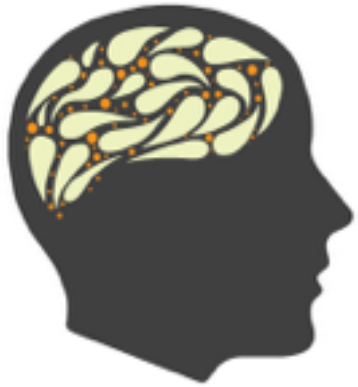

MSU NEUROSCIENCE RESEARCH FORUM

Hosted by the MSU Neuroscience Club and Neuroscience Program

September 30th, 2015

POSTER PRESENTATIONS	**5:30 - 7:00 PM**	**BPS Atrium**
AWARD RECEPTION	**7:00 - 7:30 PM**	**1420 BPS**

PRESENTER	POSTER #
Al-Qadi, Anisah	15
Batchelor, Hannah	17
Benjamin, Cassie	11
Bruce, Kate (Visual Agnosia Team)	16
Bubenheimer, Rebecca	1
Chupka, Nadia	6
Fisher, Courtney	9
Formiller, Erin	10
Kechner, Megan	8
Mecca, Andrew	14
Nwanze, Chiadika	4
Nwanze, Chiadika	5
O'Mara, Alana	13
Rathod, Sagar	2
Salatino, Joseph	12
Salvi, Jordan	3
Smith, Trevor	7

POSTER TITLES AND ABSTRACTS:

1. The role of Sirtuin-3 in regulation of oxidative stress in the enteric nervous system

Rebecca Bubenheimer

Gut inflammation causes changes to the enteric nervous system (ENS) that contribute to gut motility disorders. High oxidative stress during inflammation is one factor that significantly contributes to ENS dysfunction but the mechanisms that regulate oxidative stress in the ENS are unclear. Sirtuin-3 (SIRT3) was recently identified as a key regulator of oxidative stress in the central nervous system. We hypothesized that SIRT3 plays an important role in the regulation of oxidative stress in the healthy gut and during intestinal inflammation. We tested our hypothesis by assessing *in vivo* gut function, ENS structure and susceptibility to inflammation in SIRT3 knockout (*Sirt3*$^{-/-}$) mice. We used the dinitrobenzenesulfonic acid (DNBS) model of mouse colitis to study inflammation and assessed ENS structure using immunohistochemistry. Data were analyzed using a two-way ANOVA. Our results show that *Sirt3*$^{-/-}$ mice have similar colonic function as wild type (WT) controls. Likewise, enteric ganglia in *Sirt3*$^{-/-}$ mice contain similar numbers of HuC/D-immunoreactive neurons and a comparable distribution of inhibitory (neuronal nitric oxide synthase, nNOS) and excitatory (calretinin) subsets. We observed a similar extent of neurodegeneration in the myenteric plexus during DNBS colitis in WT (31% decrease; $p<0.01$) and *Sirt3*$^{-/-}$ (26% decrease; $p<0.05$) mice. The loss of SIRT3 also did not affect the susceptibility of nNOS and calretinin neurons to inflammation. Our results suggest that while SIRT3 contributes to the control of oxidative stress in enteric neurons, the loss of SIRT3 does not significantly contribute to inflammatory neuropathy in the gut.

2. The Effect of Calcium on the Homeostasis of Purkinje Neurons in a Mouse Model of Spinocerebellar Ataxia Type 1

Sagar Rathod, Ravi Chopra, Dr. Vikram Shakkotai

Spinocerebellar Ataxia Type 1 (SCA1) is one of nine inherited neurodegenerative diseases caused by the expansion of a CAG trinucleotide repeat encoding a polyglutamine tract. The pathogenesis of the disease is not well understood. The expansion of the CAG trinucleotide repeat causes the degeneration of Purkinje Neurons (PN) in the cerebellar cortex within the brainstem. PN send inhibitory projections to the deep cerebellar nuclei, and constitute the sole output of all motor coordination in the cerebellar cortex. The inositol-1,4,5-triphosphate type 1 receptor (Itpr1) and T-type, voltage-gated calcium channel (Cacna1g) are major intracellular Ca2+-release channel in PN. Calcium-activated potassium channel (Kcnma1) is an intracellular K+-release channel in PN. Past research used Northern Blots and Immunohistochemical staining to illustrate the difference in gene expression of certain genes in PN. To determine the precise correlation between the dysfunction of the genes and the degeneration of PN, the expression of Itpr1, Cacna1g, and Kcnma1 in 2-week-old and 6-month-old Wild-Type (A02) and Mutant (B05) SCA1 mice was measured using qRT-PCR and analyzed using T-Tests. There was no significant change in gene expression between 2-week-old A02 and B05 mice. However, there was significant change in gene expression between 6-month-old A02 and B05 mice. In conclusion, Itpr1, Cacna1g, and Kcnma1 expression

significantly declines as mice with SCA1 age. The degeneration of Purkinje neurons may be directly related to the decreased expression of genes. Currently, Western Blot analysis is being done to discover significant proteins connected to the genes to gain insight into SCA1 pathogenesis.

3. Lord of the Flies: Inducing Aversive Reaction to Sweet Taste Sensation in Drosophila

Jordan Salvi, Orie Shafer, Dylan Miller, Greg Gage

The goal of this research is to perform conditioning using optogenetics on the fruit fly, Drosophila melanogaster using low cost, open source, and widely available tools and methods such as 3D printing and off the shelf electronic component. This is in an effort to bring down the barrier to entry for optogenetics experiments, a key part of the future of neuroscience. The experiments utilized flies with the gr64 sweet taste receptor coupled with red light excitatory optogenetic channels. Through light stimulation combined with presentation of a taste stimulus, the flies are anticipated to be conditioned to be less aversive to bitter stimuli vs control flies. We designed circuits and software to detect the electric connection between a fly and taste stimulus, and trigger an LED to flash, activating the optogenetic channels in the fly?s taste neurons. The software handles variables such as LED delay and duration, as well as controls a motorized micromanipulator to deliver the stimulus with greater precision and reliability. This setup allows a wide range of optogenetics experiments to be performed efficiently and at low cost, opening up the field for students and researchers with lower budgets to perform these and similar experiments on fruit flies.

4. Motor Control in Clinical Populations: Activity and motor unit recruitment in seven proximal lower limb muscles

Chiadika Nwanze, Annette Pantall
Michigan State University College of Osteopathic Medicine

Slope walking is associated with increased instability particularly in the mediolateral plane and thus a greater risk of falling for all age groups. To compensate for this instability, motor units employ different task-specific strategies. A previous study (Lay et al., 2007) reported slope specific changes in EMG amplitude of 8 lower limb muscles. Slope dependent frequency changes have also been reported in a feline model (Hodson-Tole et al. 2012) which may indicate patterns of motor unit recruitment, with lower frequencies corresponding to smaller motor units. This study investigated intensity and frequency of human surface electromyography (EMG) in seven proximal lower limb muscles when walking on level and inclined surfaces (5o). It was hypothesized that different patterns of EMG frequency would be observed for downslope, level and upslope walking. Furthermore, due to the increased instability associated with slope walking, it was expected that there would be a higher variability in EMG amplitude during slope walking especially for muscles in the mediolateral plane (adductor magnus (AM) & tensor fasciae latae). All muscles showed significant differences from level to slope walking for both frequency and intensity. Frequency was greatest for upslope at the beginning of stance which generally corresponded with greatest intensity. Repeatability in activation patterns across muscles was lowest for downslope walking and across all conditions was lowest for AM. Results indicate different motor programs are

used for slope walking and there is higher stability in motor control during level and upslope walking compared to downslope walking.

5. Developing a T Cell Receptor null Jurkat cell line for testing effector functions of HIV-specific T cell receptors from elite controllers

Chiadika Nwanze, Priya Jani, Pedro Lamothe, Bruce Walker
Ragon Institute of MGH, MIT & Harvard

The selection of particular T cell receptors (TCRs) is associated with better anti retroviral function in HIV infection in a particular subset of HIV-infected individuals (Chen et al 2012). This subset of patients, termed elite controllers, have provided valuable insight into mechanisms of durable HIV control. This project focused on knocking out the T cell receptor-encoding gene and confirming the knockout of this gene in a Jurkat cell line, an immortalized line of human T lymphocyte cells. Upon confirmation of the knockout of the TCR-encoding gene, we will lentivirally transduce the gene-edited Jurkat cells to express recombinant TCR targeting immunodominant HIV Gag epitopes. Effector functions of these TCR clonotypes will then be analyzed to investigate the mechanisms of HIV control in elite controllers. TCR expression was disrupted using CRISPR/Cas9 RNA guided nucleases. PCR sequencing showed that five of the thirty candidates possessed frameshift mutations considered sufficiently disruptive to alter surface expression of the TCR on the Jurkat cells. These five samples will be transduced to express recombinant TCR from elite controllers targeting immunodominant HIV Gag peptides. Results could indicate potential therapies for T cell recovery in HIV infection.

6. Resting State Networks in Children with Temporal Lobe Epilepsy

Nadia M. Chupka, Joshua S. Shimony, Avi Snyder, Manish Shah, and Matt Smyth

Temporal Lobe Epilepsy (TLE) is a debilitating brain disorder that accounts for nearly sixty percent of all epilepsy cases. Symptoms vary in severity among those suffering from TLE. However, many people experience severe seizures and are often forced to resort to surgery for alleviation of symptoms. We sought to further understand TLE by employing fMRI techniques to analyze resting state networks in those afflicted. All major resting state networks and their connections were significantly disrupted in TLE patients compared to those of healthy controls, as previously demonstrated in the literature. When right focal TLE and left focal TLE were compared to each other, trending differences in the connections between the Default Mode Network and the Dorsal Attention Network were found. After further analysis, the connection between the amygdala and the right hippocampus was also observed as markedly different in right and left TLE. These identified differences could potentially aid in the process of determining epileptic foci in future TLE patients prior to surgery. With this additive measure, both physicians and patients may benefit from heightened confidence in their pre-surgical decisions.

7. Sexual dimorphism of neural response in moth antennae.

Trevor Smith, Dylan Miller, Greg Gage

The domesticated silkworm moth (Bombyx mori) use pheromone specific receptors in their antennae sensilla hair to find potential mates up to 11 kilometers away. The female moth excretes the pheromone bombykol to attract potential male suitors, whose antennae receptors contain more sensitive pheromone receptors than their female counterparts. The ability to detect the pheromone bombykol comes from very specific odor detecting cells that make up the majority of the sensilla hair on the antenna. I have evaluated responses to powerful odors such as lemon oil and peppermint extract in comparison to the pheromone bombykol. I recorded responses with a modified version of the Backyard Brains SpikerBoxes and Arduino microcontroller mounted SpikerShields. I tracked both individual action potential units and broad receptor potentials using well-documented differential electroantennogram (EAG) recording methods to isolate the actions specifically in Bombyx mori antennae. Antennae respond to an array of stimuli, such as mechanical stimulation as well as chemical stimulation, which can be compared on the basis of size and length of both action potential units and receptor potentials. Delivery of odor stimulus is delivered through a constant air stream and using Arduino controlled solenoids for controllable and repeatable results. This study provides evidence that the receptors for the pheromone bombykol react with much more robust and unique action potential units and receptor potentials characteristics than any other potential stimulus, and a low-cost and open source means of investigating and educating about an integral sensory apparatus for many organisms, the antenna.

8. Induction of dFosB following Physical and Emotional Stress

Megan Kechner, Darlyn Caraballo-Perez, and Michelle Mazei-Robison

Depression is a devastating disease and the underlying cellular mechanisms are not well understood. To study this, we have employed physical (PS) and emotional (ES) chronic social defeat stress as mouse models of depression. In ES, mice do not receive any physical stress, but witness physical subordination of another mouse. ES has been shown to produce many of the same depressive-like behaviors as PS. Exposure to PS has been shown to promote differences in dFosB induction in multiple brain regions including the nucleus accumbens (NAc); a region known to play a significant role in motivation, pleasure and reward. With this in mind, we sought to investigate if the induction of dFosB was similar between PS and ES. Eight-week-old c57BL/6J male mice were exposed to either PS or ES for 5 minutes per day for 10 days. PS mice were placed into the home cage of a CD-1 aggressor mouse, and ES mice were placed into the same cage, but were physically separated from the CD-1 and PS mouse by a perforated Plexiglas partition. One-hour following social interaction testing on day 11, mice were perfused and brains were post-fixed and cryoprotected. Brains were then sectioned and immunohistochemistry was performed for dFosB. FosB-positive cells were counted in multiple brain regions including NAc, dorsal and ventral hippocampus, prefrontal cortex and ventral tegmental area to assess whether PS and ES induce a similar pattern of induction. This work could identify brain regions important for depressive behaviors to focus on in future studies.

9. Treatment with TPPU Improves Cognitive Function in Hypertensive Rats with Bilateral Common Carotid Artery Stenosis

Courtney Fisher, Nusrat Matin, Anne M. Dorrance

Soluble expoxide hydrolase (sEH) converts epoxyeicosatrienoic acids (EETs), arachiodonic acid metabolites produced by cytochrome 450 enzymes, into less metabolically active compounds. sEH inhibitors have been proposed to have vascular and neural protective effects in middle cerebral artery occlusion models. We hypothesized that treatment of stroke prone spontaneously hypertensive rats (SHRSPs) with bilateral common carotid artery stenosis (BCAS) with the sEH inhibitor, trifluoromethoxyphenyl-3 (1propionylpiperidin-4-yl) urea (TPPU), would alleviate cognitive dysfunction. Data are shown as mean ± SEM, WKY vs WKY+BCAS, SHRSP vs SHRSP+BCAS and vehicle vs TPPU. After 7 weeks of BCAS, short-term memory, assessed by novel object recognition testing was impaired in both WKY rats (novel exploration quotient: 0.57 ± 0.04 vs 0.44 ± 0.04, Student's t-test $p<0.05$) and SHRSP (0.58 ± 0.05 vs 0.46 ± 0.04). TPPU treated rats showed improved short-term memory (0.5 ± 0.06 vs 0.06 ± 0.04). RNA extracted from the brain was reverse transcribed and qRT-PCR was used to assess the mRNA levels of the neuronal markers doublecortin and uncoupling protein 2 (UCP-2), and sEH. Data were expressed as fold change from control (housekeeping gene beta-2 microglobulin) ± SEM. Doublecortin, sEH, and UCP-2 all were upregulated in TPPU treated SHRSPs compared to vehicle treated (2.63 ± 0.53 vs 1.00 ± 0.00; 2.35 ± 0.44 vs 1.04 ± 0.02; 1.85 ± 0.37 vs 1.01 ± 0.00, unpaired Student's t-test $p<0.05$). These data suggest that TPPU improves short-term memory after BCAS and upregulates neural protective genes doublecortin and UCP-2.

10. Transient receptor potential A1 channel (TRPA1) as a Probable Mediator of Methylmercury (MeHg)-induced Extracellular Calcium (Ca2+e)-Dependent Cytotoxicity in Mouse Dorsal Root Ganglia (DRG) Primary Cultures

E.L. Formiller, H.E. Hannon and W.D. Atchison

MeHg is an environmental toxicant that disrupts neuronal function, causing distal paresthesia, visual deficits, and ataxia. MeHg increases intracellular calcium ([Ca2+]i) in a biphasic manner; this effect contributes to MeHg-induced cytotoxicity. To examine the extent to which Ca2+e contributes to decreased viability in MeHg toxicity of DRG, we exposed primary mouse DRG to MeHg in the presence and absence of Ca2+e. Cells were exposed to MeHg (200 nM-2 μM) in Hepes Buffered Saline (HBS) for 30 min; viability was assessed 1or 4 hrs later. For experiments performed in the absence of Ca2+e, Ca2+ was excluded from the HBS and EGTA was added to chelate trace amounts of Ca2+. DRG viability was both [MeHg]- and time-dependent in standard HBS. With the removal of Ca2+e, [MeHg]-dependence remained at only high [MeHg] and time-dependence was lost. The reduction in DRG viability at 1 hr was Ca2+e-independent, whereas viability at 4 hrs was Ca2+e-dependent. These results suggest Ca2+ influx through MeHg-susceptible channels contributes to MeHg-induced cytotoxicity at late timepoints. We examined the role of TRPA1, Ca2+-permeable cation channel highly expressed in sensory neurons, in mediating MeHg-induced cytotoxicity; cells were treated with channel blocker A-967079 before MeHg exposure and viability assessment. Blocking TRPA1 markedly improved cell viability at 4 hrs, with viabilities comparable to Ca2+e-free conditions. These results suggest that Ca2+ influx through TRPA1 contributes to MeHg-cytotoxicity. Supported by NIH R01ES03299, NIEHS R25ES025060 and MSU College of Veterinary Medicine.

11. Sex Differences in Animal Models of Posttraumatic Stress Disorder (PTSD)

R. Cassie Benjamin, A.E. Pooley, S.M. Breedlove, C.L. Jordan

Women are more than twice as likely to develop PTSD as men; however, the reasons for this sex difference are not well understood. One possible reason for this disparity is underlying differences in the neurobiological mechanisms mediating the response to traumatic stress in men and women. Previous research from our lab using single prolonged stress (SPS), a validated PTSD animal model, indicated that female rats do not respond to SPS with the same behavioral, physiological, or cellular phenotype established in male rats. It is clear that males and females respond to stress differently, but whether these sex-specific responses are related to the characteristics of the stressor itself is not clear. To address this concern, we used another validated animal model of PTSD, the predator exposure (PredEx) model, in which the traumatic stressor is exposure to a cat. Using this model and same outcome measures that were used in our SPS study, we found that both models yielded the same pattern of sex-specific results, indicating that this sex difference is a result of traumatic stress in general, not one particular model or stressor. To our knowledge, this is the first study to examine PredEx in female rats.

12. Local Changes in Expression of Markers of Excitability in Brain Tissue Surrounding Neuroprostheses

Joseph W. Salatino, Demetrius R. Moncrease, Matthew E. Sass, and Erin K. Purcell

A neuroprosthesis is a device that records or stimulates electrical activity in neurons. Recent advances in these devices have demonstrated tremendous potential for treating degenerative disorders and external insults afflicting the nervous system, with applications ranging from the restoration of motor function in victims of paralysis to the elimination of debilitative tremors in patients suffering from Parkinson's disease. However, the integration of these devices with surrounding brain tissue remains suboptimal, reducing their longevity and efficacy. Previous studies have documented local neuronal loss around the device as well as reactive gliosis which isolates the device from the surrounding tissue. Here, we expand on these observations by investigating alterations in markers related to intrinsic excitability of the remaining neurons and the balance of excitatory and inhibitory synaptic drive to the region local to the device. The specific markers probed included presynaptic excitatory and inhibitory markers as well as sodium channel expression as indicators of excitability, and effects were assessed as a function of post-implantation time and distance from the device surface.

By studying the impact of device implantation on markers of excitability local to neuroprostheses, we provide an initial report of the impact of neuroprosthesis implantation on the function of neurons remaining at the tissue-device interface. The tissue remodeling observed here may imply a role for plasticity in the signal loss and instability frequently observed in neural recordings obtained from chronically implanted neuroprostheses. The basic science understanding gained may inform new strategies for intervening in the tissue response to neuroprostheses to improve long-term device function.

13. Etiology of Parkinson's disease starting with Alpha-synuclein in the Gastrointestinal System

O'Mara, A., Benskey, MJ., Kuhn, NC., Manfredsson, FP.

Parkinson's disease (PD) is the second most common neurodegenerative disease with marked motor symptoms. Additionally, Parkinson's disease patients experience many symptoms involving the gastrointestinal track including constipation and drooling. The involvement of the gastrointestinal track, specifically within the enteric nervous system, has been of high interest to researchers. Within the gastrointestinal track, early on accumulations of alpha-synuclein (α-syn), known as Lewy Bodies, begin to appear years before motor symptoms arise. In the central nervous system, Lewy Bodies also appear, again after those seen in the enteric nervous system. Currently, little is known about the role of α-syn, yet many studies believe that the cytotoxicity seen in PD is thought to be caused by aggregated α-syn. Our study aimed to elucidate whether exogenous forms of aggregated α-syn were able to travel from the enteric nervous system neurons to the central nervous system, there causing the characteristic PD pathology. We injected exogenous aggregated α-syn, known as pre-formed fibrils, into the colon of rats. After 1, 6, and 12 months, rats were sacrificed; their brains were postfixed and guts were dissected. We used immunohistochemistry to scan for α-syn aggregates in the brains. We found no markers of aggregated α-syn in the brains of the rats in comparison with our control groups (saline and monomeric α-syn injected rats). Additionally, there was no obvious α-syn aggregation detected in the enteric nervous system. While our results were inconclusive, further research is necessary to elucidate the gastric system's response to α-syn in order to better manage the severe gastrointestinal symptoms for PD patients.

14. Corticotropin-releasing hormone (CRH) expression and serotonin signaling is increased in an animal model of visceral hypersensitivity.

Mecca A, El-Ayache N, Galligan J

Corticotropin releasing hormone (CRH) is an important mediator of the neuroendocrine stress response in many mammals, and has been implicated in stress-induced behavioral disorders such as anxiety and depression. Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder (FGID) that is often associated with stress-related behavioral disorders, which suggests dysfunctions in the neural circuitry between the brain and the gut. Both serotonin (5-HT) and CRH signaling may be altered in IBS patients. We studied serotonin transporter (SERT) gene knockout rats to determine if there is a difference in CRH protein expression compared to wildtype (WT) controls at the level of sensory afferent neurons. CRH protein expression in sensory neurons of SERT KO and wildtype female rats was visualized in lumbar and sacral dorsal root ganglia (DRG) using immunohistochemistry techniques. We found that female SERT KO rats display increased CRH protein expression in sensory neurons compared to female wildtypes, and increased CRH in small-diameter neurons may play a role in visceral hypersensitivity; an abdominal pain symptom characteristic of IBS.

15. Changes in muscle fiber type in plantar flexors after transection and repair of feline soleus and lateral gastrocnemius nerves

Anisah Al-Qadi, Saiishitha Nalamolu, Hannah Weatherford, Annette Pantall

Skeletal muscles have different proportions of muscle fiber types, which is partly due to the innervation patterns. Few studies have examined long-term changes in muscle fiber configuration due to impaired innervation. The aim of this study was to determine changes in the slow I and fast IIA populations in self reinnervated soleus (SO) and lateral gastrocnemius

(LG) muscles and their synergists, medial gastrocnemius (MG) and plantaris (PL) muscles in the cat. Muscles SO, LG, MG and PL were harvested from both hindlimbs during terminal surgery in 4 cats and frozen at -80C approximately 10 months after transection-repair of the nerve to right SO and LG. Samples were cut and labeled with monoclonal antibodies BA-F8, BF-35 and 2F7 (DHSB, Iowa) specific for myosin heavy chain (MHC) I, I and IIA, IIA, respectively (Unguez et al. 1996; Hyatt et al. 2010). Photos were taken and the tagged cells counted at 4x. In self-reinnervated muscles, significant changes in fiber type populations were found. The proportion of type IIB/IIX fibers (negative for BF-35) generally decreased for LG, MG and PL. Another sign of damage was cell atrophy. Ten months post-surgery there remained an increase in IIA fibers in SO and in I fibers in LG, injury, suggesting inappropriate reinnervation.