PI: Yama Akbari, MD, PhD
Co-I: Michael Rochon-Duck, MD

Brain-heart connections during cardiac arrest for early-stage prognosis and treatments to improve outcome

Spreading depolarization (SD) is a massive release of ions and energy that travels across the brain surface and is detectable using electrical recordings. SD is sometimes called a "brain tsunami," alluding to the fact that they are the largest and most powerful brain waves detected. The most common causes of SD include migraine auras (sensory disturbances that can precede the headache), seizures, traumatic brain injuries (TBI), strokes, and cardiac arrest (CA) which starves the brain of oxygen. Understanding SD is important to physicians because SD can cause brain tissue to swell and release toxic chemicals that can trigger the death of neurons.

While previous investigations have looked at how SD affects brain tissue directly, we want to see if SD can also have impacts on organ systems beyond the brain. Using a rat model of CA, our lab was the first to show SD may change the rate at which blood pressure drops off as the heart becomes progressively weaker. This may not be as surprising as it appears since the heart and brain are interconnected through a variety of pathways called the autonomic nervous system, the most famous such pathway being the vagus nerve. We suspect that SD alters how the vagus nerve communicates with the heart, perhaps triggering arrhythmias (irregular heart rhythms) that can be detected by monitoring the electrical activity of the heart. Other researchers have found that stimulating the vagus nerve with electrodes can make it harder for SD to happen in the brain, but no one has yet done experiments to see if stopping SDs can also stop arrhythmias in the heart.

Our lab specializes in a rat model of CA and cardiopulmonary resuscitation (CPR), and we want to be the first to test the hypothesis that SD induces arrhythmias due to vagus nerve signaling. We induce CA in a rat by stopping its air supply (done as humanely as possible and approved by veterinarians), which mimics choking, drowning, or drug overdoses in humans. We then will use electrical and optical recordings of brain activity and metabolism, as well as recordings of heart activity and blood pressure. This will allow us to correlate SD events in the brain to arrhythmias in the heart by using computational algorithms to extract detailed statistics. After we establish whether SD is correlated with arrhythmias, we will experimentally block or stimulate the vagus nerve with electrodes or drugs to test this relationship between the heart and brain.

Doing these experiments in animals will tell us what to look for in human patients. In parallel to these animal experiments, we will use a database of brain recordings during CA in the hospital to see if the same signatures of SD and arrhythmia occur simultaneously in patients. Even after successful CPR, most people who have CA are left in comas or have permanent brain damage. Understanding how the brain interacts with the heart during the process of dying may be the first step to allow doctors to develop better, targeted resuscitation methods.

PI: Ralph Clayman, MD
Co-I: Seyed Hossein Hosseini Sharifi, MD
Intraoperative Ureteral Dilation Using Electromotive Drug Administration (EMDA)

Ureteroscopy is a minimally invasive surgical procedure most commonly used to treat stones in the ureters (the tubes that connect the bladder to the kidneys). The limiting factor in ureteroscopy is the small diameter of the ureter and its vulnerability to the external force. Ureteral access sheaths (UAS) are commonly used during ureteroscopy to facilitate the movement of surgical instruments in and out of the ureter. Placing the largest access sheaths available results in the improved success of the procedure; however, it is accomplished in only 50% of ureters. We want to develop a regimen to acutely dilate the ureter intraoperatively, enabling urologists to safely insert larger ureteral access sheaths in the ureter, improve the success rate of surgery, and minimize the risk of ureteral surgery. Muscle relaxant drugs might cause relaxation of the ureter. However, most studies use the oral route of these drugs, which takes upwards of one or two weeks before exerting its maximal effect; administration of these drugs for a week or two may lead to complications and clearly are of no value in urgent cases. We hypothesize that direct intraluminal administration of muscle relaxants into the ureter will allow us to deliver a higher concentration of a drug acutely with fewer complications. An impediment to successful intraluminal drug administration is the ureteral wall itself, which is relatively impermeable. This is not surprising as it would be disadvantageous should the ureter or bladder absorb any waste material excreted by the kidneys. Electromotive Drug Administration (EMDA) is a technique used for enhancing drug delivery to tissues. EMDA provides a low electrical current which increases the penetrance of drugs carrying an electrical charge. We intend to use EMDA for the acute delivery of drugs capable of causing ureteral dilation. If our hypothesis is correct, we would be able to deploy a larger ureteral access sheath and, as a result, remove larger stones from the ureter in a safer and more expedited manner. To evaluate the possibility of acute drug-induced dilation of the ureter, our research team proposes to instill different muscle relaxant drugs into the ureteral lumen while simultaneously applying an electrical current to enhance the drug penetration across the ureteral wall and into the ureteral smooth muscle and, consequently, acute dilation of the ureter. Earlier work in our laboratory has shown that a test molecule, methylene blue, when instilled under active EMDA, penetrated all layers of the ureteral wall. Without EMDA, it just entered the superficial layer of the ureter. The purpose of this study is to examine whether EMDA augments the penetration of muscle relaxant drugs into the ureteral wall and provides immediate ureteral dilation during ureteroscopy.

PI: Oliver Eng, MD
Co-I: Eric Hanse, PhD
Metabolic Phenotype and Development of Novel Therapeutics for Peritoneal Metastases

Given the prevalence of disease that has spread in the abdominal cavity (peritoneum metastases, PM) in patients with metastatic colorectal cancer and the multitude of challenges related to limited treatment modalities, the development of novel therapeutic approaches can impact millions of patients worldwide. Moreover, current therapies targeting PM are restricted to general chemotherapy agents. Here, we propose foundational experiments that will drive future research aimed at targeting the metabolic tumor microenvironment. We hope to demonstrate this treatment will modulate durable changes to the cancer cells which will no longer be adapted to survive in the unique environment of the peritoneum.

PI: Andrej Lupták, PhD
Co-I: Claudia Benavente, PhD
Investigating the role of CPEB3 ribozyme in glioblastoma

This project describes a novel mechanism for expression of tumor suppressing protein using an established lead compound- an antisense oligonucleotide (ASO)- and has a high probability of success. Malignant gliomas are the most common primary brain tumors with a poor prognosis that highlights the clinical need for innovative therapeutic interventions. It is estimated that 25,000 malignant brain tumors are diagnosed in the US annually. Despite significant advances in diagnoses and multimodality therapies incorporating surgical resection, radiotherapy, and chemotherapy, the overall prognosis for patients with glioblastoma multiforme (GBM), remains poor, with a median survival time of 15-18 months. Therefore, there is an unmet medical need to develop alternative treatment strategies to improve clinical outcomes. Next-generation sequencing technologies have significantly expanded our understanding of key molecular and genetic alterations that contribute to gliomas and GBM. Studies have been undertaken to target putative molecular or cellular pathways that are implicated in cancer pathogenesis. Recent advances in genomic analysis have identified molecular biomarkers to tailor disease management by targeting the molecular aberration underlying individual patients' pathogenesis. One of the unique features of ASO technologies is that ASOs can bind to specific genes that are not accessible by small-molecules or antibodies. For example, Danvatirsen (IONOS-STAT3-2.5) was designed to target STAT3, which has been shown to promote tumor growth, and represents one of several targets of ASO therapy developments for cancer treatment. Nucleic acid-based therapies, such as ASOs, can be designed to target specific tumor suppressor gene (TSG) RNA sequences via simple base-pairing to restore their functions, if the targeted sequence inhibits expression of the TSG. By targeting the CPEB3 ribozyme, we will increase CPEB3-based tumor suppressor function.

PI: Bryce Mander, PhD
Co-Is: Elizabeth Thomas, PhD, & Douglas Granger, PhD
Assessment of relationships among sleep quality, memory, and salivary biomarkers of Alzheimer's disease, glial activation, and neurodegeneration biomarkers

There is no cure for Alzheimer's disease, the most common form of dementia. It has been estimated that over 150 million people worldwide will be suffering from Alzheimer's disease by 2050, if there is no progress in current therapeutic or preventative approaches. The personal and economic cost of this condition is staggering, and most of it is paid by the caregiver. A newly discovered risk factor contributing to the development of Alzheimer's disease, sleep disturbance, has received recent attention. Sleep disturbance is readily treatable with currently available methods. However, the complex biological links between sleep disturbance and Alzheimer's disease remains unclear. Understanding these links more fully will aid in the development of targeted, sleep-based interventions to support Alzheimer's disease prevention. One challenge with developing large scale clinical trials to test whether or not sleep interventions are effective is that Alzheimer's disease biomarkers are typically measured using radioactive imaging, spinal taps, or blood sampling. These methods are invasive, and often painful, expensive, inaccessible, difficult to repeat with frequency, or require the availability of medical services, facilities, or personnel to oversee the collection of these biological samples. Some of these biomarkers are also insensitive to change over timescales less than two years, making clinical trials impractical. The current study proposes to examine whether we can measure biologically relevant Alzheimer's disease biomarkers in saliva in humans collected before and after a night of sleep, by comparing these saliva measures to gold standard neuroimaging and fluid biomarkers already collected in our study cohort. If feasible, using saliva samples has a lot of advantages, as it is noninvasive, painless, it can be collected in a person's natural environment without medical support or invasive or expensive equipment, it is easy to collect frequently, and it is affordable. We further seek to determine whether these saliva measures are associated with sleep disturbances that can be targeted by currently available sleep interventions. We plan to next explore whether sleep disturbances that are related to Alzheimer's disease biomarkers in saliva are also associated with how much we forget over a night of sleep. In characterizing these associations, we plan to determine the feasibility of designing a clinical trial focused on improving sleep in older people with sleep disturbances to change Alzheimer's disease biomarkers in saliva in the service of aiding Alzheimer's disease prevention. While various forms of sleep disturbance have been shown in numerous studies to increase Alzheimer's disease risk, there is a lack of clinical trials showing that we can reduce Alzheimer's disease risk by improving sleep. Part of the reason for this is scalability of currently available biomarkers that have shown sensitivity to sleep. This study directly addresses this knowledge gap in support of future work aimed at treating sleep disturbance to prevent Alzheimer's disease.

PI: Farouk Nouizi, PhD
Co-Is: Nadine Abi-Joudeh, MD, & Gultekin Gulsen, PhD
Physiological Monitoring of Transarterial Embolization Outcomes using a dedicated CT-guided Optical Imaging System

Worldwide, primary liver cancer hepatocellular carcinoma (HCC) is the fifth most common solid organ malignancy, leading to more than 700,000 deaths each year. Recent statistics showed that HCC incidence and related mortality rates have doubled in the USA in the past two decades. Transarterial chemoembolization (TACE) is the standard of care for intermediate HCC. TACE is performed based on an opinion piece published by a physician in 1982 without any fundamental translational data. Two studies published in 2002 found TACE to be effective considering the increase in overall patient survival. TACE was then added to the guidelines as standard of care. However, due to lack of understanding of its mechanism of action, it has not been possible to tailor the procedure and improve its therapeutic outcome. Interventional radiologists have attempted several strategies to improve TACE outcomes. One of which is the use of embolic beads loaded with a specific drug. This combination was intended to both: a) block the blood supply to starve the tumor cells (ischemia) and b) achieve a more effective therapeutic effect due to the direct delivery of the drug to the tumor. However, this mechanism presented a discrepancy since blocking the blood supply also resulted in reduction of oxygenation (hypoxia) necessary for the drug performance.

Understanding the basic pathophysiological effects of TACE becomes crucial since it will provide the foundation to finally tailor the procedure and improve its outcomes for patients. Considering the large number of unknowns to be investigated, such studies wouldn't be possible in clinic. Thus, preclinical in vivo studies are needed to improve TACE procedures and finally establish a standard TACE protocol. Currently, BOLD-MRI is the conventional clinical tool for monitoring changes in tumor microenvironment. However, its use in preclinical studies is very limited due to its high-cost, inability to provide real-time measurements during the TACE procedures, and the burden of scheduling required to be in tandem with TACE procedures. Being significantly low-cost and an easily operational alternative in surgery settings, optical molecular imaging techniques are perfectly suitable to undertake this challenging task. We recently developed a dedicated, low-cost, preclinical CT-guided Optical molecular scanner that enables real-time, non-invasive tumor microenvironment monitoring during the TACE procedures in the operating room. Using this novel system, we will investigate the hypothesis that different size beads induce different levels of hypoxia/ischemia in rabbits with liver cancer. We will be using unloaded beads to eliminate the effect of the drug and single out the contribution of the beads' size. We expect that small and large beads will induce different levels of embolization and result in a distinct TACE outcome.

Validation of our preclinical system as a better alternative to BOLD-MRI will enable researchers to investigate different embolic agent-related aspects (size, drug concentration, and radiation dose) to optimize TACE procedures that will be directly translated into the clinic. Indeed, gaining a better understanding of the physiological effects of TACE will allow us to tailor

standard TACE procedures resulting in optimal ischemia/hypoxia. This will consequently limit angiogenesis and significantly improve patients' survival!

PI: Dorota Skowronska-Krawczyk, PhD
RGC protection in glaucoma

Aging, a universal process that affects all cells in an organism, is a major risk factor for a group of neuropathies called glaucoma, where elevated intraocular pressure (IOP) is one of the known stresses affecting the tissue causing retinal ganglion cells (RGC) death and loss of vision. Currently, lowering the IOP is the only clinically proven treatment. Nevertheless, many glaucoma patients continue to progress and lose vision, despite achieving optimal IOP levels under different therapies.

Recently, using our novel model of glaucomatous damage, we have shown that natural aging increases RGCs' susceptibility to stress and that this process is regulated, in part, on an epigenetic level. Aged animals exposed to single, mild (30mmHg), 1-hour IOP elevation respond to stress stronger than young animals, leading to severe tissue damage and pronounced visual function decline.

Interestingly, one of the first pathways induced immediately after IOP elevation in aged animals is an inflammatory response related to Interleukin 1 alpha (IL1alpha) signaling. We hypothesize that this early activation of the IL1alpha pathway has an essential role in retina damage induced by IOP elevation. We propose to use the FDA-approved antagonist of IL1 receptor immediately after the IOP elevation to inhibit the early inflammation and propagation of the stress in the tissue. We propose to inject the drug intravitreally since intravitreal injections are common, ambulatory procedures in Ophthalmology as thousands of wet age-related macular degeneration patients undergo ~monthly intravitreal drug injection (anti-VEGF therapy). If successful, our data can be used to set up foundations for a viable strategy to potentially treat glaucoma.

PI: Lisa Wagar, PhD
Co-I: Douglas Trask, MD, PhD
Tracking immune responses to live-attenuated influenza vaccine in human tonsils

The need for effective vaccines against respiratory pathogens has never been clearer. Respiratory infections are responsible for a large proportion of the world's illnesses and deaths. Influenza viruses are particularly high-risk because they easily acquire mutations that escape our immune response, which can lead to pandemics. Unfortunately, current influenza vaccines are not very effective; they range from 10-60% effective, largely depending on whether the vaccine strains are well-matched to the viruses circulating during a particular flu season. Thus, there is an urgent need for a universal influenza vaccine that elicits robust and persistent immune responses across all ages. One potential strategy to improve influenza vaccination is to stimulate a protective immune response in the upper respiratory tract, since this is the site of influenza infection in humans. There is an FDA-approved intranasal vaccine for influenza; however, we have a poor understanding of how it mediates protection. We suspect that there are tissue-specific events that lead to protection after intranasal vaccination, but it has been understandably difficult to measure correlates of protection in the tissue. Most human vaccine studies are limited to peripheral blood sampling, even though the critical cellular decisions that lead to productive adaptive immune responses occur within lymphoid tissues.

To better investigate the adaptive immune responses that occur in human lymphoid tissues, the Wagar lab uses an in vitro tonsil organoid model. We previously showed that tonsils are a good model to effectively capture the most important features of a protective vaccine response, including stimulation of vaccine-specific B and T cells, affinity maturation of the antibody response, and the ability to secrete protective, virus-neutralizing antibodies. The main novelty of the system is that we can test multiple vaccine candidates on the same individual's cells, thus allowing us to directly compare the types of immune responses that could be elicited from different vaccine formulations, but within the same individual. It is our hope that the platform could be used for high throughput and rapid identification of effective vaccines in the future. A major question that remains to be addressed in the organoid platform is how well the features we observe are replicating what happens during actual intranasal immunization in people. In this project, we will address this question by collecting data about the adaptive immune response in tonsils from adults who were recently immunized with different types of influenza vaccine, including the intranasal formulation. We will use cutting-edge techniques to characterize the antigen-specific response and directly compare these findings to the data already generated using tonsil organoids. Completion of the proposed experiments will 1) allow us to validate the strengths and weaknesses of the tonsil organoid model for testing and comparing vaccines, and 2) identify correlates of protection to guide the design and testing of a broadly cross-reactive universal influenza vaccine.

PI: Oswald Steward, PhD
Co-I: Maya Hatch, PhD
Quantitative assessment of arm movement function for nerve repair surgery prognosis

The deltoid muscle, innervated by the axillary nerve, plays a critical role in raising and extending the arm for activities of daily living and serves as a dynamic stabilizer of the shoulder, particularly when the arm is externally rotated and abducted. Importantly, the deltoid muscle is also critical to compensate for rotator cuff muscle deficiency and is required for any reconstructive procedure to improve shoulder motion and function (such as reverse total shoulder arthroplasty). In a shoulder with deficient rotator cuff integrity, the deltoid can adapt to create new movement patterns to partially compensate for rotator cuff muscle weakness. Accordingly, any loss of function of the deltoid muscle usually produces significant limitations in upper extremity function.

Nerves that control the deltoid muscle are often damaged by trauma (shoulder dislocation) and the nerves also are highly vulnerable to injury during surgical interventions. These nerve injuries are catastrophic for patients as they often result in deltoid weakness with significant, long-lasting functional deficits and disability in activities of daily living. While younger patients with intact rotator cuff musculature may be able to compensate for this permanent deltoid weakness, older patients usually experience substantial functional limitations. As such, optimizing recovery of deltoid function following injury to the nerves that control the deltoid muscle is of paramount importance for shoulder function.

Our collaborator, Dr. Ranjan Gupta, has been developing novel nerve repair procedures that are yielding remarkable results in some patients even long after the nerve injury. The breakthrough came from a combination of basic science and clinical research in which Dr. Gupta and his team have shown that specialized assessments of a muscle biopsy can help predict outcome from nerve repair, potentially identifying which patients will recover substantial function. So far, however, the remarkable recovery has only been documented by physician observations, not by standardized quantitative assessments of upper extremity movement abilities. Here, we will test feasibility of using the "Kinect" system, which can be readily deployed in the clinical setting and can provide a thorough quantitative assessment of patients' arm function in less than 5 minutes with minimal involvement of staff. The ease of use and ready deployment will allow rapid collection of outcome data that may immediately inform surgical management for these devastating injuries. In addition, the Kinect system can also serve as a powerful tool for future studies of therapeutic interventions for nerve and spinal cord injuries that lead to a loss of upper extremity motor function.