UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 17, 2013 Mary Gates Hall

Online Proceedings

SESSION 1D

MEDICAL THERAPEUTICS AND ENDOCRINOLOGY

Session Moderator: Ian Sweet, Medicine
231 MGH
1:15 PM to 2:45 PM

* Note: Titles in order of presentation.

Pathologic Onset of Megaesophagus in the Aging Mouse Model of Duchenne Muscular Dystrophy
Ladan Laurel (Ladan) Mukherjee, Senior, Biochemistry
Mentor: Jeffrey S Chamberlain, Neurology
Mentor: John Hall, Neurology

Pathologic enlargement of the esophagus, termed megaesophagus, results in a failure to complete peristalsis leading to vomiting, severe weight loss and potential death. Megaesophagus is characterized in a number of disease states including parasitic (Chaga's disease), autoimmune (myasthenia gravis), and neuromuscular (muscular dystrophies), however, a detailed cellular and mechanistic understanding is lacking. To address this deficit, we performed a comprehensive examination of the onset, pathology and cellular composition of megaesophagus in the mouse model of Duchenne muscular dystrophy (DMD). DMD affects ~1/3500 male births and is a catastrophic and ultimately fatal muscle wasting disease resulting from a mutation in the gene encoding the integral skeletal muscle protein dystrophin. Although poorly defined in human DMD patients, megaesophagus is documented in canine and mouse models of DMD. Advancements in DMD therapies has increased patient lifespan and underscored a need to assess the impact of the disease in a range of organ systems, including the esophagus. We find that older dystrophic mice can develop severe megaesophagus, but that the timing of onset depends on the nature of dystrophin expression in muscle and non-muscle tissues and the genetic background of the mice. Further studies are in progress to delineate the precise dystrophin expression pattern in different cell types of the esophagus that contribute to pathology. Work presented here will be essential for future work aimed at identifying new therapeutic targets for DMD.

SESSION 2H

NEUROSCIENCE AND GENETICS: FROM DISORDERS TO TOOLS FOR DISCOVERY

Session Moderator: Gwenn Garden, Neurology
248 MGH
3:45 PM to 5:15 PM

* Note: Titles in order of presentation.

Analysis of Altered miRNA Expression in a Transgenic Mouse Model of SCA7
Kt Nguyen, Senior, Neurobiology
Levinson Emerging Scholar, Undergraduate Research Conference Travel Awardee
Mentor: Gwenn Garden, Neurology

Spinocerebellar ataxia type 7 (SCA7) belongs to a family of neurodegenerative diseases. It is characterized by degeneration of the cerebellum and brainstem. The disease is caused by an expanded polyglutamine tract in ataxin 7. This type of polyglutamine mutation is a common feature in many neurodegenerative diseases. Other neurodegenerative disease including those involving polyglutamine expansions demonstrate altered microRNA (miRNA) expression. Given the supporting evidence that miRNA are differentially expressed in other polyglutamine diseases, I seek to determine the role of miRNAs in SCA7. My proposed research will analyze altered miRNA expression in Purkinje cell neurons, a selectively vulnerable neuronal population that degenerate in SCA7. I will first identify miRNAs that are upregulated or downregulated in Purkinje neurons from SCA7 mice. Next using predictive databases I will identify their potential mRNA targets. I will then test identified miRNAs in cell culture to see if they will inhibit expression of their predicted target. If expression is inhibited, I will then measure the level of mRNA and protein of the predicted target in transgenic mouse tissue. By identifying miRNAs that are differentially expressed in SCA7 mice and confirming their activity, we can build our understanding of the mechanisms of disease progression in SCA7.

SESSION 2N

MCNAIR SESSION - IMPROVING LIVES VIA ENGINEERING, NEUROSCIENCE, EVOLUTIONARY BIOLOGY, AND PSYCHOLOGY

Session Moderator: Gene Kim, Education, Office of Minority Affairs & Diversity

287 MGH

3:45 PM to 5:15 PM

* Note: Titles in order of presentation.

Perception and Physiology: A Neurobiological Perspective on Cognition and its Potential Consequences

Tabatha Memmott, Senior, Organismal Biology, Public Health Education, Portland State University
McNair Scholar
Mentor: Barry Oken, Neurology and Behavioral Neuroscience, Oregon Health & Science University

Studying human perception from the perspective of a biologist or physician is a recent innovation, whose aims were previously appropriated by the domain of psychology. Yet in recent years, emphasis on perception has grown in fields like psychosomatic medicine, behavioral neuroscience, stress physiology, and complementary/alternative medicine. This new perspective on perception has prevailed with increased rates of psychosomatic disorders that have physiological and cognitive components (Post Traumatic Stress Disorder (PTSD), depression, etc.), and through recent technological advances made available to researchers (EEG, fMRI, etc.) Most specifically, technological innovations have enabled less invasive and more precise studies of neuroanatomy, which enables us to connect precise physiology with corresponding cognitive change. The manner in which a stimulus is perceived, and the resulting trauma, reward, danger or stress, then cognitively processed can have positive or negative physiological outcomes for individuals. However, researchers are just beginning to understand this mechanism and its implications.My research will study activation differences during visual working memory (VWM) tasks in stressed individuals. The ability to hold and process information in the VWM is related to general alertness, and I anticipate finding that stress will impair functionality of the VWM. This can be ascertained using electrophysiology (event-related potentials). These results would help establish the cycles or mechanisms that characterize these disorders and produce their cognitive-processing malfunctions. I hope to increase awareness of the genuine and detrimental progressions of these disorders, while also identifying treatments that effectively ameliorate both symptoms and identifiable lesions (chemical, physical, or otherwise). Understanding these pathways can assist in the recovery from disorders such as PTSD and depression, or potentially shorten a patient's stay in hospital, where serious infection rates and stress are high, and overall comfort, low.

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Session Moderator: Gene Kim, Education, Office of Minority Affairs & Diversity

287 MGH

3:45 PM to 5:15 PM

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A New Approach to Controlling Epilepsy through p38 MAPK

Vicky Herrera, Senior, Biochemistry
Amgen Scholar, McNair Scholar, Undergraduate Research Conference Travel Awardee
Mentor: Nicholas Poolos, Neurology

Epilepsy is a neurological disease characterized by recurring seizures and it affects millions. We hypothesize that modulation of p38 mitogen-activated protein kinase (p38 MAPK) will influence hyperpolarization-activated cyclic nucleotide-gated (HCN) channel activity, affecting the frequency of seizures in an animal model of epilepsy. p38 MAPK is a kinase activated by conditions of cellular stress. It strongly stimulates the HCN channels, which are voltage gated ion channels that are highly expressed in the cortex and hippocampus, brain regions where seizure onset occurs. A decrease in HCN channel expression or function has previously been observed in epileptic animals associated with a loss of p38 MAPK activity, which produced neuronal hyperexcitability. We hypothesized that increasing p38 MAPK activity might decrease neuronal activity and decrease seizure frequency. In this experiment, Epilepsy was induced in Sprague Dawley rats through treatment with pilocarpine, a convulsant drug. Recurrent, unprovoked seizures were seen after three weeks of treatment. An osmotic pump was placed in the rats, delivering drugs that modulate p38 MAPK. The drug SB203580 (SB), a specific inhibitor of p38 MAPK, was administered, as was anisomycin, a non-specific activator of p38 MAPK. Animal brain activity was analyzed with video electroencephalography through electrode implants in the skulls of the rats. Inhibition of p38 MAPK by SB increased seizure frequency by 179 % as predicted, however, anisomycin also increased seizures by 236%. We hypothesize that this may be due to non-specific actions on other signaling pathways like

the Jun N-terminal kinase pathway (JNK). We're investigating the role of JNK in modulating seizure frequency, and this may point us to some new therapies. The implications of this study may indicate a new approach to the control of epilepsy via anti-epileptic drugs targeting p38 MAPK.

POSTER SESSION 3

Commons East, Easel 48

2:30 PM to 4:00 PM

Adoptively Transferred Lymphocytes Influences Immunological Profile and Myelin Composition Following Stroke

Derek Tang (Derek) Nhan, Senior, Neurobiology, Biochemistry

Howard Hughes Scholar, Mary Gates Scholar, Washington Research Foundation Fellow

Mentor: Kyra Becker, Neurology

Approximately 800,000 Americans suffer a stroke each year, making this neurological disease the leading cause of adult disability in the US. An ischemic stroke occurs when blood flow to the brain is interrupted, resulting in inadequate oxygen delivery to brain cells. Currently, only one intervention has proven to improve outcome after stroke and must be given shortly after stroke onset. Effective interventions given at delayed time points after stroke are needed. During stroke, breakdown of the blood-brain barrier allows for interactions between once-segregated central nervous system antigens and the systemic immune system. My project investigates the effects of a cellular immune response directed towards myelin basic protein (MBP) and the interactions between $CD8^+$ on myelin and stroke outcome in experimental cerebral ischemia. In this model, Lewis rats are subjected to middle cerebral arterial occlusion (2 hours) and injected with either lipopolysaccharhide, shown to elicit an immune response to MBP, or saline (control). I performed a battery of behavioral tests, including a standard neurological score for rodents and evaluation of rotarod performance at set time points before and after stroke. Using double-label immunocytochemistry, the brain sections were labeled for $CD8^+$ and MBP. Quantitative analysis of myelin between the infarcted and non-infarcted hemispheres was performed using *Metamorph*; the numbers of $CD8^+$ lymphocytes were counted in a standard fashion. Preliminary data show prominent demyelination in the infarcted regions. Additionally, we observed a correlation between myelin loss and performance on the rotarod immediately post-stroke, though further results are pending. The goals for this project are to (1) determine if animals receiving MBP specific lymphocytes adoptively at stroke onset suffer more myelin loss than those receiving lymphocytes unreacted to MBP and (2) determine if the number of $CD8^+$ lymphocytes within the infarct correlate with the extent of myelin loss, and degree of the MBP response among the adoptively transferred lymphocytes.

POSTER SESSION 4

MGH 241, Easel 135

4:15 PM to 5:45 PM

Microglia Toll-like Receptor and Interferon Signaling in Ischemic Preconditioning

Dorender Appiah (Dorender) Dankwa, Senior, Psychology, Neurobiology

Mary Gates Scholar

Mentor: Jonathan Weinstein, Neurology

Ischemic Preconditioning (IPC) is a neuroprotective phenomenon in which a brief ischemic exposure increases resistance to serious injury from subsequent ischemia. Understanding the basic mechanisms of IPC and taking advantage of this knowledge may provide better treatment options for those at high risk for stroke. We hypothesize that ischemia induces brain tissue release of endogenous Toll-like receptor (TLRs) agonists (sometimes referred to as damage association molecular patterns (DAMPS)) that stimulate microglia Toll-like receptors, thus releasing interferon (IFN) α/β to trigger the transcription of neuroprotective interferon stimulated genes (ISG). Our objective was to culture mouse microglial cells and stimulate the various microglia TLR receptors with their respective agonists to identify the receptors that mediate ISG release. We also wanted to demonstrate TLR-agonist induced release of IFN α/β, especially because IFNs are already effective FDA-approved treatments for Multiple Sclerosis and could serve to increase ischemic tolerance for patients at high risk for strokes. Due to results from previous experimental paradigms strongly suggesting that IPC-mediated neuroprotection is TLR4-dependent, we proposed that stimulation of TLR4 generates release of IFNs that in turn induce transcription of ISGs. Our preliminary results revealed that activation of multiple TLRs (TLR 3, 4, and 9) could mediate the release of ISG chemokines, with notable ISG release following TLR3 and TLR4 activation. IFN release was not detected through TLR4 but instead TLR3. We are currently exploring the possibility of a synergistic relationship between TLR3 and TLR4 in regulating IPC-mediated neuroprotection.

POSTER SESSION 4

MGH 241, Easel 159

4:15 PM to 5:45 PM

What's the Matter: Effects of Ischemic Preconditioning in White Matter

Thu Le, Senior, Korean, Biology (General)

Mentor: Jonathan Weinstein, Neurology

Ischemic preconditioning (IPC) is a neuroprotective phenomenon in which a short ischemic episode confers resistance to prolonged subsequent ischemic attacks, and is dependent upon functional Toll-Like Receptor 4 (TLR4) and possibly downstream interferon (IFN)-related signaling. Understanding this mechanism and being able to mimic it pharmaceutically may lead to better stroke therapy and treatment. In our current study, we use the optic nerve—a pure axonal tract—as our model in determining the effects of stroke in white matter. Roughly half the human brain is composed of white matter—the myelinated axons and supporting cellular milieu that connect neurons into networks. In our experimental paradigm, we induce IPC in vivo by performing common carotid artery occlusion (CCAO) and then expose ipsilateral (IPC-induced) and contralateral (control) optic nerves to ex vivo oxygen-glucose deprivation (OGD) (in order to model ischemic or "stroke" related injury). We then characterize electrophysiological properties and axonal integrity of the isolated optic nerves. In addition, we characterize glutamate receptor isoform expression and interferon stimulated gene induction. IPC-exposed optic nerves showed improved compound action potential (CAP) recovery following OGD in wild-type versus TLR4-/- mice. Using immunohistochemical techniques we show that oligodendrocytes are protected from OGD-induced death. Likewise, axonal integrity is maintained better in IPC-treated optic nerves as shown by immunohistochemistry of phosphorylated neurofilaments. Glutamate receptor (GluR) isoform variants flip and flop can exhibit different channel opening kinetics by affecting rates of receptor desensitization—possibly influencing how CAPs respond following IPC. However, our data indicated little regulation of GluR mRNA transcript isoforms. We did however observe upregulation of several interferon-stimulated genes (ISGs) following IPC in optic nerve, which may contribute to the mechanism by which IPC can mitigate the injurious effects of stroke. Studying IPC and stroke pathophysiology in the optic nerve may lead to potential therapeutics specific to white matter.

POSTER SESSION 4

MGH 241, Easel 160

4:15 PM to 5:45 PM

The Role of Interferon Signaling in Microglia in Ischemic Preconditioning-Mediated Neuroprotection

Erwin Lanier Odongo, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Jonathan Weinstein, Neurology
Mentor: Shahani Noor, Neurology

Ischemic preconditioning (IPC) refers to the neuroprotective phenomenon in which a brief ischemic exposure in the brain increases resistance to injurious effects of subsequent prolonged ischemia. Ischemia is the restriction of blood to tissues. Interferons (IFNs) are cytokines released during ischemic exposure to activate the Interferon alpha receptor (IFNAR) complex and trigger the transcription of interferon stimulated genes (ISG). ISG gene products may contribute to IPC-mediated neuroprotection. However, IFN receptors are expressed on myeloid cells as well as neural cells; the cell type-specific contribution of IFN signaling in IPC is not established. Microglia are the resident immune cells in the brain and are central in the inflammatory responses to stroke. Data from our lab strongly suggest that microglia exposed to ischemia both *in vitro* or *in vivo* demonstrate a robust expression of ISGs. We hypothesize that expression of IFNAR1 and intact IFN signaling on microglia is required for IPC mediated neuroprotection. Thus, the administration of IFNβ prior to stroke will mimic IPC in the wild type (WT) but not in myeloid cell targeted IFNAR1 (Cre/loxP) knockdown mice. We obtained IFNAR1fl/fl strains through collaboration and purchased LysM-Cre. We then used selective breeding and PCR genomic strategies to generate mice that are combined for homozygous IFNAR1fl/fl and heterozygous for LysM-Cre and wild type (WT) genes. We are also currently optimizing our method to administer IFN β using a stereotactic injection system through the lateral ventricle of the brain. We have recently confirmed the appropriate positioning of the intracerebroventicular (ICV) injection and plan to carry out ICV injection of IFN β on the WT and IFNAR1fl/fl /LysM-Cre strains. After inducing stroke, we will quantify and compare the infarct volume and neurobehavioral outcomes. This study will demonstrate the cell-type specific contribution of microglia in IFN-mediated neuroprotection. If successful, our findings can be used to develop pharmaceutical therapies for stroke and gene therapy remedies.