became much weaker (r2<=.08). We also found that muscle co-activation from the full dataset change across walking conditions to match changes in task constraints (interlimb median r2 change =.14) whereas the intertrial muscle synergies were maintained the same. Lastly anatomical muscle co-activations identified in the intertrial dataset of patients were the same (22/22 r2=.40) as controls and surprisingly symmetric across legs. Conversely full dataset analysis revealed that stroke patients have less multijoint (94/188 r2=.38) and bilateral (113/225 r2=.30) muscle co-activations than controls suggesting a deficit in patients task performance or reduced task demands (since all patients walked at slower than controls). Taken together these results suggest that only anatomical groups might receive unified neural drive but correlated activity in muscles across joints and legs (multijoint and bilateral muscle synergies) reflects task demands rather than shared neural control signals. As such differences in multijoint and bilateral muscle synergies between patients and controls may represent patients' deficits in task performance or reduced task demands and not clear differences in neural commands.

Morning Poster Session
Location: Row B
Poster #24

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Carly Sombric	Graduate	Torres-Oviedo	Bioengineering

Changes in perception of step length size after split-belt walking

Step asymmetry post-stroke significantly limits patients' mobility. It has been proposed that patients' reduced perception of their gait asymmetry contributes to their inability to recover symmetric walking (Wutzke et al. 2015). Thus there is an interest in understanding if the perception of step asymmetry can be altered. A recent study indicates that split-belt walking in which legs move at different speeds changes the perception of asymmetric walking speeds in unimpaired subjects (Vazquez et al. 2015). Here we hypothesize that the perception of asymmetric step lengths can also be altered after split-belt adaptation. To test this we investigated subjects' perception of step lengths for each leg before and after split-belt adaptation. Participants (n=7 25.9±6.1 yr.) first learned a spatial map of three distinct subject-specific step length sizes (short comfortable and long) by observing their step length and the targeted step size. All visual feedback was displayed with an Oculus Rift and Vizard software in order to remove the effect of peripheral vision on subjects' control of position. We assessed changes in step position for seven subjects during two pseudorandomly presented testing sessions: normal walking at 1m/s and split-belt walking at 1.5 m/s (fast leg) and 0.5 m/s (slow leg). Step length perception before and after walking was evaluated by recording step length accuracy while subjects walked at 1m/s with reduced visual feedback projecting only 35% of the actual step length error. Both testing sessions included 810 strides of walking before step length perception was assessed. A catch trial (both legs walk at 1m/s for 10 steps) was introduced during the walking period to identify motor after-effects induced by the split-belt walking condition. We found that subjects in the split-belt group had significantly more motor (slow leg catch: p<0.001; fast leg catch: p=0.007) and perception (short target slow leg: p<0.001; short target fast leg: p=0.019; long target slow leg: p=0.12; long target fast leg: p=0.001) after-effects compared to the control session. We also found that perception after-effects were distinct for each leg: the slow leg undershot the stepping target while the fast leg overshot it following split-belt walking. Interestingly a single rate can be used to fit the decay of perception across legs and targets (all fits R^2>0.72) even though the amplitudes of perceptual after-effects are different. Importantly split-belt walking did not disrupt subject's step size map even when no visual feedback was given (p>0.33). In sum we found that split-belt walking induces changes in the perception of step lengths. This is important since paradigms like split-belt walking could be used to alter patients' active perception of limb position and improve their awareness of asymmetric stepping.

Morning Poster Session
Location: Row B
Poster #25

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Chung-Yang Yeh	Graduate		Neurobiologu

Kv2.1-derived peptide ameliorates apoptosis in vitro and in an in vivo model of ischemic stroke

Neurodegeneration in the ischemic stroke penumbra involves caspase activation and represents an important target for therapeutic interventions. The voltage-gated potassium channel Kv2.1 has been shown to play an important role in programmed neuronal apoptosis. Previously, we have identified that Kv2.1 promotes caspase activation by generating a pronounced K+ efflux through de novo channel insertion. This process is modulated by Zn2+-dependent pathways leading to the phosphorylation of Kv2.1 at residues Y124 and S800, allowing interactions between the proximal Kv2.1 C-terminus (C1a) and the SNARE protein syntaxin 1A. Overexpression of the C1a decreases pseudo-apoptotic Kv2.1-S800E currents in CHO cells and improves neuronal survival after oxidative stress. Thus, suppressing apoptosis-permitting Kv2.1 currents through the competitive binding of syntaxin 1A represents a promising neuroprotective approach and is explored here through the evaluation of a peptide aptamer. Far-Western blot analysis of overlapping 15 a.a. segments of the C1a region found a 9 a.a. sequence with high binding affinity to syntaxin 1A. Conjugating the cell-permeable HIV trans-activator of transcription domain (TAT) to the N-terminus of this sequence resulted in an administrable peptide construct (TAT-C1aB). In vivo two-photon imaging with FitC-tagged TAT-C1aB confirmed that i.p. administration of the peptide reaches brain vessels within minutes. Whole-cell patch clamp recordings demonstrated that incubation with 10 µM TAT-C1aB significantly reduces pseudo-apoptotic Kv2.1-S800E current density (vehicle vs TAT-C1aB: 267.2±22.03 vs 189.5±19.64, Mean±SEM pA/pF, n=11; p=0.0159) in CHO cells. Further, 1 µM TAT-C1aB treatment significantly decreased lactate dehydrogenase release in rat cortical neuron cultures after excitotoxic insult (2.45±0.24 vs 1.31±0.077, Mean±SEM normalized LDH readout, n=4 each group, p=0.0038). Proximity ligation assay revealed that post-injury TAT-C1aB incubation prevents the dramatic increase in Kv2.1/syntaxin interactions 3 hr after treatment with an apoptogen. Notably, in a mouse model of transient middle cerebral artery occlusion, injections of TAT-C1aB (i.p., 6nmol/g, at 1+6h reperfusion) significantly reduced infarct size (0.20±0.019 vs 0.12±0.016, Mean±SEM infarct ratio, n=10 and n=7 respectively, p=0.0053). In all above experiments, TAT-conjugated scramble control had no detectable effects. Together, these findings characterized a promising neuroprotectant prototype and established the Kv2.1/syntaxin interaction as a novel molecular target in ischemic stroke as well as other neurodegenerative diseases.

Morning Poster Session
Location: Row B
Poster #26

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Sabya Das	Postdoctoral		Pharmacology & Chemical Biology

WNK kinase-mediated changes in GABAA receptor neurotransmission after ischemic stroke

Synaptic and extrasynaptic γ-aminobutyric acid type A receptors (GABAARs) generate inhibitory neurotransmission in the healthy adult brain through hyperpolarizing chloride ion influx and are essential in regulating brain function. Ischemic brain injury results in severe damage and death of brain tissue due to disruption of intracellular and extracellular ion homeostasis and an imbalance between excitatory/inhibitory neurotransmission. In particular ischemic injury is associated with reduced GABAAR inhibition; however the underlying mechanisms are not understood. The strength and polarity of GABAAR neurotransmission (inhibitory or excitatory) depends on the intracellular chloride concentration ($[Cl^-]i$) set by the activity of two cation-chloride transporters: chloride importer Na+-K+-Cl- cotransporter (NKCC1) and neuronal chloride extruder K+-Cl- cotransporter (KCC2). The Cl- transporters are reciprocally regulated by the chloride sensitive WNK3 kinase; WNK3 stimulates NKCC1 but inhibits KCC2 activity through phosphorylation. Recent studies show that GABAAR subtype expression depends on $[Cl^-]i$ during development but effects of ischemic stroke on altering GABAAR plasticity have not been investigated. WNK3 knockout (KO) mice exhibit smaller ischemic infarct and improved neurological function recovery after focal ischemic stroke (middle cerebral artery occlusion MCAO) in association with reduced phosphorylation and activation of NKCC1. However whether improved post-stroke recovery in WNK3 KO mice in part results from maintenance of inhibitory GABAergic neurotransmission remains unknown. Our pilot study shows that in vitro ischemia [an oxygen-glucose deprivation (OGD) model] leads to loss of KCC2 and downregulation of α1 GABAAR protein expression in cortical neurons. Furthermore initial in vivo data reveal a decrease in α1/α2 GABAAR ratio extrasynaptic α4δ GABAAR and KCC2 expression in WNK3 wild type (WT) mice at 48 h post-stroke. Importantly WNK3 KO mice exhibit an absence of ischemic stroke-induced GABAAR plasticity that could be neuroprotective. Biochemical calcium imaging and electrophysiological methods are being used to investigate WNK3 dependent mechanisms leading to ischemic alteration of KCC2 activity and GABAAR subunit composition and localization. These studies will provide critical insight into GABAergic signaling following ischemic injury and identify new therapeutic targets for stroke treatment.

Morning Poster Session
Location: Row B
Poster #27

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Arpan Prabhu	Graduate	Branstetter	Radiology

The CT Prevalence of Arrested Pneumatization of the Sphenoid Sinus in Patients with Sickle Cell Disease

Background: Arrested sphenoid pneumatization is an incidental radiologic finding on CT and MRI that may be confused with more aggressive pathologic conditions. No definite etiology for arrested sphenoid pneumatization has been established although changes in regional blood flow during childhood as is seen with sickle cell disease (SCD) have been proposed. The purpose of our study was to compare the prevalence of arrested pneumatization of the sphenoid sinus in patients with and without SCD. Methods: We retrospectively identified 146 patients with SCD who had undergone CT scans of the skull base between January 1990 and May 2015. We identified 292 control patients without SCD matched for age and sex in a 2-to-1 ratio. We tabulated the prevalence of arrested pneumatization along with the location and size of the lesions. We used Fisher's exact test to correlate SCD with arrested pneumatization of the sphenoid sinus and Student's t test to correlate SCD with lesion size. Results: Of the 146 patients with SCD 14 (9.6%) had arrested pneumatization of the sphenoid sinus. In the 292 control patients 6 (2.1%) had arrested pneumatization. Patients with SCD had a statistically significantly higher rate of arrested pneumatization compared to patients without SCD (p < 0.001). There was no statistically significant correlation between lesion size and diagnosis of SCD. Conclusions: Patients with SCD have a greater prevalence of arrested pneumatization of the sphenoid sinus than patients without SCD. This supports the theory that either regional blood flow anomalies or increased serum erythropoietin cause arrested sinus pneumatization.

Morning Poster Session
Location: Row B
Poster #28

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Mark Linsenmeyer	Postdoctoral	Galang	UPMC Department of Physical Medicine and Rehabilitation

Disorders of consciousness due to anoxic brain injury: a case series of 8 patients

Authors: Mark Linsenmeyer Shanti Pinto Gary Galang OBJECTIVE: To characterize common medical complications treatments and recovery in patients with disorders of consciousness (DOC) due to anoxic brain injury. DESIGN: Retrospective case series at a single academic inpatient rehabilitation (IPR) center. Patients with current or recent DOC due to anoxic brain injury who were admitted to IPR from 2015-2016 were considered for inclusion. Patients were excluded if there was head trauma. History and clinical course were reviewed from electronic records. Uniform Data System (UDS) data was used to determine FIM scores. RESULTS: 8 patients were identified. On admission to IPR 4 were vegetative 1 was minimally conscious and 3 had recently emerged. While at IPR 1 vegetative and 1 minimally conscious patient emerged. 1 vegetative patient became minimally conscious. FIM scores on admission were 22 or below for all patients and improved in 4 patients by an average of 40.5 points. Scores did not improve for the remaining 4. All patients were given neuropharmacologic medications for arousal and attention. Paroxysmal sympathetic hyperactivity (PSH) affected 6/8 patients and clinically resolved for 2 of these patients prior to discharge. 6/8 patients had spasticity resolving in 3 by discharge. 5/8 patients exhibited movement disorders primarily myoclonus. No patients developed seizures during IPR admission but 2/8 patients experienced status epilepticus prior to IPR. 2/8 had MRI evidence of focal ischemic stroke in addition to hypoxia. 2/8 were briefly transferred from IPR to acute care for sepsis and 7/8 had urinary tract infections while on IPR. Overall 6/8 patients were discharged home. CONCLUSIONS: DOC due to anoxia is a unique clinical entity accompanied by specific clinical and social challenges. Common limitations to rehabilitation include the severity of deficits in arousal and cognition PSH spasticity movement disorders and a high rate of infection. Further investigation into predictors of outcome and optimal medical management for this population is warranted. Disclosures: None

Morning Poster Session
Location: Row C
Poster #29

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Yalikun Suofu	Postdoctoral	Friedlander	Neurological Surgery

The role of miR-155 in ischemic/reperfusion induced hemorrhagic transformation

miRNAs are non-coding small RNA molecules and recently emerged as key regulators of pathogenic response. However whether miRNAs play a role in neurovascular disorders after ischemic/reperfusion is unknown. One of extensively studied miRNAs is miR-155 which involves in inflammation auto-immunity and cell plasticity. Recently miR-155 was found to be a negative regulator of BBB function. In miR-155 knockout mice it was reported that central nervous system extravasation of systemic tracer was reduced both in an acute systemic inflammation model and experimental autoimmue encephalomyelitis in mice. It is not known however whether miR-155 plays a role in ischemic/reperfusion-induced hemorrhagic transformation. In this study we used mouse model of 1 hour reversible MCAO and 71 hours reperfusion. The brain slices were then stained with TTC and hemorrhagic volumes were quantified by imageJ. We found that miR-155 is significantly upregulated at 72 hours after cerebral ischemic stroke in wild type mice. miR-155 knockout did protect from ischemic injury as compared to wild type at 72 hours after stroke and there is significant reduction in infarct size in mir-155 knockout mice (p<0.05). We found that miR-155 knockout mice had none or smaller hemorrhagic dots whereas WT showed larger petechial hemorrhage or hematoma at 72 hours after ischemia. The quantification of hemorrhagic volume showed that miR-155 knockout mice have significant smaller hemorrhagic volume (p<0.05) and rate of hemorrhage is lower in miR-155 knockout mice as well (WT vs miR-155 is 100% vs 62.5%). In conclusion miR-155 knockout protects from ischemic/reperfusion induced hemorrhagic transformation and inhibition of miR-155 may benefit long-term stroke recovery.

Morning Poster Session
Location: Row C
Poster #30

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Yejie Shi	Postdoctoral	Chen	Neurology

Endothelial-Targeted Overexpression of Heat Shock Protein 27 Ameliorates Rapid Blood Brain Barrier Impairment and Improves Long Term Outcomes after Ischemia and Reperfusion

Introduction: The damage borne by the blood brain barrier (BBB) during ischemic stroke disrupts the neurovascular unit and leads to poor patient outcomes. We recently discovered that Caveolin-1-independent subtle structural aberrations of brain microvascular endothelial cells (BMECs), such as abnormal actin polymerization, stress fiber formation and subsequent junctional protein (JP) disassembly, are a novel mechanism for rapid BBB breach after ischemia/reperfusion (I/R) injury. Hypothesis: Heat shock protein 27 (HSP27) attenuates BBB breakdown and neurovascular injury after I/R by inhibiting actin polymerization and JP disassembly in BMECs. Methods: We created neuron- and EC-specific HSP27-overexpressing mice, which were subjected to 1h MCAO and reperfusion. Assessments for BBB damage were performed 1-24h after I/R; infarct volume and neurobehavioral performance were assessed up to 35d after I/R. I/R-induced BBB damage was also simulated in BMEC cultures, where gene manipulations were achieved using lentiviral vectors. Recombinant HSP27 containing a cell-penetrating domain (TAT-HSP27) was i.v. injected into mice after I/R to rapidly elevate HSP27 in BMECs. Results: Targeted overexpression of HSP27 within ECs—but not neurons—was sufficient to reduce early (1-3h) and late (24h) BBB damage after I/R ($p<0.01$). Mechanistically, HSP27 suppressed I/R-induced actin polymerization, stress fibers, and JP disassembly in BMECs, but independent of its anti-cell death properties. Intracerebral infiltration of neutrophils and macrophages was attenuated in EC-HSP27 mice by 35.3% and 59.6%, respectively ($n=6$, $p<0.05$) at 48h after I/R, thereby alleviating secondary injuries. Infarct was reduced by 35% at 72h, and sensorimotor functions ($p<0.01$, cylinder and corner tests) were improved in EC-HSP27 overexpressors up to 21d. Injection of TAT-HSP27 after I/R markedly reduced BBB damage 1-24h after I/R and elicited sustained (up to 35d) protection against neurological deficits. Conclusions: HSP27 protects against BBB disruption after I/R by inhibiting actin polymerization and JP disassembly in BMECs. HSP27 has translational potential as a therapy for ischemic stroke in conjunction with reperfusion.

Morning Poster Session
Location: Row C
Poster #31

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Gregory Weiner	Graduate	Jankowitz	Neurosurgery

Is the 64-Channel Multidetector Computer Tomography Angiography Reliable for the Diagnosis of Blunt Cerebrovascular Injury - The Importance of Digital Subtraction Angiography

Blunt cerebrovascular injuries (BCVI) are potentially associated with high morbidity and mortality. Since patients with BCVI are often asymptomatic at presentation, with neurological sequelae most commonly occurring within 72 hours, timely diagnosis is essential. To date, the use of 64-slice, multi-detector computed tomography angiography (CTA) has proven itself to be a non-invasive, cost-effective, reliable means of screening; however, the false-positive rate of CTA in diagnosing patients with BCVI represents a key drawback. Our objective was to examine the reliability of CTA in accurately diagnosing patients with BCVI when performing follow-up confirmatory digital subtraction angiography (DSA). Methods: We performed a retrospective analysis of patients with BCVI from 2013-2015 at two Level I trauma centers. All patients included herein underwent initial clinical screening based on the updated Denver Screening Criteria and were subsequently evaluated for BCVI via CTA. Patients who were found to have BCVI based on CTA underwent DSA to confirm BCVI diagnosis. Patient demographics, screening indication, CTA and DSA injury subtype and laboratory values were collected. Comparison of false positive rates stratified by CTA injury subtype was performed using Chi-squared testing. Results: One hundred and forty patients, 64% males with a mean age of 50 years presented with 156 cerebrovascular blunt injuries to the internal carotid and/or vertebral arteries. After comparison with DSA, CTA was incorrect in 61.5% of vessels studied and the overall CTA false positive rate was 47%. The positive predictive value for CTA was higher amongst worse BCVI injury subtypes on initial imaging (PPV 76% to 97%, for BCVI grades 2 & 4, respectively) compared to grade 1 injuries (PPV 30%, $p<0.001$). Conclusions: The utility of 64-slice, multi-detector CTA as a screening test for BCVI is well-established; however, its high false positive rate, especially in patients with BCVI grade 1 injuries, supports the utilization of DSA following all positive CTA findings in patients with suspected BCVI. The use of DSA as an adjunctive test in patients with positive CTA findings allows for increased accuracy in correctly diagnosing BCVI, which is crucial when considering the potential implications of initiating antithrombotic therapy in the polytrauma patient.

Morning Poster Session
Location: Row C
Poster #32

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Enhua Shao	Graduate	Burton	Pittsburgh Institude for Neurodegenerative Diesase

The role of oligodendrocytes in axonal regeneration following spinal cord injury: live imaging in a novel zebrafish model

Axons in the human central nervous system (CNS) have limited capacity to regenerate after injury; consequently neurological deficits caused by axonal damage in spinal cord compression, subcortical stroke and traumatic brain injury are associated with a poor prognosis for recovery. This is thought to result from (i) a low intrinsic ability for CNS neurons to regenerate severed axons, and (ii) a CNS microenvironment that is inhibitory to axonal growth. In contrast, CNS axons in lower vertebrates such as zebrafish, robustly regenerate after injury, resulting in functional recovery. Our goal is to understand the mechanisms underlying axonal regeneration in zebrafish, since knowledge of the cellular and molecular mechanisms may be informative for development of therapies for human neurological disease. Recently we found that zebrafish mpz promoter was strongly upregulated in oligodendroglial lineage cells of the entire optic pathway following optic nerve crush injury in adult zebrafish. To elucidate the role of oligodendrocytes in the repair response, we have developed a novel spinal cord injury model in larval zebrafish. We have generated double transgenic zebrafish in which the neurons and their axons are labeled with mCherry and the oligodendrocytes are labeled with GFP. By taking advantage of the unique ability to image fluorescent proteins in the spinal cord of live zebrafish, we have been able to demonstrate: imaging of spinal cord neurons, axons and oligodendrocytes in a living animal; loss of cells at the site of the spinal cord transection; the oligodendroglial response to injury; and axonal regrowth. Importantly, imaging can be carried out in the same zebrafish on successive days, at subcellular resolution, allowing direct observation of the entire repair response. Coupled with functional analysis of recovery of swimming movements, our new model will allow us to test the role of oligodendrocytes in CNS axonal regrowth in the zebrafish and to elucidate the underlying molecular events.

Morning Poster Session
Location: Row C
Poster #33

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Jessica Wallisch	Postdoctoral	Kochanek	CCM

Aquaporin-4 inhibitor AER-271 blocks early cerebral edema in pediatric rat asphyxial cardiac arrest

INTRODUCTION: Cerebral edema is associated with poor outcome in cardiac arrest (CA) patients. Aquaporin-4 (AQP4) is a major regulator of water transport within the CNS and may play a detrimental role after CA by exacerbating tissue swelling and promoting intracranial hypertension. This is supported by evidence that (1) cortical AQP4 levels increase following asphyxial CA in rats and (2) transgenic AQP4 gene knockout mice have reduced ICP and decreased neuronal loss after global ischemia vs. wild-type littermates. We hypothesize that pharmacologic inhibition of AQP4 by AER-271 a novel selective AQP4 antagonist reduces cerebral edema and improve outcomes in a pediatric rat model of asphyxial CA. METHODS: Post-natal day 17 Sprague-Dawley rats were anesthetized intubated and mechanically ventilated. Femoral venous and arterial catheters were placed for drug delivery and monitoring. Additional measurements included EKG ETCO2 EEG and pulse oximetry. To induce a 9-min asphyxial CA rats (n=6 per group) were given neuromuscular blockade with vecuronium and disconnected from the ventilator. CPR was initiated by resuming mechanical ventilation administering bolus epinephrine and sodium bicarbonate and rapid manual chest compressions. Experimental therapy was administered immediately after return of spontaneous circulation (ROSC). Injured rats were randomized to AER-271 (5mg/kg IP at ROSC & 60 min post-ROSC) or vehicle (identical volume/time points). Control (naïve) mice were not given surgery or CA. Rats were sacrificed 3 6 and 24 h post-arrest for cerebral wet-dry-weight analysis (110o C for 72 h). Additional rats were evaluated for early outcome with Neurologic Deficit Score (NDS) at 3 24 48 and 72 h post-arrest. RESULTS: AER-271 was well tolerated and did not alter HR MAP time to ROSC pH or base deficit after CA. At this dosage therapeutic drug levels in plasma were attained quickly and maintained throughout the entire model (mean 15 min plasma level 515.25ng/mL ±118.26 SEM; 80 min 1690.75 ±313.96; 3 h 785.25 ±116.19 by LC-MS). Cerebral edema was ameliorated in AER-271 treated CA rats vs. vehicle controls and had brain water levels similar to naïve (82.95 ±0.17 naïve % brain water; 83.87 ±0.08 vehicle; 83.28 ±0.05 AER-271 p=0.0018 one-way ANOVA). However by 6 and 24 h post-CA the percent brain water had returned to naïve levels in all injury groups. NDS scoring showed a trend toward improved neurologic functioning at 3 h post-CA for animals treated with AER-271 (0.83 ±0.83 total NDS Naïve; 335.83 ±29.34 Vehicle; 261.67 ±20.56 AER-271 p<0.0001 one-way ANOVA). CONCLUSIONS: AQP-4 inhibition by AER-271 prevents early edema formation with a trend toward reduced neurologic deficit at 3 h post-arrest in a model of pediatric asphyxial CA. The anti-edema effect of AQP4 inhibition was not evident by 6 and 24 h post-arrest but this may relate to the time course of swelling in the model rather than lack of drug efficacy. Longer durations of injury may extend the swelling in this model and is an area for future study. Finally additional testing is underway to evaluate therapeutic effects on neuronal death.

Morning Poster Session
Location: Row C
Poster #34

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Jacob Mann	Graduate	Donnelly	Neurobiology

Optogenetic induction of neurodegenerative proteinopathy

Aberrant protein misfolding and aggregation has long been considered a common pathological hallmark of a number of different neurodegenerative diseases including Alzheimer's Disease (AD) Parkinson's Disease (PD) Huntington's Disease (HD) Frontotemporal Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS) amongst others (1). Current cellular models of these neurodegenerative proteinopathies often rely on the overexpression of disease-linked mutant proteins to induce pathological protein aggregation. However the vast majority of patients suffer from sporadic forms of the disease with no familial mutations. In ALS for example ~97% of all patients show pathological cytoplasmic aggregation of the DNA/RNA-binding protein TDP-43 but mutations in the TARDBP gene only account for ~1% of sporadic (sALS) and 4% of familial ALS (fALS) cases (2 3). Similarly rodent models of ALS produced from the overexpression of these mutant proteins have been historically unreliable and often fail to generate TDP-43-positive inclusions (4). Here we present a novel optogenetic-based technique to induce pathological protein aggregation using the Arabidopsis thaliana photoreceptor cryptochrome 2 (Cry2). Using this approach we show the light-induced oligomerization and aggregation of TDP-43 and disease-related truncations of the protein occur. These Cry2-TDP-43 aggregates appear to share similar pathological characteristics with TDP-43 inclusions observed in ALS patient tissue. Furthermore light-induced aggregate formation also appears to result in endogenous TDP-43 loss-of-function mechanisms that have been previously implicated in disease progression. This technique can be applied to a number of different disorders will allow for more precise temporal and spatial control over protein aggregation than has been previously possible. Additionally the ability to reliably induce protein aggregation with light alone will allow for in-depth investigations into the effects of these pathological aggregates on various cellular pathways and downstream pathological processes.

Morning Poster Session
Location: Row C
Poster #35

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Jue Wu	Postdoctoral	Escolar	Pediatrics

Improvements in brain development following stem-cell transplantation in Krabbe disease

Background: Krabbe disease is a rare but severe neurodegenerative disorder mainly affecting infants. It is characterized by the lack of a myelin-related enzyme galactocerebrosidase and this causes abnormal myelination in the central and peripheral nervous systems. Children with this autosomal recessive disease are born normal but have disease onset in early or late infancy. The neurological symptoms progress quickly and often lead to death with 2 years if treatment is not administered early. Hematopoietic stem cell transplantation (HSCT) is the only treatment available that can halt disease progression. While the benefits of transplantation have been shown in behavioral exams we set out to directly investigate the change in cerebral myelination by MRI and propose a more sensitive and objective tool to access the effects of this transplant treatment and compare them with the natural history of the disease. Method: Diffusion tensor imaging (DTI) was obtained to assess white matter integrity of the brain. DTI measures the water diffusion property in the white matter and reflects the direction of axonal microstructure. We longitudinally scanned 55 Krabbe patients with early infantile onset of which 14 were treated with HSCT after their first MRI scan. Quality of DTI images was checked and corrupted ones were excluded in the analysis. Age-specific brain atlases (neonatal 1 to 2 year-old 3 to 6 year-old) were built based on normal controls. Alignment of patient image to the atlas was made such that specific white matter tracts could be delineated. Fractional anisotropy (FA) was derived from DTI as a measure of the organization of the corticospinal tracts which relay action potential from motor cortex to spinal cord. Lower than normal FA values indicate disorganization of myelination around the tracts. Results: Patients treated with HSCT mostly followed the normal developmental trajectory of the corticospinal tract albeit in the lower part of the normal range. Patients that were not treated with HSCT started with lower than normal FA and the measure decreased significantly within two years after an initial increase. The FA values are consistent with the motor function as measured in the behavioral test. Conclusion: Diffusion based brain MRI measure indicates the compromised white matter integrity of early infantile Krabbe patients who are not treated with hematopoietic transplantation. In contrast patients who are treated with this transplantation early in life preserved the quality in the corticospinal tract as compared to normal controls and they appear to follow a normal development over time.

Morning Poster Session
Location: Row C
Poster #36

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Daniel Charek	Postdoctoral	Collins	UPMC Sport Concussion Program

Additional Sport Exposure Following Concussion has Dose Response Effect on Recovery Time

Objective: Current guidelines suggest the immediate removal of athletes from contest after sustaining a sport-related concussion (SRC). However some athletes continue to play after sustaining a SRC due to lack of awareness of signs/symptoms sport culture and limited access to medical professionals. Concussed athletes who remain in play demonstrate worse outcomes including more severe acute cognitive impairment and longer recovery time. The goal of this study was to determine if there was a dose response of post-injury sport exposure (i.e. minutes remained in game/practice play after SRC) on athletes' severity of symptoms neurocognitive impairment and recovery time. Methods: Participants included 59 athletes aged 15.3+/-1.9 who reported remaining in play for 22.9+/-26.8 minutes (range=3-160) immediately following SRC. Participants were grouped by short duration (3-15 minutes [n=25]) or long duration (>15 minutes [n=34]) of continuous play following injury. A Mann-Whitney U test compared groups on recovery time and a series of t-tests compared groups on the Post-concussion Symptom Scale (PCSS) and Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT) 1-7 days following SRC. Results: Athletes in the long duration group took longer to recover (M=43.79 days SD=26.34) compared to those in the short duration group (M=29.08 days SD=12.90 p=.049) There were no significant differences on acute outcomes at 1-7 days post injury on ImPACT scores (p>.10) or PCSS score (p=.40). Conclusion: Additional exposure to sport immediately following SRC has a dose response effect on clinical outcomes. Further aerobic activity and exposure to additional contact are two potential mechanisms that may exacerbate injury.

Morning Poster Session
Location: Row C
Poster #37

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Darik O'Neil	Undergraduate	Bondi	Department of Physical Medicine & Rehabilitation

ENVIRONMENTAL ENRICHMENT ATTENUATES TRAUMATIC BRAIN INJURY-INDUCED INFLAMMATION AND OXIDATIVE STRESS

Environmental enrichment (EE) has been shown to facilitate motor re-cov-ery and hasten spatial learning and memory when pro-vided after traumatic brain injury (TBI). These effects are ob-served in both male and female rats as well as adult and pediatric populations. Typical explanations for the EE-mediated benefits are re-ductions in hippo-campal cell loss and in-creased neuro-genesis. The goal of this study was to assess other patho-logical mecha-nisms that are pre-valent after TBI. Anesthetized male rats received a controlled cortical impact or sham injury then were housed in EE or standard (STD) conditions and subsequently evalu-ated for motor (beam-walk/balance) and cognitive (Morris water maze) per-form-ance as well as in-flam-mation via micro-glial activation (Iba1) and oxi-dative stress (3-NT). EE impro-ved both motor and cognitive per-form-ance relative to STD (p < 0.05). More-over EE down-regu-lated TBI-induced Iba1 ex-pression levels in both hemispheres (p < 0.05) and also re-duced 3-NT immuno-staining in the ipsilateral hemisphere (p < 0.05). These data sug-gest that in ad-di-tion to neuro-genesis EE may mediate benefits after TBI by attenuating in-flam-mation and oxi-dative stress.

Morning Poster Session
Location: Row C
Poster #38

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Ian Marshall	Undergraduate	Bondi	Neuroscience Physical Medicine and Rehabilitation

Frontal lobe traumatic brain injury induces executive function impairments in male rats

Introduction: More than 10 million people worldwide sustain a traumatic brain injury (TBI) each year. The majority of survivors suffer long-lasting cognitive impairments associated with frontal lobe disturbances as well as psychological consequences such as being vulnerable to developing a psychiatric disorder. Previously we demonstrated that a controlled cortical impact (CCI) injury over the parietal cortex produced significant deficits in executive function in the attentional set-shifting test (AST) in rats a complex cognitive paradigm analogous to the Wisconsin Card Sorting Test which is used to measure strategy-switching deficits in patients with frontal lobe damage TBI and psychiatric disorders. Hypotheses: This study aims to investigate complex cognitive deficits after experimental TBI in rats subjected to frontal lobe injury a clinically relevant location by testing the hypothesis that a frontal TBI will impair executive function and cognitive flexibility in a cortical deformation depth-dependent manner. Methods: Thirty-one isoflurane-anesthetized adult male rats were subjected to CCI injury (2.0 2.2 and 2.4 mm cortical tissue deformation depth at a speed of 4 m/sec) or sham injury over the prefrontal cortex region in the right hemisphere. Rats were tested on the AST at four weeks post-surgery. The AST consists of two superimposing perceptual dimensions that the rat must use to retrieve food: scent (aromatic odor on the pots) and digging medium (different materials inside the pots). The test involves a series of increasingly difficult discriminative stages to obtain food reward including simple and compound discriminations stimulus reversals and intra- and extradimensional (ED) shifts. Dependent measures include number of trials to reach criterion of six correct consecutive responses number or total errors and number of set loss errors (i.e. after 50% or more of the contingency has been achieved). Results: Frontal CCI produced significant deficits in attentional performance on the ED stage and stimulus reversals of AST at four weeks post-injury seen as increased total trials to reach criterion and significantly higher total errors compared to SHAM rats (p<0.05 for Injury n=7-8/group). These effects were particularly robust in the two more severe injury groups namely 2.2 and 2.4 mm cortical deformation depth (p<0.05). Conclusions: These results suggest that frontal lobe injury negatively impacts complex cognitive functioning. Ongoing and future studies will focus on further disentangling brain constructs and neurotransmitter alterations responsible for such attentional deficits following brain trauma. Significance: Considering that a large percentage of TBIs occur via direct impact to the frontal part of the skull (e.g. hitting the windshield during a car accident) this approach is clinically-relevant and may prove extremely valuable for successful translation from bench to bedside identifying necessary pharmacotherapies for cognitive performance and advance rehabilitation research.

Research/Grant Support: Supported in part by UPP/UPMC Academic Foundation (Corina O. Bondi Ph.D.) and NIH grants NS060005 HD069620 and NS084967 (Anthony E. Kline Ph.D.).

Morning Poster Session
Location: Row C
Poster #39

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Paul Cohen	Postdoctoral	Kontos	Orthopaedic Surgery

Do Initial Symptom Factor Scores Predict Subsequent Impairment following Concussion?

Objective: High symptom scores at initial visit (Meehan et al. 2013) and at baseline (Custer et al. 2016) are predictive of impairment and protracted recovery following concussion. However the predictive value of symptom factors (e.g. Kontos et al. 2012) on subsequent impairment is unknown. The purpose of this study was to examine the ability of patients' symptom factor scores at their initial clinic visit to predict neurocognitive and vestibular/oculomotor impairment at their second clinic visit. Participants and Methods: Participants included 72 athletes aged 13-22 with a sport-related concussion. Participants completed the Post-Concussion Symptom Scale (PCSS) Immediate Post-concussion Assessment and Cognitive Testing (ImPACT) and Vestibular/Ocular Motor Screening (VOMS) at 1 week post-injury and again approximately 2-4 weeks post-injury. PCSS scores were aggregated into symptom factors per Kontos et al. (2012). Multiple regressions were conducted with symptom factors at <1 week as predictors of ImPACT and VOMS scores at 2-4 weeks and recovery time. Results: The cognitive fatigue/migraine symptom factor predicted impairment on visual memory (β=-.348 p=.033) and reaction time (β=.003 p=.029). The affective symptom factor predicted higher scores on the horizontal (β=.398 p=.019) and vertical saccades (β=.390 p=.031) and vertical (β=.571 p=.013) and horizontal vestibular ocular reflex (VOR) (β=.471 p=.030). Symptom factor scores did not significantly predict recovery time. Conclusions: Clinicians can utilize patient's initial symptom factor scores to better predict subsequent impairment and identify patients for earlier targeted treatment. Patients with anxiety/mood symptoms following concussion may experience increased vestibular impairment following concussion.

Morning Poster Session
Location: Row C
Poster #40

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Natalie Sandel	Postdoctoral	Kontos	Orthopaedic Surgery

Discrimination of Concussed from Healthy Controls Using a Multimodal Diagnostic Approach

Objective: Evaluate the efficacy of using a multimodal approach to discriminate between acutely concussed individuals and healthy controls. Participants and Methods: Participants included 23 concussed athletes (56.5% males) and 25 healthy age and sex matched controls (68% males) aged 12 to 20 years old (M=15.21 SD=2.03). Participants completed a multimodal evaluation that included the Post-Concussion Symptom Scale (PCSS) computerized neurocognitive testing (Immediate Post-concussion Assessment and Cognitive Testing [ImPACT]) the Balance Error Scoring System (BESS) and the Vestibular/Ocular Motor Screening (VOMS) tool. A discriminant function analysis was conducted to evaluate how well the multimodal approach classified concussed participants from healthy controls. Univariate analyses identified measures in the multimodal approach that best differentiated concussed from healthy participants. Results: The discriminant function yielded a significant model that differentiated concussed from healthy groups (χ^2= 24.382 df=6 p<.001). The model accurately predicted correct outcomes for 81.3% of cases (73.9%- concussed; 88.0%- healthy controls). Univariate ANOVAs revealed that the concussed and healthy control participants differed significantly on all predictor variables in the multimodal assessment: PCSS (F=30.45 p<.001) ImPACT Memory (F=4.72 p=.04) ImPACT Speed (F=6.78 p=.01) vestibular screening (F=23.62 p<.001) and near point convergence (F=7.30 p<.01) with the exception of balance testing (F=0.91 p=.35). Conclusions: Utilization of a multimodal approach to concussion management during the acute phase of the injury correctly discriminated concussed athletes from healthy controls in 81.3% of cases. A multimodal approach should include measures of symptoms cognitive and vestibular/ocular motor function. Balance testing did not discriminate concussed from control participants.

Morning Poster Session
Location: Row C
Poster #41

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Brandon Gillie	Postdoctoral	Kontos	UPMC Sports Medicine Concussion Program

Comparison of Adolescents with Vestibular and Anxiety Clinical Profiles following Concussion

Comparison of Adolescents with Vestibular and Anxiety Clinical Profiles following Concussion Brandon L. Gillie PhD Anthony P. Kontos PhD Erin Reynolds PsyD Alicia Sufrinko PhD Valerie L. Reeves PhD Cyndi L. Holland MPH & Michael W. Collins PhD Objective: Concussed patients present with clinical profiles including vestibular and anxiety (Collins et al. 2014; in press). However there is no research on outcomes in patients with different clinical profiles. The purpose of this study was to compare clinical outcomes among concussed adolescents with vestibular anxiety and neither-vestibular nor anxiety (NVA) clinical profiles. Participants and Methods: A vestibular group (n= 11; i.e. presence of vestibular dysfunction/symptoms) an anxiety group (n= 10; i.e. presence of anxiety symptoms with or without vestibular dysfunction/symptoms) and a NVA (neither anxiety nor vestibular) group (n=12) were derived from a sample of 33 adolescents aged 12-20 (M= 15.0 SD= 1.9) with a diagnosed concussion. Participants completed the Post-concussion Symptom Scale (PCSS) Immediate Post-concussion Assessment and Cognitive Testing (ImPACT) Vestibular/Ocular Motor Screening (VOMS) Balance Error Scoring System (BESS) 2-10 days post-concussion. Univariate ANOVAs with Bonferroni corrections were used to compare the groups. Results: The anxiety group took longer to recover ($F=2.8$ $p=0.05$ $\eta2=.21$; M= 63.9 SD= 68.6 days) than both the vestibular group (M= 36.6 SD= 11.9) and the NVA group (M= 19.9 SD= 12.3). The anxiety group reported higher symptoms ($F=3.2$ $p=0.05$ $\eta2=0.18$) and lower processing speed scores ($F=5.6$ $p=0.009$ $\eta2=0.21$) than the other groups. The vestibular and anxiety groups scored worse on vestibular ($F=4.3$ $p=0.02$ $\eta2=0.23$) and ocular motor ($F=43.5$ $p=0.04$ $\eta2=0.20$) outcomes than the NVA group. Conclusions: Clinicians should employ multimodal comprehensive assessments to identify patients with clinical profiles such as anxiety that may be linked to worse clinical outcomes and longer recovery times.

Morning Poster Session
Location: Row C
Poster #42

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Kileigh Nassau	Undergraduate	Kline	Physical Medicine and Rehabilitation

Aripiprazole benefits functional outcome after experimental brain trauma and does not attenuate the benefits of environmental enrichment

Introduction: The typical antipsychotic drug (APD) haloperidol (HAL) a D2 receptor antagonist has been shown to impede functional outcome after experimental traumatic brain injury (TBI). Furthermore the deleterious effects persist for up to 3 months after drug withdrawal. Moreover a recent study showed that HAL reduced the effectiveness of environmental enrichment (EE) a preclinical model of neurorehabilitation. Because agitation is common after TBI patients are provided APDs so that they can be safely managed. However many patients in rehabilitation will only experience agitation occasionally and thus will receive APDs intermittently. Hypotheses: Aripiprazole (ARIP) a partial D2 and 5-HT1A receptor agonist will not impair recovery or reduce the effectiveness of EE regardless of whether administered once every day (i.e. chronic agitation) or once every other day (occasional agitation). Methods: Anesthetized adult male rats received a cortical impact of moderate severity or sham injury and were then randomly assigned to EE or standard (STD) housing. Treatments with ARIP (0.1 mg/kg; i.p.) or vehicle (VEH; 1.0 mL/kg; i.p.) began 24 hr after injury and continued once daily for 19 days or once every other day for the same period. Motor (beam-balance/walk) and cognitive (spatial learning) outcome were assessed on post-operative days 1-5 and 14-19 respectively. Results: Motor and cognitive function was significantly improved in the TBI+EE+VEH vs. TBI+STD+VEH group (p<0.05). Moreover the TBI+EE+ARIP groups regardless of dosing regimen performed significantly better on all endpoints relative to the TBI+STD+VEH controls (p<0.05) but did not differ from one another or from TBI+EE+VEH (p>0.05). Conclusions: The data replicate previous work from our laboratory showing the EE improves functional outcome after TBI. Furthermore ARIP unlike HAL did not impair recovery or reduce the efficacy of EE which supports the hypothesis. Significance: ARIP is beneficial on its own and does not negate the benefits of rehabilitation (i.e. EE) and thus may be used to control TBI-induced agitation and aggression without compromising recovery.

Morning Poster Session
Location: Row D
Poster #43

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Lindsay Kutash	Undergraduate	Bondi	Physical Medicine and Rehabilitation

Effects of chronic unpredictable stress on cognitive and depressive-like behaviors following experimental brain trauma

Traumatic brain injury is highly prevalent affecting nearly 2 million Americans annually. Outcomes often involve frontal lobe dysfunction resulting in cognitive impairment and increased vulnerability to neuropsychiatric disorders. Similarly chronic unpredictable stress (CUS) has been found to elicit similar consequences. Currently we are assessing the clinically relevant cognitive behavior and anxiety-like dimensions of TBI in conjunction with CUS. After implementing a controlled cortical impact (CCI: 2.8 mm cortical depth at 4 m/s) or sham injury over the right parietal cortex rats were randomly assigned to receive 21 days of CUS. Upon cessation of stress rats were tested for perceived state of anxiety (open field test) and anhedonia (preference of 1% sucrose-water versus regular water overnight). At 4 weeks post-surgery rats were tested on the attentional set-shifting test a series of increasingly difficult discriminative tasks measuring various aspects of cognitive flexibility and were lastly sacrificed for serum analysis of corticosterone and inflammatory markers. Results demonstrate an expected decrease in cognitive and behavioral performance in the TBI-CTRL and CUS-SHAM groups. However TBI-CUS group showed paradoxically ameliorated behaviors and serum markers which may have many significant implications regarding the recovery process post-TBI especially in conjunction with environmental enrichment a rodent model of neurorehabilitation.

Morning Poster Session
Location: Row D
Poster #44

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Jamie Hanson	Faculty	Hanson	Psychology & LRDC

The experience of childhood trauma and recent stress interact to influence functional connectivity of the ventral striatum and medial prefrontal cortex associated with depression

The experience of childhood maltreatment is a significant risk factor for the development of depression. This risk is particularly heightened after exposure to additional more contemporaneous stress. While behavioral evidence exists for such “stress sensitization ” little is known about biological correlates of this putative process. Identifying such correlates may not only substantiate the “stress sensitization” model but also provide biomarkers of risk for later depression. Suggestive clues have emerged from targeted neurobiological investigations that experiences of early life stress such as childhood maltreatment may influence the structure and function of a corticostriatal circuit supporting motivation and action. Moreover dysfunction of this circuit has been implicated in the pathophysiology of depression. The limited available research though informative has not investigated whether differences in reward-related corticostriatal circuit function may be associated with “stress sensitization ” or if any circuit-level effects explain subsequent risk for depression. To begin to fill in these important gaps we turned to the Duke Neurogenetics Study (DNS) an ongoing project assessing a wide range of behavioral and biological traits in a large cohort of non-patient 18-22 year-old university students. Investigating reward-related functional connectivity within the corticostriatal circuit of 926 participants we found evidence for increased connectivity between the ventral striatum and the medial prefrontal cortex (Interaction β=0.199 p<.005) in individuals exposed to greater levels of childhood maltreatment who also experienced greater levels of recent life stress. We also found that this aberrant pattern of connectivity was associated with elevated symptoms of depression specifically reduced positive affect (β=0.089 p<.005). These findings suggest a novel neurobiological mechanism linking cumulative stress exposure with later depressive symptoms and provide support to the “stress sensitization” model of depression.

Morning Poster Session
Location: Row D
Poster #45

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Gabriela Alarcon	Postdoctoral	Forbes	Psychiatry

Adolescent sex differences in default mode and cognitive control network functional coupling during cognitive control: a potential risk factor for major depressive disorder

Rates of depression increase sharply during adolescence particularly for girls who are twice as likely to experience a depressive episode compared to boys. The neural mechanisms mediating this sex difference in risk for depression have not been fully elucidated. The current functional magnetic resonance imaging study used an affective self-referential processing (SRP) induction capitalizing on the social re-structuring of identity that occurs during adolescence to measure sex differences in functional connectivity during a cognitive control task that immediately followed an SRP (Post-SRP Flanker) or Control (Post-Control Flanker) task. This approach was developed to examine the interaction between default mode and cognitive control networks (DMN and CCN) which support SRP and cognitive control respectively since aberrant interaction of these networks is implicated in major depressive disorder (MDD). Task activation was modeled and regressed; the residual time courses corresponding to the Flanker trials were used for functional connectivity analysis. Two-way ANOVA indicated a significant interaction between sex and task condition such that girls had stronger coupling between DMN and CCN during Post-SRP Flanker. CCN and DMN are generally de-coupled during cognitive control; thus the affective SRP task may have interfered with cognitive control processing in girls as has been shown in MDD.

Morning Poster Session
Location: Row D
Poster #46

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Castro Sandra	Faculty	Castro	Neurology

Environmental isolation impairs measures of brain health

Many individuals experience an impoverished lifestyle often associated with cognitive emotional and motor decline and can lead to a reduced life span. Such individuals include elderly living with little social contact the homeless and those living in jails and prisons particularly those in solitary confinement. We are assessing the impact of housing under isolation conditions using a behavioral test battery and biochemical molecular biological and anatomical methods. F344/BN male rats 18 month old at the outset of our study were housed either individually in a standard shoebox cage (18 cm W x 38 cm D x 27 cm H; SE) or in groups of 6 in a relatively enriched environment (1 m W x 1m D x 0.6m H; EE) containing running wheels tunnels platforms and toys. Body weights remained relatively stable in the SE rats but increased by an average of 10% for the EE rats over a 4-month period. The SE animals showed relatively little behavioral activity which was consistent with the small space in which they were housed. In contrast the EE rats showed a good deal of exploration climbing playing and social interaction. After 4 months all rats were euthanized brain peripheral tissues and blood collected and the brain dissected into several regions. Assays are being performed and and comparison made between SE and EE groups. Although not all the differences were statistically significant a number of promising trends have already been observed. For example we found that the SE rats housed in isolated impoverished conditions had a 72% decrease in BDNF. These rats also had a 37% decrease in the ratio of dopamine (DA) metabolites to DA and a 30% decrease in the level of phosphorylated tyrosine hydroxylase in the striatum; both of which suggested a decrease in DA synthesis and release in that structure. There was a five-fold increase in mitochondrial DNA damage levels in hippocampus in the SE rats. In addition substantia nigra from rats in the SE showed a number of significant differences in mRNA expression including changes in Azin1 Tssc4 Ddit4 Nfkb1a Pdk4 and Sgk1 (downregulated) and Cxcl13 and slc47a1 (upregulated). Additional assays are ongoing. Thus far our results indicate that isolated housing produces significant changes consistent with decreased neuroplasticity. These results suggest that isolated impoverished living conditions can produce profound changes in the brain that may be at least partially responsible for the behavioral impairments observed in people experiencing such conditions.

Morning Poster Session
Location: Row D
Poster #47

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Anna Manelis	Faculty	Manelis	Psychiatry

Anticipatory brain activation and current depression and mania symptoms predict subsequent recognition of emotional faces in depressed individuals with bipolar disorder depressed individuals with major depressive disorder and healthy controls

Title: Anticipatory brain activation and current depression and mania symptoms predict subsequent recognition of emotional faces in depressed individuals with bipolar disorder depressed individuals with major depressive disorder and healthy controls Anna Manelis Ph.D.; Tina Liu BS; Holly Swartz MD; Mary L. Phillips MD MD (Cantab) Abstract Due to the higher prevalence of depressive over hypomanic symptoms reliably differentiating unipolar from bipolar depression remains challenging in clinical practice. Therefore understanding the range of functional psychopathology in these disorders is especially important. Neuroimaging studies using dimentional approach to examine depressed individuals with bipolar disorder (BDD) and depressed individuals with major depressive disorder (MDD) may help to understand neural mechanisms underpinning impairments in emotion and cognitive processing and to identify neurobiological diagnostic markers of these disorders. According to previous studies depressive episodes are characterized by altered anticipation of positive and negative events. In this study we have examined how current and life-time depression and mania symptoms together with anticipatory activation during preparation to processes emotional faces affect subsequent recognition of emotional faces in BDD MDD and healthy controls (HC). Participants were presented with fear happy and neutral faces and had to identify the gender of the person on the picture. Each face presentation was preceded with an anticipatory cue that indicated the emotional valence of the upcoming stimulus with a symbol. A surprise memory test was conducted outside the scanner to test subjects' memory for faces. We found that lower anticipatory activation preceding presentation of happy faces in the right middle frontal gyrus right intraparietal gyrus (RIPS) and left middle temporal gyrus was related to better subsequent recognition of happy faces. A follow-up step-wise regression analyses with anticipatory brain activation and current and life-time depression and mania symptoms showed that anticipatory activation in the right intraparietal sulcus (IPS) and current depression and mania severity explained 34% of variance in recognition of happy faces. The same analysis conducted across BDD and MDD confirmed that greater anticipatory RIPS activation and greater current mania severity reduced recognition of happy faces. The IPS is involved in preparatory control. Excessive preparatory control before processing of happy faces and the presence of mania symptoms (e.g. irritability impulsivity) could inhibit formation of memory representation for happy faces. These findings suggest that previously reported memory impairments in BDD and MDD may be related to aberrant anticipatory brain functioning and mania symptoms and highlight the importance of studying anticipatory processes to better understand emotion and cognitive impairments in mood disorders. This research was supported by the NIMH K01 grant to AM (NIMH K01MH104348)

Morning Poster Session
Location: Row D
Poster #48

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Puja Parekh	Graduate	McClung	Neurobiology

Reduced excitatory synaptic plasticity of nucleus accumbens medium spiny neurons in a genetic mouse model of mania

It is well established that the circadian molecular clock regulates monoaminergic systems controlling mood anxiety and reward behavior. While disruptions in the circadian gene Clock are associated with increased risk for bipolar disorder (BD) the underlying molecular and synaptic mechanisms remain poorly understood. Using ex vivo whole cell patch clamp electrophysiology in ClockΔ19 mutant and wildtype (WT) mice we characterized alterations in excitatory synaptic transmission strength and intrinsic excitability of NAc neurons. We performed protein crosslinking and Western blot analysis to examine surface and intracellular levels and rhythm of the glutamate receptor subunit GLUA1 in the NAc. Viral-mediated overexpression of GluA1 in the NAc and behavioral analysis were used to determine whether the manic-like phenotype could be rescued. ClockΔ19 mice display reduced AMPAR-mediated excitatory synaptic responses (mEPSCs and EPSCs) at NAc medium spiny neurons (MSNs) across the light/dark cycle compared with WT littermates. We find that these alterations are likely postsynaptic as presynaptic release of glutamate onto MSNs is not disrupted in mutant mice. Additionally NAc surface protein levels and the rhythm of GLUA1 are decreased in Clock mice diurnally consistent with reduced functional synaptic response. Furthermore we observed a significantly hyperpolarized resting membrane potential of ClockΔ19 MSNs suggesting lowered excitability. Lastly overexpression of functional GluA1 in the NAc of mutant mice is able to normalize aspects of their manic-like phenotype. Together our novel findings demonstrate that NAc excitatory signaling is integral to the effects of Clock gene disruption on mania-related behaviors.

Morning Poster Session
Location: Row D
Poster #49

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Heather Acuff	Graduate	Phillips	Psychiatry

The Elucidation of Structural-Functional Relationships in Neural Circuitry Implicated in Youth at Risk for Bipolar Disorder

Background: Recent studies using diffusion tensor imaging (DTI) or functional magnetic resonance imaging (fMRI) have implicated white matter tracts and cortical regions in the pathophysiology of bipolar disorder (BD) and in healthy offspring of parents with BD. However the relationships between these structural and functional abnormalities have yet to be elucidated in offspring of parents with BD who are at high risk for developing BD themselves. In the present study we combined DTI and fMRI to identify biomarkers in neural circuitry that reflect the pathophysiology of youth at risk for BD. Methods: 30 healthy offspring of parents with BD (OBP) 30 healthy offspring of control parents with a non-BD diagnosis (OCP) and 30 healthy offspring of healthy parents (OHP) between the ages of 8 and 17 (matched for age gender and socioeconomic status) were scanned and performed a dynamic faces task. We used ANCOVAs multiple regression analyses and Least Absolute Shrinkage and Selection operator (LASSO) regressions to examine relationships among DTI and fMRI measures. Results: Compared to OCP OBP had decreased left amygdala activity in response to positive emotions but increased right ventrolateral prefrontal cortex (VLPFC) and dorsal anterior cingulate cortex (dACC) activity in response to negative emotions. In all groups right inferior longitudinal fasciculus (ILF) length predicted increased activity in the amygdala VLPFC and dACC in response to positive emotions while forceps minor volume predicted increased activity in the dACC in response to negative emotions. Group interactions also existed for the left ILF volume such that increased volume predicted increased activity in all regions for OBP but decreased activity for OCP. Conclusions: These findings suggest a pattern of emotion dysregulation caused by both abnormal neural connections and specific regional abnormalities. These structural and functional findings may serve as biomarkers for earlier diagnosis and treatment in youth at risk for BD.

Morning Poster Session
Location: Row D
Poster #50

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Adriane Soehner	Postdoctoral	Phillips	Psychiatry

Sleep Duration Variability Predicts Altered Dorsal Anterior Cingulate Activity During A Stressful Cognitive Control Task In Adolescents With Bipolar Disorder

Introduction: Altered function within fronto-limbic circuitry is observed during cognitive task performance in adults and adolescents with bipolar disorder (BD). However modifiable factors that may be impairing brain function in BD remain under-characterized. Sleep patterns are highly variable in BD but may be ameliorated with behavioral interventions. While links between disturbed sleep and altered brain function during cognitive tasks are well-established in healthy samples such relationships remain under-characterized in BD. Thus our aim was to test sleep duration variability as a predictor of neural response to a cognitive control fMRI task in adolescents with BD. Methods: Two groups of adolescents (13-22 years old) participated: 15 with BD type I II or NOS (BD; age=18.1±2.7 years; 11 female) and 25 healthy controls (CTL; age=19.4±2.7 years; 17 female). Sleep was monitored with actigraphy for 7-14 days prior to completing an adaptive version of the multi-source interference fMRI paradigm. Group status and sleep duration variability (intra-individual standard deviation) were examined as predictors of BOLD activity to a contrast of incongruent>congruent trials within a fronto-limbic region of interest. Results: A group-by-sleep duration variability interaction was observed for bilateral dorsal anterior cingulate cortex (dACC) activity to incongruent>congruent trials (p<.05 corrected): sleep duration variability and dACC activity were negatively associated in BD (r=-0.65 p<0.01) but not related in CTL (r=0.33 p=0.12). These patterns remained significant after controlling for age sex depressive symptoms and average sleep duration. Conclusion: In adolescents with BD sleep duration variability may modulate dACC engagement during cognitive control. Stabilizing sleep patterns may improve cognitive control neural circuitry function in BD which could in turn favorably improve emotional dysregulation.

Support: T32MH018269 (Soehner) The Pittsburgh Foundation (Franzen; Goldstein) UL1 RR024153 UL1TR000005

Morning Poster Session
Location: Row D
Poster #51

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Kimberly Lin	Graduate	Price	Department of Psychiatry

Changes in Visual Attention and Cognitive Function Following Attention Bias Modification for Treatment of Anxiety

Changes in Visual Attention and Cognitive Function Following Attention Bias Modification for Treatment of Anxiety Kimberly S. Lin and Rebecca B. Price University of Pittsburgh School of Medicine Background: Increased attention to threat-related stimuli in our environment serves an adaptive function in detecting and responding to danger. However in clinical anxiety this threshold for attention to threat is significantly lowered (1 3). The neural circuitry facilitating this heightened attention towards threat involves a balancing act between the amygdala and the ventral pre-frontal cortex (vPFC): early engagement of the amygdala mediates a bottom-up subcortical pathway that initially orients attention toward threat followed by the vPFC mediating a top-down cortical pathway to regulate amygdalar activity. Studies have shown anxious individuals exhibit a hypersensitive amygdala when compared to healthy individuals as well as weaker negative correlations with the vPFC in amygdala regulation. While cognitive-behavioral therapy the gold standard treatment for anxiety targets the top-down cortical component it is thought that targeting the sub-cortical component may be more effective not only because it is earlier in the cascade but because attentional perturbation may be more easily shaped here than in cortical areas (2 3). Attention Bias Modification (ABM) is a translational neurocognitive treatment that targets this implicit pathway using repetitive computer-based training methods in which anxious individuals practice an automated task that conditions their attention away from threat stimuli. Because attention is involved in cognitive processes it is relevant to consider how cognitive changes play a role in ABM outcomes (4). We examined whether changes in a) visual attention to threat and b) general cognition tracked with reduction in clinical symptoms of anxiety and depression following ABM. Methods: 62 adults with elevated clinically impairing anxiety were randomized to receive 8 sessions of ABM treatment (n=42) via a word-based 'dot-probe' task or a sham control version (n=20) over a period of one month. Self-reported questionnaires including the Mood and Anxiety Symptoms Questionnaire (MASQ) Beck Depression and Anxiety Inventories (BDI BAI) and Response Style Questionnaire (RSQ) were administered pre- and post-treatment to mark changes in clinical symptoms of anxiety and depression. Change in attentional patterns were assessed via eye-tracking during the dot-probe task. The Stroop color-word task was used to measure executive cognitive function pre- and post-treatment. Results: All symptom measures decreased from pre- to post-ABM. Following treatment eye-tracking data showed slower disengagement from threat words (relative to neutral words) in the ABM group (p=0.026 d=0.436) while there was no significant change in disengagement in the control group (p=0.539 d=0.169). In the ABM group changes in disengagement were positively correlated with reduction in self-reported clinical depressive and anxiety symptoms (p=0.001 – 0.027). The ABM group also showed significant improvement in measures of cognitive performance (p<0.001 d=0.766) while the effect was smaller and non-significant in the control group (p=0.087 d=0.404). In the ABM group Stroop improvement was negatively correlated with reduction in self-reported depressive and ruminative symptoms (p=0.014 – 0.031). Conclusions: As is consistent with existing literature reductions in clinical depression and anxiety symptoms were

observed following ABM a translational neurocognitive intervention (4). Interestingly the mechanisms of symptom decrease included slower disengagement from threat stimuli and altered executive function. One potential explanation is that ABM treatment allowed anxious individuals to overcome chronic and excessive avoidance of threat and engage a normative amygdalar-vPFC threat-detection system that has evolutionary relevance. The improvement observed in cognition following ABM suggests that ABM may have widespread effects on the frontoparietal neural circuitry underlying general executive and cognitive abilities. The paradoxical finding that lesser cognitive improvement tracked with greater reduction in depressive and ruminative symptoms may be consistent with previous findings linking perseverative thinking styles (e.g. rumination worry) to a performance benefit on certain cognitive tasks (i.e. those requiring inflexible goal maintenance such as the Stroop task). The exact neurocognitive mechanisms of ABM and its utility in treatment of psychiatric disorders remains a promising area to be explored. References: (1)\tBar-Haim Y Lamy D Pergamin L Bakermans-Kranenburg MJ and Van Ijzendoorn MH. Threat-Related Attentional Bias in Anxious and Nonanxious Individuals: A Meta-Analytic Study. Psychol Bull. 2007;133(1):1-24. (2)\tHakamata Y Lissek S Bar-Haim Y Britton JC Fox N Leibenluft E et al. Attention Bias Modification Treatment: A meta-analysis towards the establishment of novel treatment for anxiety. 2010;68(11):982-990. (3)\tPine DS Helfinstein SM Bar-Haim Y Nelson E Fox NA. Challenges in Developing Novel Treatments for Childhood Disorders: Lessons from Research on Anxiety. Neuropsychopharmacol. 2009;34:213-228. (4)\tRozenman M Weersing VR Amir N. A Case Series of Attention Modification in Clinically Anxious Youths. Behav Res Ther. 2011;49(5):324-330.

Morning Poster Session
Location: Row D
Poster #52

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Brandon Bizup	Graduate	Ahmari	Psychiatry

Stress-induced Relapse in a Mouse Model of Obsessive Compulsive Disorder

Stress plays a role in many psychiatric disorders including those that are not necessarily classified as anxiety disorders. Stressful life events can be catalytic in the emergence of psychiatric illness and anxiety can play a role in relapse in individuals that are recovering from psychiatric episodes. Using a model of compulsive-like behavior induced by cortico-striatal hyperstimulation based on human imaging data in obsessive compulsive disorder patients we investigated whether stress was capable of causing mice to revert to a symptomatic state following a period of recovery. EMX-Cre mice (n=10 per group) were stereotactically inected in the medial orbitofrontal cortex with a channel rhodopsin virus (AAV5-DIO-hSyn-ChR2-eYFP ChR2) or an inert control virus (AAV5-DIO-hSyn-eYFP) and the mice were implanted with an optical fiber just dorsal to the nucleus accumbens core. Following recovery mice were stimulated with a 473nm wavelength laser for five minutes a day (10hz 10ms pulse width) for six days and grooming was assessed before during and immediately post stimulation. Following 6 days of stimulation ChR2-expressing mice were found to be grooming significantly more than control mice. Grooming levels of ChR2-expressing mice remained elevated from their own baseline until 4 weeks after cessation of stimulation. On the day of restraint stress ChR2 groomed significantly more than baseline though not significantly more than controls. Twenty-four hours following restraint stress ChR2-expressing mice were found to be grooming significantly more than controls at similar levels to the peak effect seen during the laser stimulation. By forty-eight hours both ChR2-expressing and control animals had returned to baseline grooming. Mice were then exposed to a second restraint stress. Strikingly following the second stressor ChR2-expressing mice did not return to baseline grooming levels forty-eight hours after restraint and maintained elevated grooming grooming until 5 weeks after the second stress event. This study demonstrates that stress in the cortico-straital hyperstimulation model of compulsive behavior can cause the overgrooming phenotype to return after recovery. Furthermore additional stress events are sufficient to reinduce a long lasting overgrooming phenotype.

Morning Poster Session
Location: Row D
Poster #53

Presenting Author:	Author Type:	Mentor/Lab:	Department:
James Hyde	Postdoctoral	Ahmari	Psychiatry

In vivo calcium imaging of pharmacologically induced perseverative grooming in awake behaving mice

Title: In vivo calcium imaging of pharmacologically induced perseverative grooming in awake behaving mice Author(s): Dr. James Hyde Dr. Susanne Ahmari Obsessive compulsive disorder (OCD) is characterized by intrusive obsessive thoughts and abnormal repetitive behaviors. Studies of several independent mouse models of OCD-like behavior suggest that perseverative grooming in mice is related to the compulsive behaviors seen in OCD. Understanding the mechanisms leading to the development of abnormal grooming is therefore relevant to OCD pathophysiology. However the changes in cellular activity that are correlated with the development of perseverative grooming are unknown. Using miniaturized head-mounted microscopes and calcium imaging we therefore examined changes in cellular activity in the ventromedial striatum (VMS) during pharmacologically- induced perseverative grooming behavior. Drd1a-tdTomato mice were injected with the genetically encoded calcium indicator AAV9.hsyn.GCaMP6m and implanted with a microendoscope (6.1mm x 0.5mm GRIN lens) in VMS. 4 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 continuously for 10 minutes prior to injection and 30 minutes post injection. Calcium transient data was extracted from processed videos to analyze event frequency and time-locked activity. As expected grooming activity increased after SKF injection in VMS implanted mice. In vivo microendoscopy demonstrated that under SKF exposure the average calcium event rates decreased during grooming while event rates increased when the mouse was not grooming. Event rates during saline control experiments showed no differences between grooming and non-grooming time periods. These results suggest selective changes in firing patterns relating to perseverative grooming. Ongoing analysis is delineating the precise relationship between changes in network level activity and bouts of perseverative grooming.

Morning Poster Session
Location: Row D
Poster #54

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Jesse Wood	Postdoctoral	Ahmari	Psychiatry

Hyper-synchrony in medial orbitofrontal-ventromedial striatal circuits accompanies development of compulsive-like grooming in mice

The World Health Organization has identified OCD as a top ten cause of illness-related disability underscoring the heavy burden this disorder places on patients and the steep cost to society at large. Increased medial orbitofrontal cortex (mOFC) and ventromedial striatal (VMS) activity is thought to drive OCD symptoms though it is unclear how this circuit gives rise to compulsivity. It is therefore necessary to uncover pathological patterns of mOFC-VMS communication associated with compulsive behaviors to understand this relationship and potentially develop novel therapeutic approaches. To elucidate the relationship between compulsive-like behaviors and mOFC-VMS hyperactivity we performed repeated optogenetic stimulation of mOFC-VMS projections of mice over 10 days using a paradigm that causes progressive development of a persistent compulsive-like grooming phenotype. To understand how the networks underlying this phenotype change over time we simultaneously recorded the electrophysiological activity of mOFC and VMS neurons as we stimulated mOFC-VMS circuits for 5 minutes/day using ChR2 (473nm 10Hz 10msec pulse width). Our data suggest that during optogenetic stimulation of mOFC terminals in the VMS many mOFC neurons are activated nearly synchronously as measured by the presence of complex waveform population spikes. Prior to the first laser stimulation there was no mOFC synchrony in ChR2 animals (0/66 pairs of simultaneously recorded mOFC neurons). At the conclusion of the first day of optogenetic stimulation 3% of mOFC neuron pairs were significantly synchronous as measured by cross-correlation analysis. Synchrony continued to emerge in mOFC networks in association with repeated optogenetic stimulation. Prior to the final day of optogenetic stimulation (pre-stimulation period in session ten) 6.6% of mOFC pairs fired in synchronous fashion. Optogenetic stimulation on that day induced even greater levels of synchrony such that 14.3% of mOFC pairs fired synchronously. Significant levels of synchrony were never detected in control mice. Collectively these data suggest that development of the compulsive-like phenotype is associated with increased synchronous activity in mOFC networks. Increased synchrony in mOFC-VMS circuits could contribute to compulsive-like behavior by disrupting processing in striatal networks that subserve behavioral selection.

Morning Poster Session
Location: Row E
Poster #55

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Sean Piantadosi	Graduate	Sibille	Psychiatry

Identifying cellular mechanisms underlying the anti-compulsive properties of fluoxetine

BACKGROUND: Serotonin reuptake inhibitors (SRIs) are the first-line and most efficacious pharmacotherapeutic treatment for obsessive compulsive disorder (OCD). However complete remission following SRI treatment is rare (< 20%) and only 40-60% of patients report improvement in symptoms following monotherapy. It is therefore important to determine the neural changes that underlie responsiveness vs resistance to treatment. Aberrant striatal activity may underlie OCD symptoms evidenced by functional imaging studies in OCD patients that demonstrate hyperactivity within the striatum. Notably successful treatment of OCD symptoms with SRIs reduces hyperactivity in the striatum of treatment-responsive patients suggesting a potential mechanism for treatment response. In addition a recent meta-analysis suggests that the therapeutic effects of SRIs in treatment-responsive OCD patients may occur much sooner than previously believed suggesting that short term changes in neural activity may be important. METHODS: Sapap3 knockout (KO) mice which have both a hyperactive striatum and compulsive OCD-like grooming phenotype were injected with AAV-GCaMP6m and implanted with a GRIN lens in the centromedial striatum (CMS) to visualize striatal calcium activity during spontaneous grooming behavior. All mice received 7 days of treatment with the SRI fluoxetine and underwent imaging and grooming assessments on days 3 5 and 7 of treatment. RESULTS: Sapap3-KO mice displayed elevated grooming behavior at baseline and treatment with fluoxetine decreased grooming. Interestingly in contrast to published studies this reduction in compulsive grooming occurred more rapidly than expected after just 3 days of treatment. At baseline Sapap3-KO mice also had elevated striatal activity as measured by calcium events relative to WT animals. This increase in calcium activity during grooming behavior was reduced by successful fluoxetine treatment. Preliminary studies selectively examining D1-medium spiny neurons (MSN) in Sapap3-KO mice also suggest increased baseline activity which may be decreased following treatment. Ex vivo data suggest that fluoxetine may be modulating the activity of striatal fast spiking interneurons (FSIs) in order to normalize striatal activity. CONCLUSION: Hyperactivity of the striatum and compulsive grooming behavior can be reversed with successful SRI treatment in a valid mouse model of OCD-like behaviors. SIGNIFICANCE: Understanding cell-type specific effects of successful and unsuccessful SRI treatment may help us develop treatments for patients that have better efficacy and fewer side effects.

Morning Poster Session
Location: Row E
Poster #56

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Victoria Corbit	Graduate	Gittis	Neurobiology

Circuit-specific corticostriatal dysfunction in a mouse model of Obsessive-Compulsive Disorder

Obsessive-Compulsive Disorder (OCD) is a psychiatric disorder associated with an inability to suppress intrusive thoughts and/or compulsive behaviors. Supplementary motor cortex (SMA/pre-SMA) and lateral orbitofrontal cortex (lOFC) have been implicated in OCD because they are hyperactive in OCD patients and they are involved in response inhibition and behavioral flexibility which have been found to be abnormal in OCD. In addition studies have demonstrated efficacy of SMA/pre-SMA transcranial magnetic stimulation (rTMS) treatment for OCD. More specifically dysfunction in the projections from these cortical areas to the striatum has been implicated in OCD. We therefore used optogenetics and acute slice physiology to investigate corticostriatal synaptic dysfunction in in an OCD-relevant mouse model the Sapap3-KO mouse. Our studies focused on the centromedial region of striatum (CMS) because 1) CMS is the projection target of the mouse homologues of these cortical regions and 2) a prior study in Sapap3-KOs has shown deficient inhibition of striatal output neurons (MSNs) by fast-spiking interneurons (FSIs) in the CMS. Intrastriatal stimulation showed that KO mice exhibit selectively increased overall excitatory drive onto CMS FSIs but not MSNs. To determine which specific cortical inputs were contributing to the increased drive of interneurons we injected channelrhodopsin2 (ChR2) and recorded 470nm light-evoked excitatory post-synaptic currents (EPSCs). We first examined inputs from lOFC (the primary input to CMS) and observed that EPSCs in FSIs were no different in KOs compared to WTs. This demonstrates that lOFC inputs are not the source of overall increased excitatory drive to FSIs in Sapap3-KOs. Furthermore lOFC EPSCs onto MSNs were weaker in KO mice in contrast to the unchanged overall excitatory drive to MSNs. These results suggested that another input played a role in CMS microcircuit changes we observe in the Sapap3-KO mice. We therefore virally injected ChR2 into M2 to investigate corticostriatal synapses from this region. Light-evoked EPSCs from M2 were increased onto both MSNs and FSIs in KO mice relative to WTs. Combined with the results from LOFC inputs the increase in M2 inputs onto both cell types can explain the selective differences in overall excitatory drive. Furthermore these data suggest that M2 corticostriatal circuits may be overactive in this OCD mouse model which supports literature demonstrating hyperactive corticostriatal circuits and hyperactive SMA/pre-SMA in OCD patients. To mimic the inhibitory effects of rTMS treatment in the SMA/pre-SMA our future experiments will inhibit the hyperactive M2 corticostriatal projections in the Sapap3-KO and determine the impact OCD-relevant repetitive grooming behavior. These results in combination with our synaptic physiology data will bring new focus to the role of supplementary motor cortical regions in the pathology of OCD and will contribute valuable information towards optimizing rTMS treatments for OCD.

Morning Poster Session
Location: Row E
Poster #57

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Melanie Grubisha	Postdoctoral	Sweet	Psychiatry

A Schizophrenia-Associated Missense Mutation in KAL9 Influences Dendritic Morphology Pathways in a Mouse Model

Background: Kalirin (Kal) is a Rho GEF that is highly involved in regulation of the cytoskeleton within dendrites a pathway that is the site of convergence of multiple genetic risk factors in schizophrenia. There are several isoforms of Kal that arise from differential splicing of its 66 exons. A missense mutation located within a region shared by the two longer Kal9 and Kal12 isoforms (P2255T PTKAL9/12) has been associated with schizophrenia. When this mutation is overexpressed on the Kal9 background in vitro it results in increased activation of RhoA and reduced branching of basilar dendrites. We sought to determine the biological effects of this mutation when expressed at the endogenous locus in a mouse model. Methods: Using CRISPR/Cas9 genome editing we introduced the PTKAL9/12 mutation at the endogenous gene locus in C57/BL6 mice. Absence of off-target effects as well as confirmatory sequence analysis was done with both PCR and Sanger sequencing. Cortical tissue homogenates were prepared from 4 wildtype and 4 homozygous PTKAL9/12 mutant mice. They were subject to either RNA extraction /qPCR protein isolation/ western blotting or trypsin digestion. The resulting peptides from digestion were enriched for phospho-peptides and analyzed by differential mass spectrometry. Peptide intensities were first calculated in Chorus and MaxQuant select phospho-peptides were then manually evaluated in Skyline. Ingenuity Pathway Analysis (IPA) was used to perform functional enrichment of cellular processes most affected by changes in peptide phosphorylation in PTKAL9/12 mice. Results: A homozygous male mouse free of any off-target effects was successfully generated via CRISPR/Cas9. Selective breeding produced multiple generations of mice with the PTKAL9/12 mutation. Transcript and protein levels of Kal isoforms in PTKAL9/12 cortical homogenate did not differ from WT. Mass spectrometry analysis of cortical homogenate phospho-peptide enrichments observed >4000 phospho-peptides. Of these 589 were confidently quantified in all 8 mice. Phospho-peptides with evidence of altered levels in PTKAL9/12 mice were enriched for genes affecting neuronal morphology and microtubule assembly. MECP2 a gene known to be involved in intellectual disability and correlated with dendritic tree and spine deficits was among the most significant phospho-peptides altered in PTKAL9/12 homogenates. Conclusions: We have successfully created a mouse model of a rare schizophrenia-associated point mutation within KAL9/12. Confirming our in vitro observations of functional effects of this mutation we have identified perturbed phosphorylation of proteins involved in neuronal morphology and microtubule dynamics pathways known to be affected by kalirin. Additional analyses of pyramidal neuron morphology and electrophysiology are ongoing and will be presented. This model will facilitate developmental studies of the downstream signaling alterations due to PTKAL9/12. A better understanding of these changes in signaling will provide insight into upstream causes of the impaired dendritic morphology that has been observed in schizophrenia and ultimately how this alters neuronal function.

Morning Poster Session
Location: Row E
Poster #58

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Gil Hoftman	Postdoctoral	Lewis	Psychiatry

Altered Gradients of Glutamate and GABA Transcripts in a Distributed Cortical Circuit in Schizophrenia

Background: Visuospatial working memory (vsWM) which is impaired in schizophrenia requires information transfer from primary (V1) and association (V2) visual cortices in the occipital lobe to posterior parietal (PPC) and dorsolateral prefrontal (DLPFC) cortices via excitatory pyramidal neurons in layer 3. In primate cortex the layer 3 excitatory glutamate-containing neurons in V1 have a lower density of dendritic spines less complex dendritic arborization and smaller soma sizes compared with the homologous layer 3 pyramidal neurons in the DLPFC. The activity of layer 3 excitatory neurons is shaped by local inhibitory neurons. The relative number of these GABA-containing neurons and the expression of GABAA receptor α1 subunit mRNA are higher in V1 than DLPFC. These findings suggest that caudal and rostral cortical regions may differ in the relative amounts of glutamate and GABA inputs. Interestingly recent transcriptome studies reported the presence of rostro-caudal gradients of mRNA expression in the primate neocortex. Whether these gradients are present in cortical layer 3 for glutamate and GABA system transcripts in postmortem human tissue and if they are preserved in schizophrenia have not been studied. Therefore we sought to answer the following questions: 1) Are gradients in expression present across regions of the vsWM network? 2) Are these gradients altered in schizophrenia? 3) Is any disease effect conserved across regions? Methods: Using laser microdissection tissue samples of layer 3 were obtained from V1 V2 PPC and DLPFC from 20 matched pairs of schizophrenia and unaffected comparison subjects. Quantitative PCR was used to measure mRNA levels of functionally analogous transcripts of the following glutamate and GABA system: glutamate (GLS1) and GABA (GAD67) synthesizing enzymes; vesicular glutamate (vGLUT1) and GABA (vGAT) transporters; synaptic glutamate (EAAT2) and GABA (GAT1) transporters; NMDA receptor subunit (GRIN1); AMPA receptor subunit (GRIA2); and GABAA receptor subunit (GABRG2) in all samples. Results: To assess the presence of mRNA expression gradients we measured the levels of these glutamate and GABA transcripts in unaffected comparison subjects. Transcript levels for the glutamate markers EAAT2 (F3 56=19.2 p<0.001) vGLUT1 (F3 56=57.1 p<0.001) and GRIA2 (F3 56=59.3 p<0.001) showed a caudal-to-rostral gradient with lowest expression in visual cortices and highest in DLPFC. In contrast the GABA transcripts GAD67 (F3 56=4.5 p<0.007) vGAT (F3 56=11.8 p<0.001) and GABRG2 (F3 56=34.1 p<0.001) showed the opposite gradient with highest expression in visual cortices and lowest in DLPFC. To determine if these regional gradients were altered in schizophrenia we generated normalized composite measures for the five glutamate and four GABA transcripts studied. These measures confirmed the presence of opposite regional gradients for glutamate (F3 114=14.5 p<0.001) and GABA (F3 114=5.4 p=0.002) system transcripts in unaffected comparison subjects. In contrast in the subjects with schizophrenia the regional gradient for glutamate transcripts was diminished (F3 114=1.3 p=0.28) whereas the regional gradient for GABA transcripts was enhanced (F3 114=15.8 p<0.001). That is in the schizophrenia subjects both the glutamate and GABA system transcript levels were higher in V1 and lower in the DLPFC relative to the unaffected comparison subjects. Since the regional gradients of glutamate and GABA system transcripts were differentially altered in schizophrenia we examined whether there was a disease effect on transcript levels that was conserved across regions. Levels of vGLUT1 mRNA were significantly lower in schizophrenia in all

regions studied (V1: -18% p<0.01; V2: -14% p<0.05; PPC: -14% p<0.05; DLPFC: -22% p<0.01). Levels of EAAT2 mRNA were significantly higher in visual cortices (V1: +286% p<0.001; V2: +258% p<0.001) but not in DLPFC or PPC. All other glutamate and GABA system transcripts studied did not show an effect of illness that was conserved across regions. We also examined whether the difference between glutamate and GABA composite measures within a region was significantly different in schizophrenia subjects and found no significant effect of diagnosis on transcript expression (F1 114=0.3 p=0.56). Conclusions: In layer 3 glutamate and GABA system transcripts exhibit opposite expression gradients across a vsWM cortical network suggesting that molecular regulation of excitatory-inhibitory balance differs across cortical regions. Altered expression of some of these transcripts in schizophrenia may disrupt normal regional patterns of excitatory-inhibitory balance (i.e. markers of excitatory-inhibitory balance are downregulated in the DLPFC and upregulated in V1) contributing to the neural substrate for vsWM deficits in the illness.

Morning Poster Session
Location: Row E
Poster #59

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Susan Sonnenschein	Graduate	Grace	Neuroscience

Impact of withdrawal from prior D2 antagonist vs aripiprazole treatment on dopamine system activity in MAM model of schizophrenia

Novel target compounds for the treatment of schizophrenia have shown promise in preclinical research but failed to show efficacy in clinical trials. However preclinical research is typically performed on drug-naïve rats whereas clinical trials are performed on patients that have received only brief withdrawal from years of prior antipsychotic drug (APD) treatment despite potential pervasive changes to the DA system. We previously found that withdrawal from repeated haloperidol (HAL) treatment produces persistent changes interfering with the ability of a novel target compound to reverse the hyperresponsive state of the DA system in the methylazoxymethanol acetate (MAM) model of schizophrenia. In the current study we examined the effects of withdrawal from mechanistically distinct APDs with a focus on the D2 partial agonist aripiprazole (ARI). Saline (SAL) and MAM-treated offspring received repeated HAL (0.6 mg/kg) clozapine (CLO; 10 mg/kg or 20 mg/kg) ARI (10 mg/kg) or vehicle (0.23% glacial acetic acid) for 21 d p.o. followed by 7d withdrawal. The number of spontaneously active DA neurons in the VTA was measured using in vivo extracellular recordings from anesthetized rats. After electrophysiological sampling a subset of rats received a low dose of apomorphine (40 ug/kg i.v.) to test for removal of depolarization block followed by resampling the VTA in the opposite hemisphere. Recordings were also conducted in SAL and MAM rats 3 h following acute treatment with ARI (10 mg/kg p.o). Finally additional MAM and SAL rats withdrawn from repeated treatments were administered DA agonist quinpirole (8mg/kg i.p.) prior to measuring locomotion in an open field to test for DA supersensitivity. In contrast to D2 antagonists withdrawal from ARI treatment did not reduce the number of spontaneously active DA neurons in normal rats. Unlike the effect of ARI treatment in normal rats MAM rats withdrawn from repeated ARI demonstrated reduced DA neuron activity following both repeated and acute treatment which was maintained following administration of apomorphine suggesting that it is unlikely the result of depolarization block. Lack of evidence for depolarization block ARI-treated rats suggests that brief withdrawal from ARI treatment may not interfere with the antipsychotic efficacy of novel target compounds which remains to be tested.

Morning Poster Session
Location: Row E
Poster #60

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Felipe Gomes	Postdoctoral	Grace	Neuroscience

Prefrontal cortex dysfunction increases susceptibility to schizophrenia-like changes induced by adolescent stress exposure

Adolescence is a developmental period of complex neurobiological changes and heightened vulnerability to psychiatric disorders. In particular evidence suggests that stress during adolescence is an important risk factor in the etiology of schizophrenia a developmental disorder that typically manifests in late adolescence or early adulthood. Indeed the emergence of psychosis is often associated with stressful life events and adolescents that are at high risk for schizophrenia experience abnormally high reactivity to stress. A dysfunction of the medial prefrontal cortex (mPFC) is proposed to interfere with stress control increasing the susceptibility to stress and consequently contributing to the emergence of psychiatric disorders including schizophrenia. Thus we evaluated the impact of single and combined stressful events during adolescence on schizophrenia-like signs in rats as adults and whether disruption of prelimbic (pl) PFC during adolescence affects stress-induced pathology that emerges in adulthood. Adolescent male rats were submitted to different stressful events [restraint stress (RS; 1 h session at postnatal day (PD) 31 PD32 and PD40); footshock (FS; 25 footshocks of 1.0 mA/2s/session daily through PD31-40); or a combination of FS and RS]. At adulthood animals were tested for anxiety responses (elevated plus-maze EPM) cognitive function (novel-object recognition test NOR) and locomotor response to amphetamine. One week after the behavioral experiments the activity of VTA DA neurons was evaluated using in vivo electrophysiology. Three parameters were measured: population activity ie the number of spontaneously active DA neurons per electrode track; average firing rate; and the percentage of action potentials occurring in bursts. We also evaluated whether the exposure to the combination of FS and RS in adulthood produced behavioral and electrophysiological changes similar to the adolescent stressors. In another experiment we sought to determine if a lesion within the plPFC would increase the vulnerability to FS exposure during adolescence in the DA system activity in rats as adults. The plPFC lesion was induced by infusing ibotenic acid bilaterally into the plPFC in rats at PD25. Six days after surgery rats were submitted to FS (daily through PD31-40). At adulthood they were tested in the EPM NOR test locomotor response to amphetamine and activity patterns of VTA DA neurons. All stressors induced anxiety-like responses in the EPM. FS and FS+RS also disrupted cognitive function as assessed by the NOR test. Additionally only animals exposed to the combination of FS+RS showed a dopaminergic hyper-responsivity in terms of amphetamine hyperlocomotion and increased VTA DA population activity resembling that observed in animal models of schizophrenia. Interestingly the increased number of spontaneously active DA neurons was confined exclusively to the lateral VTA which project to associative striatal regions analogous to those found to be hyper-responsive in schizophrenia patients. In contrast no change was observed when rats were exposed to the combination of FS+RS during adulthood underscoring that adolescence is a developmental period of particular susceptibility. Unlike intact rats animals with a plPFC lesion exposed only to the FS during adolescence showed DA hyper-responsivity. However plPFC lesioned animals exposed to FS displayed a more widespread increase in DA neuron activity with significant differences in both medial and lateral VTA regions. Given that the medial and central parts of the VTA send projections to the mPFC and amygdala and these projections play a role in emotional

states an increased DA activity in these VTA subregions may reflect a mechanism related to a disruption of the plPFC control of amygdala reactivity to stress. Our results are in agreement with previous studies showing long-lasting changes induced by stressful life events during adolescence. The impact on DA system activity however seems to depend on higher-level multiple stressors. Furthermore a failure of the plPFC to regulate the impact of stress which may be present in at-risk individuals increases the vulnerability to stress consequences. Thus predisposition to stress hyper-responsivity or exposure to substantial stressors during adolescence can initiate a cascade of events that result in a schizophrenia-like profile in adults. This data can provide information with respect to identifying markers for schizophrenia vulnerability early in life and by mitigating the impact of stressors prevent the transition to psychosis in susceptible individuals. Financial Support: NIH MH57440.

Morning Poster Session
Location: Row E
Poster #61

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Matthew Rich	Graduate	Torregrossa	Psychiatry

Cocaine-cue memory extinction is associated with depotentiation at amygdala synapses.

Extinction of memories associated with cocaine use may help reduce relapse. The basolateral amygdala (BLA) has been identified as a locus for cocaine-cue memory extinction and we have previously shown that manipulations of kinase/phosphatase activity within the BLA can enhance the efficacy of extinction. Depotentiation of excitatory synapses in the BLA has been proposed as a cellular mechanism for fear extinction but it is unclear if this mechanism explains the extinction of drug-associated memories. We tested if cocaine self-administration potentiates excitatory synapses in the BLA and if cocaine-cue extinction causes depotentiation. Rats self-administered cocaine or saline paired with an audiovisual cue (CS) for ≥10 days. 24 hours after the last training day were returned to operant chambers and received either 0 60 or 120 noncontingent presentations of the CS in the absence of reinforcer. The next day rats were euthanized and brains processed for whole-cell recordings of BLA principal neurons. Neurons were voltage-clamped at -70 mV. Thalamic afferents were stimulated with a concentric bipolar stimulating electrode and excitatory postsynaptic currents (EPSCs) were recorded. Cocaine training potentiated BLA synapses as shown by an increased EPSC amplitude relative to saline-trained controls. Cocaine-cue extinction depotentiated the synapse dose-dependently as 120 CS presentations fully reversed the potentiation caused by cocaine self-administration. Furthermore AMPA:NMDA ratio was increased in cocaine-trained animals relative to saline-trained and memory-extinguished animals. Cocaine-trained animals had a larger AMPA current suggesting that upregulation of AMPA receptors plays a role in the encoding of cocaine-associated memories. Internalization of the receptors during cue extinction may explain the resulting depotentiation and is likely an important factor for relapse prevention.

Morning Poster Session
Location: Row E
Poster #62

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Jillian Weeks	Graduate	Torregrossa	Neuroscience

Casein-kinase 2 activity may mediate CamKiia-dependent effects on reconsolidation of a cocaine-associated cue memory

Drug addiction is a widespread public health issue the resolution of which depends on treatment strategies that can produce long-term abstinence from drug use. However the complex milieu of cues that come to be associated with the drug presents a persistent challenge as these stimuli gain powerful incentive salience and can lead to robust motivation to seek the drug (craving) and relapse. Understanding of the processes by which these maladaptive memories are consolidated retrieved and potentially manipulated may present a critical outlet in developing more effective and lasting treatment strategies for drug addiction. Previous research in our lab has implicated Ca2+/calmodulin-dependent protein kinase II (CaMKIIα) in the reconsolidation of a cocaine-associated memory including phosphorylation on three threonine residues that have not previously been studied in the context of memory regulation. Bioinformatic databases suggest that these threonine residues (T334 336 & 337) are substrates for casein kinase 2 (CK2). Therefore the present experiments aimed to determine if CK2 is involved in CaMKIIα-mediated effects on reconsolidation of a cocaine-associated memory. Male Sprague-Dawley rats were trained to self-administer cocaine paired with an audiovisual cue. After lever extinction rats had the cue memory reactivated by brief presentation in a novel context. After reactivation rats were given vehicle or an inhibitor of CK2 activity 4 5 6 7-Tetrabromobenzotriazole (TBB) into the basolateral amygdala (BLA). A control group was exposed to TBB in the absence of memory reactivation (i.e. no cue presentation). We found that when cue memories were reactivated treatment with TBB but not vehicle produced a significant reduction in reinstatement responding while TBB did not reduce reinstatement in the no reactivation condition. Further experiments will aim to determine whether or not CK2 inhibition can reduce CaMKIIα activity in vitro as hypothesized by expressing CaMKIIα in HEK293T cells treating cultured cells with varying doses of TBB then assessing resulting CaMKIIα activity. The behavioral results of this study suggest that CK2 through its effects on CaMKIIα function may play a critical role in drug-related memory processes and thus serve as a target for future research and ultimately therapeutic applications.

Morning Poster Session
Location: Row E
Poster #63

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Erin Kirschmann	Postdoctoral	Torregrossa	Psychiatry

Effects of age of initiation on cannabinoid self-administration and corresponding cognitive consequences in male Sprague-Dawley rats

Marijuana (Cannabis sativa) is the most commonly used illicit drug in the US. Retrospective clinical studies suggest that initiating cannabinoid use in adolescence increases risk for negative outcomes such as cognitive impairment and risk for addiction relative to initiation in adulthood; however potential pre-existing cognitive differences among individuals and poly-substance use makes attributing negative effects specifically to marijuana difficult. We examined self-administration (SA) of the selective potent cannabinoid receptor agonist WIN55 212-2 (WIN) in adolescent vs. adult male rats and compared abuse potential and long-term effects on cognitive performance. Adolescent (starting postnatal day 28; p28) and adult (>p70) male rats were trained to SA intravenous infusions of WIN (0.0125mg/kg/infusion) on an FR1 schedule in daily sessions. Following SA rats were tested for cue-induced reinstatement of WIN-seeking after increasing periods of abstinence. The adolescent SA group was trained and tested on a delayed-match-to-sample working memory (WM) task in drug-free conditions as adults; performance was compared to adults trained to SA sucrose during adolescence. The adult SA group was trained and tested on the WM task after a similar abstinence period. An additional group of adults were trained on WM prior to initiation of WIN SA to determine the acute effects of WIN SA on WM. Adolescent and adult rats acquired WIN SA and displayed stable levels of intake during the last days of training. However while all adolescent rats met acquisition criteria only a subset of adults acquired. Adolescents and adults had similar levels of cue-induced WIN-seeking early in abstinence; the adolescent SA group (tested as adults) exhibited a further significant increase in WIN-seeking in continued abstinence suggesting an “incubation of craving.” Adult rats did not increase WIN-seeking in continued abstinence suggesting that adult-onset SA does not result in an incubation effect. Finally we found that WIN SA during adolescence resulted in improved WM performance in adulthood relative to sucrose controls if rats were abstinent. WIN SA during adulthood yielded no such improvements after abstinence. Additionally acute effects of adult WIN SA impaired WM performance relative to baseline performance. Interestingly rats with better baseline performance went on to take very low amounts of WIN; however the detrimental effects of WIN SA were magnified in this group and did not recover with abstinence. Our findings suggest that adolescent-onset cannabinoid use does produce indications of abuse liability while these indicators are blunted in adult-onset. Paradoxically adolescent WIN SA and abstinence resulted in better adult WM performance.

Morning Poster Session
Location: Row E
Poster #64

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Megan Bertholomey	Postdoctoral	Torregrossa	Psychiatry

Role of estradiol in ethanol-motivated behaviors

Recent epidemiological studies have shown that women but not men have demonstrated increased levels binge drinking and alcohol dependence compared to past cohorts. These findings are consistent with both clinical and preclinical data identifying females as a population that is particularly sensitive to predisposing factors leading to drug and alcohol abuse. For example it has been widely shown that female rodents show greater motivation to seek and take drugs compared to males. We have found that female rats maintain higher levels of ethanol self-administration and reinstatement of ethanol seeking following exposure to alcohol-paired cues and to the pharmacological stressor yohimbine especially when given in combination. However the mechanism underlying these sex differences is still unclear. Evidence suggests that estradiol is critical for the acquisition of cocaine self-administration and cocaine-primed reinstatement. However the effects of estradiol in ethanol-motivated behaviors are less consistent and few if any studies have determined estradiol-mediated changes in the reinstatement of ethanol seeking. To explore the role of estradiol in ethanol drinking and seeking gonadally intact and ovariectomized (OVX) female rats were trained to self-administer (SA) a 10% ethanol/0.1% saccharin solution paired with a light+tone cue on and FR1 schedule of reinforcement for 22 one-hour sessions. Rats then underwent ~10 extinction sessions and were tested for the effects of estrogen receptor modulation on cue+yohimbine-induced reinstatement of ethanol seeking. OVX rats were injected estradiol (E2) and intact rats were injected with either the selective estrogen receptor modulator (SERM) clomiphene to block the effects of estradiol or PHTPP an estrogen receptor β antagonist. As predicted E2 levels were positively related to ethanol drinking. OVX females showed reduced reinstatement compared to intact females and E2 did not rescue this effect. Rather activation of estrogen receptors with E2 tended to reduce reinstatement of ethanol seeking in OVX rats compared to vehicle while blockade of estrogen receptors with clomiphene or PHTPP tended to reduce reinstatement of ethanol seeking in intact rats. These findings suggest that estrogen signaling is important for ethanol-motivated behaviors though with paradoxical effects during ethanol seeking as a function of gonadal status. Current studies are aimed at further clarifying the role of E2 in ethanol-motivated behavior by determining the receptor and regional specificity of these effects in the brain. These findings will lead to a better understanding of the mechanisms underlying sex differences in alcohol-motivated behavior and guide potential treatment targets for alcohol dependent women.

Morning Poster Session
Location: Row E
Poster #65

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Ian Mitch Taylor	Postdoctoral	Cui	Bioengineering

Development of novel electrochemical sensors for the real-time in vivo detection of cocaine and dopamine

The real-time in vivo detection of neurochemicals is highly intriguing due to their widespread implication in healthy and diseased brain function. Successful neurochemical sensors must be selective and sensitive for the neurochemical of interest exhibit high spatial and temporal resolution and maintain small physical dimensions to prevent insertion related tissue damage. We have developed three novel highly successful electrochemical sensors that provide clear and robust real-time detection of dopamine and cocaine. Our in vivo cocaine sensor incorporates a cocaine-selective electrochemically active DNA aptamer onto a single shank silicon neural recording probe. The sensor exhibits selective robust cocaine detection in the rat dorsal striatum in response to both local cocaine infusion and intravenous cocaine injection and clear measurement of spontaneous and evoked electrophysiological activity in the barrel cortex. We have also developed two dopamine sensors that incorporate PEDOT coatings onto carbon fiber microelectrodes (CFE). PEDOT/graphene oxide coated CFEs exhibit an 880% increase in dopamine sensitivity and a 50% decrease in LOD compared to bare CFEs whereas PEDOT/carbon nanotube coated CFEs exhibit a 4800% sensitivity increase and a potential for signal amplification by preconcentration. These sensors are a marked improvement over existing technology and will allow for greater understanding of brain function.

Morning Poster Session
Location: Row E
Poster #66

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Gregory Rompala	Graduate	Homanics	Neuroscience

Paternal preconception chronic stress exposure reduces ethanol drinking behavior in male mice

We have previously shown that paternal vapor ethanol (EtOH) exposure decreases EtOH drinking behavior increases sensitivity to an anxiolytic injection of EtOH and blunts HPA axis responsivity selectively in male offspring. Interestingly paternal chronic variable stress (CVS) has also been shown to similarly blunt HPA axis responsivity in the next generation. Since EtOH is a physiologic stressor paternal EtOH exposure and paternal CVS may have similar effects on behavior in offspring. Here we tested the hypothesis that paternal CVS impacts EtOH-related behaviors in the next generation. To test this hypothesis we exposed adult male mice to six weeks of CVS. This entailed random daily exposure to one of seven stressors (i.e. restraint novel object predator odor wet cage constant light white noise and multiple cage changes). CVS- and control (C)- males were bred with stress naïve females to produce male and female offspring to be tested for EtOH-related behaviors. For EtOH drinking tasks adult offspring were tested for two bottle choice EtOH drinking at concentrations of 3 6 9 12 and 15% (w/vol) and for binge-like EtOH consumption (20% w/vol) in a limited access paradigm. Sensitivity to an anxiolytic injection of EtOH (1.0 g/kg) was tested in the elevated plus maze. HPA axis responsivity was tested by collecting tail blood at time points 0 15 30 and 90 min from the onset of a 15 min restraint stress and measuring plasma corticosterone levels using an ELISA assay. In the two bottle choice EtOH drinking task CVS-sired male offspring exhibited reduced EtOH preference at concentrations of 3 6 and 9% and reduced EtOH consumption at concentrations of 9 and 12% vs C-sired males. Moreover when CVS-sired male offspring were tested for binge-like EtOH consumption in the limited access assay there was similarly a significant reduction in EtOH consumption vs C-sired male offspring. In contrast CVS-sired female offspring showed no difference in EtOH drinking behaviors vs C-sired females in either EtOH drinking paradigm. We did not find a difference in EtOH sensitivity or HPA axis responsivity to acute stress for CVS-sired males or females vs C-sired groups. These results show that paternal CVS attenuates intergenerational EtOH drinking behavior in mice. This suggests that paternal environmental exposures such as to alcohol or stress can lead to heritable changes in alcohol drinking behavior. Ongoing studies are exploring possible epigenetic mechanisms in sperm.

Morning Poster Session
Location: Row E
Poster #67

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Anthony Rudine	Faculty	Rudine	Newborn Medicine/Pediatrics

Antenatal Dexamethasone Exposure Differentially Affects Distinct Cortical Neural Progenitor Cells and Triggers Long Term Changes in Murine Cerebral Architecture and Behavior

Antenatal administration of synthetic glucocorticoids (sGC) is the standard of care for women at risk for pre-term labor before 34 gestational weeks. Despite their widespread use the type of sGC used and their dose or the dosing regimens are not standardized in the US or worldwide. Several studies have identified neural deficits and increased risk for cognitive and psychiatric disease later in life for children administered sGC prenatally. However the precise molecular and cellular targets of GC action in the developing brain remain largely undefined. In this study we demonstrate that a single of glucocorticoid during mid-gestation in mice leads to enhanced proliferation in select cerebral cortical neural stem/progenitor cell populations yet thinning of the cerebral cortex at birth. These alterations are mediated by dose dependent decreases in expression of cell cycle inhibitors and increased expression in genes that promote cell cycle re-entry. This leads to changes in neuronal number and density in the cerebral cortex at birth coupled to long-term alterations in neurite complexity in the prefrontal cortex and hippocampus in adolescents and changes in anxiety and depressive like behaviors in adults. Our results recapitulate outcomes observed in steroid-exposed children and provide insights into how sGCs may act at the cellular level in the embryo adolescent and adult. More research is urgently needed to develop modifications to the antenatal dosing strategies in humans so that the fetal brain is protected during critical developmental periods when exposed to a drug that has proven life-saving benefits for preterm infants.

Morning Poster Session
Location: Row E
Poster #68

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Man Wu	Graduate		Neuroscience

New calcium channel gating modifiers with therapeutic potential that prolong channel deactivation and alter transmitter release at neuromuscular synapses

Previously we have developed novel analogs (including GV-58) of (R)-roscovitine that have reduced cyclin-dependent kinase (Cdk) activity and enhanced calcium channel gating modifier activity. The goal of this work has been to develop (1) tool compounds for studies of calcium channels and transmitter release and (2) therapeutic leads for the treatment of neuromuscular diseases. In particular Lambert-Eaton Myasthenic Syndrome (LEMS) is an autoimmune disorder that attacks and removes presynaptic calcium channels from motor synapses. As a result LEMS patients show a reduction in transmitter release leading to neuromuscular weakness. Current treatment for LEMS includes the potassium channel blocker 3 4-diaminopyridine (DAP) which widens the presynaptic action potential increasing the amount of calcium influx and thus increasing transmitter release. However 3 4-DAP has dose-limiting side effects preventing full symptomatic relief for some of these patients. Our previously reported compound (GV-58) is a gating modifier that increases total calcium entry by stabilizing the open state of the channel but is dependent on voltage-dependent calcium channel opening such that very brief depolarizations modified fewer channels than longer depolarizations. Our goal here was to develop and test additional analogs based on the GV-58 structure that might be more potent and act faster. Here we report the testing of eight new analogs four of which show promise: MF-06 MF-17 KK-75 and KK76. These new compounds included modifications to the placement of nitrogens around the core of the molecule in combination with alterations to two side chains. We tested the effects of these compounds on Cav2.1 calcium channels expressed in TSA201 cells using perforated whole-cell patch clamp techniques and on transmitter release using intracellular recordings from weakened adult mouse neuromuscular junctions. One of these compounds (MF-06) slowed channel deactivation to an even greater extent than GV-58. Interestingly both MF compounds appear to be slower to act than the KK compounds. Further these four compounds had variable cdk activity as compared with (R)-roscovitine. Effects of these compounds on transmitter release at weakened neuromuscular synapses are predicted to be complicated by their varying magnitude of effects on channel gating combined with their speed of action. Taken together these data increase our understanding of the structure-activity relationship for these gating modifiers with therapeutic potential and provide new tools for the study of calcium channels and calcium-triggered transmitter release.

Morning Poster Session
Location: Row F
Poster #69

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Ross Carson	Graduate	DeFranco	School of Medicine

Effects of Statins on the Proliferative Response of Neural Stem/Progenitor Cells

Although statins have proven to be safe and effective drugs in combating cardiovascular disease they are currently contraindicated in pregnancy due to potential teratogenic effects. Statins reduce serum cholesterol by inhibiting the rate-limiting enzyme of cholesterol synthesis HMG-CoA reductase (HMGR). In the developing neocortex products of the cholesterol biosynthesis pathway (CBP) including cholesterol and isoprenoids play essential roles in proliferation and differentiation of neural stem-progenitor cells (NSPCs); therefore we sought to investigate the effect of statins on the developing brain. To determine whether statins impact proliferation embryonic day 14.5 NSPCs were treated with pravastatin or simvastatin in vitro and cell viability was monitored over the course of 5 days. We found that both statins dose dependently decrease cell viability and neurosphere size. NSPCs treated with low doses of statins (1uM of Pravastatin or 0.1uM Simvastatin) were able to continue to grow but higher doses of statins (25uM Pravastatin or 5uM Simvastatin) caused a nearly complete inhibition of growth. In order to understand whether this phenotype was due to decreased proliferation or cell death we analyzed cell cycle phase and apoptosis by propidium iodide staining analyzed by flow cytometry. We found that while statins cause a dose dependent increase in cells progressing through the G1 phase of the cell cycle there is also a dose dependent increase in apoptosis. These findings were reinforced by significantly increased Cyclin D1 mRNA and protein and increased cleaved PARP protein in statin treated NSPCs. To understand how NSPCs respond to statins at a transcriptional level we treated NSPCs with pravastatin and examined global mRNA expression using RNA sequencing technology. Bioinformatics analysis revealed an upregulation of 17 CBP genes and a number of genes involved fatty acid and cholesterol metabolism. This upregulation was validated by qPCR in a handful of robustly upregulated CBP genes (HMGCS1 ACLY and DHCR24). To understand the regulation of this transcriptional phenotype we used our RNA-seq data to identify potential upstream regulators. We discovered that three of the top identified regulators (SCAP INSIG and SREBP2) form a complex known to regulate CBP genes in peripheral tissues. To validate this observation we measured activation of SREBP2 protein and found an increase in activation after treatment with either pravastatin or simvastatin. We found that SREBP2 activation occurs rapidly after treatment with statins and induces expression of CBP genes that plateaus around 24hr after treatment. Because NSPCs are able to continue to survive low doses of statin treatment we asked if statin induced transcription would be sufficient to rescue the cell growth phenotype. We found that a 24hr pretreatment with pravastatin not was able to rescue cell growth in NSPCs treated with statins compared to controls which indicates that the transcriptional response of NSPC's is not protective against statin induced cytotoxicity. This study suggests that chronic exposure to statins causes apoptosis in rapidly dividing NSPCs despite a robust compensatory upregulation of CBP genes. It remains to be elucidated whether statin induced cytotoxicity is due to decreases in cholesterol or other CBP end products.

Morning Poster Session
Location: Row F
Poster #70

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Tija Jacob	Postdoctoral	Jacob	Pharmacology and Chemical Biology (& CNUP)

Balancing neurotransmission: rapid agonist induced GABAergic synaptic and functional plasticity

γ-aminobutyric acid (GABA) begins as the key excitatory neurotransmitter in newly forming circuits with chloride efflux from GABAA receptors (GABAARs) producing membrane depolarization which promotes calcium entry dendritic outgrowth and synaptogenesis. As development proceeds GABAergic signaling switches to inhibitory hyperpolarizing neurotransmission. Despite the evidence of impaired GABAergic neurotransmission in neurodevelopment disorders little is understood on how agonist dependent GABAAR activation controls the formation and plasticity of GABAergic synapses. We have identified a weakly depolarizing and inhibitory GABAAR response in cortical neurons with well-established GABAergic synapses that occurs during the transition period from GABAAR depolarizing excitation to hyperpolarizing inhibitory activity. We show here that GABAAR agonist treatment at this stage mediates structural changes that diminish GABAergic synapse strength through postsynaptic and presynaptic plasticity via intracellular Ca2+ stores ERK and BDNF/TrkB signaling. We show that GABAAR stimulation results in delayed activation of the ERK pathway a cellular response distinct from early excitatory depolarizing GABAAR activity. Application of the GABAAR agonist muscimol decreases synaptic localization of surface γ2 GABAARs and gephyrin postsynaptic scaffold while β2/3 non-γ2 GABAARs accumulate in the synapse. Concurrent with this structural plasticity muscimol treatment decreases synaptic currents while enhancing γ2 containing GABAAR tonic currents in an ERK dependent manner. We further demonstrate that GABAAR activation leads to a decrease in presynaptic GAD-65 levels via BDNF/TrkB signaling. Together these data reveal a novel mechanism for agonist induced GABAergic synapse plasticity that can occur on the timescale of minutes contributing to rapid modification of synaptic and circuit function.

Morning Poster Session
Location: Row F
Poster #71

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Joshua Lorenz-Guertin	Graduate	Jacob	Pharmacology and Chemical Biology

Characterizing a Novel Tool to Monitor Pharmacologically-Induced Changes in GABA(A)R Trafficking

Surface regulation of the γ-aminobutyric acid type-A receptor (GABA(A)R) is a critical aspect of both baseline inhibitory neurotransmission and responsiveness to pharmacological treatments. We recently engineered a novel GABA(A)R γ2 subunit that is capable of tracking receptors through nearly all phases of trafficking. The fluorogen-activating peptide dual L5 (DL5) was inserted into a previously characterized γ2 subunit construct already encoding a pH-sensitive green fluorescent protein (pH-GFP) (γ2pH-DL5). DL5 is an antibody variable fragment which selectively binds and activates malachite green (MG) dyes that are otherwise non-fluorescent in solution. MG dyes have distinct characteristics including cell permeability pH-sensitivity and fluorescence properties. We find that γ2pH-DL5 is fully expressed at the cell surface in transfected cortical neurons and forms synaptic clusters. Additionally GABA(A)Rs incorporating γ2pH-DL5 respond to the endogenous ligand GABA and exhibit positive modulation via the γ2 subunit-requiring benzodiazepine type drug Diazepam in electrophysiological recordings. Imaging studies demonstrate that γ2pH-DL5 is able to bind and activate the fluorescence of the MG dyes MG-βT and the pH-sensitive dichromphore pH-se-Red. Neurons pulse-labeled with cell membrane impermeable MG-βT exhibit a time-dependent accumulation of fluorescent signal colocalized with the lysosomal marker LAMP-1 RFP indicating surface γ2pH-DL5 can be tracked to lysosomes. This work aims to use advanced live-imaging approaches to identify pharmacologically-induced changes in GABA(A)R regulation and ultimately provide critical information about the receptor as a clinical target.

Morning Poster Session
Location: Row F
Poster #72

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Juliann Jaumotte	Faculty	Jaumotte	Neurology

Isolated housing decreases the immune response in sera and brain following exposure to a bacterial toxin in older rats.

As people age they often are more likely to become ill. Among the many factors that could explain their reduced health span is the increasing isolation commonly experienced by the elderly as well as other segments of our society. This in turn can be associated with an impaired immune system including a decline in B- and T-cell production which is reflected by changes in the expression of cytokines produced in response to exposure of viral or bacterial agents. In this experiment we examined the immune response elicited by exposure to lipopolysaccharide (LPS) an endotoxin produced by bacteria. We first examined a small dose-response curve for LPS (0-2 mg/kg i.p.) in 28-month-old male Fisher 344/Brown Norway hybrid rats (F344/BN) to determine the highest tolerable dose of a single intraperitoneal injection of LPS. We then used an intermediate dose from that analysis (empirically determined to be 1 mg/kg) and delivered it to male F344/BN rats housed housed in our facility for 8 months beginning at 19 months of age. The two groups studied were either singly housed in a standard shoebox cage (18 cm W x 38 cm D x 27 cm H; SE) or a relatively enriched environment consisting of a large cage (1 m W x 1m D x 0.6m H) containing 6 rats running wheels tunnels platforms and toys (EE). Seven days after the LPS injection all animals were euthanized and brain and serum collected. Using a Luminex multiplex assay kit we observed that several cytokines and chemokines were significantly altered in both sera and brain from isolated animals in comparison to those in enriched housing. The pattern of change indicated that in isolation led to a reduction in the immune response. Cytokines and chemokines that changed in response to isolation included G-CSF (sera) IL-1 alpha (sera and brain) IL-1-beta (sera and brain) IL-4 (sera and brain) IL-6 (sera) IL-10 (sera) IP-10 (sera) INF-gamma (sera and brain). These data suggest that older isolated animals have a less reactive immune response than their more enriched counterparts which could indicate a lowered ability to fight off infections or stave off neurological disease that have an immunological component including Alzheimer's and Parkinson's disease.

Morning Poster Session
Location: Row F
Poster #73

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Gabrielle Kaplan	Graduate	McClung	Psychiatry

CLOCK represses the expression of tyrosine hydroxylase via recruitment of the metabolic sensor SIRT1

Many studies strongly implicate alterations or disruptions to circadian rhythms as contributors to the pathophysiology of mood and addiction disorders. We have shown previously that Clock mutant mice (Clock□19) display a behavioral repertoire similar to human bipolar mania with a particular sensitivity to rewarding stimuli. Clock□19 displayed enhanced cocaine conditioned place preference (CPP) along with increased dopamine cell firing in the VTA. mRNA and protein levels of tyrosine hydroxylase (TH) the rate-limiting enzyme in dopamine synthesis was also increased in the VTA of Clock□19 mice suggesting TH is a direct target of CLOCK. We investigated how CLOCK represses TH expression in the VTA and whether these mechanisms are involved in the hyperhedonic phenotype. We focused on two particular proteins that dynamically interact with CLOCK across the light-dark cycle phosphoactive CRE-element binding protein (P-CREB) and the histone deacetylase sirtuin 1 (SIRT1) a sensor of intracellular changes in metabolism. CLOCK typically drives circadian rhythms in gene transcription. However we found that CLOCK is a transcriptional repressor of TH in the VTA through interactions with P-CREB and SIRT1 at particular diurnal phases. CLOCK and P-CREB bind the TH promoter in antiphase. SIRT1 interacts with CLOCK to inhibit CREB-mediated transcription of TH. P-CREB binding and TH expression were constitutively elevated in the VTA of Clock mutants while SIRT1 protein levels were significantly reduced. Both mCREB and SIRT1-OX in the VTA of Clock mutants reduced TH expression and attenuated cocaine CPP suggesting CREB-inactivation and restoring SIRT1 levels in mutant mice reversed the hyperhedonic phenotype. Excess NAD and NAM blocked the ability of CLOCK to suppress TH expression. These studies demonstrate a link between metabolic and circadian pathways and how disruption to these pathways are important for behavioral phenotypes relevant to addiction.

Morning Poster Session
Location: Row F
Poster #74

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Chenxiao Tang	Graduate	Chenxiao	Pharmaceutical sciences

SCREENING 20-HETE INHIBITORS IN MICROSOMAL INCUBATES USING UPLC-MS/MS

Introduction: 20-hydroxyeicosatetraenoic acid (20-HETE) is a metabolite of arachidonic acid (AA) by cytochrome P450 (CYP) 4A11 and CYP4F2 in human with potent microvascular constriction activity. Inhibition of 20-HETE formation is neuroprotective in subarachnoid hemorrhage cardiac arrest and thromboembolic stroke preclinical models. This suggests that inhibition of 20-HETE formation is a potential therapeutic strategy for neuroprotection after brain injury. At this time a clinically relevant 20-HETE inhibitor is not available to evaluate as a therapeutic intervention. Our goal is to identify a selective metabolic stable and potent 20-HETE inhibitor. Hypothesis: Novel selective and specific 20-HETE formation inhibitors can be identified and confirmed by scaffold-hopping and human CYP4F2 homology model. Methods: Test compounds were obtained either via virtual screening against a CYP4F2 homology model or from a proprietary library available to our laboratory. 1.AA microsomal incubation assay: four different microsomal systems including human liver microsome (HLM) recombinant CYP4F2 (rCYP4F2) rat liver microsome (RLM) and rat kidney microsome (RKM) were used. AA was incubated with microsomes with/without compound for 20 min. 20-HETE formation rate was quantified using a validated UPLC-MS/MS assay and normalized by vehicle control group. Other eicosanoids including 15- 12-HETEs epoxyeicosatrienoic acids (EETs) and dihydroxyeicosatrienoic acids (DHETs) were monitored simultaneously. 2. Metabolic stability assay: selected compounds were incubated with HLM during a 60-minute incubation time. Remaining amount of compounds was quantified using UPLC-MS/MS and normalized to corresponding 0 min values. Results: Among 26 compounds that we screened comp 10 and 26 both inhibited 20-HETE formation in a dose-dependent manner. At 2500nM comp 10 reduced 20-HETE formation to 19.9±1.8% 24.0±5.5% in rCYP4F2 and HLM compared with control; comp 26 decreased 20-HETE formation to 32.4±6.5% 34.8±5.1% in rCYP4F2 and HLM respectively. After structure modification comp 19 and its hydrochloride salt comp 18 were the most potent and possessed dose-dependent inhibition against 20-HETE formation. At 2500nM comp19/18 brought down 20-HETE formation to 4.4±0.4%/6.0±0.3% 8.6±1.3%/9.8±1.6% in rCYP4F2 and HLM respectively without inhibitory effect on 15- 12-HETE EETs or DHETS formation. Comp 10 19 had 91.4±11.0% 100.4±1.7% remaining compound at 30min in HLM compared to 35.1±5.7% of 3-(4-n-butoxyphenyl)pyrazole. Comp 10 19 were more stable than 3-(4-n-butoxyphenyl)pyrazole which already had better stability than HET0016. Conclusions: These results suggested that compound 10 18/19 26 are potent 20-HETE formation inhibitors with better solubility microsomal stability and can serve as leads for further structure modifications that may lead to novel 20-HETE formation inhibitors. Significance: We have a rationally designed novel compound library and a CYP4F2 homology model for identification and lead compound optimization against 20-HETE formation. Selected lead compound with better solubility metabolic stability and potency could be used in preclinical animal model to evaluate its PK/PD and neuroprotective effect and could potentially serve as a clinically relevant drug for critically ill patients.

Morning Poster Session
Location: Row F
Poster #75

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Jenna Parrish	Graduate	Sibille	Psychiatry

Estradiol modulation of the renin angiotensin system and the regulation of fear extinction

Low estradiol levels during fear extinction impair extinction consolidation resulting in increased fear expression during extinction recall in women and female rats. However the mechanism by which this occurs is unknown. Estrogen modulates the renin angiotensin system (RAS) by downregulating the hypertensive axis (including angiotensin II type I receptors; AT1R) of the RAS. Our lab has found that systemic administration of the AT1R antagonist losartan prior to fear extinction enhances extinction consolidation and reduces fear during extinction recall in female rats with low estradiol levels. Next we investigated potential mechanisms by which estradiol interacts with the RAS to enhance extinction consolidation. Adult female Sprague Dawley rats received injections of levonorgestrel (0.5mg/kg/day) a hormonal contraceptive (HC) that lowers circulating estradiol levels or vehicle for 5 days. Blood and brains were collected for further analysis. Estradiol and angiotensin II levels were measured in serum. Brain sections were mounted on slides and AT1R autoradiography was performed to compare AT1R binding between groups. In a separate cohort brains were collected from rats treated with HC or vehicle. Tissue punches were taken from brain regions associated with fear and qPCR was performed to compare AT1R mRNA expression between groups. Finally another cohort of rats was run through a cued fear conditioning paradigm and angiotensin II or vehicle was administered systemically before or immediately after the extinction session in female rats with high estradiol levels. Extinction recall was tested 24 hours later. The HC treated group had significantly decreased levels of estradiol and significantly increased levels of angiotensin II compared to the vehicle treated group. No significant differences in AT1R expression or binding were found between HC and vehicle groups. Systemic angiotensin II had no effect on extinction acquisition. However pre-extinction session treatment with angiotensin II produced a non-significant increase in freezing during extinction recall. In conclusion angiotensin II which is part of the hypertensive axis of the RAS and an agonist of the AT1R was increased in rats with low estradiol levels but no differences were found at the receptor level. This suggests that extinction consolidation deficits in rats with low estradiol may be due to increased angiotensin II but additional behavioral studies are needed to clarify this relationship. Understanding the mechanism by which circulating hormones affect extinction learning could aid in the development of better treatments for people who suffer from anxiety disorders.

Morning Poster Session
Location: Row F
Poster #76

Presenting Author:	Author Type:	Mentor/Lab:	Department:
Rebecca Krall	Graduate	Tzounopoulos	Neurobiology

Endogenous extracellular zinc is neuroprotective against glutamate excitotoxicity mediated via NMDA receptors

Zinc is an endogenous modulator of neurotransmission, notably through its inhibition of NMDA and AMPA receptors (NMDARs and AMPARs), and potentiation of glycine receptors (GlyRs).The majority of free zinc in the brain is loaded into vesicles by the zinc transporter Znt3, and co-released with glutamate. However there is an additional, Znt3 independent extracellular tonic pool of zinc that inhibits extrasynaptic NMDARs and potentiates GlyRs (Anderson et al., PNAS 112:E2705; 2015; Rosello et al., Neurobiol Dis 81:14; 2015). Because extrasynaptic NMDARs receptors are implicated in excitotoxicity (Parsons & Raymond, Neuron 82:279; 2014),we investigated whether the tonic zinc pool limits excitotoxic injury via its inhibition of NMDARs. To quantify tonic zinc levels, we used the extracellular ratiometric zinc probe LZ9. We measured nanomolar levels of extracellular zinc in rat mixed cortical cultures, similar to those measured in fresh brain slices of the dorsal cochlear nucleus. DL-threo-β-benzyloxyaspartate (TBOA; 75 µM), a glutamate transporter inhibitor, induced glutamate toxicity and caused a ~30% decrease in cell viability as measured by LDH cytotoxicity assays ($p < 0.05$). Chelation of endogenous extracellular Zn^{2+} with ZX1 (3 µM), a high-affinity extracellular zinc chelator, increased the toxicity of TBOA treatment, reducing viability to ~50% of control ($p < 0.05$). In both cases, the NMDAR antagonist memantine (30 µM) blocked cell death. These results indicate that extracellular tonic zinc is neuroprotective via its inhibition of NMDA receptors.