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SUMMER 2017

AUGUST 4, 2017

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Last Name	First Name	Home University	Summer Program Name	Project Title	Mentor Full Name & Department	Faculty Mentor's Department
Allen	Austin	Denison University	Chemistry NSF REU	RNA Binding Properties of an hnRNP A1 Domain Swap Mutant	Blanton Tolbert, Chemistry	Chemistry
Armes	Tonya	Case Western Reserve University	Heart, Lung & Blood Program	The Role of Protein S-nitrosylation Regulated by S-nitroso-CoA Reductase in Myocardial Ischemic Injury	Jonathan Stamler, Biochemistry and Medicine, University Hospitals	Department of Medicine
Bomani	Nichola	Case Western Reserve University	MetroHealth Chester Scholars Program	Biomechanical Analysis of Strong and Weak Term Fetal Membranes	John Moore, Pediatrics and Reproductive Biology, MetroHealth	School of Medicine
Bonilla Fernandez	Koby	San Juan Bautista School of Medicine	Heart, Lung & Blood Program	Differences in pulmonary hypertensive changes between wild-type and GSNOR KO's mice exposed to hyperoxia conditions.	Benjamin Gaston and Raffay Thomas, Pediatrics	Department of Pediatrics
Bonner	Daphney	Howard University	Chemistry NSF REU	Synthesis of High Dielectric Constant Materials for Organic Photovoltaics Application	Genevieve Sauve, Chemistry	Chemistry
Boruah	Abhilasha	Case Western Reserve University	SOURCE	Evaluation of Non-Accidental Trauma in Infants with Skull Fractures: A Retrospective Review	Krystal Tomei, Pediatric Neurosurgery, UH Rainbow Babies & Children's Hospital	Pediatric Neurosurgery - UH Rainbow Babies & Children's Hospital
Calhoun	Cody	Case Western Reserve University	P-SURG	Generating a Drosophila Model of XX Female Gonadal Dysgenesis via CRISPR-Cas9	David Buchner, Genetics and Genome Sciences	Department of Genetics and Genome Sciences, Case Western Reserve University
Chen	Keying	Case Western Reserve University	Advanced Platform Technology (APT) Center	The Effect of Topographical Modification on Neuro-inflammation	Jeffrey Capadona, Biomedical Engineering	Department of Biomedical Engineering
Chu	Joyce	Case Western Reserve University	P-SURG	The Development of Gait Dynamics in a Mouse Model for Down Syndrome	Alberto Costa, Pediatrics and Psychiatry	Pediatrics
Darville	Joshua	Vanderbilt University	ACES+	Sensory Feedback of Compliant Modular Worm-like Robot	Roger Quinn, Mechnical and Aerospace Engineering	Mechanical & Aerospace Engineering

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Del Rio Montesinos	Teresa	Universidad Central del Caribe	Heart, Lung & Blood Program	Effect of opioids on CD8+ T cell activation and Opioid Receptor expression	Alan Levine, Molecular Biology and Microbiology	Department of Molecular Biology and Microbiology
Dona	Keith	Case Western	Advanced Platform Technology (APT) Center	An Adaptable Computer Vision System to Quantify Rat Behavioral Tests.	Jeffrey Capadona, Biomedical Engineering	Biomedical Engineering
Fowler	Jahmel	Fisk University	ACES+	Analyzing Fluorine Labelled Cysteine Mutants of The Protein UP1	Blanton Tolbert, Chemistry	Biochemistry
Gordon De Jesús	Adriana	San Juan Bautista School of Medicine	Heart, Lung & Blood Program	Upregulation of ABCA1 in lipid-loaded macrophages is dependent on autophagy	Jeffrey Deiuliis, General Medical Sciences, Case Cardiovascular Research Institute	Case Cardiovascular Research Institute
Haley	Rebecca	Case Western Reserve University	ENGAGE	Release of Resveratrol through Affinity-Based Drug Delivery to Reduce Inflammation at the Site of Intracortical Electrode Implantation	Horst von Recum, Biomedical Engineering	Biomedical Engineering
Heath Borrero	Kimberly	University of Puerto Rico in Ponce	Heart, Lung & Blood Program	Characterization of Genetic Ancestry Among Chronic Kidney Disease Patients	Dana Crawford, Epidemiology and Biostatistics	Population and Quantitative Health Sciences and Institute for Computational Biology
Hemmingsen	Christina	Case Western Reserve University	P-SURG	Synthesis of Functionalized Graphene Oxide and Analysis of Inter-Sheet Interactions	Emily Pentzer, Chemistry	Chemistry
Hermosillo Guzman	Eduardo	University of Rhode Island	Chemistry NSF REU	Studies of the Successive Protonation of Silylated Zirconophosphaalkene [Cp2Zr{C6H4C(OSi(CH3)3)P}]2 to yield Benzoylphosphine	John Protasiewicz, Chemistry	Chemistry
Lewis	Christina	University of Florida	Heart, Lung & Blood Program	Lumbar Sympathetic Nerve Activity Responds to Physiologic Challenges in Isoflurane-Anesthetized Rats	Stephen Lewis, Pediatrics	Department of Pediatrics

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Li	Amanda	Case Western Reserve University	P-SURG	Can we judge a newt by its spots? Determining if newt patterns are a reliable method of identifying individuals with the aid of pattern recognition software.	Michael Benard, Biology	Biology
Liu	Fangze	CWRU	APT at VA	Optimizing Paraspinal Electrode Implantation Techniques Through 3D Modeling Analysis	Ronald Triolo, Biomedical Engineering, VA Advanced Platform Technology Center	VA Advanced Platform Technology Center
Lu-Diaz	Michael	University of Puerto Rico at Mayaguez	Chemistry NSF REU	Functionalization of AZO Surfaces with Silanes	Emily Pentzer, Chemistry	Chemistry
Mahajan	Uma	Case Western Reserve University	CLiPS	Developing a More Robust Method to Generate Cerebral Organoids	Luke Bury, Genetics and Genomic Sciences	Genetics and Genomic Sciences
					Anthony Wynshaw-Boris, Genetics and Genoic Sciences	Genetics and Genomic Sciences
Manuck	Carolyn	Case Western Reserve University	P-SURG	Evaluation of a Play-Based Intervention for Children with Prader-Willi Syndrome	Anastasia Dimitropoulos, Psychological Sciences	Psychology
Michel	Keisha	Emory University	Heart, Lung & Blood Program	Inhibition of the Growth of Triple Negative Breast Cancer Cells by Novel HEXIM1 Inducers	Monica Montana, Pharmacology	Department of Pharmacology
Morris	Aaron	Morehouse College	SOURCE	Modeling the Effect of 20% Dietary Fructose on NOX4 Expression and Implications on Salt-Sensitive Hypertension	Jeffrey Garvin, Physiology and Biophysics	Physiology and Biophysics
Moxey	D'Andra	Fisk University	ACES+	The observation of the effect the concentration of DTT has on the ATP dependent protease ClpXP	Irene Lee, Chemistry	Chemistry
Nelson	Melanie	Wesleyan University (Middletown, CT)	Heart, Lung & Blood Program	Cdk5 Expression in Crisper Knockout PDL1 (crPDL1) and Wildtype (WT) Rhabdomyosarcoma Cells	Agne Petrosiute, Pediatrics (Hematology/ Oncology)	Department of Pediatrics (Hematology/ Oncology)
Ononuju	Ucheze	Wayne State University School of Medicine	Heart, Lung & Blood Program	An evaluation of PCNA expression in neonatal mice lungs exposed to CPAP therapy	Richard Martin, Pediatrics (Division of Neonatology)	Department of Pediatrics, Division of Neonatology

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					Anjum Jafri, Pediatrics (Division of Neonatology)	Department of Pediatrics, Division of Neonatology
Rosenberg	Josh	Case Western Reserve University	Advanced Platform Technology (APT) Center	Simulating Sensory Axons to Predict Action Potentials in the Vagal and Splanchnic Nerves	Matthew Schiefer, Biomedical Engineering	Biomedical Engineering
Santiago	Raymond	The Ohio State University	Heart, Lung & Blood Program	Determining the Relationship between Brainstem Inflammation and Changes in Ventilatory Pattern Variability (VPV) in Healthy and Septicemic Rats	Thomas Dick, Medicine and Neuroscience	Medicine and Neurosciences
Saunders	Ashley	Oakwood University	Heart, Lung & Blood Program	Hypoxia Inducible Factor-1 in Peripheral Vascular Smooth Muscle Cells is Critical for Vascular Phenotype	Diana Ramirez-Bergeron, Cardiology (CRI)	Cardiovascular Research Institute
Starks	Maurryce	The College of Wooster	Heart, Lung & Blood Program	Optimization of Membrane Protein Reconstitution for Membrane Scaffolding Protein 2N2 to Create Larger Nanodisc Complexes	Sudha Chakrapani, Physiology and Biophysics	Physiology and Biophysics
Stewart	Eric	University of Illinois College of Medicine at Chicago	Heart, Lung & Blood Program	High Throughput Screening for Uracil-DNA Glycoyslase Activity Modulators	Stanton Gerson, Case Comprehensive Cancer Center	Case Comprehensive Cancer Center
Suzuki	Takayuki	Case Western Reserve University	P-SURG	Developing a Collagen Scaffold for Surgical Repair of Bone, Tendon, and Muscle	Ozan Akkus, Mechanical and Aerospace Engineering	Department of Mechanical and Aerospace Engineering
Thompson	Matthew	Case Western Reserve University	P-SURG	Identification of CHIR-99021 as a Novel Therapy for Huntington’s Disease	Drew Adams, Genetics and Genome Sciences	Genetics and Genome Sciences
Villagrasa Mendez	Carlos	University of Puerto Rico at Cayey	SOURCE	Using SynthoPlatesTM to Enhance Platelet Aggregation in Patients Undergoing Antiplatelet Drug Therapy	Marvin Nieman, Pharmacology	Pharmacology

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Walker	Jasmine	Emory University	Heart, Lung & Blood Program	Examining the Relationship Between CDK5 and γδ T cells Using γδ Leukemia Cells	Mari Dallas, Pediatrics (Hematology/ Oncology) and Pathology	Department of Pathology/ Department of Pediatric Hematology and Oncology
Whiting	Emma	Washington University in St. Louis	Chemistry NSF REU	Synthesis of 2-[(3-cyano-6-methyl-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)carbamoyl]bicyclo[2.2.1] hept-5-ene-3-carboxylic acid	Greg Tochtrop, Chemistry	Chemistry
Whittsette	Angela	Case Western Reserve University	Heart, Lung & Blood Program	Pathogenic mechanism of epilepsy-associated gamma-amino butyric type A receptors	Tingwei Mu, Physiology and Biophysics	Physiology and Biophysics
Williams	Rachante	Fisk Universsity	ACES+	Histological characterization of wild type and Thoc6 mutant mice during neurogenesis	Ashleigh Schaffer, Genetics and Genome Sciences and Center for RNA Science and Therapeutics	Genetics and Genome Sciences and Center for RNA Science and Therapeutics
Wolkoff	Alexandra	University of Rochester	Advanced Platform Technology (APT) Center	Restoring Sensation in the Lower Extremities of Amputees Through Electrical Stimulation of Peripheral Nerves	Hamid Charkhkar, Biomedical Engineering	Biomedical Engineering
Yang	Jingyi	Case Western Reserve University	P-SURG	Neural dynamics of a feeding pattern-generating circuit in the marine mollusk Aplysia californica	Hillel Chiel, Biology	Biology

RNA Binding Properties of an hnRNP A1 Domain Swap Mutant

Austin Allen[1], Jeffrey D. Levengood[2], Niyati Jain[2], Le Luo[2], and Blanton S. Tolbert[2]

[1]Denison University Department of Chemistry
[2]Case Western Reserve University Department of Chemistry

Heterogenous nuclear ribonucleoprotein A1 (hnRNP A1) is an RNA binding protein implicated in both normal and pathological cellular functions. HnRNP A1 consists of two domains, an N-terminal RNA binding domain (UP1), and a glycine-rich C terminal domain. The UP1 domain contains two nearly identical RNA recognition motifs (RRMs) that are connected by a short linker. Previous structural studies from the Tolbert lab have found that only the first RRM (RRM1) binds to the HIV Exon Splicing Silencer 3 (ESS3) stem loop. The beta sheet surface of RRM1 and the inter-RRM linker fold to form a nucleobase pocket that interacts with an AG dinucleotide located within the ESS3 apical loop. Despite being nearly identical in structure and sequence, RRM2 does not come in contact with ESS3. Thus, to investigate if RRM2 can support ESS3 recognition within the context of UP1, a domain swap mutant of UP1 was prepared in which RRM1 and RRM2 were structurally transposed. NMR spectroscopy revealed that the core structure of UP1 is preserved in UP1swap. Moreover, biochemical and calorimetric titrations show that UP1swap still binds to ESS3 albeit with reduced affinity compared to the native protein. Collectively, this work suggests that RRM1 and RRM2 likely have non-redundant RNA sequence specificities. Crystal screens of UP1swap are underway to further understand the atomic implications brought about by the RRM transposition.

Project Mentor: Dr. Jeffrey D. Levengood, Department of Chemistry
Faculty Sponsor: Dr. Blanton S. Tolbert, Department of Chemistry

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The Role of Protein S-nitrosylation Regulated by S-nitroso-CoA Reductase in Myocardial Ischemic Injury

Tonya Armes[1,2], Hualin Zhou[1,2] and Jonathan S. Stamler[1,2,3]
[1]Department of Medicine and [2]Institute for Transformative Molecular Medicine, Case Western Reserve University, Cleveland, OH
[3]Harrington Discovery Institute, University Hospitals Cleveland Medical Center, Cleveland, OH

Protein S-nitrosylation, the covalent modification of a Cys thiol by nitric oxide (NO) to generate a protein S-nitrosothiol (SNO-protein), largely conveys the ubiquitous cellular influence of NO. SNO-proteins are in equilibrium with low-molecular-weight SNOs (LMW-SNO) including SNO-CoA, and those equilibria are governed by denitrosylases that degrade LMW-SNOs, including SNO-CoA reductase (SNOCoAR) (Figure 1). NO is produced in the heart by three NO synthases (eNOS, iNOS, and nNOS). It is generally held that cardio-protection is conferred by nNOS/eNOS via vasodilation and amelioration of oxidative stress. We have focused on the role of protein S-nitrosylation in a well-established mouse model of myocardial infarction (MI), induced by transient occlusion of a coronary artery (ischemia/reperfusion injury, I/R). Here we examine specifically the role of SNOCoAR, employing mice in which SNOCoAR was knocked-out genetically. We first observed that SNOCoAR KO was cardio-protective during I/R, as assessed by standard echocardiographic measures including left ventricular ejection fraction and fractional shortening, as well as by levels of serum troponin, in wild-type (WT) and SNOCoAR KO mice (Figure 2). Levels of SNO-protein and cGMP were assessed in the myocardium of WT and SNOCoAR KO mice employing SNO-RAC and ELISA respectively. Levels of myocardial SNO-protein were similar across WT and SNOCoAR KO mice under basal conditions and WT mice following I/R (30 min occlusion; 24 hr survival) (Figure 3). However, SNO-protein levels were markedly enhanced following I/R in SNOCoAR KO mice (Figure 3). It is known that transient changes in cGMP levels following ischemic insult are associated with myocardial injury/protection. We observed that myocardial cGMP levels decreased following I/R (30 min occlusion; 10 min survival), but cGMP levels did not differ between WT and SNOCoAR KO mice under either basal conditions or after I/R (Figure 4). Our findings indicate that enhanced protein S-nitrosylation in the absence of SNOCoAR protects against MI-induced injury, without a significant role for cGMP.

Project Mentor: Jonathan S. Stamler, M.D., Professor of Medicine and of Biochemistry and Director, Institute for Transformative Molecular Medicine, Case Western Reserve University; President, Harrington Discovery Institute, University Hospitals Cleveland Medical Center

Project Supervisor: Hualin Zhou, Ph.D., Senior Research Associate, Department of Medicine and Institute for Transformative Molecular Medicine, Case Western Reserve University

Biomechanical Analysis of Strong and Weak Term Fetal Membranes

Nichola Bomani, Department of Biochemistry, Olla Khalid, MetroHealth, Anudeepa Sharma, MD, MetroHealth, Robert Moore, School of Medicine, Deepak Kumar, MD, School of Medicine, and John J Moore, MD, School of Medicine

Preterm premature rupture of membranes (PPROM) results in preterm labor and increased neonatal morbidity/mortality. The etiology of fetal membrane (FM) rupture remains unclear. Inflammation, infection, and placental abruption cause increases in factors leading to cellular apoptosis, collagen degradation, resultant PPROM and fetal membrane rupture. This study examines differential protein expression of factors associated with biomechanically "strong" versus "weak" amnion (AM) & choriodecidua (CD), separated and analyzed following strength testing. FM from freshly delivered placentas were obtained from term elective cesarean sections. FM were cut into pieces and the strength required to rupture and deflection during testing were recorded. Biomechanical rupture data showed that intact membranes are stronger than re-approximated membranes, and weaker FM have greater deflection differences. The strongest and weakest FM pieces were compared to one another biochemically by Western Blotting. We found that weak FM express more Integrin αX and GM-CSFRα when compared to strong FM, and that strong FM exhibit much more mPRα and mPRγ than their weak counterparts. Further study of the interactions between GM-CSF and membranous progesterone receptors may provide medical interventions for those at risk for PPROM and thus, preterm labor.

Project Mentor: Dr. John J Moore, School of Medicine

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Differences in pulmonary hypertensive changes between wild-type and GSNOR KO's mice exposed to hyperoxia conditions

Koby Bonilla, MD candidate, San Juan Bautista School of Medicine; Dr. Thomas Raffay, MD, Department of Pediatrics, Div. of Neonatology; Dr. Benjamin Gaston, Department of Pediatrics, Div. of Pulmonology; Laura Smith, Department of Pediatrics; and Anjum Jafri, Department of Pediatrics

Premature infants frequently require treatment with high levels of supplemental oxygen, increasing their risk of developing bronchopulmonary dysplasia (BPD) and pulmonary arterial hypertension (PAH), a serious consequence of BPD that increases the morbidity and mortality of preterm infants. PAH models have shown that exposure to hyperoxia conditions causes pulmonary vascular remodeling and right ventricular hypertrophy. S-nitrosoglutathione (GSNO) is an endogenous smooth muscle relaxant that is downregulated in hyperoxic conditions by the increased activity and expression of GSNO reductase (GSNOR) in the lung tissue. In this study, we analyzed the pulmonary vascular and cardiac remodeling of newborn mice exposed to 3 weeks of 60% O_2. We hypothesized GSNOR$^{-/-}$ mice would be protected from PAH changes. Hyperoxia induced pulmonary hypertensive changes (right ventricular hypertrophy, arterial smooth muscle proliferation, and diminished vessel density and VEGF expression). This was, in-part, attenuated in the GSNOR$^{-/-}$ mice. These data support the need for further investigation of GSNO-based therapies as a novel treatment for neonatal PAH, for which current interventions are lacking.

Project Mentors; Dr. Thomas Raffay, Department of Pedriatics, Div. of Neonatology, and Dr. Benjamin Gaston, Department of Pediatrics, Div. of Pulmonology, and.

Synthesis of High Dielectric Constant Materials for Organic Photovoltaics Application

Daphney Bonner, Department of Chemistry; Chunlai Wang, Department of Chemistry; and Dr. Genevieve Sauvé, Department of Chemistry

Organic photovoltaic devices are very attractive because they are lightweight and can be fabricated at a low-cost. In recent decades, research in the field of organic photovoltaics has focused on developing organic semiconductors with broad absorption and tunable energy levels, as well as optimizing film morphology and device fabrication. However, less studies have looked into increasing the dielectric constant of organic semiconductors. A recent theoretical study shows that increasing the dielectric constant of organic semiconductor from ~3 to ~10 would increase power conversion efficiency of organic photovoltaics from ~10% to ~20% (Torabi, 2015). This is possible because an increased dielectric constant should minimize charge carrier recombination and reduce the exciton binding energy. Previous research has concluded that the dielectric constant of organic semiconductors can be increased using dipolar polarization. However, this type of polarization can only affect slow processes such as charge carrier recombination, because it is limited to how fast polar groups can rotate in a film. Alternatively, electrons have the capability to move much faster. We therefore seek a way to increase the dielectric constant of organic semiconductors by increasing electronic polarization. To accomplish this, we have designed a zwitterion molecule with separated positive and negative charges. It is hypothesized that the negative charge will move quickly under an external field, alternating between two resonance structures. This will increase electronic polarization as well as the dielectric constant, and allow for lower exciton binding energy. To test these hypotheses, I have synthesized and characterized this zwitterion molecule. The availability of the molecule will enable further studies, including measurement of the dielectric constant.
Project Mentor: Chunlai Wang, Department of Chemistry
Faculty Sponsor: Dr. Genevieve Sauvé, Department of Chemistry

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Evaluation of Non-Accidental Trauma in Infants with Skull Fractures: A Retrospective Review
Rainbow Babies and Children's Hospital
University Hospitals Cleveland Medical Center

Abhilasha Boruah, Department of Cognitive Science; Dr. Berje Shammassian, Neurological Surgery; Dr. Byron Hills, Neurological Surgery; Dr. Lolita McDavid, Child Advocacy and Protection; Dr. Michael Dingeldein, Pediatric Trauma Surgery; Dr. Krystal Tomei, Pediatric Neurological Surgery

Non-accidental head trauma is the most common mechanism of traumatic death in infants and the most common serious injury attributable to child abuse. While non-accidental trauma (NAT) is a frequent cause of emergency department visits for children, it often difficult to determine if the injury is due to abuse because of nonspecific symptoms, unreliable histories, and potential lack of verbal ability. Children under the age of one year are the most high-risk age group for abusive head trauma, as their limited mobility and lack of self-protected reflexes change injury profiles sustained from a fall. Therefore, improving identification of children at risk is critical to child protection. Historical studies have demonstrated varied practice patterns for evaluation of NAT in infants with skull fractures. While previously no regimen existed to properly examine these infants in our institution, our trauma committee developed a standard protocol for the evaluation wherein all infants under the age of 12 months, and non-ambulatory patients over the age of 12 months, with a diagnosed skull fracture, underwent a full non-accidental trauma workup upon presentation. This workup included a trauma surgery evaluation, social work consults, child protective team consult, laboratory tests, skeletal survey, and ophthalmologic exam. We conducted a retrospective chart review to determine the rate of adherence to our institutional protocol, compare patterns of both non-accidental and accidental trauma in our patient population, and measure the effectiveness of our hospital system in the evaluation and outcome of these patients. These findings will ultimately lend themselves to refining our current protocol to develop the most cost and resource effective algorithm to evaluate these infants and ensure their future protection.

Project Mentor: Krystal Tomei, MD, MPH, Director of Pediatric Neurological Surgery

Generating a Drosophila Model of XX Female Gonadal Dysgenesis via CRISPR-Cas9

Cody Calhoun[1], Anlu Chen[2], Laura Shapiro-Kulnane[3], Helen K. Salz[3], David A. Buchner[2,3,4*]

[1]Department of Biology, Case Western Reserve University, Cleveland, OH 44106
[2]Department of Biochemistry, Case Western Reserve University, Cleveland, OH 44106
[3]Department of Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH 44106
[4]Research Institute for Children's Health, Case Western Reserve University, Cleveland, OH 44106

XX female gonadal dysgenesis (XX-GD) is a rare disorder characterized by primary amenorrhea, hypergonadotrophic hypogonadism, and delayed pubertal development. Mutations in *FSHR, NUP107, BMP15* and other genes have been associated with the disorder. However, the genetics of this disorder, and therefore pubertal ovarian development remain poorly understood.

To identify novel genetic causes of XX-GD, we studied a consanguineous family of Israeli-Arab origin with XX-GD due to a mutation in the gene encoding mitochondrial ribosomal protein S22 (MRPS22). MRPS22 encodes a mitochondrial ribosomal small subunit protein that is found in species including mammals, fruit flies and nematodes. Therefore, we studied the role of mRpS22 in ovarian development, using an (RNAi)-mediated knockdown approach in Drosophila. Germ cell specific knockdown of the MRPS22 ortholog in Drosophila were infertile and failed to develop germ cells. This demonstrates that the Drosophila ortholog mRpS22 is required for female germ cell development.

To further study the role of *MRPS22* in XX-GD, we will generate a Drosophila model with the same mutation in mRpS22 as was seen in MRPS22 in the family described above. This mutation (p.R202H) will be introduced into Drosophila using CRISPR-Cas9. The CRISPR-Cas9 technique utilizes an endonuclease that interacts with a guide RNA sequence to create specific double strand breaks. A donor sequence containing the desired mutation can be co-injected into Drosophila embryos to be incorporated at the double stranded breaks. I have confirmed the target sequence in Drosophila, designed the targeting strategy, and generated and purified the plasmid vectors to be injected into the Drosophila embryos. Future work will entail generating the genetically altered Drosophila and examining the resulting phenotype to better understand the role of MRPS22 in ovarian development and XX-GD.

Project Mentor: David A. Buchner, Department of Genetics and Genome Sciences

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The Effect of Topographical Modification on Neuro-inflammation

Keying Chen, Department of Biomedical Engineering; Evon S. Ereifej, Department of Biomedical Engineering; Cara Smith, Department of Biomedical Engineering; Seth Meade, Department of Biomedical Engineering

Neuro-inflamamtion is considered as one of the critical factors that lead to failure of intracortical microelectrodes. It is hypothesized that a contributor to the inflammatory response is the discontinuity between the rough architecture of the native brain tissue and the smooth implant surface. The goal of my summer project is to investigate the effect of surface modification, based on the 3D matrix structure of brain native tissue, on the intensity of neuro-inflammation around the implant site. Sprague Dawley rats received were implanted in the cortex with Michigan-style, non-functional, nanopatterned, silicon shank microelectrodes, and compared to control group of animals which had non-patterned microelectrodes implanted into the cortex. Immunohistochemical (IHC) staining was performed to assess the effect of surface modifications, including NeuN, glial fibrillary acidic protein (GFAP), cluster of differentiation 68 (CD68), and immunoglobulin (IgG), which gave insights about neurons viability, astrocytes reactivity, activated microglia/macrophages, and blood brain barrier permeability respectively.

There were significantly more neuronal density around the nanopatterned implants at four weeks post implantation compared to control implants at a distance of 100-150µm from the implant site. In order to record neurons, they must be within 150µm from the implant site. There were no significant differences found between control and nanopatterned implants' astrocyte and microglia /macrophage response or the blood brain barrier permeability.

Project Mentor: Professor Jeffrey Capadona, Department of Biomedical Engineering

The Development of Gait Dynamics in a Mouse Model for Down Syndrome

Joyce Chu, Department of Biology; Ines Basten, Visiting Resident; Melissa Stasko, Laboratory Manager; Dr. Alberto Costa, Department of Pediatrics

Delayed motor development has been observed in humans with Down syndrome (DS), as well as in the Ts65Dn mouse model for DS. A better understanding of the genesis of motor dysfunction in the Ts65Dn model can potentially lead to developing a pharmaceutical drug therapy to enhance motor development in children with DS. The immediate aim of this research is to add the necessary points to a baseline curve representing the development of the motor system in young mice. Longitudinal studies of both trisomic and euploid mice were conducted beginning at 13 days of age until adulthood at 35 days of age. These data were used to plot two developmental curves, one for the trisomic mice and the other for the euploid mice. In the future, the same method of longitudinal testing will be done on a new set of trisomic mice, which will be naive or treated with memantine. Data will be collected using the assessment of gait. The aim of this future study is to determine whether memantine can enhance motor development in Ts65Dn mice, so that the new motor developmental curve is more similar to the curve of a euploid mouse.

Faculty Sponsor: Dr. Alberto Costa, Department of Pediatrics
Project Mentor: Melissa Stasko, Laboratory Manager

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Sensory Feedback of Compliant Modular Worm-like Robot

Joshua Darville, Akhil Kandhari, Kathryn A. Daltorio, Dr. Hillel J. Chiel, Dr. Roger Quinn
Department of Mechanical and Aerospace Engineering

Soft body robotics is becoming an ever more popular field of study that focuses on mitigating the hazards associated with rigid body robotics while retaining the functionality. An example would be how steel tips in needles have been replaced with sharp plastic tips; blood can still be drawn but there is less damage to the vein as the plastic conforms to it. In the lab of biologically inspired robots, the mechanism that an animal uses to locomote is mimicked by a robot that will attempt to integrate a similar synthetic mechanism. The aim of this research is attaining sensory feedback as the robot locomotes to determine its orientation. The method includes creating a testing environment to receive data from a stretch sensor that will simulate longitudinal expansion and contraction along the worm robot. Rstudio statistical language will be used to manipulate and interpret data from the framework generated through experimental trials. The result of this research should lead to a correlational between microcontroller output data and the change in length of the stretch receptor.

Faculty Mentor: Roger Quinn, Mechanical and Aerospace Engineering
Project Mentor: Akhil Kandhari, Mechanical and Aerospace Engineering

Effect of opioids on CD8+ T cell activation and Opioid Receptor expression

Teresa Del Rio, Medical Student, Universidad Central del Caribe, Braulio Llorens, Dr. Alan Levine, Department of Molecular Biology and Microbiology

Synthetic opioids are being used worldwide at alarmingly increasing rates, both in the clinical and illegal setting due to their analgesic and euphoric properties. Opioids are peptides that are synthesized endogenously, such as ®-endorphin, dynorphins, and enkephalins, while others are synthetically produced chemicals, such as morphine and DPDPE. Opioids mediate their effects via a family of G protein-coupled receptors, classified as the ⌈, ™, and⌉ opioid receptors (MOR, DOR and KOR). It has recently been reported that the use of opioids affects the body's immune system, making the host more susceptible to viral infections, such as Herpes or HIV. Since the CD8+ subset of cytotoxic T lymphocytes is critical for protection from a viral infection, our aim is to characterize the effects of opioids on their activation, proliferation, differentiation, and function. To initiate this project, we first investigated the regulation of opioid receptor expression in resting T cells and T cells activated via the T cell receptor and each individual opioid receptor. Primary T cells were obtained by isolating PBMCs (peripheral blood mononuclear cells) from volunteers and then purifying the CD8+ T cell population using antibody-based negative selection. 85% CD8+ T cell purity was routinely achieved. Purified T cells were rested or activated with cross-linked anti-CD3 and anti-CD28 antibodies (i.e., via the T cell receptor) or the opioid receptor agonists: ®-endorphin (DOR and MOR) or DPDPE (selective for DOR). T cell phenotype and opioid receptor expression were assessed by cell surface protein staining and flow cytometer. Preliminary results indicate that ®-endorphin increases DOR expression at 24 and 48 hr, while the other stimulants have no discernable effect. Future aims include defining the effects of other opioid receptor agonists and if ®-endorphin is mediating its effects via the DOR or MOR pathways. Understanding the process of opioid receptor regulation will help elucidate the mechanism by which opioids affect immune cells and, in the long term, define the effect of legal and illegal opioid use and abuse on the function of the immune system.

Project Mentor: Dr. Alan Levine, Department of Molecular Biology and Microbiology.

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An Adaptable Computer Vision System to Quantify Rat Behavioral Tests.

Keith R. Dona[1,2]**,** Monika Goss[1,2], Justin A. McMahon[1,2], Andrew J. Shoffstall[1,2], Evon S. Ereifej[1,2], Jeffrey R. Capadona*[1,2]

[1]Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH, USA;
[2]Advanced Platform Technology Center, Rehabilitation Research and Development, Louis Stokes Cleveland VA Medical Center, Cleveland, OH, USA

Intracortical microelectrodes have shown great success in enabling locked-in patients to interact with computers, robotics, and their own electrically driven limbs. The recent advances have inspired world-wide enthusiasm resulting in billions of dollars invested in federal and industrial sponsorships to understanding the brain for rehabilitative applications. The current study aimed to quantify any motor deficit caused by microelectrode implantation in the motor cortex of healthy rats compared to non-implanted controls. Following electrode insertion, rats were tested on an open-field grid test to study gross motor function and a ladder test to study fine motor function. In order to quantify results from the both tests I aimed to create a custom adaptable tracking algorithm using MATLAB's vision toolbox. The system thresholds the video of the rat's performance for intensity, color and motion, then tracks the rat's position based on a combination of these readings and returns the statistics required by either test. For the grid test, the system analyzes the path and returns the rat's total distance traveled, maximum velocity achieved, left and right turns and the number of grid lines crossed. For the ladder test, the system will return whether the rat completed the test and his time to completion or distance traveled in failure along with any potential paw slips off the rungs.

Project Mentor: Jeffrey R. Capadona, Department of Biomedical Engineering

Analyzing Fluorine Labelled Cysteine Mutants of The Protein UP1

Jahmel Fowler, Department of Biochemistry, Jeffrey D. Levengood, Ph.D., Department of Biochemistry, Blanton Tolbert, Ph.D., Department of Biochemistry

The element fluorine has been used in NMR studies to aide in the elucidation of protein structure and function. It can be incorporated into proteins either by growing cells in the presence of a fluorinated compound, or through post-translational labeling. In our experiments, post-translational labeling was carried out at the cysteine residues of UP1, a protein that is the N-terminus of the heteronuclear ribonucleic protein (hnRNP) A1. UP1 contains two cysteine residues, one in each of its RNA Recognition Motif (RRM) domains. These cysteines were mutated to serine residues to allow for site-specific fluorine labelling. Genes for the mutants were cloned into pMCSG vector and sequence confirmed. Recombinant proteins were overexpressed in BL21 DE3 cells and purified through sonication of the cells followed by nickel column and FPLC purification. SDS-PAGE was carried out to determine the purity of the protein. Previous studies have shown the HIV-1 RNA exonic splicing silencer (ESS3) binds to UP1. A gel shift was performed to ensure that the cysteine mutants did not affect this binding. For site-specific incorporation of fluorine, the proteins were labelled with a tri-fluorinated molecule known as Bis Trifluororoacetamide (BTFA) and will be analyzed using NMR.

Project Mentor: Jeffrey D. Levengood, Ph.D., Department of Biochemistry,
Principal Investigator: Blanton S. Tolbert, Ph.D., Department of Biochemistry

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Upregulation of ABCA1 in lipid-loaded macrophages is dependent on autophagy

Adriana Gordon De Jesús, MD Candidate San Juan Bautista School of Medicine; Palanivel Rengasamy, Case Cardiovascular Research Institute; Rafay Syed, Division of Cardiovascular Medicine, University of Maryland; Xiaoquan Rao, Case Cardiovascular Research Institute; Sanjay Rajagopalan, Case Cardiovascular Research Institute; Andrei Maiseyeu, Case Cardiovascular Research Institute; and Jeffrey A. Deiuliis*, Case Cardiovascular Research Institute

Retinoic acid (RA) has anti-atherosclerotic benefits in animal models, likely in part to its ability to enhance macrophage cholesterol efflux (CE) *in-vivo* via upregulation of cholesterol transporter protein ABCA1 in macrophages. RA has a similar effect on cultured macrophages from humans and mice potentiates CE from lipid-loaded murine macrophages to soluble cholesterol acceptors. We hypothesize that RA enhances macrophage ABCA1 expression in a manner dependent on autophagy. We found that RA increased ABCA1 levels in non- and acetylated-LDL-loaded macrophages and RA-potentiated CE in functional assays using lipid-loaded murine macrophages. Inhibition of late phase autophagy by bafilomycin A1 blocked RA-induced ABCA1 and ABCG1 expression, reduced CE by functional assays, and resulted in lipid in macrophages after an efflux protocol. Rapamycin induced ABCA1 expression; RA in combination with rapamycin did not increase ABCA1 beyond RA-induced levels. Our findings demonstrate that RA-potentiated cholesterol efflux in macrophage foam cells is autophagosome-formation dependent.

Project Mentor: Jeffrey A. Deiuliis, Ph.D., Case Cardiovascular Research Institute

Release of Resveratrol through Affinity-Based Drug Delivery to Reduce Inflammation at the Site of Intracortical Electrode Implantation

Rebecca M Haley, Biomedical Engineering; Sean T Zuckerman, Biomedical Engineering

The implantation of intracortical electrodes results in chronic inflammation at the implantation site. This inflammation can affect measurements, and significantly decrease the overall reliability of the electrode. The usage of resveratrol, a naturally-derived antioxidant, has been shown to help alleviate this inflammation on an acute scale. Resveratrol has been successfully administered through both intraperitoneal (IP) injection and chemical conjugation directly onto the electrode surface. However, IP injections require large dosages to reach significant concentration in the brain, and surface coating only shows effects short term.

In order to achieve the positive anti-inflammatory effects of resveratrol on the chronic time scale, new delivery methods must be explored. This project tests the validity of using the polymer cyclodextrin (CD) to release resveratrol at the site of implantation long term.

To prove the efficacy of this method, the release of resveratrol using CD is examined both in vitro and in vivo. In vitro, resveratrol is consistently released from CD disks for upwards of a month, and the specific loading conditions for this release are examined. It is also shown that intracortical electrodes can be coated with CD and loaded with resveratrol. To prove the viability of this model in vivo, these electrodes are implanted and examined in animal models, to show delivery of resveratrol and resulting reduction in inflammation.

The administration of resveratrol at the implantation site using CD has the potential to reduce inflammation on a chronic scale, improving the reliability of intracortical electrodes.

Project Mentor: Professor Horst von Recum, Biomedical Engineering

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Characterization of Genetic Ancestry Among Chronic Kidney Disease Patients

Kimberly N. Heath, Biology Department, University of Puerto Rico, Ponce, PR; William S. Bush, Department of Population and Quantitative Health Sciences and Institute for Computational Biology; Jessica N. Cooke Bailey, Department of Population and Quantitative Health Sciences and Institute for Computational Biology; Kristy Miskimen, Department of Population and Quantitative Health Sciences and Institute for Computational Biology; Penelope Miron, Department of Population and Quantitative Health Sciences and Institute for Computational Biology; John O'Toole, Department of Medicine; John Sedor, Departments of Medicine and Physiology and Biophysics; and Dana C. Crawford, Department of Population and Quantitative Health Sciences and Institute for Computational Biology.

Genetic ancestry is clinically important in predicting risk and tailoring treatments. Genetic ancestry is also a major tool in genetic mapping or admixture studies for common diseases such as chronic kidney disease (CKD). The higher CKD risk among African Americans is due in part to genetic loci common in African-descent populations but rare in other populations. CKD is the gradual loss of the kidney function, and these patients are at high risk for developing cardiovascular disease. This study aims to characterize the genetic ancestry of African American and European American CKD patients from the MetroHealth System in Cleveland, OH as part of a larger study to identify factors associated with worsening CKD status and the development of cardiovascular events. The average age of this sample is 60.5 years, and more than half (61%) are female. Half of the participants self-identify as African American, with the remaining self-identifying as European American (46%) or Hispanic (4%). To determine CKD stage, we extracted creatinine from the patients' electronic health records and calculated estimated glomerular function (eGFR) using the CKD-EPI equation. All consented patients donated biospecimens for DNA extraction and genome-wide genotyping using the Illumina Mega^{EX}, and underwent quality control using PLINK. Global genetic ancestry was estimated for each participant using ADMIXTURE, and principal components were estimated using EIGENSOFT. Consistent with previous literature, global genetic ancestry was highly correlated with self-identified race/ethnicity. Overall, half of the participants had ≥72% African ancestry, and the other half had 99% European ancestry. Analyses are ongoing to determine if global ancestry is associated with CKD progression and/or risk of cardiovascular disease in this patient population.

Project Mentor: Dana C. Crawford, Ph. D., Department of Population and Quantitative Health Sciences and Institute for Computational Biology

Synthesis of Functionalized Graphene Oxide and Analysis of Inter-Sheet Interactions

Christina Hemmingsen, Department of Chemistry; Dr. Al de Leon, Department of Chemistry; Bradley Rodier, Department of Chemistry

Graphene oxide (GO), interesting for its applications from electronics to medicine, is a sheet of carbon atoms with various oxygen functionalities on the surface. Because GO is easily functionalized, it has great potential in accessing materials with unprecedented properties not possible with current state-of-the-art systems. This study aims to analyze how GO containing various facial functionalizations interact with each other at the air-water interface in Langmuir Blodgett (LB) films. By creating solubility profiles with alkylamines of different alky lengths C4, C6, C9, and C12, we were able to ascertain that C9 functionalization resulted in the greatest shift in polarity and ease of post-functionalization processing. By slight variations in procedure, three distinct functionalizations were generated: basal plane and edge, mostly basal plane, and mostly edge. All materials were characterized by thermogravometric analysis (TGA), X-ray photoelecron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), and atomic force microscopy (AFM) to assure the functionalization occurred as predicted. LB isotherms were then used to observe how the difference in functionalization affects particle-particle interactions by measuring the change in surface pressure of a water subphase during the compression of the nanosheets at the surface.

Project Mentor: Dr. Emily Pentzer, Department of Chemistry

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Studies of the Successive Protonation of Silylated Zirconophosphaalkene $[Cp_2Zr\{C_6H_4C(OSi(CH_3)_3)P\}]_2$ to yield Benzoylphosphine

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The addition of a zirconophosphaalkene salt polymer $[Cp_4Zr_2\{C_6H_4C(ONa(THF)_2\}_2]_n$ to Me_3SiCl yields an unusual, silylated dimer $[Cp_2Zr\{C_6H_4C(OSi(CH_3)_3)P\}]_2$. Upon hydrolysis with excess water, the zirconophosphaalkene complex releases benzoylphosphine, $PhC(=O)PH_2$. In order to better assess the reaction pathway between the two aforementioned molecules, the zirconophosphaalkene is protonated stepwise with one equivalent of either H_2O or D_2O at a time. This renders a sequence of products differing only by the location of deuterium or hydrogen. Ultimately, the arrangement of these isotopes in benzoylphosphine can help characterize the mechanism of hydrolysis and the structures of the intermediates.

Project Mentor: Professor John Protasiewicz, Department of Chemistry

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Lumbar Sympathetic Nerve Activity Responds to Physiologic Challenges in Isoflurane-anesthetized Rats

Christina M. Lewis, University of Florida College of Nursing; Martin S. Muntzel, Department of Biological Sciences, Lehman College

Recording of autonomic nerve activity in humans and experimental animals such as the rat can be used to study the role of the sympathetic nervous system in the control of the cardiovascular system. The sympathetic nerves within the lumbar sympathetic chain play a dominant role in controlling blood flow in the legs. The objective of this study was to ascertain the baroreceptor reflex-mediated changes in lumbar sympathetic nerve activity (LSNA) that occur in response to changes in mean arterial blood pressure (MAP) elicited by the vasopressor agent, phenylephrine, and the vasodepressor agent, sodium nitroprusside, in isoflurane-anesthetized Sprague-Dawley rats. The lumbar nerve was accessed near the bifurcation of the abdominal aorta and rested perpendicular to the bipolar leads of the recording electrode throughout the recording. ABP, HR and LSNA were recorded via a TR56SP Millar Rat SNA/Pressure transmitter in two Sprague-Dawley rats (wt: 308g and 343g). Drugs were given via a catheter in the jugular vein. The phenylephrine-induced increases in MAP were associated with pronounced decreases in LSNA. The sodium nitroprusside-induced decreases in MAP were associated with relatively minor increases in LSNA. These findings suggest that the pressor arm of the baroreceptor reflex plays a dominant role in the control of LSNA in isoflurane-anesthetized rats.

Project Mentor: Dr. Stephen J. Lewis, Division of Pulmonology, Allergy and Immunology in the Department of Pediatrics

Can we judge a newt by its spots? Determining if newt patterns are a reliable method of identifying individuals with the aid of pattern recognition software.

Amanda Li, Department of Biology; David Dimitrie, Department of Biology ; Hilary Rollins, Department of Biology; Kacey Dananay. Department of Biology

The ability to consistently identify individuals of a species makes in-depth demographic analysis through capture-mark-recapture studies possible. These studies can gauge a population's size, growth, and provide insight on specie's life history. Information gained from these types of studies can then be applied in population management and conservation recommendations. For amphibians, the most common forms of identifying individuals are invasive techniques, like toe-clipping, passive identification transponder implantation (PIT), and visible implant elastomer (VIE). Unfortunately, there are practical and health drawbacks to these methods. Some species of amphibians can regenerate lost limbs, making toe-clipping ineffective in some situations, and the elastomers in VIE may migrate within the body, obscuring marks. Additionally, all of the invasive procedures carry an infection risk. Therefore, we hope to determine the reliability of computer assisted identification, a non-invasive method where photos are analyzed by a computer program (Hotspotter), in order to reduce the risks associated with invasive identification methods. There are two goals in this study. The first is to determine if Eastern newts (*Notophthalmus viridescens*) can be reliably identified with Hotspotter. Newts will be captured and photographed multiple times, and these photographs will be analyzed by Hotspotter for matches. We will be looking for low rates of two types of error: false positives and real matches missed. Our second goal is to determine if patterns on adult newts are stable. To do this, adult newts will be VIE tagged, held in mesocosms, and have their patterns observed over time for any changes.

Project Mentor: Professor Michael Benard, Department of Biology

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Optimizing Paraspinal Electrode Implantation Techniques through 3D Modeling Analysis

Fangze Liu, Department of Biomedical Engineering; Dr. Ronald Triolo, Department of Biomedical Engineering

Activating the paralyzed paraspinal muscles with stimulating electrodes implanted at T12–L3 spinal nerves can significantly improve seated posture and stability, reachable workspace, and ability to withstand external disturbances in wheelchair-dependent individuals with spinal cord injuries. Yet, clinical results vary across subjects because of our lack of knowledge regarding the underlying neuroanatomy. This research aims to identify the ideal location for electrode implantation by developing a system to quantitatively describe and compare the locations of electrodes in current subjects. 3D mesh models of the spinal cord were established from CT scans using a series of open-sourced software packages before importing into Matlab for further analysis. Different approaches were made to describe the area the electrodes may be implanted, such as fitting ellipsoids to the space between the transverse processes of two vertebrae. This approach can yield satisfying result for a single subject, however, since the fitting algorithm requires the 3D model to be manipulated and trimmed manually, it is difficult to define a standardized and repeatable process for data analysis across all subjects and evaluators. Our most recent approach involves obtaining the location of electrodes by establishing a coordinate system for the 3D model based on readily identifiable anatomical landmarks. Once a repeatable processing and normalization methods are in place, we hypothesize that all the electrodes functioning well in current subjects will cluster in one general area, separated from those with undesired responses. We can then relate the ideal target area to the fluoroscope images available in the operating room and help standardize insertion techniques, reduce the risks of complications, ensure consistency of clinical outcomes and prepare for translation to other practitioners for widespread dissemination.

Project Mentor: Dr. Ronald Triolo, Department of Orthopedics & Biomedical Engineering

Functionalization of AZO Surfaces with Silanes

Michael Lu-Diaz, Department of Chemistry; Nolan Kovach, Department of Chemistry; Dr. Ina Martin, Department of Materials Science and Engineering; and Dr. Emily Pentzer, Department of Chemistry

Transparent conductive oxides(TCO) are a type of transparent semiconductive material that have been widely used for many applications inside the photovoltaic and optoelectronic fields. Aluminium-doped zinc oxide(AZO) films have gained popularity inside this area due to their non toxicity, high radiation resistance, low cost, and the abundance of their composition elements. However, AZO films have demonstrated to degrade over time under damp heat, as a consequence of their chemisorption of water in their grain boundaries. Previous studies have shown that modifications with organosilane compounds have improved the long term surface stability of films while offering great hydrophobic properties. In this study we investigated different AZO modifications utilizing silanes with varying functional group character that were chosen for developing optimal deposition methodologies.

Project Mentor: Dr. Emily Pentzer, Department of Chemistry

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Developing a More Robust Method to Generate Cerebral Organoids

Uma Mahajan, Systems Biology

Cerebral organoids are three-dimensional structures generated from human induced pluripotent stem cells (iPSCs) that recapitulate various steps in human embryonic cortical development. This model system enables these unique processes to be studied in neurodevelopmental disorders such as autism, where human embryonic phenotypes are extremely difficult to assess due to disease diagnoses occurring postnatally. Previous experiments conducted have revealed high variability in the structure and organization of organoids, even within the same patient cell line. This variability makes it difficult to accurately compare phenotypes in control versus disease organoids. Since unwanted variability can be observed in the initial stages of organoid formation, we hypothesized that modulating various aspects of the protocol (such as the initial reprogramming factors utilized) at this early stage would lead to more homogeneous organoid production. Reprogramming pathways that were modulated included the canonical Wnt and SMAD signaling pathways. In addition, bioengineered constructs of gelatin beads and collagen fibers were utilized to provide a scaffold for organoid growth. Various methods for removing the iPSC colonies from feeder-dependent plates and dissociating colonies into single cells were also attempted. The goal of the project was to develop a robust method to grow organoids that do not disintegrate or stagnate, have low variability within and between cell lines, and produce large, robust progenitor regions characteristic of human cortical development. Once this protocol is optimized, it will be used to grow organoids derived from autistic and control iPSC lines for long term (15-week) analysis.

Project Mentor: Anthony Wynshaw-Boris, Department of Genetics and Genomics Sciences, and Luke Bury, Department of Genetics and Genomics Sciences

Evaluation a Play-Based Intervention for Children with Prader-Willi Syndrome

Carolyn Manuck, Department of Biology; Dr. Anastasia Dimitropoulos, Olena Zyga, Ellen Doernberg, and Dr. Sandra Russ, Department of Psychological Sciences

The goal of this project is to evaluate the efficacy of an intervention to improve play skills in children with Prader-Willi Syndrome (PWS). PWS is a genetic disorder characterized by hyperphagia and food preoccupations, which often cause obesity. People with PWS frequently display maladaptive behavioral traits similar to those seen in autism spectrum disorder including tantrums, stubbornness, compulsive or repetitive behaviors, and resistance to change. Additionally, children with autism and PWS share an impaired capacity for pretend play, a type of play that uses make-believe and symbolism, and is important for intellectual, emotional, and social development. The intervention in this study, similar to play-based interventions used in autism, aims to enhance pretend play skills and ultimately to improve maladaptive behaviors in children with PWS. It utilizes telehealth to make the intervention more accessible to children nation-wide who have this rare disorder. Through a series of twelve videoconferences, children engage in play with a facilitator who promotes positive play behaviors. Currently, only baseline and post-intervention assessments have been used to evaluate the intervention. For my project I will develop a new coding system to assess the child's pretend play skills within each intervention session. Through these sessions, we will be able to collect additional information not obtainable in the baseline and post-intervention assessments, including the child's ability to play over a long period of time, to play out multiple different storylines, and to interact with the interventionist. This data will allow for a more detailed understanding of the development of the child's play skills throughout the intervention.

Faculty Mentor: Dr. Anastasia Dimitropoulos, Department of Psychological Sciences

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Inhibition of the Growth of Triple Negative Breast Cancer Cells by Novel HEXIM1 Inducers

Keisha Michel, Pre-Medical Track, Sociology and African-American Studies; Monica Montano, Department of Pharmacology

Triple-negative breast cancer (TNBC) is an aggressive type of breast cancer that is characterized by its lack of receptors for the hormones estrgogen (ER) and progesterone (PR), or the protein HER2. Because TNBC lacks these three receptors, traditional chemotherapy and targeted therapies do not work as well, and make TNBC more likely to reoccur. TNBC are more common in women of African descent than women of other ethnic origins. Extensive research is being conducted to find new ways to effectively treat TNBC. Hexamethylene bisacetamide inducible protein 1 (HEXIM1) has been identified as a potential tumor suppressor in breast and prostate cancer by the Montano laboratory. Increased expression of HEXIM1 or treatment with small molecule HEXIM1 inducers resulted in the inhibition of metastasis and angiogenesis. The goal of this project is to test novel compounds (HM5, HM6, HM7, HM8, KDX) for their ability to induce HEXIM1 expression and inhibit growth of TNBC. We tested these compounds using different TNBC cell lines, MDA-MB-231, MDA-MB-468, MDA-MB-453, and BTB549. These cell lines represent 4 out of the 6 TNBC subtypes that have been shown to respond differently to a variety of targeted therapies. We performed western blots to test their ability to induce HEXIM1 protein expression, as well as cell proliferation assays to assess inhibition of TNBC growth. Thus far, two of the inducers, HM8 and KDX, have worked best in inducing HEXIM1 expression and inhibiting proliferation of all 4 cell lines.

Project Mentor: Monica Montano, Ph.D – Department of Pharmacology

Effect of 20% Fructose consumption on NOX4 Expression in Rat Proximal Tubules

Aaron Morris, Major: Biology

Agustin Gonzalez, Department of Physiology and Biophysics; Nianxin Yang, Department of Physiology and Biophysics

Hypertension is the leading cause of loss of health worldwide. In America, roughly 35% of people are hypertensive and half of them display sensitivity to salt. Angiotensin II (Ang II) is an important regulator of blood pressure, in part by controlling salt reabsorption in the kidney. Upon increases in salt intake Ang II levels drop to allow renal excretion of salt. Malfunction in Ang II signaling is associated with elevated oxidative stress, salt retention and hypertension. The effects of Ang II on the proximal tubules are mediated in part through the production of superoxide from NOX4 which leads to increased sodium transport. NOX4 is the main form of NADPH Oxidase found in the proximal tubules of the kidneys where Na reabsorption occurs in the renal system. An increase in proximal tubule transport contributes to an increase in blood pressure. Fructose increases renal sensitivity to Ang II. Therefore, the increased sensitivity to Ang II displayed in fructose fed animals may be increasing superoxide production by NOX4 and thereby Na transport. We hypothesize that a 20% fructose diet will lead to the stimulation of NOX4 leading to increased sodium transport in the proximal tubules. To test our hypothesis, proximal tubules suspensions were prepared from rats on either a normal diet or a 20% fructose diet. Protein samples were put through a gel electrophoresis, and then a blotted for NOX4. We found that NOX4 expression in tubules from rats in fructose was ~61% greater than in controls. Our findings indicate that a 20% fructose diet heightens NOX4 expression in proximal tubules. This likely elevates superoxide production leading to an increase in salt absorption and contributing to hypertension.

Jeffrey L. Garvin, Department of Physiology and Biophysics

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The effect of dithiothreitol (DTT) on the activity of the ATP- dependent protease ClpXP

D'Andra Moxey, Department of Chemistry; Zhou Sha, Department of Chemistry; Sarah Krul, Department of Chemistry

ClpXP is an ATP-Dependent protease that is formed in the matrix of the mitochondria when ClpP and ClpX interact with each other.[1] Mitochondria are the site of respiration in the cell which is a process in which glucose and oxygen are converted to adenosine triphosphate (ATP) or energy. ClpP, a tetradecameric protease, has a cylindrical shape with two 7-membered symmetric rings, which makes it a double heptameric ring.[2] The hexameric ClpX, an AAA+ ATPase, interacts with either or both sides of the ClpP. This three dimensional structure enables the protein to carry out its purpose of digesting misfolded proteins into peptides with the use of energy from ATP binding and hydrolysis. ClpX recognizes misfolded proteins and unfolds them so that ClpP can degrade them into small peptide fragments. Abnormal amounts of ClpXP are implicated in a couple severe diseases, such as, Friedreich ataxia (FA)[3] and Perrault syndrome (PS)[4]. Recombinant human ClpP and ClpX were expressed in Escherichia coli (E. coli) and purified in the lab. In order to determine the activity of ClpXP under physiological conditions, DTT, a reducing agent, was used at varying concentrations.

Project Mentor: Dr. Irene Lee, Department of Chemistry

References

1. De, M. R., I. Mayo, S. Marco, S. Rodríguez-Vilariño, J. Oliva, J. L. Carrascosa, and J. G. Casta. "Mitochondrial localization and oligomeric structure of HClpP, the human homologue of E. coli ClpP." Journal of molecular biology. U.S. National Library of Medicine, 01 Oct. 1999. Web. 26 July 2017.
2. Wang, Jimin, James A. Hartling, and John M. Flanagan. "The Structure of ClpP at 2.3 Å Resolution Suggests a Model for ATP-Dependent Proteolysis." The Structure of ClpP at 2.3 Å Resolution Suggests a Model for ATP-Dependent Proteolysis - ScienceDirect. N.p., 25 Sept. 2000. Web. 21 July 2017.
3. "Friedreich ataxia - Genetics Home Reference." U.S. National Library of Medicine. National Institutes of Health, n.d. Web. 21 July 2017.
4. "Perrault syndrome - Genetics Home Reference." U.S. National Library of Medicine. National Institutes of Health, 18 July 2017. Web. 21 July 2017
5. "Gel electrophoresis (article)." Khan Academy. N.p., n.d. Web. 24 July 2017.

Cdk5 Expression in Crisper Knockout PDL1 (crPDL1) and Wildtype (WT) Rhabdomyosarcoma Cells

Melanie Nelson, Department of Neuroscience, Psychology, and Science in Society (Wesleyan University, Middletown, CT); Daniel Kingsley, Agne Petrosiute, MD

PD-L1 (Programmed Death Cell Ligand-1) is an immune regulatory molecule that binds to its receptor PD-1, causing an inhibitory signal to be transmitted into the T cell and suppressing T-cell proliferation, which can be adopted by tumor cells to suppress antitumor immune response. Recent discovery of PDL1 blocking agents has caused a breakthrough in treatment of various tumors. Rhabdomyosarcoma (RMS) is a type of cancer that manifests in any soft tissue. RMS is a very common childhood cancer, with survival rates as low as 20% for metastatic RMS. In the present study, we examined the role of the PD-1-PDL1 axis in RMS. The data shows that PDL1 is upregulated by IFN gamma in murine RMS76.9 cell line. PDL1 was silenced in the RMS76.9 line using CRISPR/Cas-9, then the mice were injected and the tumor free survival rate of RMS 76.9 crPDL1 tumor cells and WT controls were also compared. A cohort of mice bearing RMS76.9 WT and RMS76.9crPDL1tumors were subjected to the PD-L1 blockade, starting on Day 0 or Day 7. We noted tumor free survival was best in mice subjected to RMS76.9 crPDL1 tumors that received PDL1 antibody treatment starting on Day 7. When mice were analyzed by flow, MHCII and MHCI expression increased in WT tumors subjected to PDL1 blockade. When the tumor cell lysates were analyzed with Western Blot, it was shown that RMS 76.9 crPDL1 tumor cells have higher Cdk5 expression compared to WT controls. The observations highlight the importance of timing of the PDL1 blockade.

Project Mentor: Dr. Agne Petrosiute, Department of Pediatrics (Hematology/Oncology)

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An evaluation of PCNA expression in neonatal mice lungs exposed to CPAP therapy

Uche Ononuju, Department of Pediatrics; Dr. Richard Martin, Department of Pediatrics, Division of Neonatology; Anjum Jafri, Department of Pediatrics

The administration of continuous positive airway pressure (CPAP) is commonly used for preterm infants in respiratory distress in the neonatal intensive care unit. However, the long-term effects on airway function are still unknown. Supplemental oxygen treatment, however, is believed to be a major contributor to the development of bronchopulmonary dysplasia (BPD) and other respiratory morbidities. Research has identified that CPAP therapy increases the presence of lung smooth muscle (via actin markers), but it is not well understood if this is due to hypertrophy or hyperplasia. Therefore, proliferating cell nuclear antigen (PCNA) can be used to study the differences between hypertrophy or hyperplasia. A neonatal mouse model (P8 pups) were exposed to CPAP and its response to the PCNA in bronchial epithelial and smooth muscle cells was studied. Preliminary data (n=3-5) have not shown a significant difference in the expression of PCNA in lungs of the control versus CPAP treated animals, potentially suggesting that hypertrophy may be occurring. Ultimately, using a larger sample in the evaluation of PCNA expression in neonatal mice lungs could provide a better understanding of the potential long-term benefits or consequences of CPAP therapy in the clinical setting.

Project Mentors: Dr. Richard Martin, Department of Pediatrics, Division of Neonatology, UH Rainbow Babies & Children's Hospital; Anjum Jafri, Department of Pediatrics, Case Western Reserve University

Simulating Sensory Axons to Predict Action Potentials in the Vagal and Splanchnic Nerves

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By 2014, 22 states had an obesity rate over 30% and no states had less than 20%. Obesity-related costs exceed $190B/yr. Neuromodulation can provide a means of reducing excess body weight (EBW) that is less invasive than bariatric surgery. The vagal and splanchnic nerves relay information about the stomach. Vagal and splanchnic nerve stimulation have reduced EBW, but trial results have been inconsistent and stimulus parameters vary widely. This project seeks to simulate sensory axon responses to varied patterns of electrical stimulation in these nerves. Of particular importance is the rate and pattern of action potential propagation. Action potentials are a propagating change in electrical potential that transmit information and commands between and within the central and peripheral nervous systems. The goal of electrical nerve stimulation is to restore the proper neural code ("biomimetic"); therefore predicting action potentials is necessary to optimize stimulation parameters and achieve the desired outcome. To build our model we altered an existing motor axon model, accounting for the electrical dynamics of sensory axons. Nerves are modeled as an electrical circuit with myelin as an imperfect insulator; ion channels leading in and out of the nerve as resistors with varying conductances; and the charge separation across the membrane as a capacitor. This circuit repeats at every node of Ranvier which occur regularly along the length of a nerve fiber. When the potential across the membrane is sufficiently high an action potential occurs causing current to flow into and out of the nerve at each of the nodes of Ranvier, propagating the change in voltage down the fiber. Based on whether given parameters generate an action potential, the model will be used to fine tune stimulation to work towards producing desired neural patterns of activity, such as those associated with satiety.

Project Mentor: Matthew Schiefer, PhD, Louis Stokes VA Medical Center Advanced Platform Technology Center

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Determining the Relationship between Brainstem Inflammation and Changes in Ventilatory Pattern Variability (VPV) in Healthy and Septicemic Rats

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Septicemia evokes sickness behavior – fever, increases in respiratory and heart rates, and decreased locomotion. We have identified brainstem inflammation during septicemia. Our objective was to correlate central (dorsomedial Medulla & dorsolateral Pons) and peripheral (serum, and lungs) inflammation to ventilatory pattern variability (VPV). We hypothesized that this inflammation would lead to a decrease in VPV. Sterile or bacterial (*Escherichia coli*) pellets were implanted surgically in the abdomen of male rats (~275 g, n=8). The rat's breathing pattern was recorded using whole-body plethysmography at 18, 20, 22 and 24 h. At 24h, rats were euthanized and tissues were harvested. VPV was determined from the coefficient of variation, Poincaré plots, and nonlinear dynamics. Inflammation was determined by measuring the levels of pro-inflammatory cytokine markers IL-1β, TNFα, and IL-6 using ELISAS. The pellets had either no bacteria (n=2), or *E. coli* ($x10^6$) 10 (n=1), 25 (n=2), 50 (n=2), and 100 (n=1). At 24h, the 2 rats with sterile pellets had VPV values of (TTOT=790±85 & 890±140 ms, CV=10.8 & 15.9, TPV_{TD}= 0.072-0.089 & 0.113-0.147); *E. coli* ($x10^6$) 10 (TTOT=470±35 ms, CV=7.2, TPV_{TD}= 0.030-0.035); 25 (TTOT=460±30 & 490±30ms, CV=7.1&6.7, TPV_{TD}= 0.022-0.031 & 0.029-0.034); 50 (TTOT=410±25 & 430±40ms, CV=6.3&8.6, TPV_{TD}= 0.021-0.028 & 0.025-0.040); and 100 (TTOT=410±20 ms, CV=4.8, TPV_{TD}= 0.019-0.021). Development of VPV between 18 and 24 h was insignificant. The cytokine levels will be measured and be presented at the poster. We conclude that VPV analysis indicates that the rats became septicemic by 18h with VPV decreasing little during 18-24h.

Project Mentor: Dr. Thomas E. Dick, Division of Pulmonary, Critical Care and Sleep, Department of Medicine and Department of Neurosciences.

Hypoxia Inducible Factor-1 in Peripheral Vascular Smooth Muscle Cells is Critical for Vascular Phenotype

Ashley Saunders, Department of Biology; Anna Henry Borton, Cardiovascular Research Institute, CWRU Department of Medicine

Peripheral vascular disease (PVD) is a blood circulation disorder that causes vessels in the limbs to narrow, block, or spasm. Decreased vessel diameter limits blood flow to tissues, creating a hypoxic environment. The transcription factor hypoxia inducible factor-1 (HIF-1) is activated by low oxygen levels, stimulating the angiogenic growth of new vessels. We hypothesize that responses driven by HIF in vascular smooth muscle cells (VSMCs) are critical for proper remodeling of vessels. Our previous data using an in vivo murine hindlimb ischemia model of PVD shows that mice with tissue specific deletion of the β-subunit of HIF (*Arnt*$^{SM22\triangle SMC}$) have decreased vascular reperfusion following femoral artery ligation. In order to determine the effect of VSMC HIF on vascular phenotype, we quantified the number of vessels. Unligated limbs of *Arnt*$^{SM22\triangle SMC}$ mice show a significant increase in perfused blood vessel numbers compared to control ($P \leq 0.01$). Hypoxic molecular signatures were examined in primary human iliac artery VSMCs exposed to hypoxia (2% oxygen) for 24 hours. Analysis indicates induction in expression at the transcriptional level of proliferative genes (*PAI-1, THBS-1)* and contractile genes (*ACTA-2, CNN-1*) in response to hypoxia. The role of HIF in driving these responses was assessed using digoxin, a HIF-1 inhibitor. Digoxin inhibited the transcriptional induction observed with hypoxia treatment. This data demonstrates the importance of the HIF pathway in regulating peripheral VSMCs' responses to hypoxia and suggests a role for VSMC HIF in determining vascular phenotypes in vivo. Understanding VSMCs responses at cellular and molecular levels will contribute to the development of medical therapies and surgical interventions to treat peripheral vascular diseases.

Project Mentor: Dr. Diana Ramírez-Bergeron, Cardiovascular Research Institute, CWRU Department of Medicine

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Optimization of Membrane Protein Reconstitution for Membrane Scaffolding Protein 2N2 to Create Larger Nanodisc Complexes

Maurryce Starks, Department of Neuroscience; Sandip Basak, Department of Physiology and Biophysics; Soumili Chatterjee, Department of Physiology and Biophysics; Yvonne Gicheru, Department of Physiology and Biophysics; and Yi Ziyue, Department of Physiology and Biophysics

The study of membrane proteins outside of their native environment proves to be unfavorable for researchers who seek to isolate their membrane protein of choice. Artificial structures like nanodiscs allow researchers to emulate a natural, stable environment to study target membrane proteins more explicitly. Nanodiscs are soluble, self-assembling membrane bilayers composed of phospholipids, a target membrane protein, and two membrane scaffolding proteins. Membrane scaffolding proteins are synthetic proteins that emulate the function of the natural protein Apolipoprotein A-1. The non-polar (interior) of the membrane scaffolding protein allows it to interact with the "tails" of a phospholipid bilayer, while the polar (exterior) of the protein allows it to be soluble in a solution. This amphipathic nature of the protein allows it to wrap around the phospholipid bilayer thus creating the nanodisc structure. Previous research has determined that different membrane scaffolding proteins constitute various sizes of nanodisc structure. Membrane scaffold protein 2N2 has been shown to create nanodisc in size up to 16.5nm in diameter. This increase in size allows the insertion of larger membrane proteins inside the nanodisc. Once the complete nanodisc structure is assembled, we can then isolate a target membrane protein, in our case a serotonin receptor, to study the mechanisms that underlie the opening and closing of ligand-gated ion channels. We are currently performing experiments to discover the structural mechanisms that open and close serotonin receptors.

Project Mentor: Dr. Sudha Chakrapani, Department of Physiology and Biophysics

High Throughput Screening for Uracil-DNA Glycoyslase Activity Modulators

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Our focus is on the human uracil DNA glycosylase (UNG) as a chemotherapeutic target. UNG is the primary repair enzyme used to recognize misincorporated uracil bases in DNA and removes these incorrect bases by initiating base excision repair. The ability of cancer cells to rapidly and efficiently repair DNA base lesions induced by the chemotherapeutic drugs pemetrexed and 5-fluorodeoxyuridine (5FdU), both of which inhibit thymidylate synthase and induce UMP and ultimately dUTP accumulation and DNA incorporation, is due to UNG activity. UNG specific inhibitor could improve response rates for patients treated with PEM or 5FdU.

We have characterized two enzymes (*E. coli* UNG and Human UNG) over two concentrations to validate previous lab work with a high-throughput biochemical assay. This assay measures the activity of the enzyme in removing uracil from DNA based on relative fluorescence un-quenching. As uracil is removed from the DNA, the quenching effect is decreased allowing for a greater fluorescence to be detected. Concentrations used for the *E. coli* UNG were 20pM and 100pM and the K_m was determined to be 22.45 ± 13.6 and 74.07 ± 32.6 respectively. Concentrations used for the Human UNG were 5nM and 10nM and the K_m was determined to be 30.0 ± 25.2 and 53.3 ± 8.1 respectively. This assay was also used to screen 15,680 compounds in which 22 of the compounds showed significant inhibition. We rescreened the 22 compounds and confirmed the inhibition in four of those compounds out of the larger 15,680.

Project Mentor: Dr. Stanton Gerson, Case Comprehensive Cancer Center

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Developing a Collagen Scaffold for Surgical Repair of Bone, Tendon, and Muscle

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Tendon, muscle, and bone injury is very common in the United States. Particularly, athletes and military personnel often suffer from tendon injury. According to some sources there are more than 33,000 tendon repair procedures in the US and 100,000 ACL reconstruction annually in the USA. Despite tendon repair being such a common process, tendon repair has a high failure rates. According to some sources, 94% of repaired massive rotator cuff tear fails and 56% of ACL reconstruction patients experience knee pain 1 year post surgery. These high failure rates are due to the bone tendon connection (enthesis) or the tendon muscle connection failing to reintegrate post-surgery. The goal of my current project is to develop a continuous collagen scaffold with regional chemical doping to promote enthesis formation that can help the healing process during tendon repair surgeries. My summer research project focused on investigate the effect of biochemical cues on the differentiation of cells into bone, muscle, and tendon *in vitro*. To this end, we used a muscle precursor cell line, C2C12, cultured in either basic culture media or osteogenic medium supplemented with BMP-2, CaCl2, Calcium Citrate, and NaCl. Muscle differentiation was assessed by counting and measuring myotubles and bone differentiation was determined via alizarin red staining for *de novo* calcium deposits. We also created a continuous scaffold prototype using braided biphasic electro compacted collagen threads embedded in a cell laden collagen gel. Future projects for this study will involve differentiating cells on collagen scaffold and eventually using these scaffolds on various animal models.

Project Mentor(s): Dr. Ozan Akkus & Dr. Victoria Webster-Wood Tissue Engineering and Mechanobiology lab Department of Mechanical and Aerospace Engineering, Case Western Reserve University

Identification of CHIR-99021 as a Novel Therapy for Huntington's Disease

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Huntington's Disease (HD) is a fatal genetic disorder characterized by an expanded polyglutamine tract in the first exon of the huntingtin (Htt) gene. Accumulation of mutant Htt (mtHtt) leads to the death of medium spiny neurons (MSNs) in the basal ganglia, and as a result, HD patients suffer progressive motor and cognitive deficiencies, vision and speech loss, and an elevated suicide risk. Recent evidence suggests that mtHtt causes mitochondrial defects within affected neurons, causing decreased mitochondrial membrane potential (MMP) and oxygen consumption, and increased neuronal death. Despite our understanding of HD's genetic and cellular mechanisms, current therapies merely alleviate HD symptoms but cannot halt or even slow disease progression. Our recent success in reversing HD-induced mitochondrial deficits via a peptide inhibitor led us to conduct a high-throughput screen to discover small molecules capable of mitochondrial rescue. CHIR-99021 (CHIR), a GSK3α/β inhibitor, emerged as a leading candidate capable of restoring MMP in HD striatal neurons. Follow-up studies showed CHIR enhanced mitochondrial oxygen consumption and neuronal viability *in vitro*. In the R6/2 HD mouse model, CHIR increased the density of MSNs and prolonged survival compared to a vehicle control. Subsequent mechanistic studies have shown that CHIR likely does not modulate HD pathology through inhibition of its canonical target, GSK3, and we are currently working to determine its therapeutic mechanism. Ultimately, further optimization of CHIR as an HD therapeutic and elucidation of its mechanism could open new avenues for HD drug development and provide much needed therapies capable of reversing disease progression.

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

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Using SynthoPlates™ to Enhance Platelet Aggregation in Patients Undergoing Antiplatelet Drug Therapy

Carlos A. Villagrasa Méndez, Department of Biology; María de la Fuente, Department of Pharmacology

Platelet function is crucial to maintaining hemostasis without causing unwanted blood clots within blood vessels that could lead to heart attack and stroke. Antiplatelet therapy is a primary treatment for individuals with these detrimental risks. The use of antiplatelet drugs requires a delicate balance to prevent clots without causing bleeding. This is particularly difficult to achieve as many platelet inhibitors are irreversible. Synthetic platelets could be an alternative solution to platelet transfusion for emergency situations where platelet function needs to be restored. Synthoplates™ are liposomes synthesized to mimic platelets which have platelet resembling peptides in their membranes like von Willebrand factor binding peptides (VBP), collagen binding peptides (CBP), and active GPIIb-IIIa-binding Fg-mimetic peptides. Platelet aggregation studies such as Light Transmission Aggregometry (LTA), are used to monitor patients' response in the clinic and testing new therapies in the laboratory. However, this technique uses platelets in plasma isolated from whole blood, which potentially removes some contributing factors. My project's goal was to establish a whole blood aggregometry assay to measure platelet response in their native environment. I measured platelet aggregation and granule secretion in response to stimulating platelet receptors (PAR1, PAR4, and ADP). Importantly, I was able to show inhibition of aggregation using platelet antagonists. These studies established a system to assess aggregation of inhibited and active platelets complemented with SynthoPlates™. Testing whole blood or platelet rich plasma (PRP) inhibited with 2MeSAMP after adding SynthoPlates™ will allow us to determine if synthetic platelets can restore hemostasis to irreversible platelet antagonists.

Project Mentor: Marvin Nieman, Department of Pharmacology

Examining the Relationship Between CDK5 and γδ T cells using γδ Leukemia Cells

Jasmine Walker, Department of Human Health, Emory University; Suzanne Tomchuck, Ph.D., Pathology; Brooke Holland, MS, Pathology; Mari Dallas, MD, Pathology

Cyclin-dependent kinase 5 (CDK5) is a kinase that phosphorylates several proteins. CDK5 is essential for brain development, and helps regulate some adult brain functions. CDK5 has an impact on T cell migration, development, proliferation, and survival. CDK5 is often upregulated in cancer cells, causing heightened proliferation, and cancer cell survival. Thus far, there is little information known about the impact of CDK5 on γδ T cells. γδ T cells are responsible for initiating innate immune responses, and can also act as antigen presenting cells for the adaptive immune response. The ability of γδ T cells to directly recognize antigens from viruses, microbes and tumors and contribute to their destruction make these T cells ideal candidates for immunotherapy.

This project focused on discovering if there is a role for CDK5 in γδ T cell biology. We first examined the expression of CDK5 protein and mRNA from several γδ T cell leukemia cell lines using western blotting and polymerase chain reactions (PCR). Additionally, we utilized an inhibitory peptide that was developed by the Letterio lab, that successfully and specifically inhibits CDK5 expression. We then performed western blotting on different γδ T cell leukemia cell lines lysates treated for 48 hours with varying amounts of CDK5 inhibitory peptide (CIP).

Using the western blotting technique, we observed that CDK5 expression decreased with increasing amounts of CIP. We also looked at p35 expression, which cleaves into p25 to become a CDK5 activator, and found no protein expression in these leukemia cell lines. Based on the western blot results, we next plan to look at CDK5 and p35 expression on an RNA level using PCR. Because γδ T cells are a part of both the innate and adaptive immune response, further understanding of CDK5 expression in γδ T cells could potentially help cancer patients using cancer immunotherapy. More research will need to be done to further explore the relationship between CDK5 and γδ T cells.

Project Mentor: Dr. Mari Dallas, MD, Department of Pathology/ Department of Pediatric Hematology and Oncology

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Synthesis of 2-[(3-cyano-6-methyl-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)carbamoyl]bicyclo[2.2.1]hept-5-ene-3-carboxylic acid

Emma Whiting, Chemistry Major, Washington University in St. Louis; *Dr. Gregory Tochtrop*, Department of Chemistry

Retinoids, which have been commonly studied for their function in vision, play an essential role in gene transcription. Retinoic acid (RA), a derivative of Vitamin A, is processed in two distinct pathways which result in opposing responses: either cell growth as a result of activation of PPARdelta or apoptosis due to the activation of RAR (Retinoic Acid Receptor). Research that has been conducted in the Tochtrop lab has focused on iLBPs (intercellular lipid binding proteins) binding to RA in the cytoplasm and delivering RA into the nucleus. Within the RA signaling pathway, two iLBPs are responsible for transporting RA, CRABP-II and FABP5: CRABP-II delivers to RAR and FABP5 delivers to PPARdelta. Through analysis utilizing high throughput screening, 2-[(3-cyano-6-methyl-4,5,6,7-tetrahydro-1-benzothiophen-2-yl)carbamoyl]bicyclo[2.2.1]hept-5-ene-3-carboxylic acid was found to be a potential inhibitor of FABP5. Organic synthesis of a racemic mixture of this target inhibitor was completed using inexpensive as well as accessible starting materials and followed the mechanistic steps of the Diels Alder reaction, the Gewald reaction, and amide bond formation. Currently, biological experiments are underway to determine if the target molecule binds irreversibly to FABP5 without activation of PPARdelta, in addition to directing retinoic acid to RAR. If the racemic mixture is found to inhibit FABP5, additional research will need to be conducted to determine which stereochemical possibilities irreversibly bind to FABP5.

Faculty Mentor: Dr. Gregory Tochtrop, Department of Chemistry

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Pathogenic mechanism of epilepsy-associated gamma-amino butyric type A receptors

Angela Whittsette, Department of Nutritional Biochemistry; Xiojing Di, Department of Physiology and Biophysics; Kate Fu, Department of Physiology and Biophysics; Dr. Tingwei Mu, Department of Physiology and Biophysics

Recent advances in genetics identified many mutations in gamma-amino butyric type A (GABAA) receptors that are associated with Idiopathic Epilepsy. Such mutations lead to loss of their function on the plasma membrane and thus disrupt neural circuits in the brain. Here, we aimed to elucidate the molecular mechanism of how these mutations lose their function. We focused on identifying mutations that lead to their misfolding and excessive protein degradation in cells and thus loss of their protein levels in their functional location, the plasma membrane. As previous studies have been done on mutations on alpha1 subunit of GABAA receptors, the study at hand was done to investigate the gamma2 subunit mutations. We used cellular assays that evaluate protein aggregation and total protein levels to find out the cause of these epilepsy-associated mutant gamma2 subunits. In the experiment we cultured human embryonic kidney cells also known as HEK293 cells, expressed GABAA receptor variants in HEK293 cells by transfection, performed SDS-PAGE protein electrophoresis, and concluded with a Western blot analysis. The results of the blots were visualized by developing films. This was all done for the purpose of analyzing the bands produced by the Western blot that were displayed on the films. The amounts of proteins were compared, information was taken from relative protein content compared between the soluble and insoluble fraction to determine which mutations were more prone to aggregation due to their protein misfolding in cells in comparison to the wild type case as a positive control. We demonstrated that two specific mutations in gamma2 subunits, R177G and G257R, caused excessive misfolding of the mutant subunits. Our mechanistic work will enable future efforts to find strategies to correct their misfolding in cells to restore function, as a new therapeutic strategy to ameliorate idiopathic epilepsy.

Project Mentor: Dr. Tingwei Mu, Department of Physiology and Biophysics

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Histological characterization of wild type and *Thoc6* mutant mice during neurogenesis

Rachante Williams, Department of Genetics and Genome Sciences and Center for RNA Science and Therapeutics

The Beaulieu-Boycott-Innes Syndrome is an autosomal recessive developmental disorder causing intellectual disability and cardiac abnormality. The disease is attributed to loss-of function mutations that occur in the *Thoc6* gene which plays a vital role in mRNA processing and export. My research seeks to investigate how the mutations effect the development of neural tissue. To identify the expression of the *Thoc6* gene, V5 tagged *Thoc6* was labeled with Alexa 488 in e8.5-10.5 mice. Additionally, *wild type* and *Thoc6* mutant mice were compared, by proliferation, neural, cell death and progenitor markers, to test the hypothesis that the lack of proliferation or cell death of neural cells produces the mutant phenotype. Through a series of microscopy images, the *wild type* and *Thoc6* mutant embryonic neural tissue was found to vary dramatically in composition as measured by cellular count and size.

Project Mentor: Dr. Ashleigh Schaffer, Department of Genetics and Genome Sciences and Center for RNA Science and Therapeutics

Restoring Sensation in the Lower Extremities of Amputees through Electrical Stimulation of Peripheral Nerves

Alexandra Wolkoff[1]; Hamid Charkhkar[2]; Courtney Shell[1,3]; and Ronald Triolo[2]

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2. Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH
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People with limb loss in their lower extremities have difficulties in navigating unfamiliar terrains in part due to lack of sensory feedback from the lost limb. For example, going up stairs or walking around at night are considered challenging tasks among most lower-limb amputees. Restoring sensation could potentially allow an amputee to regain a more balanced, faster, and efficient gait, which would then improve an amputee's quality of life. Our team has shown, for the first time, that natural sensation in the missing limb can be generated by providing an electrical stimulation to peripheral nerves in the residual limb of below-knee amputees via 16-contact C-FINEs (Composite Flat Interface Nerve Electrodes). One aspect of this on-going research is concerned with mapping and threshold testing to determine the locations, modalities, and intensities of the elicited sensations. I have developed a method to quantitatively represent the qualitative acquired data and to show how the stimulus parameters affect described sensations. From this information, a subset of stimulus parameters was chosen to investigate the participant's postural adjustment in a standing position. My analysis demonstrates how the center of pressure in the foot changes in response to different sensory inputs. My findings highlight the association between sensory input from the foot and postural adjustment during standing. This work could be considered as an initial step in developing advanced prosthetics that provide sensory feedback to lower limb amputees.

Project Mentor: Hamid Charkhkar[2], Biomedical Engineering

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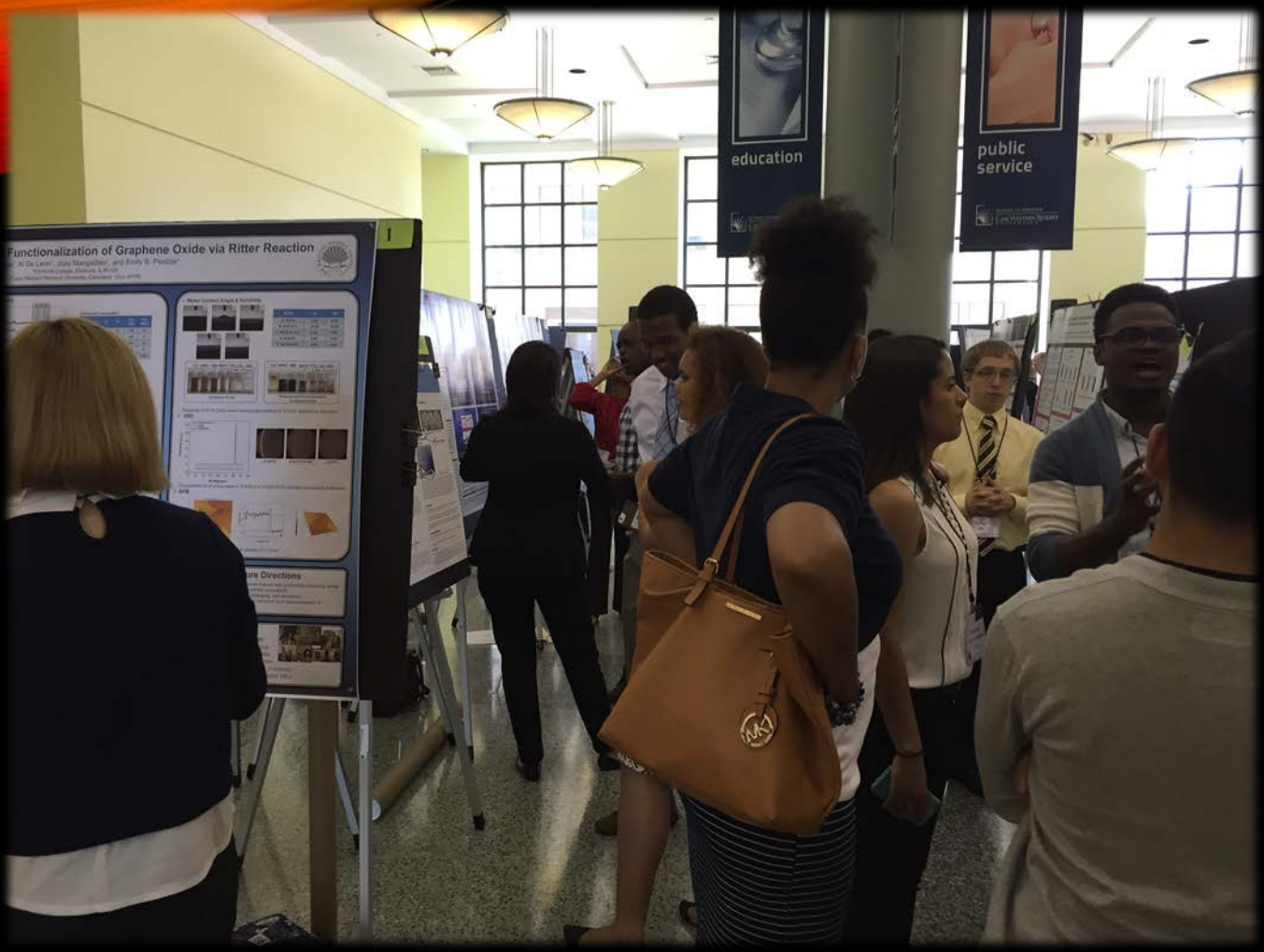
Neural dynamics of a feeding pattern-generating circuit in the marine mollusk Aplysia californica

Jingyi Yang, Department of Biology; Hui Lu, Department of Biology; Nathan X. Kodama, Department of Electrical Engineering and Computer Science; Tianyi Feng, Department of Biology

Because of its large, identified neurons, the buccal ganglion of Aplysia californica is a model system for understanding the neural basis of feeding behavior, which provides insights into motivated behavior and multi-functionality. We are monitoring in real time the activity of multiple key neurons in the neural circuit that controls feeding behavior. To this end, we are combining nerve recordings of the circuit's motor output with recordings of cell bodies of neurons in the neural circuit using a two-dimensional, high-density (100 um pitch) microelectrode array (MEA, 120 electrodes). The majority of previous studies have focused on recordings from single neurons, or small groups of neurons. However, many interesting aspects of neural dynamics can only be understood by looking at large populations of neurons and their relationships to each other. We are using the MEA and at the same time recording from buccal nerves 2 and 3, the radular nerve and the I2 muscle to identify motor neurons on the array during motor patterns, and distinguish them from interneurons. We have successfully recorded from large identified neurons in the ganglion while recording one to one extracellular action potentials in the nerves. At the same time, we can monitor the extracellular potentials and current densities throughout the ganglion. Our preliminary data suggests this approach will provide deeper insights into a pattern-generating circuit.

Project Mentor: Professor Hillel Chiel, Department of Biology; Professor Roberto F. Galán, Department of Electrical Engineering and Computer Science



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