SUMMER 2022 INTERSECTIONS

Thursday, August 4
10 a.m. - 12 p.m.
Tinkham Veale University Center, Ballrooms A and B

CASE WESTERN RESERVE UNIVERSITY EST. 1826

Post-Graduate Planning and Experiential Education, SOURCE
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Last Name	First Name	Summer Program	Home University/ School	Project Title	Mentor's Last Name, First Name	Faculty Mentor's Department	Page #
Apostolakis	Aristotle	Independent of a Program/Working Directly With a Faculty Member	Brown University	Traumatic Brain Injury accelerates pathology in a mouse model of Alzheimer's disease.	Pieper, Andrew	Psychiatry	1
Badat	Saad	Independent of a Program/Working Directly With a Faculty Member	Case Western Reserve University	A Segmentation Pipeline for Analyzing Dynamic Cellular Stress Responses	Luna, Joseph	Biochemistry	1
Bambakidis	Peter	SOURCE (AHSS, STEM, P-SURG, SURES)	St. Olaf College	The role of neuronal primary cilia in neurodegeneration after brain injury	Miller, Emiko	Neurosciences	2
Castro	Marianita	Heart, Lung and Blood Minority research Training Program	Case Western Reserve University	Impact of Hydrogen Peroxide on the ATP-dependent Peptidase Activity of Human Lon Protease	Lee, Irene	Chemistry	2
Cheng	Cheng-En	SOURCE (AHSS, STEM, P-SURG, SURES)	Case Western Reserve University	Combination of 5-fluorouracil with photodynamic therapy: Enhancement of innate and adaptive immune responses in a murine model of actinic keratosis	Maytin, Edward	CCF Lerner Research Institute	3
Coggan	Peter	SOURCE (AHSS, STEM, P-SURG, SURES)	Case Western Reserve University	The Impact of Neuronal Coordination of Wing Flapping and Olfactory Tracking in Free-flying Manduca sexta moths.	Willis, Mark	Biology	4
Dullur	Prateek	Independent of a Program/Working Directly With a Faculty Member	Case Western Reserve University	Using machine learning for classification of rhythmic auditory stimuli in Parkinson’s disease	Baker, Ken	CCF, LRI, NEU	5
Fabian	Matthew	Advanced Platform Technology Center Summer Internship	Youngstown State university	Mechanical Testing of High-Density Connector	Gbur, Janet	Materials Science and Engineering	6

Last Name	First Name	Summer Program	Home University/ School	Project Title	Mentor's Last Name, First Name	Faculty Mentor's Department	Page #
Goldfarb	Ralston	SOURCE (AHSS, STEM, P-SURG, SURES)	Case Western Reserve University	Examining Colon Cancer Cell Sensitivity to Lipid Biosynthesis Inhibition in Varying Lipid Environments	Adams, Drew	Genetics and Genome Sciences	6
Guci	Johana	Pharmacology SURP	Case Western Reserve University	The PAR4 Variant P322L Directly Impacts Thrombin Generation	Nieman, Marvin	Pharmacology	7
Guisse	Soda	Heart, Lung and Blood Minority research Training Program	University of Cincinnati	The role of KLF6 in Inflammatory Response and Pathogenesis of Atherosclerosis	Mahabaleshwar, Ganapati	Pathology	7
Hajihassani	Arian	Independent of a Program/Working Directly With a Faculty Member	Duquesne University	Role of IDH1 on chemotherapy efficacy in pancreatic cancer	Winter, Jordan	Biochemistry	8
Han	Lindsey	SOURCE (AHSS, STEM, P-SURG, SURES)	Case Western Reserve University	Alteration of Renal Phenotype in Lupus-prone B6.Nba2 Mice with IFNAR-deficiency on Myeloid Cells	Jorgensen, Trine	CCF Lerner Research Institute	9
Harvey	Germaine	Heart, Lung and Blood Minority research Training Program	Case Western Reserve University	Studying Calcium Signaling on Protease Activated Receptor-1 (PAR1) Between Glycosylated Wild Type and Glycosylation Deficient Chinese Hamster Ovary (CHO) Cells	Nieman, Marvin	Pharmacology	10
Hsu	Caitlyn	Wharton Summer Student Research (Nutrition)	Case Western Reserve University	Comparing the microbiota profiles associated with various dietary patterns and lifestyle factors	Bebek, Gürkan	Nutrition, Computer and Data Sciences	11
Huff	Douglas (DJ)	Heart, Lung and Blood Minority research Training Program	Northwestern University	Effects of Phospho-Mimetic Mutations on Drp1 Function	Ramachandran, Rajesh	Physiology & Biophysics	12

Last Name	First Name	Summer Program	Home University/ School	Project Title	Mentor's Last Name, First Name	Faculty Mentor's Department	Page #
Hyatt	Gabby	Advanced Platform Technology Center Summer Internship	Binghamton University	Development of sensorized glove to indicate pressure changes	Gbur, Janet	Materials Science and Engineering	12
Kandjoze	Uapingena	American Heart Association	Earlham College	Role of Voltage-Gated Calcium Channel Cav1.2 in Pericytes on the Blood-Brain Barrier Stability	Pieper, Andrew	Psychiatry	13
Kassaie	Hannah	SOURCE (AHSS, STEM, P-SURG, SURES)	Case Western Reserve University	Transcranial Direct Current Stimulation (tDCS) to the Dorsolateral Prefrontal Cortex (DLPFC) to Evaluate Functional Connectivity with the Reward Network	Nock, Nora	Population and Quantitative Health Sciences	14
Kraiselburd	Franco	ENGAGE	Case Western Reserve University	Studying the Cross-talk between MSCs and Osteotropic Cancers	Somoza, Rodrigo	Biology	15
Krishnan	Varsha	Wharton Summer Student Research (Nutrition)	Case Western Reserve University	Benchmarking Obesity-Related Competencies in Dietetic Supervised Practice Programs	Watowicz, Rosanna	Nutrition	16
Lee	Hana	SOURCE (AHSS, STEM, P-SURG, SURES)	Case Western Reserve University	Effect of Charge Residues on the Glycosylation of Thr 57 of the PSGL-1 Glycoprotein by the Core-1 Transferase	Gerken, Thomas	Biochemistry	17
Leon-Alvarado	Rose	American Heart Association	Earlham College	Blocking aberrantly high mitochondrial fission is neuroprotective after traumatic brain injury	Pieper, Andrew	Psychiatry	18
Martinez	Angela	Heart, Lung and Blood Minority research Training Program	The University of Texas at Rio Grande Valley	An in vitro assay to assess cellular selectivity of adeno-associated viral vectors enhanced by polymeric adjuvant ePL	Maiseyeu, Andrei	Cardiovascular Research Institute & Biomedical Engineering	19

Last Name	First Name	Summer Program	Home University/ School	Project Title	Mentor's Last Name, First Name	Faculty Mentor's Department	Page #
McShine	Alannah	Heart, Lung and Blood Minority research Training Program	Dartmouth College	The ability of isobutyric tropine ester to blunt fentanyl-induced respiratory depression is independent of its activation of carotid body chemoafferents	Lewis, Stephen	Pediatrics, Pulmonology, Pharmacology	20
Metz	Grace	SOURCE (AHSS, STEM, P-SURG, SURES)	Case Western Reserve University	Advancing Optical Data Storage Systems Using Fluorescent Materials	Saini, Anuj	Physics	20
Moorthy	Skanda	Independent of a Program/Working Directly With a Faculty Member	Case Western Reserve University	Air Pollution Exposure Induces Cognitive Decline in Wild type Mice	Rajagopalan, Sanjay	School of Medicine	21
Muralidharan	Ani	Wharton Summer Student Research (Nutrition)	Case Western Reserve University	Vitamin E Can Elicit Unique Transcriptional Responses Via its Antioxidant-Independent Properties	Manor, Danny	Nutrition	21
Norville	Malyka	Bio-Inspired Materials Systems (REU in Polymers)	Howard University	Emulating Polychaete Worm Anatomy With a Worm Robot Constructed from Compliant Plastic Meshes	Daltorio, Kathryn	Mechanical and Aerospace Engineeering	22
Nworie	Joshua	Heart, Lung and Blood Minority research Training Program	University of Cincinnati	Nuclear Localization of Regulator of G-Protein Coupled Receptors in Human Embryonic Kidney 293 Cells	Osei-Owusu, Patrick	Physiology & Biophysics	23
Ochoa	Elizabeth	Wharton Summer Student Research (Nutrition)	Case Western Reserve University	Development of a Research Protocol for Examining the Effects of Gamification in an Introductory Nutrition Course	Cavallo, David	Nutrition	24
Okafor	Chiemeka	Heart, Lung and Blood Minority research Training Program	Case Western Reserve School of Medicine	Demographic and Clinical Factors Affecting Keratoconus Risk in Multi-Ethnic Veterans Using Electronic Health Records	Iyengar, Sudha	Population and Quantitative Health Sciences	25

Last Name	First Name	Summer Program	Home University/ School	Project Title	Mentor's Last Name, First Name	Faculty Mentor's Department	Page #
Reynero	Kalob	Ingalls Immunology	Case Western Reserve University	Distinct Intracellular IgG Trafficking Route Restricts B cell's Role in IgG Sialylation	Cobb, Brian	Pathology	26
Rozen	Valery	Heart, Lung and Blood Minority research Training Program	Michigan State University College of Human Medicine	Role of STX1A in mediating Cathepsin G entry into HTC116 cells	Wang, Zhenghe	Case Comprehensive Cancer Center	26
Shaffer	Charles	SOURCE (AHSS, STEM, P-SURG, SURES)	Case Western Reserve University	Effects of Inhibition of Src Signaling in Toxoplasma gondii Infection	Subauste,Carlos	Infectious Diseases and HIV Medicine	27
Shah	Krish	CanSUR (Case Comprehensive Cancer Center)	Case Western Reserve University	Role of glucocorticoid receptor in prostate cancer stress response and epigenetic alterations	Sharifi, Nima	CCF, Genitourinary Malignancies Research Center & CWRU Molecular Medicine	28
Shelton	Devine	Heart, Lung and Blood Minority research Training Program	Cleveland State University	Neonatal rat brain model demonstrates implications for important proteins In extracellular matrix related to abnormal cardiorespiratory functions	MacFarlane, Peter	UH RBC Pediatrics, Neonatology	29
Sunil	Achintya	Independent of a Program/Working Directly With a Faculty Member	Case Western Reserve University	Calcium chloride drug dissolution imaging by single molecule microscopy	Saini, Anuj	Physics	30
Taylor	Juliana	Heart, Lung and Blood Minority research Training Program	John Carroll University	Contribution of mutations in the ORF9b gene to the increased infectivity of SARS-CoV-2 variants of concern	Levine, Alan	Molecular Biology and Microbiology, Pharmacology, Pathology, Medicine, Pediatrics	31
Tripette	Jaden	Heart, Lung and Blood Minority research Training Program	Morehouse College	Transporting RNA aptamers into HeLa Cells	Fortenberry, Yolanda	Biology	32

Last Name	First Name	Summer Program	Home University/ School	Project Title	Mentor's Last Name, First Name	Faculty Mentor's Department	Page #
Tsang	Samantha	SOURCE (AHSS, STEM, P-SURG, SURES)	Case Western Reserve University	Generating Isogenic Lines Using Primary Microcephaly with Simplified Gyral Pattern, Epilepsy, and Permanent Neonatal Diabetes Syndrome (MEDS) Patient-Derived iPSCs	Schaffer, Ashleigh	Genetics and Genome Sciences	33
Van der Linden Costello	Paola	SOURCE (AHSS, STEM, P-SURG, SURES)	Case Western Reserve University	Climate Change Attitudes and Perceptions	Martin, Ina; Taylor, Cyrus ; Gran, Brian; Corbett, Stephanie	Materials Science and Engineering; Physics; Sociology; Energy and Sustainability	34
Walker	Joel	Heart, Lung and Blood Minority research Training Program	Morehouse College	Serotonergic Input to R5 Neurons Plays a Role in Shaping Wakefulness and Sleep Architecture	Tabuchi, Masashi	Neurosciences	34
Washington	Christopher	Heart, Lung and Blood Minority research Training Program	Lewis Katz School of Medicine at Temple University	Understanding the Role of TANs in Tumorigenesis using a VTE Pancreatic Adenocarcinoma Mouse Model	Nayak, Lalitha	Case Comprehensive Cancer Center	35
Wheeler	Vivian	SOURCE (AHSS, STEM, P-SURG, SURES)	Case Western Reserve University	The Role of Wing Fanning in Odor Detection of the Oriental Fruit Moth	Willis, Mark	Biology	36
Wilkerson	Jasmine	Heart, Lung and Blood Minority research Training Program	Oberlin College	Myocardial Fibrosis Assessment in Hypertensive Individuals with Chronic Kidney Disease	Dobre, Mirela	UH Nephrology and Hypertension	37
Woods	Kamila	Heart, Lung and Blood Minority research Training Program	Pacific Lutheran University	Correlation Between Urine and Serum Measurements of Serotonin Pathway Biomarkers	Hibbs, Anna Maria	Pediatrics	38

Last Name	First Name	Summer Program	Home University/ School	Project Title	Mentor's Last Name, First Name	Faculty Mentor's Department	Page #
Yoon	Christopher	SOURCE (AHSS, STEM, P-SURG, SURES)	Case Western Reserve University	Development of Micron-Thin Polymer Films to Observe Transport in the Selective Layer of Membrane Absorbers Using Single Molecule Spectroscopy	Duval, Christine	Chemical Engineering	39
Zhang	Jichu (Michael)	Advanced Platform Technology Center Summer Internship	Case Western Reserve University	Antioxidant Dimethyl Fumarate Lacks Long-term Efficacies Against Neurodegeneration and Neuroinflammation Induced by Intracortical Microelectrode Implants	Capadona, Jeffrey	Biomedical Engineering	40
Zielke	Cecelia	Wharton Summer Student Research (Nutrition)	Case Western Reserve University	Protein Crystallography of a Cortisol Nanobody to Pinpoint Molecular Mechanism for Cortisol Binding for Use in a Real-Time Biosensor for Stress Metabolites	Lodowski, David	Nutrition	41
Zinsser	Anna	SOURCE (AHSS, STEM, P-SURG, SURES)	Case Western Reserve University	Identifying Target for Novel Selective Anti-Cancer Small Molecule	Adams, Drew	Genetics and Genome Sciences	41

Traumatic brain injury accelerates pathology in a mouse model of Alzheimer's disease.

Aristotle Apostolakis[1], Sarah Barker[2,3,4,5,6], Min-Kyoo Shin [2,3,4,5], Edwin Vázquez-Rosa[2,3,4,5], Kathryn Franke[4], Coral J Cintrón-Pérez[2,3,4,5], Preethy Sridharan[2,3,4,7], Li Gan[8], Andrew A. Pieper[2,3,4,5]

(1) Brown University, Biology (2) Harrington Discovery Institute, University Hospitals Cleveland Medical Center (3) Department of Psychiatry Case Western Reserve University(4) Geriatric Research Education and Clinical Center (GRECC), Louis Stokes Cleveland VA Medical Center (5) Institute for Transformative Molecular Medicine, School of Medicine, Case Western Reserve University (6) Department of Pathology, School of Medicine, Case Western Reserve University (7) Department of Neuroscience, School of Medicine, Case Western Reserve University (8) Weill Cornell Medicine, Helen and Robert Appel Alzheimer's Disease Research Institute

Traumatic brain injury (TBI) is the leading environmental risk factor for the development of Alzheimer's disease (AD). Importantly, both conditions share strikingly similar pathology with regard to acetylated tau and amyloid aggregates. We hypothesized that TBI plays an escalatory role in the development of AD. Using the 5xFAD mouse model, we studied the effect TBI has on AD both behaviorally and pathologically. Using a jet-flow overpressure chamber to model TBI, we delivered a TBI or Sham injury to mice at 8 weeks of age. Next, we performed object recognition and water maze behavioral experiments at 10 weeks of age. Finally, we euthanized the mice at 11 weeks of age to assess acetylated tau and beta amyloid plaque levels in the brain via western blotting and congo red staining respectively. We found that TBI accelerates AD pathology in mice. TBI accelerated age of onset of learning and memory deficits in 5xFAD mice. Furthermore, TBI increased the levels of acetylated tau and amyloid beta plaques in AD mice. These findings not only establish a supported relationship between TBI and AD acceleration, but also highlight acetylated tau as a potential key player in the mechanism of AD. Because of our promising preliminary data, we plan to further investigate the acetylated tau mechanism and explore ways to reduce acetylated tau levels as a possible treatment for the accelerated onset of AD after TBI.

Project Mentor: Dr. Andrew Pieper Harrington Discovery Institute

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A Segmentation Pipeline for Analyzing Dynamic Cellular Stress Responses

Saad Badat, Department of Biochemistry; Dr. Joseph Luna, Department of Biochemistry

Image analysis for microscopy data is a challenging task that typically requires programming experience, access to specialized software and equipment, and is difficult to reproduce. Tools such as CellProfiler and CellPose have helped bridge this gap as accessible and open source software platforms for image analysis. Here we present an image analysis pipeline using CellProfiler to quantitatively analyze dynamic stress responses, in the form of stress granules (SGs), within living cells. Stress granules are cytoplasmic condensates composed of non-translating mRNPs, RNAs and proteins that form when protein synthesis is repressed during the integrated stress response. We applied this pipeline across live cell image sequences of cells undergoing stress and observed periodic oscillations of SG formation and dissolution using a fluorescent SG reporter. Cells were accurately segmented with CellPose and tracked through a multi day image sequence even in the absence of a nuclear channel. Using CellProfiler, we created a cytoplasmic mask and measured the reporter intensity where we observed that a majority of cells exhibit oscillating SGs. We are currently working on a means to supplement this pipeline to quantify the number, size and intensity of stress granules for each cell across the image sequence. This image analysis pipeline can be easily replicated and is accessible to anyone with minimal software experience.

Project Mentor: Dr. Joseph Luna, Department of Biochemistry

The Role of Neuronal Primary Cilia in Neurodegeneration After Brain Injury

Peter Bambakidis[1], Emiko Miller[2], Preethy S. Sridharan[2], Min-Kyoo Shin[3], Andrew A. Pieper[2,3,4,5]

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The primary pathological causes and mechanisms of neurodegeneration after traumatic brain injury (TBI) are poorly understood. This active area of investigation holds promise for discovering new therapeutic opportunities for patients, which would fill a critical unmet need in medicine today. We are exploring the role of neuronal primary cilia in TBI-mediated neurodegeneration. TBI is the 3rd leading cause of Alzheimer's disease (AD), behind genetics and aging, and neuronal primary cilia collapse has recently been implicated in the pathogenesis of AD. Primary cilia are microtubule-based organelles that act as an antenna for neurons by detecting changes in the extracellular environment. When patients lose primary cilia in the brain, they present with reduced hippocampal and cortical mass, leaving patients with enlarged ventricles and severe cognitive deficits. Previous research has suggested a link between disruption of the primary cilia and the onset of neurodegeneration. We are taking an in vivo approach to monitor neuronal primary cilia length after TBI, using a well-characterized clinically-relevant model of multimodal TBI in our laboratory. Using a marker for primary cilia in neurons, adenylate cyclase 3, I have observed a decrease in the length of primary cilia in the hippocampus of the brain 24 hours after injury. Notably, this decrease in length precedes the onset of both neurodegeneration and cognitive deficits in these mice. This is compatible with a loss of primary cilia serving an early role in neurodegeneration after brain injury, which could lead to new therapeutic opportunity for neuroprotection in TBI or AD.

Project Mentor: Emiko Miller,

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Impact of Hydrogen Peroxide on the ATP-dependent Peptidase Activity of Human Lon Protease

Marianita Castro, Department of Chemistry; Dr. Irene Lee, Department of Chemistry

Lon is an ATP-dependent serine protease. It consists of an ATPase site and a proteolytic site. ATP hydrolysis is needed to maintain protease activity. In mammals, Lon functions primarily in the mitochondrial matrix to eliminate oxidatively damaged proteins that can otherwise aggregate and compromise mitochondrial integrity. In rat models subjected to transverse aortic constriction (TAC), Lon has been shown to be oxidatively inactivated.[1] The long-term goal of this project is to examine the mechanism by which mitochondrial Lon is oxidatively damaged. The approach entails measuring the effect of hydrogen peroxide on the ATP-dependent peptidase activity of human mitochondrial Lon *in vitro* using a continuous fluorogenic peptidase assay. By altering the order of ATP versus hydrogen peroxide addition, we observed that ATP protected Lon from hydrogen peroxide inactivation. The results generated in this study establish a quantitative framework for further evaluating the possibility of using the reductant DTT and glutathione to rescue Lon from hydrogen peroxide inactivation. This research approach may be expanded to study the impact of oxidative damage on other ATP-dependent proteases such as ClpXP, which consists of a regulatory domain (ClpX) and two heptameric serine protease rings (ClpP).[2] The effects of other oxidants, such as nitrogen species, may also be examined.

Project Mentor: Dr. Irene Lee, Department of Chemistry

References:
[1] Hoshino A, Okawa Y, et al. Oxidative Post-translational Modifications Develop LONP1 Dysfunction in Pressure Overload Heart Failure. *Circulation: Heart Failure*. 2014; 7(3):501
[2] Vass R, Nascembeni J, Chien P. The Essential Role of ClpXP in Caulobacter crescentus Requires Species Constrained Substrate Specificity. *Frontiers in molecular biosciences*. 2017; 4:28

Combination of 5-fluorouracil with photodynamic therapy: Enhancement of innate and adaptive immune responses in a murine model of actinic keratosis

Cheng-En Cheng[1], Sanjay Anand[1, 2, 3], Lauren Heusinkveld[3], Lefatshe Lefatshe[4], Tayyaba Hasan[5], and Edward V Maytin[1, 2, 3, 5]

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Previous studies showed that with the combination of differentiation-inducing agents, such as 5-fluorouracil, vitamin D3, or methotrexate, and aminolevulinate (ALA)-based photodynamic therapy (PDT) improves clinical responses by increasing the concentration of protoporphyrin IX within the cell. With the previous research, we review the studies and show that 5-fluorouracil (5-FU) can enhance PDT-induced anti-tumor immune mechanisms. We conduct the research on murine actinic keratosis (AK), which is the precancers of squamous cell carcinoma. The AK lesions were treated topically with 5-FU or vehicle for three days prior to the ALA application, followed by blue light illumination (~417 nm) for PDT. Lesions were harvested for time-course analyses of innate immune cell, including neutrophils (Ly6G+) and macrophages (F4/80+), which peaked at 72 hours and 1 week post PDT, respectively, and was greater in 5-FU treated lesions. Enhanced infiltration of activated T cells (CD3+) and cytotoxic T cells (CD8+) also occurred in 5-FU treated lesions throughout the time course and at 1 – 2 weeks post PDT, respectively. Our research also show that 5-FU pretreatment reduced the presence of cells expressing the immune checkpoint marker PD-1 at about 72 hours post PDT, which favors the cytotoxic T cell activity. With the combination of 5-FU and PDT, which both known to induce long-term anti-tumor immune responses in addition to their more immediate effects on cancer cells individually, may synergize to provide better management and result of squamous precancer.

Project Mentor: Dr. Edward V. Maytin, Department of Molecular Medicine, School of Medicine

The Impact of Neuronal Coordination of Wing Flapping and Olfactory Tracking in Free-flying *Manduca sexta* moths.

Peter Coggan, Department of Biology; Kim Thompson, Department of Biology; Vivian Wheeler, Department of Biology; Laura Chin, Department of Astrophysics, Wellesley College; Mark Willis, Department of Biology

An organism's location in its environment has the potential alter their perception of the world, however, the effects of its own movements on this perception is seldom noticed or studied. For example, it is critical for any organism to know if the environment looks like it is spinning because they are executing a turning maneuver or just turned their head. Information about the upcoming head movements is transmitted to the eye allowing the visual system to perceive the scene as stable. These motor-to-sensor pathways are known as corollary discharge circuits (CDCs). Compared to other senses, there has been no research focused on how CDCs influence olfaction. One of the only studied olfactory based CDCs is found in the tobacco hornworm moth, *Manduca sexta*. In this system, two neurons connect the flight control circuits in the thorax to the antennal lobes, the odor processing circuits in the brain. When the flight muscles activate, the CDC neurons release histamine into the antennal lobe. Previous studies with restrained moths showed that histamine improves how they process odor and the concentration sensitivity to odor. We can block histamine's effect by injecting an antihistamine(cimetidine) directly into the moths' antennal lobes. Silencing the CDC and testing how well the free flying animals can track an odor in a wind tunnel will provide valuable information on the behavioral importance of CDC's role in olfactory processing. We have established a protocol to test how inhibition of the histaminergic neurons impacts the ability of free flying male moths to track a plume of female sex pheromone in a wind tunnel. This involves a minimally invasive surgery to inject antihistamine into the antennal lobe. Moths are then challenged to fly and track an attractive odor in a wind tunnel. Control moths that experienced injection of saline alone continued to fly and track the attractive odor plume in a manner like untouched controls. Current experiments are underway with antihistamine injections.

Professor Mentor: *Mark Willis, Department of Biology*

Using machine learning for classification of rhythmic auditory stimuli in Parkinson's disease

Prateek Dullur[ae], *Joyce Bore-Norton, Ph.D.*[a], *Kai Abitbol-Pierce*[ae], *Julio Almeida, MD*[a], *Kyle Baker*[a], *Carmen Toth*[ad], *Andre Machado, MD, Ph.D.*[abc], *Kenneth Baker, Ph.D.*[ab]

[a]Department of Neurosciences, Lerner Research Institute, [b]Center for Neurological Restoration, Cleveland Clinic Neurological Institute, Cleveland, OH, and [c]Department of Neurosurgery, Neurological Institute, Cleveland Clinic, Cleveland, OH, 44106, United States, [d]Department of Biomedical Engineering, Case Western Reserve University, [e]Department of Neurosciences, Case Western Reserve University

Introduction: Both rhythmic auditory stimulation (RAS) and deep brain stimulation (DBS) of the Subthalamic Nuclei (STN) can ameliorate the motor symptoms of Parkinson's Disease (PD). Using machine learning (ML) to decode the neural correlates of RAS can elucidate the connection in therapeutic benefit pathways between RAS and other treatments, including DBS. We investigated the feasibility of using ML to decode fast vs. slow RAS in PD patients using magnetoencephalography (MEG). *Methods:* MEG recordings were collected in PD patients using the 306-channel, whole-head MEG system. Patients withheld anti-PD medications for at least 12 hours prior to testing. During testing, they were instructed to sit quietly with their eyes open and listen to auditory tones of varying frequency (slow at 1 and 2 Hz; fast at 4 and 5 Hz). The tones were presented in randomized blocks. Trials were collected first without DBS and repeated with their bipolar-converted, clinical therapeutic settings. In this work, ML was used to decode 2 Hz slow RAS from 5 Hz fast RAS from common spatial patterns (CSPs). 114 epochs of 0.7 s each were generated from the filtered data. A support vector machine (SVM) with a radial-basis kernel was used for classification with C=2 and gamma=0.001. Moreover, we performed a 10-fold cross-validation on the data to assess the performance of the ML algorithm. *Results*: Slow RAS could be classified from fast RAS with high accuracy (averaged over 100 shuffled iterations). Evoked response analysis demonstrates a higher amplitude response from slow RAS compared to fast RAS after stimulus onset. *Conclusion*: These findings support the feasibility of decoding the neurophysiology of RAS in PD patients and using it to inform future combinations of therapy. The data show that machine learning can accurately decode RAS from MEG data. The data show that the neural signatures for fast and slow RAS are meaningfully distinguishable from each other and from resting state. Using machine learning (ML) to adaptively adjust DBS parameters to account for auditory stimuli could be used to create a more effective closed-loop therapeutic system. This may include optimizing therapy, reducing stimulation side effects, and improving battery life.

Project mentors: Dr. Joyce Bore-Norton and Dr. Kenneth Baker, Department of Neurosciences, Lerner Research Institute, Cleveland Clinic

Mechanical Testing of High-Density Connector

Matthew L. Fabian, Mechanical Engineering, Youngstown State University

Recent biomedical advances have shown the potential for prosthetics capable of sending signals back to the brain through nerve stimulation, creating the potential for the sensation of touch to be restored to amputees. This development creates the need for an implantable system that takes up the least space and has the least surface area possible to ensure comfort, safety, and a minimal immune response. For this purpose, a 32-channel connector and lead body were designed and are currently being developed. The reliability of the connector and lead body is paramount to the functionality of the system; therefore, a firm understanding of the mechanical behavior of the components is necessary. Static and cyclic mechanical testing, materials analysis, and functional characterization of the connector and lead body were the focus of this work. The development of custom fixtures was necessary to enable successful and repeatable component-level testing under tension, static bending, and flex bending fatigue. Electrical resistance measurements were performed on the lead body as-received, following pre-conditioning at 37°C for ten days, and post-test (i.e. tension, flex bending fatigue) to ensure viability. Six of six specimens passed twenty fully-reversed cycles at a cyclic strain of 0.0102 which represented worst-case handling. All six specimens also passed 1 million fully-reversed cycles at a cyclic strain of 0.0025 to simulate the *in vivo* conditions. One specimen was tested to 4 million total cycles without failure. The 1X7 35N LT drawn filled tube insulated strands exhibited an average maximum tensile load of 6.3 ± .02 N.

Aerosol printing was chosen as the fabrication method to create the flexible silicone circuit housed inside the connector body due to its ability to print high resolution traces over topological surfaces. Early characteristic techniques including digital optical microscopy, scanning electron microscopy, and optical profilometry were used for the development of early printing parameters. The static bending test fixture will be used to observe cracking in printed traces as a function of applied strain. Data collected from this work will support the optimization of the printing parameters for the circuit and future directions will be discussed.

Project Mentor: Dr. *Janet L Gbur, PhD*, Case Western Reserve University Department of Materials Science and Engineering

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Examining Colon Cancer Cell Sensitivity to Lipid Biosynthesis Inhibition in Varying Lipid Environments

Ralston Goldfarb, Genetics, Department of Genetics and Genome Sciences, School of Medicine

Cholesterol inhibition has been a target of cancer research for years. Cancer cells require more cholesterol than normal cells, making cholesterol biosynthesis a target for reducing cancer growth. Novel drugs like TASIN-1 have been found to target this biosynthesis by selectively blocking cholesterol biosynthesis in certain cancers containing a tumorigenic APC mutation. However, research has not investigated other lipid biosynthesis pathways besides cholesterol. In this study, cell death in colon cancer cells was examined in both high and low lipid levels. Cells are traditionally supplemented with 10% fetal bovine serum, which provides essential lipids and nutrients to the cells. Cells were grown in both normal FBS conditions as well as FBS that had been delipidated. In these delipidated conditions, cells were sensitive to treatment with cholesterol biosynthesis inhibitors. However, supplementation of cholesterol into this media desensitized cells to the inhibitors. Since cholesterol eliminates toxicity from these inhibitors, cholesterol addition to cells in delipidated conditions provides a method to find sensitivities that cancer cells have to other lipid biosynthesis pathways. Lipid-based dependencies can be found by comparing cell death between cancer cells grown in high and low lipid levels; cholesterol can be excluded from this by the addition of cholesterol to the low lipid medium. Therefore, a high throughput screen, which treated cells grown in both conditions with over to 3,000 drugs of known targets, can elucidate lipid biosynthesis and regulation mechanisms that cancer cells require to survive. This screen will provide greater information on essential lipids in cancer growth in the future.

Project Mentor: Dr. Drew Adams, Department of Genetics and Genome Sciences, School of Medicine

The PAR4 Variant P322L Directly Impacts Thrombin Generation

Johana Guci, Department of Biochemistry; and Elizabeth Knauss, Department of Pharmacology, Case Western Reserve University

Thrombin is an enzyme that plays an important role in blood coagulation, cleaving the N terminus of protease activated receptor 4 (PAR4) on the surface of platelets, and creating a tethered ligand. Extracellular loop 3 (ECL3) swings out and allows the ligand to bind to the revealed pocket. Upon activation, phosphatidylserine on the inner platelet membrane will flip to the outside, creating a surface for prothrombinase to assemble and convert prothrombin to thrombin. Mutations in ECL3 can disrupt PAR4 reactivity and subsequent platelet activation. A single-nucleotide polymorphism (PAR4-P310L) has recently been associated with a lower risk of developing venous thromboembolism (VTE). We are asking how thrombin generation affects coagulation in wild type mice in comparison to mice with a homologous mutation (PAR4-P322L), and if platelet-expressing mutant PAR4 have a difference in phosphatidylserine exposure. Four types of mice are being examined: wild type, P/L (one allele has a replacement of Proline with Leucine in ECL 3 of PAR4), L/L (both alleles have that replacement), and KO (the gene that codes for PAR4 is knocked out). Our hypothesis is that we will observe progressively lower levels of blood coagulation from wild type mice (highest), to P/L, L/L, and KO mice (lowest). The results show that thrombin generation gets progressively lower as PAR4 reactivity decreases, with wild type mice generating the most thrombin and KO mice the least ($p<0.01$). Our findings suggest a key role of PAR4 and ECL3 in platelet activation thrombin generation.

Project Mentor: Dr. Marvin Nieman, Department of Pharmacology
Faculty Sponsor: Dr. Marcin Golczak, Department of Pharmacology

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The Role of KLF6 in Inflammatory Response and Pathogenesis of Atherosclerosis

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Monocytes-derived macrophages are the effector cells of the innate immune system that play central role in maintaining tissue homeostasis. There are increasing evidences which support that transcriptionally dynamic macrophages play key role in the pathogenesis of vascular diseases. In this context, accumulation of pro-inflammatory macrophages in the subendothelial layers leads to atherosclerotic plaque development. Thus, macrophage pro-inflammatory gene expression should be tightly regulated to restrain inflammatory response and avert host tissue damage. The goal of this project is to understand the impact of macrophage- Kruppel-like transcription factor 6 (KLF6) deficiency on inflammatory gene expression and experimental atherogenesis. In this study, we identified that myeloid-KLF6-deficient mice on the *Apoe*$^{-/-}$ background are protected from high-fat diet -induced atherosclerotic lesions formation. At the molecular level, our study identifies that mRNA as well as protein expression of KLF6 increases in Lyz2$^{cre/cre}$ mice BMDMs after inflammatory challenge. Further, inflammatory agent exposure robustly increased the macrophage pro-inflammatory gene expression (*Vcam1, Icam1, Il1α, Il1β, Il6, Cd44, Cox2, Ptgs2*) in Lyz2$^{cre/cre}$ mice BMDMs. However, the expression of these pro-inflammatory genes were significantly attenuated in Klf6$^{fl/fl}$:Lyz2$^{cre/cre}$ mice BMDMs. Collectively, our results provide evidence that KLF6 enhances macrophage pro-inflammatory gene expression and experimental atherogenesis in vivo.

Project mentor: Dr. Ganapati H. Mahabaleshwar; Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, Ohio

Faculty Sponsor: Prof. Monica M. Montano, Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland, Ohio

Inhibition of Isocitrate Dehydrogenase 1(IDH1) sensitizes pancreatic cancer cells to chemotherapy

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Abstract

Introduction:
Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer mortality in the United States. The poor prognosis is in part due to the development of chemotherapy resistance, despite improvements in multiagent regimens. Our previous work demonstrated that oxidative stress plays an essential role in drug resistance. Wild-type isocitrate dehydrogenase 1 (IDH1) is an important enzyme that generates cytosolic NADPH to maintain redox homeostasis and protect cancer cells from oxidative damage. Additionally, we demonstrated that ivosidenib (AG-120), an FDA-approved mutant IDH1 inhibitor, is actually a potent inhibitor of wild-type IDH1, under low magnesium and nutrient levels that are present in the tumor microenvironment.

Methods:
We evaluated IDH1 expression in PDAC using The Cancer Genome Atlas (TCGA). Cell viability was assessed by Trypan blue and PicoGreen in drug combination assays. Cellular reactive oxygen species (ROS) levels were determined by the DCFDA method. To further assess the therapeutic potential of AG-120 in combination with chemotherapy, tumor volume analyses were performed using patient-derived xenografts (PDX) in athymic nude mice, and survival studies were performed in C57BL/6J mice transplanted with orthotopic murine pancreatic cancer.

Results:
Analysis of TCGA data indicated that IDH1 is overexpressed in pancreatic cancer tumors. Treatment of MiaPaca2 and Panc1 cancer cells with 5- fluorouracil (5-FU) induced expression of wild-type IDH1 in vitro. Short-term cell viability data demonstrated that targeting IDH1 with AG-120 when combined with DNA-damaging agents (5-FU, oxaliplatin) had a synergistic effect with a positive synergy score and Bliss score greater than 1. Additionally, we assessed long-term cell survival using colony formation assays, which yielded a dramatic reduction in cell survival for both Panc1 and MiaPaCa-2 cells when 5-FU was combined with AG-120, as compared to single-agent controls. Inhibiting IDH1 impairs the ability of pancreatic cancer cells to scavenge ROS levels and enhances chemotherapy-induced apoptosis in pancreatic cancer cells via ROS-mediated damage in vitro. Both PDX tumor volume studies and overall survival analyses revealed that the combination of these AG-120 and chemotherapy synergistically enhanced anti-tumor activity and doubled the survival benefits as compared to single-agent alone.

Conclusion:
IDH1 plays a critical role in tumorigenesis and chemoresistance in pancreatic cancer. Our data demonstrate that IDH1 inhibition with AG-120 may enhance chemotherapy efficacy and represents an important area for future investigation in the form of clinical trials.

The undergraduate student is Arian Hajihassani. He is an undergraduate Biochemistry student at Duquesne University.

Alteration of Renal Phenotype in Lupus-prone B6.Nba2 Mice with IFNAR-deficiency on Myeloid Cells

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Systemic Lupus Erythematosus (SLE) is an autoimmune condition characterized by widespread inflammation of multiple body systems, including the skin and kidneys. The B6.Nba2 lupus-prone mouse model presents with alterations in renal phenotype that mimic the disease progression of lupus nephritis, a common manifestation of SLE involving damage to the kidney filtration system. Although type I interferon (IFN) and type I interferon receptor (IFNAR) are known to be key players in the disease progression of SLE, whether or not there will be changes to the renal phenotype that would indicate further disease progression in B6.Nba2 with IFNAR-deficiency on myeloid cells remains unknown.

Based on previous unpublished data of spleen cells from the Jorgensen lab, the myeloid cells in the kidney were predicted to be altered and become more pathogenic in the absence of IFNAR on myeloid cells. The kidneys of age-matched 9 month-old female B6, B6.Nba2, and myeloid-cell specific B6.Nba2 conditional IFNAR knockout (cKO) mice were taken and kidney inflammatory cells were isolated for flow cytometry. Using flow cytometry, specific myeloid and lymphocyte cell populations were analyzed in proportion to the total leukocytes count.

B6 to B6.Nba2 displayed a trend down in both the proportion of neutrophilic/n-myeloid derived suppressor cells (MDSCs) and B cells that was further amplified in B6.Nba2.IFNAR cKO mice, indicating potential further disease progression in the absence of IFNAR on myeloid cells. Surprisingly, the proportion of monocytic/m-MDSCs in B6.Nba2.IFNAR cKO mice was significantly elevated compared to the B6.Nba2, bringing the proportion closer to that of the B6 non-autoimmune mice.

The proportion of CD8+ cytotoxic T cells of CD45+ leukocytes had a significant trend down between B6 and B6.Nba2 but little to no change between B6.Nba2 and B6.Nba2.IFNAR cKO, indicating that inflammation by CD8+ T cells may be IFNAR-independent. Similarly, the proportion of CD4+ T cells displayed a trend up from B6 to B6.Nba2 but little to no change between B6.Nba2 and B6.Nba2.IFNAR cKO mice.

Ultimately, these findings provide potential insights into the role of IFNAR in the progression of SLE in the kidney, as some inflammatory cell subsets seem to indicate IFNAR-independent renal phenotype alteration while others indicate IFNAR-dependent renal phenotype alteration. Studies are ongoing to increase the number of animals to be analyzed and to determine the intra-renal location and function of both myeloid and lymphoid immune cells.

Faculty Project Mentor: Dr. Trine N. Jørgensen, Department of Molecular Medicine, Case Western Reserve University and Department of Inflammation and Immunity, Lerner Research Institute

Studying Calcium Signaling on Protease Activated Receptor-1 (PAR1) Between Glycosylated Wild Type and Glycosylation Deficient Chinese Hamster Ovary (CHO) Cells

Germaine Harvey, Department of Biochemistry, Case Western Reserve University

Protease Activated Receptor-1 (PAR1) is a subclass of related GPCRs that is found on platelets and endothelial cells. PAR1 plays a critical role in regulating openings that occur in vascular barriers through initiating thrombosis. To activate PAR1, the N-terminus gets proteolytically cleaved to expose a tethered ligand. The ligand binds with the receptor causing a conformational change, and thus a specific cellular response. Moreover, extensive research in the past decade attributes PAR1's structure and function to the glycoproteins attached during post-translational modification, but their effect on PAR1 signaling has not yet been investigated. The purpose of this research is to determine if the glycosylation of PAR1 influences downstream signaling. In the previous summer, we showed that there may be a link between PAR1 and their glycoproteins by using two extreme groups of Chinese Hamster Ovary (CHO) cells—wild type, fully glycosylated CHO-K1, and glycosylation deficient CHO-Lec8. The signaling through the pathway responsible for intracellular calcium release, Galpha-q (Gαq), showed differences in calcium fluorescence between both groups when treated with the protease thrombin. A Western Blot for PKC substrate phosphorylation also supported those results. This summer, I expanded the number of cell lines to two more glycosylation deficient cells—CHO-Lec1 and CHO-Lec2. All four cell lines were suspended in a Fura-2 dye that binds to calcium within the cells and placed in a 96-well plate to be analyzed by a microplate reader that measured calcium fluorescence of the cells once treated with thrombin. The ratio between unbound and bound calcium to the Fura-2 was compared to see if there were any response differences between the cells. The microplate reader data indicated the glycosylation deficient cells CHO-Lec1, CHO-Lec2, and CHO-Lec8 did not respond to the PAR1 agonist thrombin to induce intracellular calcium release but wild-type, fully glycosylated CHO-K1 did. The differences witnessed in each experiment suggest that glycosylation of PAR1 does have an effect on the signaling cascade in the Gqα pathway. The next steps would be to examine the other pathways associated with PAR1—Galpha-i or Galpha-12/13—to see if their downstream signaling is also influenced by glycosylation as well as try a different peptide other than thrombin to see if the protease was the reason for the difference.

Project Mentor: Dr. Marvin Nieman, Department of Pharmacology

Comparing the microbiota profiles associated with various dietary patterns and lifestyle factors

Caitlyn Hsu, Department of Nutritional Biochemistry and Metabolism; Gürkan Bebek
Department of Nutrition, Center for Proteomics and Bioinformatics; Computer and Data Sciences Department

The human microbiome encompasses trillions of microbes such as bacteria, fungi, and viruses living within the body's ecological niches. Microbial dysbiosis (imbalance) results from a number of causative factors including diet. The average American diet—rich in saturated fat, sodium, added sugars, and meat based proteins— has been associated with a number of diseases including cardiovascular disease, diabetes and cancer whose etiologies are thought to be related to disruptions of the microbiome (1). Using data from the The Human Microbiome Project (HMP)—the largest publicly available database containing nearly 81-99% of the microbes inhabiting the healthy Western human body—researchers have defined a standard, "healthy" microbial gut profile (2). Phase two of the HMP further investigated host-microbiome interactions and revealed that microbiome dysbiosis is also related to Inflammatory Bowel Disease (IBD), Preterm birth and Type 2 Diabetes (3). Recently, mycobiome dysbiosis has also been observed in patients with various diseases including IBD, HIV and cystic fibrosis (4). Therefore, fungi appear to play an integral role in disease pathogenesis and state of health as well. Based on the healthy gut profile defined by the HMP, we hypothesized that the average American gut displays a unique microbial profile indicative of an unhealthy state. Using data collected from approximately 3300 participants, we examined the effect of diet on microbial order and their potential relationship to health using the statistical T test. We show that specific dietary patterns and lifestyle factors (such as sleep, stress, level of physical activity) are associated with unique microbial profiles. This data suggests that nutritionally based therapies such as dietary intervention and probiotic/prebiotic supplementation are promising therapeutic alternatives to classical pharmaceuticals which often result in adverse health effects. Therefore, by targeting specific pathogenic microbes via dietary alterations, we can re-establish gut homeostasis and improve the overall health of individuals.

Project Mentor: Professor Gürkan Bebek, Department of Nutrition; Center for Proteomics and Bioinformatics; Computer and Data Sciences Department

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Effects of Phospho-Mimetic Mutations on Drp1 Function

Douglas (DJ) Huff, Department of Physiology and Biophysics; Rajesh Ramachandran, Department of Pharmacology; Jason Mears, and Post-Doctoral Scholar; Ashutosh Prince

Mitochondria are dynamic organelles that continuously undergo fission and fusion. Mitochondrial fission and fusion lie in equilibrium, and any disruption to this balance can result in diseases ranging from neurodegenerative to cardiovascular. Various proteins are required to maintain this equilibrium, among them is our protein of interest, Drp1. Drp1 is a mechanoenzymatic GTPase pertinent for mitochondrial fission. This protein harbors interfaces that allow for helical self-assembly. With this capability, Drp1, with the help of partner and receptor proteins on the outer mitochondrial membrane, can oligomerize around mitochondria and hydrolyze GTP to perform scission. Drp1 possesses various domains with distinct structures and functions, but an unknown that remains is the unstructured region known as the variable-domain (VD). It appears the VD has an auto-inhibitory function that doesn't allow for premature self-assembly; it also plays some role in membrane interactions, and possibly other functions. Most post-translational modifications (PTMs) occur within this region and offer conformational changes that have some impact on the function of Drp1. Knowing that most PTMs occur within the VD, two residues are of particular interest—S579 and S600 (isoform 3). Both these residues are phosphorylated post-translationally, and previous literature reports that phosphorylation of S579 promotes Drp1-mediated mitochondrial fission. The effect that phosphorylation of S600 has on Drp1 is controversial and could be dependent on which kinase is involved in the PTM. Either way, there is possible crosstalk between the two. This research explores the effects of PTMs at S579 and S600 on the interactions of Drp1 with membranes and microtubules, along with exploring their effects on oligomerization state and GTPase activity. This is all done using phospho-mimetic mutants (S579E & S600E) that were created using site-directed mutagenesis. This research could garner a deeper understanding of the effects of PTMs on the function of the VD and the overall mechanism of Drp1-mediated mitochondrial fission.

Project Mentor: Professor Rajesh Ramachandran, PhD

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Development of sensorized glove to indicate pressure changes

Gabrielle Hyatt, Department of Biomedical Engineering at Binghamton University

A prosthetic hand that can "feel" pressure and tell the user the strength of their grip would enable prosthetics to complete more delicate tasks such as using tweezers or holding an ice cream cone. While a mechanical prosthetic hand that can hold objects is beneficial, it is missing a key component: sensory feedback. For those with upper extremity limb amputations, this would significantly increase quality of life and improve the use of their prosthetic hand during activities of daily living. These sensorized gloves will require flexible circuits to serve as integrated pressure sensors. Initial work focused on the development of a prototype glove using pre-fabricated strain gauges and resistors. Success was shown by using a monitoring integrated circuit that displays real-time strain measurements. While a variety of fabrication techniques are available to custom print flexible circuits including inkjet and screen printing, the limiting factor is that these techniques cannot be used on three-dimensional surfaces. Aerosol printing is a potential solution and as a part of this work the technique will be used to fabricate a strain gauge and bridge circuit. This will allow for the sensor to be integrated onto the glove itself, rather than an external device added to the surface. Development of the aerosol printing parameters for this application is ongoing and preliminary data suggests resistances are nearing half the desired resistance value. Future work will focus on determining the optimum number of layers and curing parameters to print strain gauges with higher resistance values.

Project Mentors: Dr. Janet L. Gbur, CWRU Department of Materials Science and Engineering and Dr. Douglas B. Shire, VA Northeast Ohio Medical Center Advanced Platform Technology Center

Role of the Voltage-Gated Calcium Channel Cav1.2 in Pericytes on Blood-Brain Barrier Stability

Uapingena Kandjoze, Department of Neuroscience, Earlham College; Hua Fang, Hathaway Brown School

This study focuses on understanding the role of the pericyte voltage-gated calcium channel Cav1.2 (encoded by the cacna1c gene) on the stability of the blood-brain barrier (BBB). The BBB comprises microglia, astrocytes, pericytes, and endothelial cells, which work together to maintain the integrity of the BBB. The function of the BBB is to maintain homeostasis of the brain by regulating the transport of biological substances between the blood and brain. One of its principal functions is to prevent entry of exogenous toxins from the blood, and BBB deterioration leads to dangerously increased permeability in several forms of neurodegenerative disease, including Alzheimer's disease. Loss of BBB integrity exacerbates neuroinflammation, oxidative stress, loss of neuronal synaptic connections, and neurodegeneration leading to neuropsychiatric impairment. While loss of pericyte number is correlated to BBB deterioration in neurodegenerative disease, the specific role of pericytes in regulating BBB stability is unclear. Notably, aberrant balance of the intracellular second messenger calcium (Ca2+) has been linked to BBB dysfunction. However, the specific mechanism for this relationship has not been established. We hypothesize that Ca2+ signaling through the Cav1.2 channel in pericytes functions to stabilize the structure and function of the BBB. To test our hypothesis, we are evaluating the effects of pericyte Cav1.2 on the BBB through genetic deletion or mutation of the cacna1c gene. Specifically, we are using two mouse models: the Pdgfr-b-cacna1c knock-out (KO) model in which Cav1.2 has been completely removed from pericytes, and the Timothy syndrome (TS2) mouse model in which the Cav1.2 carries a mutation associated with autism that also causes overactivation of this channel. In these models, cre-recombinase drives the mutations. To validate the Cre expression in these two models, either genotyping using DNA or cre-staining using the protein expression was used. As a measure of BBB integrity, IgG and fibrinogen staining of the brain was performed. IgG and fibrinogens are peripheral substances that should not be present in the brain with a healthy BBB, so their presence in the brain tissue indicated deterioration of this structure. Additionally, IBA1 and GFAP staining was performed to evaluate possible neuroinflammation, which typically accompanies degradation of the BBB.

Project Mentor: Yeojung Koh, PhD candidate, Department of Pathlogy, Case Western Reserve University *in the laboratory of Dr. Andrew A. Pieper, Department of Psychiatry.*

Transcranial Direct Current Stimulation (tDCS) to the Dorsolateral Prefrontal Cortex (DLPFC) to Evaluate Functional Connectivity with the Reward Network

Hannah Kassaie, College of Arts and Sciences; Dr. David Cunningham, Department of Physical Medicine and Rehabilitation, Dr. Nora L. Nock, Department of Population and Quantitative Health Sciences.

The rates of severe obesity (Class III: BMI ≥ 40.0 kg/m^2) and endometrial cancer (EC) incidence and mortality have been increasing significantly in the U.S. over the past several years. Women with severe obesity have a higher risk of EC development and mortality than women with Class I or II obesity (BMI: 30.0 – 39.9 kg/m^2). Behavioral lifestyle interventions involving exercise and nutrition programs including the REWARD (Revving-up Exercise for Sustained Weight loss by Altering Neurological Reward and Drive) trial have been effective in initiating clinically relevant weight loss in obese EC survivors; however, additional strategies are needed to maintain long-term weight loss. Transcranial direct current stimulation (tDCS), a non-invasive neuromodulation technique, has shown some promise in modifying food behavior and inducing modest weight loss in obese non-cancer patients. However, it is not known if tDCS coupled with other intervention components can induce and sustain clinically relevant weight loss in obese adults with EC. Thus, as part of a larger set of studies, we are conducting a secondary analysis of resting-state functional magnetic resonance imaging (rs-fMRI) data before and immediately following active stimulation to the right DLPFC using tDCS as well as a sham condition in 10 healthy (non-cancer) individuals. We hypothesize that stimulation of the dorsolateral prefrontal cortex (DLPFC) using tDCS will result in greater functional connectivity with reward regions of the brain compared to the sham (control) condition. We are in the process of conducting functional connectivity analyses of these data using independent component analysis (ICA) with FMRIB Software Library (FSL). Dual regressions between the tDCS and sham conditions are also being completed to compare differences between conditions and pre and post data. Analysis of Functional NeuroImages (AFNI) is being used to visualize the data and results. Analyses are currently underway and results will be reported in the final written project and corresponding poster presentation. Our intent is to use these results as preliminary data for additional studies in obese EC survivors.

Project Mentor: Dr. Nora L. Nock, Department of Population and Quantitative Health Sciences

Studying the Cross-talk between MSCs and Osteotropic Cancers

Franco Kraiselburd, Department of Biomedical Engineering*; Rodrigo Somoza*, Department of Biology; *Arnold Caplan*, Department of Biology

Mesenchymal Stem Cells or Medicinal Signaling cells (MSCs) have been the target of several innovations in the regenerative medicine field due to their immunomodulatory and anti-inflammatory features and their supporting role in the stem cell niche, which make them an effective therapeutic tool in treating several chronic diseases[1]. MSCs have been identified *in-vivo* as pericytes in the perivascular space, known as perivascular MSCs (pMSCs). The bone marrow's microvasculature has an elevated presence of pMSCs, whose main function is to provide trophic support to HSCs mediated by the secretion of specific factors such as CXCL12 and KITL. These factors attract HSCs to specific sites where they can extravasate into the bone marrow stroma and to become activated. We have shown that the melanoma extravasation mechanism is also mediated by pMSCs[2]. This function *in-vivo* has been found to be critical in the regulation of bone cancer metastasis since the bone marrow microenvironment is very rich in nutrients, growth factors, and cytokines, which can promote cancer metastasis[2]. In previous studies, we assessed how CXCL12 and KITL can influence cancer's invasiveness by selecting cancer cells (melanoma and breast cancer) that highly express those factors and testing their invasion levels when in contact with MSCs. This was done through an *in-vitro* model that simulates the invassive process. Although MSCs have shown potential in cancer therapy, controversial results have recently emerged evaluating the therapeutic potential or homing efficiency of MSCs, as both antitumor and protumor effects were reported[3]. This project aims to study the cross-talk between osteotropic breast, melanoma, and prostate cancer cells and MSCs by: *i*) exposing MSCs to cancer cell conditioned medium (to test cancer's influence on MSC populations) & *ii*) testing direct influence of cancer cells on MSCs by co-culturing cells in transwells with 3 uM pore size (testing the cancer-MSC cross-talk). The MSCs that are influenced by cancer cells indirectly (conditioned medium) and directly (co-culture) are then used in our invasion assay to test their effect on invasion of osteotropic cancer cells (PC-3, MDA231, and A375). Our hypothesis is that MSCs influenced by cancer cells promote osteotropic cancer invasion.

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Benchmarking Obesity-Related Competencies in Dietetic Supervised Practice Programs

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Registered Dietitians are one of the many healthcare professionals that advise and guide patients with obesity. Education on weight stigma and many other factors related to obesity care and maintenance is important for healthcare professionals to properly assist and guide patients. This research assessed the level of obesity education and awareness in Dietetic supervised practice programs.

This study was a cross-sectional survey sent to 319 program directors of supervised practice programs across the country.The competencies published in the interprofessional 2017 Provider Competencies on the Prevention and Management of Obesity served as the foundation of the survey. The survey was created using Qualtrics, and then Questions in the survey asked the program directors about their personal perceived importance of various competencies as well as the level of incorporation of each competency in their program using a 4-point likert scale for each category.

At the time of our interim data analysis, one week after survey distribution,, there were 34 responses, about 10% of the total program directors. The mean years as a program director between all the respondents was around 19 years. 32% (n=11) of the respondents felt that their students were “very prepared” to provide nutrition care for patients with obesity, while 64% (n= 21) of respondents included the prevention and treatment of obesity as an intentional objective in their programs. The largest barriers to integrating obesity education among the respondents were lack of room in the curriculum (38%, n= 13), and a lack of obesity-related rotation sites (29% n= 10). One of the competencies that was integrated into most programs to a “great extent” was evaluating BMI and other anthropometric measurements routinely (82% n= 28). One of the competencies that were integrated into most programs to a “very little extent” was identifying access-to-care barriers for patients with obesity and solutions to mitigate those barriers (35% n=12).

We believe that this is the first study to look at the incorporation of obesity competencies in dietetic supervised practice programs. Educators can use these results to adapt programs to prepare dietitians effectively for treating patients with obesity.

Project Mentor: Dr. Rosanna P Watowicz, Department of Nutrition

Effect of Charged Residues on the Glycosylation of Thr 57 of the PSGL-1 Glycoprotein by the Core-1 Transferase

Hana Lee, Department of Biochemistry; Haley Aharoni, Department of Biochemistry; Collin Ballard, Department of Biochemistry; Miya Paserba, Department of Biochemistry; Dr. Thomas Gerken, Department of Biochemistry

The P-selectin glycoprotein ligand 1, PSGL-1, is a glycoprotein on the surface of lymphoid and myeloid cells that binds to P and E selectins on endothelial cells to induce leukocyte rolling towards areas of inflammation, the first step in an immune reaction. This binding requires a specifically elongated O-glycan, sialyl-Lewis X glycan, at Thr 57, initiated by a family of N-acetylgalactosaminyltransferases, or GalNAc-Ts. The second step in this glycan synthesis requires the Core-1 transferase that elongates the glycan by adding a β-Galactose to the peptide GalNAc. In this research, we hypothesized that negative flanking charges around Thr 57 of PSGL-1 glycoproteins help play a role in targeting the glycosylation by the Core-1 transferase. This is supported by the recently produced structure of the dmCore-1 enzyme, which displays positive surface charges at the peptide binding site. To test this, we designed an assay consisting of five model PSGL-1 glycopeptides with altered flanking charges surrounding Thr 57 (including the wild type) as substrates for the Core-1 transferase. In initial studies, the reactions were saturated, and differences between glycopeptides could not be observed. After lowering enzyme concentrations and reaction times, optimal conditions were obtained to show the difference between the peptides in the assay. The results did not agree with the Core-1 transferase electrostatic surface charges in that both the glycopeptides with negative and positive flanking charges yielded similar percent glycosylations while the neutral glycopeptide showed close to no activity. In studies within the Gerken lab on a different set of charged substrates, prior heating of the glycopeptides at 93°C in a reaction buffer resulted in activities that better correlated with the Core-1 transferase surface charges. Therefore, the PSGL-1 glycopeptides were heated in the same manner, and the previously low activity of the neutral glycopeptide showed a significant increase. The highest activity was ultimately seen in the glycopeptide with a -4 charge, followed by the wild-type peptide, with a -6 charge, while the remaining neutral and positively charged glycopeptides (0, +2, and +4 charges) showed lower activity, which corroborates the predictions from the x-ray structure. However, the glycopeptide with the +2 charge shows the reverse trend of decreased activity. Although the reason why prior heating alters glycopeptide specificity is unknown, we believe that the glycopeptides may form aggregates under normal conditions, which are broken up by heating in the reaction buffer. Further research is being performed to investigate the cause of changed glycosylation by Edman amino acid sequencing, mass spectroscopy, and NMR techniques. These results confirm that flanking charge does affect the Core-1 activity on PSGL-1 glycopeptides.

Project Mentor: Dr. Thomas Gerken, Department of Biochemistry
Graduate Student: Collin Ballard, Department of Biochemistry

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Blocking aberrantly high mitochondrial fission is neuroprotective after traumatic brain injury

Rose Leon-Alvarado: B.A candidate, Department of Neuroscience, Earlham College,

A traumatic brain injury (TBI) is any injury or blow to the head that damages the brain. TBIs vary in severity and are a leading worldwide cause of neurodegenerative disability. TBIs can result in both acute and chronic symptoms, and it is therefore important to study the full spectrum of progression of neurodegenerative disease after TBI in the lab. Some of the neuropsychiatric manifestations reported by TBI patients include personality changes, depression, anxiety, and impulsivity, with depression being one of the most frequently reported symptoms. Neurocognitive impairment is also prominent with TBI. Mitochondrial dysfunction has recently been identified as an important pathological instigator across several neurodegenerative diseases, including acute and chronic TBI. The tightly-regulated dynamic process of mitochondrial fission and fusion is important for mitochondrial health in nerve cells, and neurodegeneration occurs when this balance is pathologically disrupted. In this project, we hypothesized that preventing excessive mitochondrial fragmentation, using a peptide inhibitor of mitochondrial fission (P110) developed and shared collaboratively by the Xin Qi laboratory at Case Western Reserve University, would be protective against neurodegeneration and resulting symptoms after TBI. We used a murine model of jet-flow overpressure TBI, which incorporates components of blast wave exposure, acceleration/deceleration, and global concussive injury. Wild type mice (C57bl6/J) were subjected to either TBI or sham injury and treated daily with either P110 or vehicle control for the subsequent two weeks, starting 3 hours after injury. They were then evaluated in both the novel object recognition test of cognition and the tail suspension test of depression-like behavior, at three different time points: 2 weeks, 6 months, and 9 months post-injury. Treatment with P110 blocked pathologically high mitochondrial fission and prevented neurodegeneration and cognitive impairment at all time points. Unexpectedly, no statistically significant difference in the tail suspension test was found among groups at any time point. This suggests that while this form of TBI in mice readily recapitulates the neurodegeneration and cognitive impairment seen in human TBI, it does not model the depression that people frequently experience after TBI.

Project Mentor: Preethy Sridharan, MD/PhD candidate. Department of Neuroscience. Case Western Reserve University, Laboratory of Andrew A. Pieper, Department of Psychiatry

An *in vitro* assay to assess cellular selectivity of adeno-associated viral vectors enhanced by polymeric adjuvant ePL

Angela Martinez, Department of Biomedical Sciences student, The University of Texas at Rio Grande Valley; Andrei Maiseyeu, PhD, Department of Medicine, Cardiovascular Research Institute and Department of Biomedical Engineering; and Lauren Switala, Department of Medicine, Cardiovascular Research Institute and Department of Biomedical Engineering

Previous studies have shown the clinical safety and efficacy of adeno-associated virus (AAV) vectors for gene therapy in the cardiovascular field. Yet, the therapeutic efforts have been hampered by the intrinsic high uptake of AAV in the liver, which limited sufficient dose delivery to the heart. Our laboratory has developed a nanoparticle (NP) enhancer polymer (ePL) that facilitates AAV delivery to the heart while limiting liver AAV uptake. However, the *in vivo* experiments that allow us to understand the biodistribution of AAV with ePL are laborious, costly, and ethically contentious. This study aimed to develop an *in vitro* assay that allows for better understanding of ePL's ability to direct AAV uptake. Here, we investigated whether serum from mice injected with ePL can facilitate the transduction of AAV in different cell lines. AAV Serotype 1 (AAV1), carrying cytomegalovirus (CMV) promoter and luciferase (Luc) transgene, was used at a dose of 50,000 multiplicity of infection (MOI). The following cell types were cultured in 96-well plates (n=6): HEK293, L6 Myocytes, and H9C2 cardiomyocytes. The different doses (5%, 1.5%, 0.5%) of ePL and normal mouse serum (NMS) were added to the cells simultaneously with AAV1. Following 24 h incubation, transduction efficacy was determined through luciferase reporter assay and normalized by bicinchoninic acid (BCA) protein assay. Statistical analysis was carried out through ANOVA. Transduction efficacy in HEK293 was not statistically different with ePL compared to NMS, irrespective of the dose. In contrast, a statistically significant increase in the transduction efficacy was observed in L6 myocytes and H9C2 cardiomyocytes, particularly when comparing 5% ePL and 5% NMS. Differentiated L6 myotubes exhibited a 60-fold increase ($p=0.0077$) compared to non-differentiated L6 myocytes which showed a 3.75-fold increase ($p=0.0000029$). Furthermore, H9C2 cardiomyocytes showed a 1.73-fold increase ($p=0.0053$).
In conclusion, ePL is advantageous for viral delivery in heart and muscle cells compared to nonmuscle cells. The cell type selectivity and a dose-dependence observed with ePL recapitulated findings from previous *in vivo* studies, thus validating our *in vitro* assay and supporting the previously theorized mode of action of ePL.

Project Mentor: Andrei Maiseyeu, PhD, Department of Medicine, Cardiovascular Research Institute and Department of Biomedical Engineering

The ability of isobutyric tropine ester to blunt fentanyl-induced respiratory depression is independent of its activation of carotid body chemoafferents

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Within the context of surgery or alleviating chronic pain, a significant amount of importance is placed on finding a drug that can be used to reverse opioid-induced respiratory depression (OIPD) without reducing the analgesic effect when using opioids. Intravenously injecting isobutyric tropine ester (ibutropin) has been shown to overrule the negative effects of an intravenous fentanyl injection in male Sprague-Dawley rats. The rats were divided into two groups: one group had a carotid sinus nerve transection surgery (CNSX) while the other group had a sham operation (SHAM). After a 6 day period of recovery, whole body plethysmography (WBP) was used to measure ventilatory parameters while a 200 **μ**mol/kg bolus of ibutropin was injected, followed by an injection of 75 **μ**g/kg of fentanyl. Findings show that upon the injection of the ibutropin, SHAM rats showed more immediate and increased responses in breathing frequency, tidal volume, minute ventilation, peak inspiratory and expiratory flows, and inspiratory and expiratory drives, compared to CNSX rats. Furthermore, there were smaller responses in reaction to the fentanyl injection in both the SHAM and CNSX rats that were given ibutropin, when compared to the rats given the vehicle saline solution injection. An assessment was performed on the analgesic effect of the fentanyl after the administration of ibutropin, and it doesn't appear to impact its pain reducing abilities. The data collected for the sham surgery and then compared to the CNSX rats indicates that fentanyl's effects work independently of the carotid sinus nerve.

Project Mentor: Stephen J. Lewis (Faculty Advisor) Department of Pediatrics, Case Western Reserve University

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Advancing Optical Data Storage Systems using Fluorescent Materials

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Over the past decade, the total amount of data produced per year has increased from 0.9 zettabytes (ZB) to 20 ZB. It has been predicted that this will rise to a staggering 175 ZB by 2025 because of the massive amount of data being generated by the Internet of things, mobile technology, artificial intelligence, and social media. Currently, there is a huge demand for replacing data storage materials involving magnetic materials with optical data storage methods, which provide lower energy consumption, higher capacity, along with longer lifetimes. It is not possible to store large amounts of data generated onto traditional optical and electronic data storage media (tapes and USB flash drives). A polymeric optical data storage (ODS) medium has been combined with microscopy technologies to provide advantages in cost, performance, and durability. However, there has been a fundamental limitation imposed by far-field diffraction physics that creates a restriction on the current state-of-the-art in ODS systems. There are also limitations imposed by the large amount of laser power required to write in such a media. Here, we present multiple methods that will change the face of the ODS systems by solving these problems using plasmonic absorption enhancement by metal nanoparticles and techniques from single molecule microscopy. Spherical metal nanoparticles have been shown to increase the absorption and fluorescence of molecules placed in their close vicinity. By doping the polymeric ODS medium with these nanoparticles and a fluorescent dye (Rhodamine 6G), the absorption and fluorescence of the material could be enhanced in such a way that an effective data writing process could require a lower intensity laser. Stimulated Emission Depletion (or STED) microscopy can be used to overcome the limitation posed by the diffraction limit of light to the written spot size. Photoswitching properties of spiropyran and diarylethene dyes will be used to achieve STED-based data writing. Like STED microscopy, a donut shaped photoswitching laser can be used to reduce the point spread function and create written data spots for ODS that are much smaller than the diffraction limit of the writing laser.

Project Mentor: Professor Anuj Saini, Department of Physics, Case Western Reserve University

Air Pollution Exposure Induces Cognitive Decline in Wild type Mice

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INTRODUCTION: Particulate matter $\lesssim$ 2.5 ($PM_{2.5}$) or fine inhalable particles is arguably the leading environmental pollutant worldwide, implicated in death and disability. Most urban $PM_{2.5}$ results from anthropogenic activities such as transportation and power generation but can also originate from natural sources. Extensive empirical evidence links $PM_{2.5}$ with chronic non-communicable disease such as cardiovascular disease and cancer, with evidence suggesting that exposure accelerates aging in cardiovascular tissues. In this study, we explored the association between exposure to ambient air pollutants on cognitive function, hypothesizing that exposure to $PM_{2.5}$ through aging related pathways in neural and endothelial cell populations may accelerate neurocognitive decline. **METHODS**. We employed a novel Versatile Aerosol Concentrator and Exposure System (VACES) that allows unprecedented in-vivo inhalational exposure to relevant concentrations of ambient $PM_{2.5}$ in Cleveland, to test the impact of chronic exposure to $PM_{2.5}$ on the cognitive abilities of wild type mice (C57BL/6J). We additionally tested the impact of reversal/cessation of exposure (36 weeks exposure to filtered air (FA) or $PM_{2.5}$ followed by a 10 week reversal (PM-Rev) for a total of 46 weeks). Neurocognitive testing included the Barnes Maze Test and the Object Recognition Test and behavioral testing included the Open Field Test, Light Dark Transition Test, Tail Suspension Test, and a motor coordination Beam Walk Test. **RESULTS.** 36 weeks of exposure significantly reduced cognitive function with reversal alleviating. Results on endothelial and neural cell transcriptomic and epigenomic signatures are currently being investigated using unbiased approaches and will be presented when available. **CONCLUSION**. We demonstrate the significant impact of air pollution exposure on cognitive decline with a short-term amelioration on cessation of exposure. Our results, once confirmed and replicated, may provide potential mechanistic pathways and may have substantial implications for regulation and the recognition of $PM_{2.5}$ as a potential risk factor for cognitive dysfunction.

Project Mentors: Dr. Palanivel Rengasamy, School of Medicine, Dr. Sanjay Rajagopalan, School of Medicine

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Alpha-tocopherol Elicits Unique Transcriptional Responses Via Antioxidant-Independent Properties

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Vitamin E is a lipid-soluble antioxidant known to mitigate the harmful effects of oxidative stress by inhibiting the propagation of reactive oxygen species (ROS) reactions in biological lipids and membranes. However, the potential antioxidant-independent functions of vitamin E's biologically active constituent, α-tocopherol, are poorly understood. Previous RNA-seq experimentation comparing α-tocopherol and an antioxidant-inert analog of vitamin E (6-hydroxymethyl α-tocopherol; 6-HMTC), indicated that vitamin E may possess antioxidant-independent biological activities that underlie its health-promoting benefits. Here we aimed to verify the impact of α-tocopherol on the expression of a select subset of genes, namely *ENHO*, *GSTT2*, *INE4*, and *TTPα* using real-time RT-polymerase chain reaction (RT-PCR). Further results and conclusions are pending.

Project Mentor: Dr. Danny Manor, Departments of Nutrition, School of Medicine, and the Case Comprehensive Cancer Center

Emulating Polychaete Worm Anatomy With a Worm Robot Constructed from Compliant Plastic Meshes

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The purpose of this research is to redesign the current worm-like robot. When completed the worm-like robot will have a novel capability of versatile locomotion. The multiple types of movement allow it to be capable to traverse different types of terrain. The previous model had difficulties changing direction because the segments interfered with each other's movements. We proposed a new design of the worm-like robot inspired by the anatomy of polychaete worms. Historically, when ancestor Annelids of segmented worms evolved into polychaete worms, their circular muscles become vestigial or disappeared entirely. The body segment contraction is performed by straight longitude muscle bands while the rest of the horizontal contraction is carried out by small bands of oblique muscles. Thus, this allows the worms to adapt to dynamic modes of locomotion and active predatory lifestyles. While the original design relied on a mesh of tubes which rotate at multiple pivots to translate circular contraction to longitudinal expansion with a contracted resting state, the new model relies on a flexible woven mesh of plastic strips to emulate the hydrostatic skeleton. The structure is then actuated by parallel tendon cables running along the length of the worm through 3D printed channels. The new model instead of relying on the translation of contraction to bodily extension to perform the peristaltic locomotion now allows the actuator to perform direct contraction of bodily length from an extended resting state much similar to the musculoskeletal structure in polychaete worms. This allows for a more compliant structure and more efficient force distribution. The new method allows the worm to be constructed from multiple separated segments, which not only makes it easier to manufacture but also prevent interference between segment during high deformation states such as turning, allowing for a sharper faster turn. Moreover, in addition to peristalsis, the ability to rapidly change direction allows the worm to perform more dynamic modes of locomotion such as undulation, specifically swimming and crawling. These modifications were made without the expense of the important structural property of the worm which is the internal channel for transporting payloads and substrate.

Faculty Mentor: Kathryn Daltorio, Department of Mechanical Engineering
Project Mentor: Huy Pham, Department of Electrical, Computer and Systems Engineering

Optimizing the Heterologous Expression of FLAG-tagged Human Regulator of G Protein Signaling (RGS) 2 Variants in Human Embryonic Kidney (HEK) Cells

Joshua Nworie, Alethia Dixon, Patrick Osei-Owusu

The largest and most diverse family of membrane receptors are guanine nucleotide-binding protein (G protein)-coupled receptors (GPCRs). Stimulated GPCRs act as guanine nucleotide exchange factors, facilitating the activation of heterotrimeric G proteins upon switching of GDP for GTP on the Gα subunit. This leads to the dissociation of Gα from Gβγ obligate dimer to initiate various downstream cellular signaling. One mechanism involved in the termination of G protein signaling is the hydrolysis of the bound GTP by the intrinsic GTPase activity of the Gα subunit. This termination step is accelerated by a family of GTPase activating proteins called Regulators of G protein signaling (RGS), thus restoring the Gα subunit to its inactive, GDP bound state. Failure to regulate this process can lead to increased G protein signaling, which has been implicated in various diseases, such as hypertension and heart failure. One potential mechanism facilitating increased G protein activity is low RGS protein levels, particularly RGS2. Low levels of RGS2 are often the consequence of increased proteolytic degradation. Studies have shown that RGS2 contains four N-terminal methionine residues, Met^{1}, Met^{5}, Met^{16} and M^{33}, that can act as alternative translation initiation sites. M^{16} and M^{33} are shorter translation variants which have been shown to protect against increased degradation. Therefore, the overall goal of this project is to determine the effect of Met^{1}, Met^{5}, Met^{16} and M^{33} RGS2 variants on G protein signaling in the presence of structurally and functionally similar RGS proteins, including RGS5. First, we examined the transfection efficiency of FLAG-tagged human RGS2 variants Met^{1}, Met^{5}, Met^{16} and M^{33} in HEK cells. To this end, we used bacterial transformation and DNA midi-prep to amplify the plasmid containing the RGS2 variants, followed by transfection of the plasmid into cultured HEK cells. We then examined the efficiency of the transfection using western blot and immunofluorescence. Our results indicated successful transfection of the different RGS2 variants. In the future, the lab plans to explore how the different RGS2 variants are compartmentalized in various subcellular locations and whether different levels of these variants influence G protein activity.

Faculty Mentor: Patrick Osei-Owusu, Ph.D., FAHA, Department of Physiology & Biophysics

Development of a Research Protocol for Examining the Effect of Gamification in an Introductory Nutrition Course

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Gamification is commonly defined as the use of game-like mechanics in non-game activities, which has been shown to produce motivational and cognitive benefits in educational settings. The purpose of this study was to develop a research protocol for testing the effect of gamification elements (e.g., points, leader boards, teams) on student performance, engagement, and satisfaction in a college-level introductory nutrition course. A literature review of gamification in nutrition education was conducted, which identified 97 research articles, 23 of which were included in the review based on a priori criteria. Results from the literature review were used to develop a conceptual model of the effects of gamification based on Self Determination Theory (SDT) and an intervention mapping approach was employed to create course elements that would operationalize key constructs in the conceptual model. These course elements will be tested in a two-group posttest-only randomized controlled trial (RCT) developed as part of the current study. The primary hypotheses are that students with access to gamification features will evaluate the course more positively, have higher levels of motivation and engagement, and earn higher scores when compared to students without access to gamification elements. In addition, theoretical mediators of SDT, including identified and integrated motivation, will be examined to test the theoretical assumptions that form the basis of the conceptual model.

Project Mentor: Dr. David Cavallo, Department of Nutrition, Case Western Reserve University

Demographic and Clinical Factors Affecting Keratoconus Risk in Multi-Ethnic Veterans Using Electronic Health Records

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Keratoconus is a non-inflammatory corneal genetic disorder which causes corneal thinning. As a result, increased internal pressures on a weakened corneal surface leads to its characteristic coning or doming of the eye. Most of the knowledge for this genetic disorder has been centered around European and Asian populations. To widen perspective on how keratoconus affects other populations, we diversified our approach by utilizing the Million Veteran Program (MVP). We created an Electronic Health Record (EHR) based algorithm to reliably identify Keratoconus cases and controls. Our purpose is to evaluate the accuracy of the algorithm predicting keratoconus case status and determine how that relates to lifestyle, demographics, and comorbidities.

To pinpoint keratoconus cases and controls, we first had to generate an algorithm that could recognize keratoconus. This was constructed through International Classification Disease Clinical Modification (ICD-CM) and Current Procedural Terminology (CPT) codes in the VA's Computerized Patient Health Records (CPRS). Once the algorithm phenotype had been built, we manually reviewed a small sample of patient records to assess its viability. We calculated positive and negative predictive values as measures of accuracy, and the algorithm was adjusted for errors and refined to attain a better result. The final algorithm was highly accurate, with positive and negative predictive values of 94% and 98%, respectively. It was then used to extract data from the entire nation's VA network system. Data relating to sex, genetic ancestry, and comorbidities, was extracted from EHR and structured veteran interviews of over 100,000 veterans. These variables together with the Charlson Comorbidity Index, a measure of higher mortality, were used to test for differences between cases and controls in lifestyle, systemic diseases, and demographics via single and multiple regression models.

Keratoconus is rare in all ancestries, but some groups are more at risk than others (African Americans > Hispanic Americans > European Americans). Women are at greater risk than men. Cases and controls show the same co-morbidity burden, including heart, lung and blood conditions. Additional analyses are ongoing.

Project Mentor: Dr. Sudha Iyengar, Department of Population and Quantitative Health Sciences, School of Medicine

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Distinct Intracellular IgG Trafficking Route Restricts B cell's Role in IgG Sialylation.

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Immunoglobulin G (IgG) is an antibody subtype that when α2,6 sialylated can be associated with anti-inflammatory effects. When beta-galactoside α2,6-sialyltransferase 1 (ST6Gal1) was knocked out of B cells in a murine model, the sialylation of surface glycoproteins was found to be decreased, while IgG proteins circulating in the plasma were still sialylated. This suggests that knocking ST6Gal1 out of B cells has different outcomes on glycosylation of different B cell-produced glycoproteins. Consistent with this notion, we first discovered that IgG secreted from wild type murine B cell hybridomas had low sialylation but high "core" fucosylation, mediated by α1,6-fucosyltransferase 8 (FUT8). In contrast, flow cytometry revealed that the surface glycoproteins on the same hybridomas were highly sialylated and poorly fucosylated, similar to results analyzing whole cell extracts by ELISA. This suggested that IgG takes a different intracellular trafficking route which leads to differential glycosylation. Using confocal microscopy, we further discovered that IgG colocalized poorly with intracellular regions high in α2,6 sialylation and ST6Gal1, but colocalized well with regions rich in core fucose and FUT8. This supports a model in which IgG takes a divergent trafficking route intracellularly relative to other glycoproteins to leave the cell without being sialylated, thereby providing a possible mechanism for the reason why B cell ST6Gal1 is dispensable for IgG sialylation *in vivo*.

Project Mentor: Brian A. Cobb, Department of Pathology

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Role of STX1A in mediating Cathepsin G's entry into HTC116 cells

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Neutrophils with anti-tumor potential have the capability to kill cancer cells in a contact-dependent manner. It has been reported that Cathepsin G (CG), a serine protease mainly expressed in neutrophils, aids in the targeting of cancer cells. Nevertheless, the molecular mechanisms underlying NETosis released CG remain unexplored. This study aims to focus attention on the prospective mechanisms that could offer soluble CG the potential to kill cancer cells. Our previous CRISPR knockout (KO) screen targeting membrane trafficking found that several KO genes, including STX1A, suppress CG's induced apoptosis in cancer cells, indicating its role in assisting CG cell entry. Based on the results, special attention was directed towards STX1A and its effect on CG's role in anti-cancer activity. STX1A KO cells were generated, and protein levels in these cells as well as in wild type HCT116 will be determined. Immunofluorescence (IF) will be utilized to visualize the localization of CG in both cell types, and CCK8 assay will be employed to measure cell viability.

Project Mentor: Zhenghe J Wang, PhD, Case Comprehensive Cancer Center, Case Western Reserve School of Medicine
Project Sponsor: Monica M. Montano, PhD, Department of Pharmacology, Case Western Reserve School of Medicine

Effects of Inhibition of Src Signaling in Toxoplasma gondii Infection

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Toxoplasma gondii is a parasite that causes retinitis and encephalitis. The parasite hijacks host cell signaling to avoid targeting by autophagy because it cannot survive lysosomal degradation. Current antibiotic regimens against *T. gondii* are suboptimal. Previous research demonstrated that *T. gondii* activates host cell Epidermal Growth Factor Receptor (EGFR) to prevent killing by autophagy. However, EGFR is not widely expressed in retinal and cerebral cells frequently infected by *T. gondii*, decreasing treatment efficacy. Src is a signaling protein that has been identified as part of the autophagy avoidance pathway and is ubiquitously expressed. We hypothesize that Src directly phosphorylates the signaling molecule Akt downstream from EGFR in the autophagic targeting pathway. This research focuses on the blockade of Src Akt cascade to induce autophagic targeting and killing *of T. gondii* as a therapeutic target for treatment. We have found that incubation of Wild Type and Dominant Negative (inactive) EGFR mouse brain endothelial cells challenged with RH strain *T. gondii* in Saracatinib renders a reduction in percentage of infected cells and parasite replication. Experiments show that *T. gondii* triggers the activation of Src at 1 hr and 2 hr post-infection. Western blot analysis of preliminary data indicates incubation with Saracatinib, a Src family kinase inhibitor, leads to a decrease in activated (phosphorylated) Src and Akt expression, allowing autophagic clearance of the parasite. *T. gondii* infected cells treated with Saracatinib shows significant accumulation of autophagic and lysosomal markers, LC3 and LAMP1, respectively, around the parasite, indicating successful autophagic targeting. Further experimentation is currently investigating the mechanism by which Src activates Akt to prevent autophagic targeting of the parasite. With further data, we will establish the mechanism by which Src inhibition leads to autophagic targeting of *T. gondii*, thus allowing Src inhibition to be a possible therapeutic target.

Project Mentor: Carlos Subauste, School of Medicine Division of Infectious Diseases and HIV Medicine

Role of glucocorticoid receptor in prostate cancer stress response and epigenetic alterations

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A cornerstone of treating metastatic prostate cancer (PCa) is androgen-deprivation therapy (ADT), but tumors often progress to a more aggressive form known as castration-resistant prostate cancer (CRPC) through increased androgen receptor (AR) activity. Despite the success of next-generation antiandrogen drugs such as the AR antagonist enzalutamide (Enz), treatment resistance develops, leading to poor patient outcomes. Among mechanisms such as intratumoral androgen synthesis, AR point mutations, and AR splice variants, glucocorticoid receptor (GR) upregulation has been proposed to contribute to therapy resistance. Our group has recently shown the aberrant transcriptional activation of repeat elements (REs) by antiandrogen treatment leads to the formation of double-stranded RNA (dsRNA) which initiates interferon (IFN) response and leads to antitumor activity. In response to stress, the secretion of glucocorticoids leads GR to function as a ligand-dependent transcription factor and regulate the expression of glucocorticoid-responsive genes. By measuring GR expression under stress-based conditions such as hypoxia, irradiation, and Enz treatment, which regulate dsRNA formation, we sought to discover if GR expression is associated with a generalized stress-based response in PCa cells. We also aimed to establish if GR regulates hormone response elements implicated in PCa such as REs. To date, we recorded no change in GR expression in an AR-positive and GR-negative PCa cell line under acute stress, suggesting GR expression may not be impacted by acute stress. To understand the potential impact of GR expression on RE activation, we generated GR-positive cell lines transduced with lentiviruses to inactivate GR using CRISPR-Cas9. We performed quantitative real-time polymerase chain reaction and Western blot analyses and showed a decrease in the expression of long interspersed nuclear element-1 (LINE-1) at both the transcript and protein levels with chronic stress, but not with acute stress. This suggests GR may be associated with the transcriptional activation of REs and be implicated in a possible mechanism of treatment resistance. Our work lays a preliminary foundation for exploring the role of GR in PCa stress response and epigenetic alterations.

Project Mentor: Nima Sharifi, MD, Director, Genitourinary Malignancies Research Center, Lerner Research Institute, Cleveland Clinic

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Chronic postnatal hypoxia exposure causes brainstem serotonergic abnormalities: implications for Sudden Infant Death Syndrome (SIDS).

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Sudden Infant Death (SID) is the leading cause of infant mortality up to one year of age. SIDS pathophysiology is complex, multifactorial, and still largely anecdotal although chronic hypoxia and abnormalities in serotonin (5-HT) neurotransmitter (5-HT_{1A} subtype) expression in key brainstem cardiorespiratory control regions are consistent observations in SIDS infants at autopsy. The cause for the 5-HT1A abnormality is unknown so in this study, we used a rat model of SIDS to investigate whether chronic hypoxia neonatal exposure is sufficient to cause brainstem 5-HT_{1A} receptor deficits. Rat pups were exposed to hypoxia (24h/day) between postnatal (P) days 11-15 and brains were removed for 5-HT_{1A} expression in the nucleus tractus solitarius (NTS) using immunohistochemistry. Compared to normoxia treated rats, hypoxia tended to decrease 5-HT1A receptor expression in the NTS, although it wasn't statistically significant. We also observed an increase in NTS microglia expression, which has also been observed in subsets of SIDS cases. These data demonstrate that the 5-HT abnormalities in distinct cardiorespiratory neural control regions can be initiated by prolonged neonatal hypoxia exposure and may be modulated by microglia. These observations share several commonalities with the risk factors thought to underlie the pathophysiology and etiology of SIDS, including: (1) a vulnerable neonate; (2) a critical period of development; (3) evidence of hypoxia; (4) brainstem gliosis (particularly the NTS and DMNV); and (5) 5-HT abnormalities. These findings could offer significant insight into the long-standing mystery behind the cause of brainstem neurochemical abnormalities in SIDS and the associated autonomic and respiratory control dysfunction.

Faculty Mentor: Pete MacFarlane, UH RBC Pediatrics, Neonatology

Calcium chloride drug dissolution imaging by single molecule microscopy

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The most common mode of drug delivery - Oral administration, brings with it an uncertainty in the variation of bioavailability of the drug. This is a common problem faced by the pharmaceutical industry. Current in-vivo models for drug dissolution are based on the phenomenological Whitney-Noyes equation. They provide inconsistent and unreliable results because of dependence on hydrodynamics and the testing apparatus. Most importantly, these models ignore the heterogeneities of dissolution at the molecular level. Single-molecule fluorescence microscopy can lead to more efficient and predictable drug absorption models and can help speed up the drug development process. This research aims to unveil the heterogeneities in the molecular mechanism of drug dissolution by using this technique. In this study, we use fluorescence microscopy to image the dissolution of $CaCl_2$ (our active pharmaceutical ingredient or API) in water at the single molecule level. Changes in the fluorescence of Rhod-2 dye bonded with aqueous Ca^{2+} can be used to detect changes in the concentration of aqueous $CaCl_2$ at ensemble levels. An increase in "turn-on" events of Rhod-2 as $CaCl_2$ is dissolved in water, is observed at the single molecule level using a Total Internal Reflection Fluorescence microscope. This proof-of-principle single molecule detection method, applied to the dissolution of our API, will be used to develop a pharmacokinetic model for molecular drug dissolution. Integration of Van der Waal's forces and interfacial effects in this model will enable us to simulate the impact of certain internal and external physiological conditions on the dissolution process. Further investigations involving super resolution techniques will be used to develop a pharmacokinetic model through various barrier layers (hydrogels, polymeric coatings, etc.), thereby investigating the roles of different tablet coating materials in drug dissolution.

Project Mentor: *Prof. Anuj Saini*, Department of Physics, Case Western Reserve University

Contribution of mutations in the ORF9b gene to the increased infectivity of SARS-CoV-2 variants of concern

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SARS-CoV-2 is the pathogen at the root cause of the ongoing world-wide pandemic, COVID-19. Both SARS-CoV-1 and SARS-CoV-2 partially escape the host's anti-viral response by inhibiting the production of Type I interferon (IFN). The mitochondrial antiviral signaling (MAVS) surveillance pathway plays a vital role in an innate immune response to viral infection by stimulating interferon synthesis. This project focuses on a non-structural gene of this coronavirus family, known as Open Reading Frame 9b (ORF9b), which is proposed to block the MAVS surveillance pathway by binding to Tom 70 (translocase of the mitochondrial outer membrane 70), an adaptor protein on the outer membrane of the mitochondria linking MAVS to its downstream signaling complex of TBK1/IRF3. Recent SARS-CoV-2 variants of concern (VOC): Gamma, Delta, and Omicron are highly infectious. We hypothesized that mutations in ORF9b in these VOCs contribute to their higher rate of infection. Four mutations in of ORF9b in these VOCs have been identified, and mammalian expression vectors, containing a *c-myc* tag, for three of these mutations were previously constructed. In this project the fourth expression vector for the two mutation sites in the Omicron variant was constructed. To investigate the ability of the three previously constructed VOC ORF9b mutations to inhibit the MAVS surveillance pathway, we optimized the expression of the ORF9b mutations in HEK293T cells transfected with the plasmid vectors using lipofectamine P3000. Successful expression of ORF9b at 24 and 48 h post transfection was demonstrated by immunoblot for the *c-myc* tag. To induce the MAVS pathway HEK293T cells were transfected with a synthetic polyinosinic-polycytidylic acid double-stranded RNA (poly-IC) to model viral infection, using lipofectamine P2000. Phosphorylation of IRF-3 at 2,4, and 6 h post-transfection will be followed by immunoblot to validate the model system. Once these two processes have been optimized, we will investigate the relative ability of each ORF9b mutation to inhibit the MAVS pathway, by measuring the degree of IRF3 phosphorylation in poly-IC treated cells. In conclusion, this study will supply essential details on ORF9b's ability to block Type I IFNs after viral infection.

Project Mentor: Dr. Alan D. Levine, Department of Molecular Biology and Microbiology, Pharmacology, Pathology, Medicine, Pediatrics

Transporting RNA aptamers into HeLa Cells

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Sickle cell disease (SCD) is the US's most common inherited genetic disorder. Sickled hemoglobin contains a single-point mutation within the beta-globin subunit. This mutation codes for valine instead of glutamic acid. This singular mutation results in sickle hemoglobin polymerizing, forming long chains that change the shape of the red blood cells when deoxygenated. Currently, the only available cure for SCD is a bone marrow transplant; however, only approximately 18% of patients are eligible for this treatment. This research focuses on using RNA aptamers, which are RNA molecules that bind to their target protein with immense affinity and specificity. Dr. Fortenberry and her colleagues have previously designed two RNA aptamers that prevent the polymerization of sickle hemoglobin. This research investigates a mechanism to transport the anti-polymerization aptamers into cells. For these studies, we focused on transporting the molecules into HeLa cells. Two aptamers were investigated in this study; the transferring receptor aptamer (Tfr) binds to the transferrin receptor on the cell's surface and is internalized into the cells. We used the Tfr aptamer to transport the anti-polymerization aptamer (DE3A) into the cells. Transferrin, a protein that contains iron, binds to the transferrin receptor and transports iron into cells by receptor-mediated endocytosis. Thus, we hypothesize that the transferrin receptor aptamer can be used to transport the anti-polymerization aptamers into the cells. My project was to use HeLa cells, which also express the transferrin receptor on their surface, to demonstrate that the transferrin receptor aptamer can transport across the membrane when complexed with the anti-polymerization aptamer.

HeLa cells were seeded into 24-well cell culture plates and allowed to grow to approximately 80% confluency. Then, the aptamers were incubated with the cells at various concentrations overnight. The experiment was duplicated, including control wells that did not contain aptamers. The following day, the cells were washed two times with PBS before incubating the cells with RIPA and preparing cell lysates. The cell lysates contain everything inside of the cells, including the cell membrane. The lysates were then subjected to RT-PCR, where the RNA was converted to DNA via reverse transcription, and PCR then amplified the DNA. The RT-PCR reaction was then run on a 5% agarose gel stained with Ethidium Bromide (EtBr). Our results show that the transferrin receptor aptamer and the transferrin receptor-aptamer/anti-polymerization aptamer were expressed inside the HeLa cells. This data suggest that the transferrin receptor aptamer can transport the anti-polymerization aptamer into cells. These initial results indicate that using the transferrin receptor aptamer to chaperone the anti-polymerization aptamer into cells is a viable method to get the anti-polymerization aptamer into red blood cells and potentially prevent the polymerization of sickle hemoglobin. Consequently, the next step is to test these aptamers in patients with sickle cell disease.

Project Mentor: Yolanda Fortenberry, Department of Biology

Generating Isogenic Lines Using Primary Microcephaly with Simplified Gyral Pattern, Epilepsy, and Permanent Neonatal Diabetes Syndrome (MEDS) Patient-Derived iPSCs

Samantha Tsang, Department of Neurosciences; Lucie Yeongran Ahn, Department of Genetics and Genome Sciences; Dr. Ashleigh Schaffer, Department of Genetics and Genome Sciences

Primary microcephaly with simplified gyral pattern, epilepsy, and permanent neonatal diabetes syndrome (MEDS) is an extremely rare autosomal recessive neurodevelopmental disorder characterized by significantly smaller head size, early-onset epileptic encephalopathy, persistent neonatal insulin-dependent diabetes mellitus (PNDM), and severe global developmental delay. It is caused by a homozygous missense mutation in the *Immediate-Early-Response-3 Interacting Protein-1* (*IER3IP*) gene. This gene is thought to code for an Endoplasmic Reticulum (ER) stress response protein that is linked to mediate cell differentiation and apoptosis. However, little is known about this gene's mutation in MEDS patients. This study aimed to model the microcephaly phenotype seen from the *IER3IPI* gene mutation in MEDS patients using isogenic MEDS patient-derived cortical brain organoids. Primary fibroblasts from Patient 1578-41-A1 were reprogrammed into induced pluripotent stem cells (iPSCs) using episomal vectors, then clonally selected, karyotyped, and characterized for expression of pluripotency markers. After maintaining the iPSCs, DNA transfection was performed to test whether we can correct the p.Leu78Pro gene mutation. Based on the primer design, an expected PCR amplification size of the wildtype *IER3IP1* gene of about 800 bp was observed. As expected, a second band around 400bp in the gel electrophoresis was found supporting the observation that a restriction digest enzyme cut was introduced in the middle of the amplification. Since a correction of p.L78P was made, our next step would be to develop cortical brain organoids using the isogenic iPSC line to investigate the pathogenesis of the microcephaly phenotype seen in MEDS patients.

Project Mentor: Dr. Ashleigh Schaffer, Department of Genetics and Genome Sciences
Funded by: SOURCE, CWRU, and the Flora Stone Mather Center for Women

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Climate Change Attitudes and Perceptions

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With rising concerns of Climate Change effects around the world, more research has been conducted that demonstrates that vulnerable populations are at a higher risk of experiencing more severe consequences. Therefore understanding how community members experience climate change, what they know or perceive and how they work to improve the quality of life for their communities are key factors to start addressing these issues. This research focuses on the analysis of data from 3 focus groups conducted in East Cleveland and Glenville which aim to answer the previous points. Demographically, 80% of the participants were Black, 5% White, 5% Asian and 10% Mixed. Additionally 75% of respondents were female and 25% of the respondents were male. The qualitative data gathered from these focus groups are then being analyzed using a coding framework to better understand and compare the respondents' answers. A coding platform, Dedoose, is being used to code the transcripts using a framework developed by the faculty and students on this project. Further coding and analysis is underway to reach conclusions based on this analysis.

Project Mentors: Dr. Ina T. Martin, Department of Materials Science and Engineering; Dr. Cyrus Taylor, Department of Physics; Dr. Brian Gran, Department of Sociology; Stephanie Corbett, Department of Energy and Sustainability.

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Serotonergic Input to R5 Neurons Plays a Role in Shaping Wakefulness and Sleep Architecture

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Sleep is an important biological process for most organisms. It allows both the mind and body to rest and recharge. Two interconnected processes: circadian rhythm and homeostasis regulate sleep. These processes depend on numerous chemicals in the brain, one of which is serotonin. The current study focuses on the interaction between serotonin and sleep-wakefulness in Drosophila melanogaster. Sleep and its physiological role are the subjects of constant research because there is so much that is not known about what happens when we sleep. Nearly 70 million people in the United States alone suffer from chronic sleep problems. Insufficient sleep and disruption of the circadian rhythm cause serious health issues, including metabolic disorders, exacerbated mental illnesses, and even physical accidents. The architecture of Drosophila sleep is very similar to human sleep, making them an excellent model to observe and provide insight into human sleep. Additionally, our lab isolated a single serotonergic neuron, EXR3, using the Gal4UAS system to target transgenic expression and manipulate neural function. EXR3 is one of many specific neurons responsible for serotonin production and regulation throughout the brain and body. The serotonin produced here is used in biological processes, including the circadian rhythm. In the current study, the sleep behavior of a mutant circadian clock strain was compared to wild-type flies in a series of 12-hour light/dark and dark/dark cycles. The target times for observation and comparison were determined by serotonin profiling as ZT 8-10 and ZT 16-18. The second part of our study examined the loss of serotonin production by comparing wild-type flies with toxins to inhibit serotonin production to 5-HT serotonin receptor knockdown/knockout flies. Gain of function was examined using thermogenetics and optogenetics. Flies with heat-activated receptor potential (dTRPA1) were activated by an increase in temperature on the second night of LD. CsCrimson flies were affected by the presence of light. Overall, the results revealed expected trends of serotonin promoting wakefulness. Additional experiments will help to identify if there are any other functions of serotonin in sleep-wake architecture.

Project Mentor: Assistant Professor Masashi Tabuchi, PhD, Department of Neurosciences, School of Medicine

Understanding the Role of TANs in Tumorigenesis using a VTE Pancreatic Adenocarcinoma Mouse Model

Christopher Washington, Division of Hematology and Oncology; Kenneth Kalikasingh, Division of Hematology and Oncology; Asha Thomas, Division of Hematology and Oncology; and Lalitha Nayak, Division of Hematology and Oncology, Case Western Reserve University, Cleveland Ohio

Given that immune cells have been shown to play a pivotal role in both Venous Thromboembolism (VTE) formation and tumor progression, this study investigates VTE that occurs co-morbidly with pancreatic adenocarcinoma from an immune centric perspective. Studies have shown that during neoplasm progression, tumor infiltrating neutrophils (Tumor-associated Neutrophils or TANs) can undergo protumorigenic phenotypic changes. The impact of TAN phenotype manipulation on tumor progression is largely dependent on the presence of CD8 T-cells. Interestingly, a series of unspecified mechanisms induced in the Tumor Microenvironment (TME) have been identified as agents of CD8 T-cell exclusion. Consequently, it was hypothesized that VTE modulates neutrophil activity to facilitate the promotion of pancreatic cancer growth; more specifically, the apparent drive of VTE modified TANs to induce CD8 T-cell apoptosis, evidently, self-imparts their characterization as indubitable contributors to cancer progression. A pancreatic cancer (PANO2) and VTE mouse model (VTE-PANO2) was developed to further evaluate our hypothesis. Data indicates that VTE+ mice actively develop larger tumors and greater thromboses than mice without (VTE-). Additionally, it was found that VTE-PANO2 mice propagate TANs with a heightened propensity to induce CD8 T-cell apoptosis. Similarly, neutrophil depleted VTE-PANO2 mice developed notably smaller tumors relative to the control VTE-PANO2 mice. Finally, RNA sequencing data collected from the peripheral blood neutrophils of our mice suggests that VTE -PANO2 neutrophils exhibit their own unique phenotype. Our findings indicate that neutrophils do in fact mediate the enhanced tumor growth associated with VTE. More specifically, it seems as though VTE promotes a neutrophil phenotype that has a higher capacity to induce CD8 T-cell apoptosis in the TME.

Project Mentor: Lalitha Nayak MD, Division of Hematology and Oncology

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The Role of Wing Fanning in Odor Detection of the Oriental Fruit Moth

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In the natural world, animals frequently adapt to their environment through modifying their behaviors to increase information input. For instance, when more information is necessary to detect or identify an odor, many animals, including humans, sniff to increase the amount of odor molecules they intake. Insects may exhibit a similar behavior by fanning their wings to increase the flow of air and odorants over their odor-detecting antennae. During the process of mating, male *Grapholita molesta*, the oriental fruit moth (OFM), exhibit a behavior called wing fanning. Rather than flying, the male OFM land and track a plume of female pheromone while walking and fanning their wings. This behavior increases the flow of wind-borne pheromone across the moths' antennae. To test the idea that wing fanning is an important odor sampling behavior in insects, we removed the wings of OFM males and compared their ability to track a female moth's sex pheromone up a wind tunnel in varying wind speeds to OFM males with intact wings. So far in our research, all groups located the pheromone source at the highest wind speed. As the wind speed decreased to 0 m/s, the importance of wing beat induced flows became apparent. In 0 m/s wind, males with no wings found the source 56% of the time while males with intact wings located the source 93% of the time. These results suggest that the wing fanning behavior of OFM males does aid in odor detection and tracking by increasing air flow and delivery of pheromones to their antennae. In other words, OFM males sniff with their wings.

Project Mentor: Professor Mark Willis, Case Western Reserve University Department of Biology

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Myocardial Fibrosis Assessment in Hypertensive Individuals with Chronic Kidney Disease

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Background:
Chronic kidney disease (CKD) is associated with an increased risk of myocardial fibrosis, a process of extracellular matrix remodeling, leading to abnormal heart function. Myocardial fibrosis can be assessed non-invasively by cardiac magnetic resonance (CMR) T1 mapping or by circulating biomarkers; however, the performance of such biomarkers in the setting of CKD has not been rigorously tested.

Methods:
The Systolic Blood Pressure Intervention Trial (SPRINT), a multicenter randomized controlled trial conducted in the US, enrolled participants aged ≥50 years, at increased risk for cardiovascular disease and randomized them to intensive systolic blood pressure lowering (<120 mm Hg) or standard blood pressure lowering (<140 mm Hg). In a randomly selected group of SPRINT participants, myocardial fibrosis was assessed by CMR T1 mapping, two collagen-derived serum peptides: C-terminal propeptide of procollagen type I (CICP) and N-terminal propeptide of procollagen type 3 (P3NP), and the protein Galectin 3 (Gal-3) at study baseline. Chronic kidney disease was defined as eGFR<60ml/min/1.73m^2. Unadjusted and age-adjusted correlations were performed between each of the circulating biomarkers, CMR T1 values, and kidney function as measured by eGFR and log transformed urine albumin/creatinine.

Results:
A total of 337 SPRINT participants were included in the study. The mean age was 64.3 [SD 8.9] years, 44.8% were women, and 21% had CKD. The average concentrations of circulating myocardial fibrosis markers at the study baseline were 14.1 ng/ml [SD 7.1] for Gal-3 and 7.4 ug/L [SD 2.4] for PIIINP. The median concentration of CICP at baseline was 94.7 ng/ml [IQR 74.8, 118.1]. There was an inverse correlation between eGFR and Gal-3, PICP, and PIIINP, respectively (p-value < 0.001 for all). The levels of the three biomarkers were positively correlated with log-transformed spot albumin/creatinine ratio. No significant correlation was found between the concentrations of the three biomarkers and the CMR T1 values.

Conclusion:
In a cohort of individuals with and without CKD, no correlation was found between circulating fibrosis biomarkers and T1 mapping relaxation times. CKD status broadly alters the diagnosis value of myocardial fibrosis biomarkers. Future clinical trials should include gold standard CMR techniques when assessing myocardial fibrosis in CKD.

Project Mentor: Dr. Mirela Dobre, MD, Department of Medicine, Division of Nephrology and Hypertension, University Hospitals Cleveland Medical Center

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Correlation Between Urine and Serum Measurements of Serotonin Pathway Biomarkers

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Background: In previous studies, serotonin (5-HT) has shown to be a modulator of respiratory drive and serum serotonin may be linked to intermittent hypoxia events in preterm infants. A non-invasive method of measuring serotonin status would decrease patient burden. We aimed to determine whether urine serotonin pathway biomarker measurements correlate with serum measurements.
Methods: This is a secondary analysis of biomarkers obtained in the CWRU Pre-Vent site study of infants <30 weeks GA enrolled in the Pre-Vent study between 2019-2021 at UH Rainbow Babies & Children's Hospital in Cleveland, Ohio. Samples were analyzed for serotonergic/kynurenic biomarkers using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Analysis focused on determining correlations between urine and blood (platelet poor plasma, PPP) for each biomarker (serotonin; tryptophan, TPH; 5-hydroxyindoleacetic acid, 5-HIAA; and kynurenic acid, KYN).
Results: 83 infants at DOL 7 and 84 infants at DOL 30 had both serum and urine data for each biomarker. Of the four biomarkers tested, DOL 7 and DOL 30 of serotonin (R^2 =0.017, R^2 =0.011), tryptophan (R^2 =0.005, R^2 =0.012), and 5-HIAA (R^2 =0.000, R^2 =0.020) showed little to no correlation between serum and urine. DOL 7 and DOL 30 of kynurenic acid showed a correlation between serum and urine biomarker concentrations (R^2 =0.423, R^2 =0.171).
Conclusions: Urinary biomarker concentrations were not reflective of serum biomarker values. Kynurenic acid was the only biomarker that showed moderate linear correlation between serum and urine samples. Serum sampling of the serotonin pathway continues to be recommended.

Project Mentor: Dr. Anna Maria Hibbs, Department of Pediatrics

Development of Micron-Thin Polymer Films to Observe Transport in the Selective Layer of Membrane Absorbers Using Single Molecule Spectroscopy

Christopher Yoon, Department of Chemical and Biomolecular Engineering

Lanthanides and actinides, also known as f-elements, are widely used due to their versatility in industrial applications. As practical as these elements are, they also come with processing and supply issues that create economic disadvantages and environmental harm. Therefore, recycling them from industrial waste would be a sensible way to minimize the problem. To improve the recycling process of industrial waste, we studied single-molecule imaging, which allows for the observation of exactly one molecule on a surface. This helps us reveal detailed information about the heterogeneous interactions that occur between a single molecule and a surface that are mimicked by a fluorescent dye, Rhodamine 6G, and a polymer film, respectively, in this research. Using single-molecule spectroscopy, we aim to visualize diffusion within the selective layer of a membrane absorber and understand how the morphology of the selective polymer layer affects the diffusion.

Polymer films are prepared to be micron-thin and optically transparent. They are, then, coated with ligands attached using two different methods of polymerization: thermal polymerization and Activator Generated by Electron Transfer Atom Transfer Radical Polymerization (AGET ATRP). As a result, each polymerization technique produces surfaces with different physical properties, and this would reveal the difference in retention time of elements of interest. Thermal polymerization results in polydisperse ligands, whereas AGET ATRP causes the ligands to be monodispersed. The films with the polymer physisorbed onto them are then imaged by a Total Internal Reflection Fluorescence microscope to observe the activities of the attached ligands.

Over the summer, thermal polymerization was successfully achieved with 99% polymerization of ethylene glycol methacrylate phosphate (EGMP). The average polymer film thickness was approximately 3 microns. Depending on the concentration of the polyEGMP solution, the overall film thickness would increase from 4 microns to 6 microns.

Project Mentor: Dr. Christine Duval, Department of Chemical and Biomolecular Engineering; Dr. Lydia Kisley, Department of Physics

A New Methodology for Quantifying Histological Markers Reveals Confounded Antioxidant Capacity of Dimethyl Fumarate to Reduce Chronic Neuroinflammation Induced by Intracortical Microelectrode Implants

Jichu Zhang, Department of Biochemistry; George Hoeferlin, Department of Biomedical Engineering

Intracortical microelectrodes (IMEs) demonstrate great therapeutic potentials, but their performance is limited due to neuroinflammation and oxidative stress around the implant site. Since reactive oxygen species are a main culprit driving failure, mitigation via antioxidants is necessary to improve recording quality and neuronal health.

Dimethyl fumarate (DMF) is an antioxidant that treats multiple sclerosis in patients. It is found to protect neurons from oxidative stress by up-regulating the expression of antioxidant glutathione and reduce the level of pro-inflammatory cytokines by inhibiting the inflammatory regulator NF-κB. So, we hypothesize that the use of DMF will improve neuronal health and alleviate oxidative stress and neuroinflammation. Rats implanted with IMEs are gavaged daily with either DMF (60 mg/kg) or vehicle for control. Then, immunohistochemistry is performed on brain tissues to detect neuronal and inflammatory markers. The cellular responses at different distances from the implant are quantified through a novel, streamlined workflow. Specifically, the neuron population is calculated by Cellpose, a cell segmentation algorithm based on deep learning. The intensities of inflammatory markers are calculated by high-performance Python programs. In this study, the new workflow shows a four-fold increase in speed compared to the old one.

Our results show that DMF does not show protective effects against the death of neurons, the breaching of blood-brain barrier (BBB), or neuroinflammation within 50 μm of the implant ($p > 0.1$). However, DMF shows an adverse effect ($p < 0.05$) on the integrity of BBB between 150–450 μm from the implant. This suggests that DMF as an antioxidant has confounded efficacies in chronic cases of brain implants. One possible explanation is that the frequent oral delivery alters the composition of gut microbiome, which could in turn exacerbate the oxidative stress and inflammation in the brain. Also, the long-term effect of DMF varies across studies that use different brain injury models, ranging from traumatic brain injury to Parkinson's Disease. To conclude, evidence suggests efficacies of DMF are more confounded in the long-term and require more investigation with consistent injury and animal models.

Project Mentor: Jeffrey Capadona, Department of Biomedical Engineering

Protein Crystallography of a Cortisol Nanobody to Pinpoint Molecular Mechanism for Cortisol Binding for Use in a Real-Time Biosensor for Stress Metabolites

Cecelia Zielke, Department of Nutrition; Dr. David Lodowski, Department of Nutrition

When faced with threatful stimuli, cortisol grants humans an increased ability to escape threat. However, when elevated for prolonged periods of time, cortisol acts as a muscular and neurological inflammatant, inhibiting competent "fight or flight". In military populations and collegiate athletes subjected to prolonged physical and mental stress, monitoring cortisol levels will inform the maintenance of a maximal long-term physical response. In the long-term, we aim to develop a biosensor to monitor serum cortisol levels in real-time by measuring the binding efficiency of cortisol nanobodies to serum cortisol. To do so, the precise molecular mechanism for cortisol binding to a VHH cortisol nanobody (CorNB) must be determined, the steps for which I describe in my poster. Employing transformation of e. coli and purification of CorNB via gel filtration chromatography, we successfully expressed and isolated CorNB. We Conducted several rounds of crystal growth screening, and refined the solvent, buffer, and precipitant conditions, eventually arriving at conditions that grow large and well-defined three-dimensional crystals of CorNB. In continuation of the project, we harvested and froze CorNB crystals and will collect X-Ray diffraction data to determine the precise molecular recognition 127 mechanism for CorNB binding to cortisol for use in the real-time biosensor. Both are confidential, and the PI/Student will not see and vice versa.

Project Mentor: Dr. David Lodowski, Department of Nutrition

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Identifying Target for Novel Selective Anti-Cancer Small Molecule

Anna Zinsser, Department of Biochemistry; Matthew Pleshinger, Department of Pharmacology; Drew Adams, Department of Genetics and Genome Sciences

The treatment of cancer has been a prevalent issue for doctors and researchers for many years now. The highly similar nature of cancerous and noncancerous cells makes it difficult to develop treatments that selectively target and kill cancer cells without harming healthy cells. One attempt to combat this issue involves the use of high-throughput small-molecule screens to identify compounds that are specifically toxic to cancerous cells. The compound 96505 was found in a previous screen to selectively target cancer cells, specifically certain hematopoietic cancer cells. While the compound is known to have selective toxicity, the intracellular target has not yet been identified. In order to determine the prospective cellular target of 96505, our lab has employed a forward genetic screening approach. This approach involves treating cells that have a high mutation rate with 96505 over multiple weeks in order to stimulate the formation of resistant clones. Once resistant clones have been obtained, exome sequencing can be performed in order to identify DNA mutations that are shared amongst all resistant clones but not found in the parent cell line. The mutation that is shared amongst all clones and absent in the parent is likely to be related to the compound's cellular target. Determining the cellular target of 96505 would provide crucial insight into the possibility of developing this compound into a therapy in the future.

Project Mentor: Professor Drew Adams, Department of Genetics and Genome Sciences