SCHOOL OF MEDICINE & DENTISTRY
UNIVERSITY of ROCHESTER MEDICAL CENTER

2024 PREP Symposium

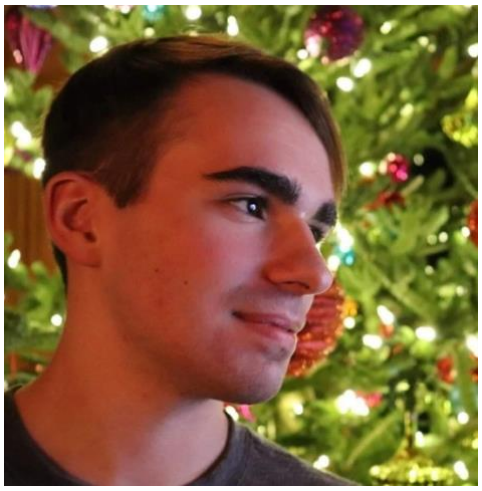
June 11, 2024

Program Directors

Jacques Robert, PhD & Elaine M. Smolock, PhD

Presentations

Time	Name	Title; *Advisor*
1:00pm-1:15pm		**Introduction**
1:15pm-1:30pm	**Hunter Houseman-Eddings**	**Searching for Poxvirus Restriction Factors** ***Advisor: Brian Ward, PhD***
1:30pm-1:45pm	**Alesandra Martin**	**Examining the Interplay of Spatial and Feature-based Attention in Modulating Visual Neurons** ***Advisor: Farran Briggs, PhD***
1:45pm-2:00pm	**Jacob Morales**	**Beneficial Off-Target Effects of Verapamil in a Non-Conducting DHPR Mouse Model** ***Advisor: John Lueck, PhD***
2:00pm-2:15pm	**Lily Mussallem**	**Potential Effects of Microbiome Extracellular Vesicles on Myeloid-derived Suppressor Cells** ***Advisor: Minsoo Kim, PhD***
2:15pm-2:30pm	**Evelyn Pineda**	**Using Virtual Reality to Study How Trauma Exposures in Childhood and Adulthood Affect Learning and Attention in Threatening Environments** ***Advisor: Benjamin Suarez-Jimenez, PhD***
2:30pm-2:45pm	**Pavel Rjabtsenkov**	**Detecting Heart Rate Using a Virtual Reality Headset and its Head Representation in Three-dimensional Space** ***Advisors: Cristiano Tapparello, PhD***
2:45pm-3:00pm	**Maeve Sheehy**	**Locus Coeruleus Orexin Modulation** ***Advisor: Lauren Hablitz, PhD***
3:00pm – 4:00pm		**Reception in the Microbiology Break Room** ***2nd floor MRBX***



Searching for Poxvirus Restriction Factors

Hunter Houseman-Eddings and Brian Ward

The host's innate immune system is an early line of defense against infection. An important component of this is the interferon response, which induces expression of a wide array of interferon-stimulated genes upon sensing an infection. These interferon-stimulated genes can have specific or broad antiviral activity through a wide range of mechanisms. Many viruses have evolved mechanisms of avoiding and antagonizing the interferon pathway and its effectors, including poxviruses. Poxviruses represent a major public health concern, as exemplified by the 2022 mpox epidemic. Poxviruses are large, double-stranded DNA viruses that encode approximately 200 genes, which are expressed in three distinct temporal stages: early, intermediate, and late. Many early genes have been shown to directly antagonize both innate and adaptive immune responses. This makes poxviruses an excellent tool to uncover host restriction factors. An siRNA screen was performed to identify interferon-stimulated genes that altered each stage of vaccinia virus gene expression. Through this screen, we have identified several candidate restriction factors with a strong impact on poxvirus gene expression. One of these genes is GLIPR2, a protein with described functions in regulating autophagy and TLR4 signaling. To further validate and characterize the impact of GLIPR2 on vaccinia virus infection, we generated a GLIPR2 knockout cell line. Using this model, we show that loss of GLIPR2 increases viral gene expression. Preliminary data also show an increase in production of infectious virions. Work is ongoing to further characterize the effect of GLIPR2 on vaccinia virus infection.

Examining the Interplay of Spatial and Feature-based Attention in Modulating Visual Neurons

Alesandra Martin and Farran Briggs

Visual attention refers to the brain's process of selecting the most pertinent aspects of a visual scene according to the current task demands. Attention modulates activity throughout the entire visual system by selectively altering activity in individual neurons and circuits. The neuronal mechanisms underlying visual attention effects remains unclear. However, several models propose candidate mechanisms by which attention changes the activity of individual neurons. One such model is the feature selectivity model (Martinez-Trujillo and Treue, 2004), which suggests that feature-based attention modulates neuronal activity in a manner that complements known effects by visual spatial attention. Our study tested the feature selectivity model to examine the unique contributions of feature-based attention in modulating neuronal activity by observing single-unit recordings from the behaving macaque monkey. We hypothesized that attentional modulation of neuronal activity (facilitation or suppression) can be predicted by the match between the neuron's feature tuning and the specific feature being attended to. To explore this, we utilized electrophysiological recordings to track neuronal activity in response to drifting gratings varying across multiple feature dimensions, independent of the attention tasks. Additionally, we recorded from the same neurons while monkeys perform three different versions of a feature discrimination task utilizing a Posner cueing paradigm, such that spatial attention is held constant across all tasks. Monkeys were required to attend to the color, contrast, or orientation of the stimulus and report a change with an eye movement. We observed that neurons were facilitated on tasks requiring attention to the preferred stimulus feature and suppression on tasks requiring attention to the non-preferred feature. These results further support the feature selectivity model and provide more insight into the way visual neurons are modulated by attention.

Beneficial Off-Target Effects of Verapamil in a Non-Conducting DHPR Mouse Model

Jacob Morales and John Lueck

Myotonic Dystrophy type-1 (DM1) is an autosomal dominant, progressive disease, and the systemic clinical presentations of DM1 are variable and wide ranging. Despite its variability, approximately 60% of cases of early mortality in DM1 are due to respiratory failure. This may be due to atrophy and weakness of crucial respiratory muscles; however, the mechanisms of skeletal muscle weakness and wasting are not clear in DM1. In DM1, the genetic mechanism is a genetic lesion that leads to aberrant splicing of transcript throughout the whole body. In skeletal muscle, excitation contraction coupling is altered, with many key transcripts being aberrantly spliced and the downstream protein products having altered function. Of the many proteins impacted in the process, it was recently found that the combination of myotonia, the namesake delay in muscle relation seen in DM, and DM1 isoform of the dihydropyridine receptor (DHPR), a voltage-gated calcium channel which gives a gain of function, is synthetically lethal in mice. Beyond this, these mice failed to thrive, had prolonged myotonia and time of righting reflex, severe transient and generalized muscle weakness, and altered respiration. To test if blocking this gain of function in the DHPR had therapeutic benefit in these mice, we chose verapamil, an FDA approved calcium channel blocker. Although verapamil significantly improved survival, transient weakness, and myotonia in mouse models expressing DM1, there may be beneficial off-target effects associated with its efficacy. To further investigate the direct impact of verapamil blocking calcium conduction through DHPR and its unknown indirect impact, we used CRISPR-cas9 to generate a mouse model that expresses a non-conducting DHPR (ncDHPR) isoform. Any improvement seen in muscle function of the genetically altered mice after verapamil treatment would demonstrate the presence of off-target effects. To measure this, we isolated extensor digitorum longus (EDL) muscles from ncDHPR and wild-type (WT) mice, then harvested them for *in vitro* muscle contraction. We found transient weakness and myotonia were significantly reduced in ncDHPR EDL muscle treated with verapamil; thereby revealing the presence of its off-target effects. In a separate study, we investigated the long-term impact of high doses of verapamil (200 mg/kg/day) on WT and ClC-$1^{-/-}$ homozygous mice. At weaning, we weighed and measured the time of righting response (TRR) of the two lines of mice weekly for 30-weeks. In ClC-$1^{-/-}$ mice treated with verapamil, we found that body weight and TRR were similar to WT mice. As expected, untreated $ClC^{-/-}$ mice had greatly increased TRR and decreased body weight when compared to WT mice. By deepening our understanding of how verapamil beneficially impacts skeletal muscle function, we hope to reveal DHPR as a therapeutically relevant target or unveil another target for the future treatment of transient weakness and myotonia.

Potential Effects of Microbiome Extracellular Vesicles on Myeloid-derived Suppressor Cells

Lily Mussallem and Minsoo Kim

Neutrophils are the most abundant type of white blood cells in the human body and serve as the first line of defense against invading pathogens. However, in response to the cancer microenvironment, neutrophils can transform into myeloid-derived suppressor cells (MDSCs), which exhibit immunosuppressive activity. The presence of MDSCs is often associated with negative prognosis and outcomes for cancer patients, but the mechanisms that trigger MDSC differentiation are not fully understood. The microbiome and its products have been linked to cancer progression, often due to gut dysbiosis caused by cancer treatments. However, there is a gap in research regarding the role of the microbiome in a steady state. We hypothesize that microbiome-derived extracellular vesicles that cross the gut barrier into circulation can influence neutrophil heterogeneity and lead to distinct differentiation of neutrophils into MDSCs in cancer microenvironments. To test this, we used *ex vivo* mouse models of microbiome training. We found that naïve bone marrow neutrophils from microbiome-free mice and microbiome-induced mice did not exhibit heterogeneous responses to the cancer microenvironment, as expected. However, in circulating neutrophils, we observed higher levels of activation and arginase production in microbiome-trained mice in response to cancer microenvironments. Overall, this suggests that the microbiome may play a role in neutrophil differentiation into MDSCs. Studies are ongoing to determine the specific effects of microbiome training on MDSC development *ex vivo.*

Using Virtual Reality to Study How Trauma Exposures in Childhood and Adulthood Affect Learning and Attention in Threatening Environments

Evelyn Pineda and Benjamin Suarez-Jimenez

Trauma exposure at any life stage negatively affects one's mental, emotional, and physical health. Additionally, children with a history of trauma sometimes have difficulty learning and maintaining attention. However, little is known about how the life stage in which one experiences trauma affects learning and attention in a threatening environment. We used a virtual reality (VR) task to investigate how trauma exposures in childhood and adulthood affect learning and attention in a threatening environment. Participants include individuals with no exposure to trauma (trauma naïve; TN); trauma-exposure in childhood (TE-C); and trauma exposure in adulthood (TE-A). We hypothesized that in a threatening environment, 1) TNs would exhibit higher learning and lower attentional bias toward threatening cues than participants exposed to trauma, and 2) TE-Cs would perform worse on learning than TE-As. Before and after the VR Flower Task (VRFT), participants completed an eye-tracking task to assess attentional bias. In the VRFT, participants picked flowers (n=80; CS) in a circular environment. Picking the flowers in one half would result in a shock (threat; CS+) with a 50% reinforcement rate; flowers in the other half would never result in a shock (CS-). Once the participant approached a flower, they were asked to rate how likely (0-9) they were to receive a shock and their skin-conductance level was collected using electrodes. We also collected their skin-conductance response throughout the task. Post-task, participants answered questions about the task to assess if they had been successful at threat learning. This research intends to highlight the cognitive effects of trauma at a more individual level, especially during childhood. Investigating how trauma impacts cognitive processes in adulthood can inform mental health professionals and lead to improved interventions and treatments for trauma exposed populations.

Detecting Heart Rate Using a Virtual Reality Headset and its Head Representation in Three-dimensional Space

Pavel Rjabtsenkov and Cristiano Tapparello

As virtual reality (VR) devices become ubiquitous in healthcare, the ability to monitor a patient's heart rate without additional equipment, training, or supervision may diversify clinical VR applications, improve accessibility, and cut staffing costs. Previous research has already shown how heart rate can be inferred from head motion data taken directly from motion sensors in wearable devices. However, no research has been able to account for the motion inherent to wearing VR headsets. This work is the first work to investigate the possibility of inferring heart rate from the virtual representation of the patient's head in three-dimensional (3D) space without restricting the VR headset wearer's motion. We asked ten healthy participants to wear a Oculus Quest VR headset, stay seated, and follow a guided mindfulness exercise. Heart rate inferred from a participant's virtual head representation was compared to the heart rate inferred from motion sensor data and photoplethysmography (PPG) readings. We found traces of cardiovascular signal in motion data collected using VR headset. However, the signal was insufficient to accurately deduce a participant's heart rate. Heart rate inference using motion sensors directly approximated the heart rate readings using PPG. However, compared with the heart rate estimation obtained from the motion sensors, VR headset's virtual representation of a wearer's head was from 2 to 4 times less accurate and practically unusable. Therefore, we advise using the data directly from the motion sensors or a third-party device to assess a wearer's heart rate

Locus Coeruleus Orexin Modulation

Maeve Sheehy and Laruen Hablitz

Tools that offer greater spacial-temporal resolution for the detection of neuropeptides are especially helpful when they can be used in the context of complex behaviors *in vivo.* G protein coupled receptor activation-based (GRAB) sensors have been developed for this purpose[i] Orexin (also known as hypocretin) is an endogenous neuropeptide best known for its function in promoting and maintaining wakefulness.[ii] There is a gap in the literature when it comes to *in vivo* measurement of Orexin, particularly surrounding one of its strongest projection sites, the Locus Coeruleus (LC). Over the course of my time in the PREP program I have attempted to validate a previously unpublished Orexin-2 receptor specific GRAB sensor for future use in the LC during complex behaviors.

[1] Sun et al. 2018
[1] Xia et al. 2023

Thank you to everyone involved in URMC-PREP!

Mentors, Bench Mentors, & Committee Members

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