UNIVERSITY OF MINNESOTA

2016 SUMMER UNDERGRADUATE RESEARCH SYMPOSIUM

Life Sciences Summer Undergraduate Research Program (LSSURP)

Faculty Director: Dr. Colin Campbell

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Presenter: Anthony Acholonu
Poster Number: 1
Home Institution: Hampton University
Program: LSSURP
Faculty Mentor: Dr. Lucy Vulchanova-Hart
Poster Title: **The Neuropeptide VGF in Neuropathic Pain**

Abstract: Expression of the protein VGF is associated with the development of chronic neuropathic pain. The specific protein, VGF, has been shown to be involved in the expression of the peptide TLQP-21 and TLQP-62 that has been demonstrated in rodent models of chronic pain. My lab and I have been mainly focusing in the dorsal horn of the lumbar region of the spine, which receives sensory input from the hind limbs. The dorsal horn is involved in sensory processing and contains nociceptive specific circuitry composed of both excitatory neurons and inhibitory neurons that work together to facilitate or inhibit the transition of nociceptive signaling. Following peripheral nerve injury this circuitry undergoes change in plasticity that facilitates the transmission of nociceptive signals and the development of chronic pain. By determining which neurons in the dorsal horn express VGF we aim to increase our understanding of how VGF-derived peptides may affect the circuitry of the dorsal horn to during the development of chronic pain. To this end, we have used immunofluorescence to stain for a population of excitatory neurons, inhibitory neurons, all neurons, and the VGF protein. In all, we want to use the research we have conducted to hopefully gain understanding of this circuitry that may lead to future developments of therapeutics for the treatment of chronic pain.

Presenter: Alejandra Albino-Ramírez
Poster Number: 2
Home Institution: Interamerican University
Program: LSSURP
Faculty Mentor: Dr. Donald Simone
Research Advisor: Iryna Khasabova
Poster Title: **ROS Scavengers Counteract the Neurotoxic Effect of Cisplatin in Dorsal Root Ganglia Neurons**

Abstract: Cisplatin is a platinum based chemotherapeutic drug effectively used for the treatment of different types of cancer. Cisplatin is unable to cross the blood-brain barrier, however, since dorsal root ganglion (DRG) neurons are not protected by a blood-brain barrier peripheral neurotoxicity is a common complication of cisplatin treatment. It was shown previously that cisplatin produced a bilateral hyperalgesia in mice. Accumulation of reactive oxygen species (ROS) in DRGs may contribute to cisplatin-evoked neurotoxicity that underlies the hyperalgesia. The goal of our study is to determine the impact of ROS on cisplatin-evoked neurotoxicity in DRG neurons. Primary cultures of DRG neurons were used to define neurotoxic effects of cisplatin by measuring intracellular calcium and neurite outgrowth. Small DRG neurons (cell body area <500 □m2), likely nociceptors, treated with cisplatin (13 M) for 24 hours demonstrated an increased level of intracellular basal calcium as well as an increased responsiveness to subthreshold concentration of KCl (25 mM, 15 s). Co-treatment of cisplatin with phenyl N-tert-Butylnitrone, an ROS scavenger (PBN, 100 mM) stabilized the level of intracellular basal calcium and decreased responsiveness to KCl, reflecting the normalization of neuronal excitability. Treatment of DRG neurons with cisplatin decreased the length of the PGP9.5- and peripherin-immunoreactive neurites. Co-treatment of cisplatin with PBN attenuated the decrease in neurite length produced by cisplatin. Collectively these results suggest that ROS contribute to the mechanism of cisplatin-evoked neurotoxicity in DRG neurons. Pharmacological elimination of ROS is a promising strategy for attenuating cisplatin-associated peripheral neurotoxicity.

Presenter: Gerardo Arroyo-Martinez
Poster Number: 3
Home Institution: University of Puerto Rico-Ponce
Program: LSSURP
Faculty Mentor: Dr. Nathan Springer
Poster Title: **Molecular Genotyping of Transposable Elements Insertions in a Population of Uniform Mu Events**

Abstract: Uniform Mu is a maize strain that was developed as a resource for genetic studies. Mutator transposable elements have been activated within an inbred W22 background and the movement of these transposons results in novel mutations. Researchers can identify Uniform Mu events that contain transposon insertions within a gene of interest. TE insertion events within the organism's genome often result in loss-of-function mutant alleles. This study aimed to genotype the presence of a TE insertion in several genes predicted to be involved in regulation of gene expression or chromatin structure. Molecular genotyping was performed by using Polymerase Chain Reaction (PCR) and gel electrophoresis. We determined that 43.89% of the population showed to be a homozygous wild type. In the other hand, 7.24% of the individual scored had a homozygous genotype, while 11.08% of the rows assessed presented heterozygous alleles. In future experiments, we hope to analyze these genes further and identify their possible role in causing abnormalities in the chromosomes.

Presenter: Ted Bebi
Poster Number: 4
Home Institution: Macalester College
Program: LSSURP
Faculty Mentor: Dr. David Potter
Poster Title: **Testing EET sensitivity in SSM2 Mouse Mammary Carcinoma Cells with Excised Cyp3a11**

Abstract: There has been rising interest in cytochrome P450 (CYP) proteins due to their implication in tumor progression. CYP3A4 expression in breast cancer correlates with overall decreased survival. CYP3A4 silencing in ER+ breast cancer cells was shown to decrease levels of epoxyeicosatrienoic acid (EET) biosynthesis and reduce tumor growth. Cyp3a11 is homologous to the human CYP3A4 in mice. While there are studies demonstrating the effects of knocking down the CYP3A4 gene in human breast cancer, the role of its mouse homolog Cyp3a11 remains to be determined. CRISPR/Cas 9 technology was used to excise Cyp3a11, in SSM2 mouse mammary carcinoma cells. Genotyping of excised clones showed knockdown of exon 3 with some level of heterozygosity. An MTT assay will be performed to show the sensitivity of Cyp3a11-excised SSM2 cells to EETs. We predict that EETs will restore the growth suppressed from Cyp3a11 excision, establishing the epoxygenase activity of Cyp3a11 and confirming its similarity to human CYP3A4. This result would allow the creation of a mouse tumor model to further study the role of CYP3A4 in breast cancer as well as the intrinsic effects of EETs in tumor growth.

Presenter: Brionna Bennett
Poster Number: 5
Home Institution: Winthrop University
Program: LSSURP
Faculty Mentor: Dr. Julia Davydova
Poster Title: **The Toxicity of the Fiber-Knob Modified Human Oncolytic Adenovirus (hOAds-5/3) on Immune Cells**

Abstract: Our fiber-knob modified human oncolytic adenoviruses (hOAds-5/3) have been shown to kill human pancreatic cells in vitro and reduce tumor growth in immunocompromised mice models better than unmodified Ad5 wildtype viruses. Recent studies suggest that in mice you can elicit an inflammatory response with Ad5. However, very few studies have been devoted to investigating Adenoviruses and their elicited immune response in reference to the modifications of the fiber knob. More specifically, we want to investigate which mode of virus inoculation would be optimal—intratumorally or systemically. Consequently, the safety of the Human adenovirus (Ad) vector is the main concern when moving forward to preclinical trials. Therefore, in the present study, we analyzed the four Ad vectors—Ad5CMV, Ad5WT, Ad5/3WT and Ad5/3Cox2 to asses their toxicities by using two immune cell lines—huPLB985 and THP-1. We hypothesize that the vectors would not kill nor replicate in the immune cells but a fraction of the virus would bind to the immune cells. We found that the virus does not kill the immune cells using an MTS assay, in both cell lines. We also found that the immune cells do not demonstrate any replication of the virus using a cell flow cytometry assay. Lastly, we found that the virus does bind to the immune cells. In summary, this study shows that the hOAds-5/3 is not toxic to immune cells.

Presenter: Lucas Bertram
Poster Number: 6
Home Institution: Normandale Community College
Program: LSSURP
Faculty Mentor: Dr. Geoff Ghose
Poster Title: **Exploring the Substructure of Cortical Areas using Ultra-High Field Imaging**

Abstract: Although past studies have revealed areas in the brain that are responsible for recognizing faces, the internal organisation and specific function of these areas remains unknown. We attempted to better understand the cortical regions involved in face perception by addressing where and how these areas might discriminate between two categories of faces (Male vs. Female and Smiling vs. Neutral). We imaged the temporal cortices of awake human subjects while they viewed a video of faces that varied within these categories on a morph continuum. By collecting images within an ultra-high magnetic field (7T), we could record cortical activity at 1.5mm resolution. This compact size was significant: analyzing such small sections of cortical face areas allowed us to more precisely define the computational properties of these regions than previous studies have permitted. We found that within the previously known face areas, there are clusters of neurons responsible for identifying certain facial features, such as gender and emotion. Pixels within a putative face area may show as large as a 7% signal difference for one or another such category of faces. These results suggest that the substructure of cortical areas contains discrete groups of neurons capable of identifying specific visual features.

Presenter: Kayla Bohlke
Poster Number: 7
Home Institution: University of Maryland-College Park
Program: LSSURP
Faculty Mentor: Dr. Rusen Yang
Research Advisor: Kory Jenkins
Poster Title: **Noninvasive Sensory Substitution And Testing Platform For Prosthetic Hands**

Abstract: Sensory substitution has been shown to improve quality of life for upper limb amputees but is lacking in commercially available prosthetics. Moreover, the few devices that do use tactile sensors can only detect normal forces. The project goal is to develop a piezotronic sensor utilizing zinc oxide (ZnO) nanowires to detect both normal and shear forces, thereby improving shape and texture discrimination. The nanowires are highly sensitive to crystal structure deformation due to the piezoelectric properties of ZnO. Nanowires are grown using chemical vapor deposition and the sensors are synthesized using microfabrication techniques. According to FEA simulations, the nanowires should be sensitive to forces in the nanonewtons range. A fabricated sensor responded to normal forces on the order of 10 newtons, however, its nonlinear Schottky response shows potential for higher force sensitivity with further refinement. An open source prosthetic hand was built for exploring surfaces in preliminary psychophysics discrimination studies using off-the-shelf sensors. Fourier analysis was conducted showing distinct frequency spectra for different surfaces. This demonstrates that off-the-shelf normal force sensors can be used to extract information about surface textures. The same tests will be performed with the nanowire sensors to examine the effect of shear force information. Because shear sensing is an important component of physiological surface exploration, the nanowire sensors are expected to improve sensory substitution systems for prosthetic limbs by delivering additional tactile information to the user.

Presenter: Sarah Brotman
Poster Number: 8
Home Institution: Arizona State University
Program: LSSURP
Faculty Mentor: Dr. Scott Dehm
Research Advisor: Yingming Li
Poster Title: **Inhibition Of The Androgen Receptor N-Terminal Domain By Novel Compounds In Prostate Cancer Models**

Abstract: Androgens, such as testosterone and dihydrotestosterone, are essential for sexual differentiation and for the maintenance of sexual tissues in males. These androgens serve as the main ligands for the androgen receptor (AR). Ligand-bound AR is a transcription factor that interacts with androgen response elements in the promoter and enhancer regions of genes essential for the growth and maintenance of normal and cancerous prostate cells. Therefore, androgen deprivation therapies (ADT) for locally advanced and metastatic prostate cancer rely upon depleting circulating androgens. ADT targets the ligand-binding domain at the C-terminus of the AR protein, and if the first line of treatment fails, the prostate cancer is considered castration resistant and treated with a second line of ADT. These second line treatments, also targeting the C-terminus, are often ineffective, which drives our study to find novel compounds to target the transactivation unit 5 (TAU5) domain in the N-terminus of the AR protein. We developed a yeast-based assay to evaluate the ability of small drug-like molecules to inhibit the transcriptional activity of a Gal4-TAU5 fusion protein. We used this system to conduct dose-response assays with 16 different compounds that were identified as TAU5 inhibitors in a high-throughput screen. Our results showed that none of the compounds inhibited AR TAU5, but some displayed dose-dependent inhibition of the AR N-terminal domain (NTD). The discovery of new compounds that inhibit the AR NTD rather than the ligand-binding domain could circumvent patient resistance to ADT and help improve prostate cancer survival.

Presenter: Amanda Brunick
Poster Number: 9
Home Institution: Bucknell University
Program: LSSURP
Faculty Mentor: Dr. Mark Masino
Poster Title: **Characterization Of Transgene Expression In Spinal Motor Neurons Of Larval Zebrafish**
Abstract:

Transgenic lines that drive GFP expression are commonly used to target particular subtypes of cells. Transgenic zebrafish that express fluorescent proteins in motor neurons have been utilized in many studies, however the expression patterns in these lines have not been systematically compared to motor neuron identity. In this study, the specificity of spinal motor neuron labeling was assessed in several zebrafish lines (Tg(nrp1a:GFP), Tg(mnx1:GFP), and Gt(IRES-Gal4ff)213A/Tg(UAS:GFP)). To characterize the transgene expression in motor neurons, these lines were outcrossed to WT fish, GFP positive larvae were fixed at 5 days post fertilization (dpf), and larvae were immunohistochemically labeled with antibodies to choline acetyl transferase (ChAT), an enzyme in the synthetic pathway for acetylcholine. The degree of colocalization of GFP with anti-ChAT was obtained by confocal imaging of a midbody segment and analyzed using ImageJ. The number of ChAT labeled cells remained consistent across all lines observed, reinforcing its use as a reliable marker of cholinergic neurons. The Tg(nrp1a:GFP) line had the greatest amount of overlap with anti-ChAT followed by the Tg(mnx1:GFP) line, while the Gt(IRES-Gal4ff)213A/Tg(UAS:GFP) line had the least. The ChAT positive cells also remained consistent on their distribution over the dorsal-ventral axis while the GFP positive cells differed between lines. Although ChAT has been shown to be a consistent indicator of spinal cholinergic neurons in fixed tissue, there is a need for another in vivo marker that labels a greater number cholinergic neurons with more specificity than the lines investigated in this study.

Presenter: William Cao
Poster Number: 10
Home Institution: Williams College
Program: LSSURP
Faculty Mentor: Dr. Douglas Yee
Research Advisor: Xihong Zhang
Poster Title: **Investigating the Viability of SMASh-Tagged IRS1 in MCF-7L Breast Cancer Cells**
Abstract:

Insulin Receptor Substrate 1 (IRS1) is a crucial element of the Insulin-like Growth Factor-1 Receptor (IGF1R) pathway and, accordingly, is critically involved in cell growth, proliferation, and survival. IRS1 expression has been found to be elevated in many human malignancies, including breast cancer, and its upregulation is associated with resistance to anticancer drugs. However, there has been scarce progress in the development of anticancer agents targeted for IRS1. Small Molecule-Assisted Shutoff (SMASh), developed by Chung et al. (2015), allows for direct, adaptable drug control of protein production through the fusion of a self-excising degron, known as a "SMASh tag". Protease inhibitor drugs block this removal, and successive copies of the SMASh-tagged protein are rapidly degraded. In this experiment, we explored the viability and effectiveness of SMASh-tagged IRS1 in breast cancer cells. The SMASh tag was genetically engineered onto IRS1 in MCF-7L breast cancer cells, and the most stable clone variant, #34, was selected for treatment. Clone 34 demonstrated successful integration of the tag, displaying efficient self-excising behavior in the absence of drug and dose-dependent inhibition in response to the protease inhibitor drug Asunaprevir. The SMASh tag did not appear to affect the IGF1R pathway, as expression of IRS1, IRS2, insulin receptor, and IGF1R-beta did not deviate significantly from those in the MCF-7L control. Furthermore, MTT assays showed normal cellular proliferation and activation in response to IGF-1, insulin, and estradiol. Thus, SMASh possibly offers an effective and nonintrusive method for post-translational IRS1 protein regulation.

Presenter: Carla M. Cardona-Valle
Poster Number: 11
Home Institution: University of Puerto Rico-Mayaguez
Program: LSSURP
Faculty Mentor: Dr. Michael Georgieff
Research Advisor: Phu Tran
Poster Title: **Determination of Transcription Factors in Iron Deficiency-Induced Neuronal and Hematopoietic Lines via CRISPR-ChAP-MS Technique**

Abstract: According to the World Health Organization, iron deficiency affects approximately 30% of the world's population, including 20-30% of pregnant women and their offspring, and causes long-term deficits in learning and socio-emotional behavior despite prompt iron treatment. Although the mechanism is unknown, iron uptake from transferrin transport protein is prioritized among tissues, favoring red blood cells over neurons. Identifying this mechanism is potentially instrumental for developing therapeutic strategies to shuttle iron preferentially to the brain for neurodevelopment, thereby ameliorating long-term negative neurobehavioral effects for at risk fetuses and neonates. Differential regulation of transferrin receptor (TfR-1) gene promoter is proposed and will be tested using a novel CRISPR-ChAP-MS technique. GuideRNAs, targeting various TfR-1 promoter regions encompassing suspected transcriptional factor (TF) binding sites (-1K, -3K and -4K upstream of transcription start site of TfR-1 gene), were inserted into pLE016.6 plasmids, which carry a HALO tag and deactivated CAS9 (dCAS9). Engineered vectors were then used to transform DH5α competent bacterial cells for plasmid amplification. Purified expression vectors will be used to transfect hematopoietic (K562) and neuronal (HT-22) cell lines. Following iron deficiency induction by an iron chelator (Deferoxamine), quantitative Real-Time PCR (qPCR) will be used to determine TfR-1 expression. The promoter-TFs-complexes will then be purified using Halo tags. TF identification will be assessed using protein-sequencing LC-MS/MS technique.

Presenter: Kevin Chen
Poster Number: 12
Home Institution: Univ. of Maryland-Baltimore County
Program: LSSURP
Faculty Mentor: Dr. Martina Bazzaro
Poster Title: **Polymerization May Be Necessary For UNC-45A Destabilization Of Microtubules In Cancer Cells**

Abstract: In our laboratory, we have recently shown that UNC-45A is a cytoskeletal-associated protein overexpressed in paclitaxel resistant cancer cells. Furthermore, we have shown that UNC-45A directly binds to microtubules (MTs) and destabilizes them. Thus, our working hypothesis is that UNC-45A overexpression in paclitaxel resistant cancer cells causes excessive destabilization of MTs de-facto antagonizing paclitaxel effect on cancer cells. Recent data obtained in lower organisms (worm) suggest that UNC-45A oligomerization is required for its function possibly by being required for its binding to microtubules. Thus we aim to answer the question: is polymerization of the mammalian UNC-45A required for it function? To do so we will a) determine whether the human UNC-45A forms oligomers in vivo (cancer cells) and in vitro (recombinant protein) and b) identify the domain of UNC-45A that is required for oligomerization. To achieve our two research goals, we first identified UNC-45A polymer formation using disuccinimidyl suberate (DSS) crosslinking agent. DSS agent is able to maintain preexisting polymer formation interactions as they occur in cells. Therefore, if UNC-45A does form polymers in cancer cells, DSS will allow us to preserve the polymer interactions and visualize them via western blot. Our western blots show that UNC-45A does To determine whether specific terminals or regions on UNC-45A are also required for UNC-45A polymerization, we created recombinant UNC-45A protein with either N or C terminal deleted. Using plasmids to introduce recombinant DNA into cells to produce recombinant protein, we resulted in four recombinant protein forms: empty, full-length, C terminally deleted, and N terminally deleted. Using western blots, we determined that UNC-45A forms polymers in cells containing full-length protein. There also seems to be indication of polymerization with the deleted C terminal form of UNC-45A, indicating that the N terminal is largely involved in polymerization.

Presenter: Patricia Claudio-Vázquez
Poster Number: 13
Home Institution: University of Puerto Rico-Cayey
Program: LSSURP
Faculty Mentor: Dr. Michael Kyba
Research Advisor: Darko Bosnakovski
Poster Title: **Analyses of Downstream Targets of DUX4, Gene Involved in Facioscapulohumeral Muscular Dystrophy**

Abstract: The process underlying Facioscapulohumeral Muscular Dystrophy (FSHD) involves a deletion of a subtelomeric repeat sequence on chromosome 4q called D4Z4. When contracted, this region fails to suppress the expression of DUX4, a transcription factor that in turn induces the expression of germline genes in muscle cells. Downstream targets genes of DUX4 include TRIM43 and MBD3L2, and are usually expressed between the embryonic stem cell and germline cell stages, but not afterwards. In patients with FSHD, these genes have been found to be expressed, but their function in the muscle cells is unknown. Through the doxycycline inducible system, we expressed these genes in LHCN-M2, human myoblast cells. RT-qPCR shows that the expression of TRIM43, and MBD3L2 is upregulated after induction. Performing cell viability assays, we found that the number of cells did not vary throughout different concentrations of doxycycline. After inducing the cells for differentiation, it was found that MBD3L2 is inhibiting the differentiation of the cells into myotubes. We confirmed myogenic differentiation by immunofluorescence against myosin heavy chain (MHC), which is a marker for terminal differentiation and gene expression analysis. We concluded that the expression of TRIM43 does not appear to be toxic to human myoblast cells, and MBD3L2, while not toxic, does hinder proper differentiation. We hope further investigation will lead to a better understanding of FSHD.

Presenter: Duncan Claypool
Poster Number: 14
Home Institution: Macalester College
Program: LSSURP
Faculty Mentor: Dr. Craig Henke
Research Advisor: Adam Gilbertsen
Poster Title: **The Impact of Collagen Thermal Stability on Sircol Assay Output and Potential Assay Modifications**

Abstract: The Sircol assay is used to quantify collagen content as a metric indicating degree of fibrosis and thus can be a powerful tool in the study of various fibrotic diseases. Primary literature lacks information regarding frequent laboratory sample conditions and the impact they have on collagen concentrations determined by the Sircol assay. This study sought to address thermal denaturation due to storage temperature and freeze-thaw cycles, photostability, and alternate reagent usage. Neither storage temperature (-195.79°C, -80°C, -20°C, 4°C, or room temperature) nor number of thawing cycles (1,5, or 10) significantly impact assay readout. Well plates left light-exposed saw little change in absorbance by day 4 but a modest decrease by day 10. Additional tests examined assay function when using reduced volumes of reagents. Reducing the volumes of both the dye and the acid-salt wash used by 75% significantly increased assay output. The same results were observed when only the volume of dye was reduced. However, reducing only the volume of the acid-salt wash did not significantly alter results. The Sircol assay appears unaffected by the tested collagen storage conditions and brief light exposure but compromised by losses in protein tertiary structure and extended light exposure. The volume of only the acid-salt wash can be reduced while maintaining assay accuracy.

Presenter: Sarah Cone
Poster Number: 15
Home Institution: Northwestern University
Program: LSSURP
Faculty Mentor: Dr. Chris Kim
Research Advisor: Luke Everson
Poster Title: **Developing a Low Bandwidth Self Navigating System Using Neural Networks**
Abstract:
In the present era, self-navigating cars are no longer bound to the realm of science fiction. Companies such as Google are already using autonomous vehicles for various purposes. However, the equipment needed for these vehicles is often extremely bulky. We hope to provide a practical, low-bandwidth alternative that can be widely used in both self-navigating cars and drones. Our model for this technology is an omnidirectional 4WD robot which communicates with a computer through bluetooth. The computer makes a decision based on visual data from the robot via a neural network. This method requires minimal processing and data transfer, making it ideal for small self-navigating machines such as drones.

Presenter: Reina Desrouleaux
Poster Number: 16
Home Institution: Carleton College
Program: LSSURP
Faculty Mentor: Dr. Romas Kazlauskas
Research Advisor: Bryan Jones
Poster Title: **Understanding the Enantioselectivity of Hydroxynitrile Lyases through X-ray Crystallography**
Abstract:
Plants use hydroxynitrile lyase catalyzed cyanohydrin reactions to produce (hydrogen) cyanide as a defense mechanism. The hydroxynitrile lyase AtHNL is an R-selective enzyme as opposed to its S-selective family members. To enantiomerically study AtHNL and its relative, HbHNL, we combined features from both to make two mutant hybrids. Despite having similar active sites, these hybrid enzymes preserved their enantiomeric specificity. To try to understand these unexpected results, we decided to crystallize the mutant enzymes to solve its structure through x-ray diffraction. We prepared hanging drop plates in which the pH, concentration of precipitants and proteins were varied according to literature. Based on our previous results, we hypothesize their enantioselectivity is determined by residues outside of the active site.

Presenter: Benjamin Dralle
Poster Number: 17
Home Institution: Iowa State University
Program: LSSURP
Faculty Mentor: Dr. Jill Siegfried
Research Advisor: Mariya Farooqui
Poster Title: **Estrogen Regulates Inflammation in NNK-Induced Lung Cancer in a Mouse Model**

Abstract: Considerable evidence has demonstrated that the primary sex hormone estrogen (E2) plays a role in regulating inflammation. Estrogen has previously been described as pro-inflammatory as well as anti-inflammatory depending upon the microenvironment and tissue type. This flexibility in function is of particular interest in lung cancer, a disease in which inflammation levels and local E2 production may influence disease progression. The project was designed to understand how estrogen modulates inflammation at an early time point in the development of lung cancer. Mice were given nicotine-derived nitrosamine ketone (NNK) injections to induce lung cancer. To evaluate the effects of E2, one group of mice was fed estradiol and another group was fed ethanol (vehicle control) in their drinking water beginning 3 days prior to the first NNK injections. This treatment regimen continued for 4 weeks before the mice were euthanized. Following euthanasia, immunohistochemistry (IHC) and Western blotting were used to examine samples from each group. IHC staining was performed for angiogenic, proliferative, and inflammatory factors present in the lung tissue collected. Western blotting was completed for several markers of inflammation. The results showed that estrogen induced inflammation by increasing the expression of COX-2 and VEGF, activating NF-κB, and decreasing the expression of IL-6. We propose a model that describes estrogen's role in regulating inflammation after exposure to a carcinogen. This study also adds to the rationale of using anti-estrogen drugs as anticancer treatments either alone or in combination with other therapies.

Presenter: Alec Vallota-Eastman
Poster Number: 18
Home Institution: Oregon State University
Program: LSSURP
Faculty Mentor: Dr. Mike Smanski
Research Advisor: Maciej Maselko
Poster Title: **Programmable Gene Expression in Zebrafish Using CRISPR/Cas9-Mediated Transcriptional Activation**

Abstract: Within the last decade, several technologies have emerged which provide unparalleled accuracy for engineering gene expression. Although past studies demonstrate targeted gene expression in several human, mouse, and fly cell lines, these systems have yet to be developed in the model organism, Danio rerio (zebrafish). In this project, we aim to demonstrate programmable gene expression in zebrafish using an RNA-programmable reporter gene system. We also hypothesize that in-vivo overexpression of seven targeted genes – gata5, slit2, actb1, ern1, wif1, shha, and rabac1 will result in embryonic lethality, showing that tight regulation of these genes is important for viability. Our reporter gene system consists of one or more transcriptional activators (VPR, EDLL, TAD, VP64, Gal4) linked to a catalytically dead CRISPR associated protein (dCas9). Localization of the protein complex is accomplished using short guide RNAs (sgRNA). Each sgRNA is designed using a self-made computational algorithm which locates 20-mer target sites that are unique to 5' promoter regions of each gene and are in the presence of an 'NGG' motif, required for dCas9 to bind. Each dCas9-activator complex is transcribed in-vitro and the resulting mRNA is combined with sgRNA strands, synthesized by PCR extension of annealed oligo pairs. mRNA and sgRNA components are then co-injected into single-cell stage zebrafish embryos for in-vivo translation and activator system efficiencies are analyzed by quantitative real-time PCR (qRT-PCR). This work will be the first example of using these tools in zebrafish, providing reliable control of gene expression in a principal model for studying development and genetics.

Presenter: Sydney Fine
Poster Number: 19
Home Institution: College of Wooster
Program: LSSURP
Faculty Mentor: Dr. Reuben Harris
Research Advisor: Artur Serebrenik
Poster Title: **(A3)Beating Cancer: Over-expression of APOBEC3B Sensitizes Cells to 5-Fluorouracil Treatment**

Abstract: The APOBEC3 family of cytosine deaminases consists of seven members that function as part of the innate immunity. One family member, APOBEC3B (A3B), has been implicated as an endogenous source of mutation in cancer cells, specifically in head/neck, lung, cervical and breast cancers. A3B increases the rate of mutation in DNA through its ability to deaminate cytosine into uracil. The aim of this study was to determine if A3B over-expression synergizes with 5-Fluorouracil (5-FU), an FDA-approved chemotherapeutic agent, to promote cell death. 5-FU promotes cell death through two mechanisms. First, 5-FU inhibits thymidylate synthetase, an enzyme that converts deoxyuridylate to deoxythymidylate. Second, 5-FU becomes incorporated into DNA, which causes significant DNA damage. To investigate the hypothesis, A3B was over-expressed in a mammary epithelial cell line, MCF10A, via transfection, transduction, or with PMA. Then, the cells were treated with various concentrations of 5-FU. Cell viability in response to 5-FU treatment was assessed by clonogenic survival assays, which measure cells' ability to proliferate. It was determined that up-regulation of A3B increased the efficacy of 5-FU at low concentrations of drug. This research has implications in regards to personalized treatment in cancer patients with A3B-high tumors because treating these tumors with 5-FU may widen the therapeutic window and allow for more effective disease treatment.

Presenter: Nihan Gencerliler
Poster Number: 20
Home Institution: Macalester College
Program: LSSURP
Faculty Mentor: Dr. Kevin Wickman
Poster Title: **VTA Dopamine Neuron Excitability Regulates Sensitivity to Drugs of Abuse: The Role of GIRK3**

Abstract: Drugs of abuse exert their reinforcing properties via enhancement of the mesocorticolimbic dopamine projection from the ventral tegmental area (VTA) to downstream targets such as the nucleus accumbens and prefrontal cortex. The excitability of VTA neurons is regulated, among others, by G protein-coupled inwardly-rectifying potassium (GIRK) channels. When activated by their respective GPCRs, GIRK channels produce an outward K+ current that hyperpolarizes the cell, thereby making the cell less likely to produce an action potential. Selective ablation of the GIRK3 subtype from the VTA has been shown to reduce opioid-induced motor activity. However, it has not yet been determined whether this occurs by way of GABAergic or dopaminergic neurons, the two main neuronal populations of the VTA. We induced GIRK3 overexpression selectively in DA neurons of the mouse VTA using a cre-dependent AAV and ran a locomotor activity test in order to elucidate this subtype's role in opioid-induced motor activation. Preliminary data suggests that limiting GIRK3 overexpression to DA neurons is sufficient for the enhancement of opioid-induced motor activity. This supports the contention that GIRK3 plays a regulatory role in trafficking inhibitory GIRK channels away from the plasma membrane in DA neurons, thereby enhancing dopamine neurotransmission along the mesocorticolimbic projection. Additional trials will be needed to solidify the pattern observed in this study and further clarify the role played by GIRK channels in drug addiction.

Presenter: Graham Giesting
Poster Number: 21
Home Institution: St. Olaf College
Program: LSSURP
Faculty Mentor: Dr. Nathan Springer
Poster Title: **Genotyping Maize for the Establishment of a Uniform Mu Transposon-Positive Study Population**

Abstract: We are pursuing the epigenetic basis for phenotypic differences between plants by identifying the genes responsible for the processes governing expression, knocking them out, and detecting phenotypic responses. Previous work has identified genes that code for proteins involved in the chromatin complex, and in methylation and methyl-transfer reactions, understood to be integral to the storage and expression of DNA; these we have designated our genes of interest. We obtained seeds for plants that have activated transposons inserted into our genes of interest. Plants from the Uniform Mu line of W22 have heritable transposon insertions in an inbred background. Ten seed samples from second generation sibling crosses of fully mapped, selfed individuals are available in small samples from the Maize Genetics Cooperation Stock Center at the University of Illinois. We genotypically screened, then selected and bred, individuals homozygous for our gene of interest. By propagating these homozygous mutants we will create a strain in which the genes which putatively code for the mechanisms of epigenetic regulation have been knocked out.

Presenter: Mayra Gonzalez-Torres
Poster Number: 22
Home Institution: University of Puerto Rico-Ponce
Program: LSSURP
Faculty Mentor: Dr. Bryce Binstadt
Research Advisor: Lee Meier, Jennifer Auger
Poster Title: **The Role of Tumor Necrosis Factor Receptor 2 (TNFR2) in a Mouse Model of Valvular Inflammation and Fibrosis**

Abstract: Fibrosis is increased production of extracellular matrix that results from chronic inflammation. Acquired cardiovascular diseases are the most significant source of morbidity and mortality worldwide and virtually all exhibit underlying fibrosis. The K/B.g7 mouse develops mitral valve (MV) inflammation and fibrosis with complete penetrance, spontaneous onset, and with many features that mirror human disease and thus provides a powerful tool for studying the effects of chronic inflammation on the cardiovascular system. We have observed evidence for a mechanism of fibrosis that is dominated by recruitment of circulating inflammatory monocytes rather than local self-renewal of resident macrophages that has been observed in other systems. Previous results lend support to a TNF/IL6-VCAM1-VLA4 axis mediating inflammatory cell recruitment and subsequent fibrogenesis in the K/B.g7 system. We next sought to determine the role for tumor necrosis factor receptor 1 (TNFR1) vs. TNFR2 in mediating endothelial activation in this pathway. Based on recent studies highlighting a role for TNFR2 in inflammatory monocyte recruitment, we hypothesized a similar role for TNFR2 in this system. We performed inhibition studies using anti-TNFR2 monoclonal antibody injected biweekly beginning at the onset of valve disease. After 4 weeks of treatment, hearts were collected and frozen in liquid nitrogen. Coronal sections at 10 m were stained with hematoxylin and eosin. MV thicknesses were measured using ImageJ and compared to IgG control treated animals. These studies will enhance our understanding of cardiovascular fibrosis and could inform potential therapeutic strategies that target the inflammatory cell recruitment process in human fibrotic diseases.

Presenter: Glorimar Guzmán-Pérez
Poster Number: 23
Home Institution: University of Puerto Rico-Humacao
Program: LSSURP
Faculty Mentor: Dr. Louis Mansky
Research Advisor: Jessica Martin, Morgan Schuck , Rachel Marusinec
Poster Title: **Development Of A Fluorescent Reporter That Monitors And Allows The Visualization Of HIV-1 Gag Expression In Living Cells**

Abstract: Human immunodeficiency virus type 1 (HIV-1) causes acquired immune deficiency syndrome (AIDS) by infecting immune cells and decreasing host defense against diseases. Virus assembly processes are still not understood, including the assembly and budding of viral progeny. The retroviral Gag polyprotein coordinates much of this process. This makes production of virus-like particles (VLPs) possible by expressing Gag by itself. In the Mansky lab, we fluorescently label Gag with a carboxy-terminal green fluorescent protein (GFP) to visualize viral assembly and budding. In this project, our aim was to replace the GFP gene with an mTurquoise gene in a codon-optimized Gag plasmid. This will facilitate visualization of Gag with a distinct turquiose color compared with other viral and host proteins stained with DAPI, GFP, or actin red. N3-HIV-1 Gag-GFP and N3-HIV-1 Gag were digested with BamHI and NotI (NEB) before gel purification to create vector backbones. The mTurq gene was PCR amplified from N1-mTurq before digestion to create the insert. To confirm successful ligation products, HeLa cells were transfected with candidate plasmids and were observed with confocal microscopy. The most successful results were obtained with the N3-HIV-1 Gag-GFP plasmid, adding calf intestinal phosphatese (CIP). Overall, we created a tool that can help visualize Gag with a unique staining so that future studies can contribute to understanding HIV-1.

Presenter: William Hail
Poster Number: 24
Home Institution: University of Alaska-Anchorage
Program: LSSURP
Faculty Mentor: Dr. Mathew Johnson
Poster Title: **Directional Simulation of the Globus Pallidus Interna and Gobus Pallidus Externa**

Abstract: Parkinson's patients, who make up 1% of the elderly population, face a variety of debilitating symptoms which are not all treated effectively with available therapy. Parkinson's is usually treated with Sinemet (LDopa) or stimulation of the Subthalamic Nucleus or stimulation of the Globus Pallidus. Deep Brain stimulation of approved targets effectively treats some symptoms of Parkinson's disease but many patients still experience gait disturbances and postural instability. Studies have shown that stimulation of the Peduncle Pontine Nucleus (PPN) has improved postural stability and gait disturbances in Parkinson's patients with varied success. Evidence suggests that stimulating in both GPe and GPi could achieve a therapeutic effect on patience with gait disturbances and with more consistent targeting and success than PPN stimulation. To explore the effects of directional stimulation of the GPe and GPi to treat Parkinson's disease, we will compare the therapeutic effect of existing treatment to therapeutic effect of combined GPe and GPi stimulation. We have constructed a habit trail which allows a non human primate model to walk freely back and forth. This will allow us to collect electrophysiological data during gait. We will compare the gait of MPTP treated Parkinsonian non-human primates to understand the effects of combined GPe and GPi stimulation to existing treatment. We hypothesize that stimulation of GPi and GPe will help reduce postural instability and gait disturbances and lead to a significant increase in mobility for Parkinson's patients. Further research is needed to determine the optimal surgical placement of the DBS between the GPi and GPe and the optimal stimulation settings for directional stimulation of the GPi and GPe to maximize therapeutic effect. If this therapy is unsuccessful, stimulation of the PPN for treating gait disturbances will need to be further explored.

Presenter: Madeleine Hart
Poster Number: 25
Home Institution: Beloit College
Program: LSSURP
Faculty Mentor: Dr. David Bernlohr
Research Advisor: Amy Hauck
Poster Title: **Oxidative Stress In Mitochondria Of TKD Adipocytes Increases Histone Carbonylation In Nuclei By Retrograde Signaling**

Abstract: Insulin resistance is a precursor to the development of type II diabetes and is highly associated with obesity. One causal mechanism of insulin resistance is thought to be protein carbonylation, the post-translational modification of proteins by reactive lipid aldehydes such as 4-hydroxy-trans 2,3 nonenal (4-HNE) and 4-hydroxy trans 2,3 hexenal (4-HHE). Such reactive aldehydes are generated in response to oxidative stress and reactive oxygen species (ROS) modification of mitochondrial phospholipids. Indeed, in the obese, insulin resistant state the expression of mitochondrial antioxidant genes, Prdx3, Gpx4 and Gsta4, are all down regulated in response to inflammation leading to increased aldehyde synthesis. Triple knockdown adipocytes (TKD) were generated from concomitant shRNA mediated silencing of Prdx3, Gpx4 and Gsta4 in cultured 3T3-L1 adipocytes and represent a cell based model of the obese, insulin resistant state. This project tested the hypothesis that higher oxidative stress in the mitochondria of TKD cells will increase histone carbonylation in the nucleus via retrograde signaling. Histones are the primary components of chromatin, important for many genetic and cellular regulatory processes. Histones, as well as nuclear, cytoplasmic, mitochondrial fractions, and whole cell lysates were obtained from TKD and control adipocytes, and standard western blot protocol was administered on all the samples using anti 4-HNE primary antibodies. Results showed that HNE carbonylation is slightly higher in the histones and mitochondria. This suggests that adipocytes under high levels of oxidative stress are more prone to protein carbonylation which may be induced by intracellular communication between mitochondria and nuclei.

Presenter: Arielle Hay
Poster Number: 26
Home Institution: Carthage College
Program: LSSURP
Faculty Mentor: Dr. Walter Low
Research Advisor: Vibha Harindra Savanur
Poster Title: **Characterizing Receptors for Zika Virus Infection of Retinal Tissue**

Abstract: Due to its rapid spread throughout Asia and South America and the 2015 outbreak in Brazil, Zika virus (ZIKV) has been declared a global health concern. ZIKV is transmitted primarily through Aedes aegypti mosquitoes, and although it results in mild illness in adults, it is able to cross the placental barrier in pregnant women and infect fetuses. There is evidence that the virus causes neural stem cell dysfunction and death in the brains of these fetuses, often causing congenital defects such as microcephaly. A member of the Flavivirus genus, ZIKV is an RNA virus composed of a capsid, membrane and envelope protein. The envelope protein has been found to attach to host cell surface receptors, initiating endocytosis and allowing replication and increased virulence. The specific receptors and cell types susceptible to ZIKV remain elusive, although neural stem cells appear most vulnerable. Recent studies have identified four membrane receptors, AXL, DC-SIGN, DTK and TIM-1, that bind ZIKV in human skin cells. Clinical studies now demonstrate that the virus additionally can cause ocular malformations. It is hypothesized that these putative receptors may allow viral entry into the developing neural retina. Immunohistochemistry was used to identify and characterize these receptors in retinal tissue of fetal mouse models at varying gestational stages. These findings demonstrate the route the virus takes when infecting fetal retinal cells, and give insight into how retinal lesions occur. These receptors may be targets for drug development, as there are currently no treatments available for Zika virus.

Presenter: Cristal Hernandez-Hernandez
Poster Number: 27
Home Institution: University of Puerto Rico-Mayaguez
Program: LSSURP
Faculty Mentor: Dr. Li-Na Wei
Poster Title: **Enhanced Crabp1 Protein Stability Guided by Differential Scanning Fluorimetry**
Abstract:

All-trans Retinoic acid (atRA) is a potent therapeutic agent for cancer. Unfortunately, off target effects through retinoic acid receptors (RAR) limit the clinical application of this treatment. Independent of RAR's, the cellular retinoic acid binding protein 1 (Crabp1) can mediate events to slow cancer cell growth. The mechanism through which Crabp1 can slow growth is thought to be through its non-genomic activity. Cellular retinoic acid binding protein 1, Crabp1, mediates the non-genomic activity of atRA since the complex of Crabp1-atRA activates kinase 1/2 (ERK 1/2). Understanding the mechanism through which this protein can act as a tumor suppressor is interesting given that there are several genetic association studies that associate reduced Crabp1 expression in tumors to poorer patient outcomes. Differential Scanning Fluorimetry (DSF) can be used to visualize conditions that favor protein stability by shifting the melting temperature (Tm). It works as a screening method that can rapidly determine and optimize protein stability conditions. Among the factors that may influence protein stability are buffers, salts, and detergents, which have no specific interaction with the protein. With DSF, the conditions that are favorable for the stability of Crabp1 can be determined. The results of the screen indicated that Crabp1 protein stability was enhanced in 0.1M HEPES pH 7.5. Stability was further increased by altering the ionic strength of the buffer and by including osmolytes. Obtaining stabilized Crabp1 allows for further biochemical study to elucidate the mechanism of non-genomic action of Crabp1 that may be associated with the proposed tumor suppressor function.

Presenter: Eustacia Ikeri
Poster Number: 28
Home Institution: University of Wisconsin-Madison
Program: LSSURP
Faculty Mentor: Dr. Margaret Titus
Research Advisor: Alexander McQuown
Poster Title: **Rescue of *Dictyostelium discoideum* Myosin 7 null spore morphology**
Abstract:

Microorganisms form spores in response to adverse conditions. *Dictyostelium* (Dicty) cells form spores upon starvation that then germinate only under favorable conditions. Xu et al (Eukary. Cell, 2004), found that mutant Dicty lacking Myosin 7 (myo7) germinate prematurely, as opposed to wild type, by autoactivation. To reproduce these findings, myo7 null spores were stained with neutral red dye; spores which were dyed red indicated the onset of germination. Surprisingly, the myo7 null mutant spores could not be stained with neutral red. These findings suggest that the myo7 protein may not actually play a role in germination of spores. Sameshima et al (Cell Str. Func., 1994) found that the typical oval shape of a spore depends on a core of actin filaments in the nucleus. Interestingly, the myo7 null mutant has round spores.The spore shapes of other actin cytoskeleton protein mutants were also examined. The Vasp null mutant was also found to have round spores, while the TalA null mutant was found to have longer spores compared to the wild type. This indicates that these actin binding proteins may play a role in actin filament length and are required for normal spore shape. It is known that wild type Dicty pre-spore cells secrete substances that contribute to its oval shape; the presence of these secretions amongst myo7 null cell lines may rescue their shape. It is possible that the spore shape defect in the mutant spores may be related to a lack of secretion of this substance. This was tested by performing cell mixing experiments. Myo7 null cells and GFP tagged wild type cells were mixed in three different proportions: 80% to 20%, 50% to 50%, and 20% to 80%. This resulted in a more oval shape in the mutant spores that were collected from those mixtures. Together, these experiments suggest that myo7 mutant pre-spore cells may lack a secreted factor which may lead to a morphological defect.

Presenter: Donovan Inniss
Poster Number: 29
Home Institution: St. John's University
Program: LSSURP
Faculty Mentor: Dr. Michael Lee
Poster Title: **UNC-45A Expression in Mouse Brain and Peripheral Tissues**

Abstract: UNC-45A is a member of the UCS protein family (UNC-45/CRO1/She4p) and is required for motor protein assembly1,2. Studies show that UNC-45A is required for a variety of cytoskeletal-dependent functions including motility, adhesion, exocytosis, and cytokinesis3-6. Importantly, Dr. Bazzaro's laboratory has recently discovered that UNC-45A plays a crucial role in regulating growth cone dynamics. Specifically, UNC-45A is expressed in neuronal cell lines and mouse primary neurons and enriched at the growth cone. Furthermore, while UNC-45A expression was not required for cell survival, it is crucial for neuronal differentiation. Specifically, UNC-45A knockdown interferes with the initiating and growth of neurites. Given the important role of UNC-45A in neuronal cytoskeletal functions, we sought to determine the pattern of UNC-45A expression in mouse brain and peripheral tissues via Western blot and via immunohistochemical (IHC) analysis. Our preliminary data shows that UNC-45A is widely expressed in mouse brain, and is particularly present in the cortical region. Our data also shows that brain tissues may be expressing different UNC-45A isoforms. This is consistent with the highly specialized role of cytoskeletal proteins in neurons. Further research is needed to definitively determine expression trends of UNC-45A within the body, and to differentiate between isoforms of the protein found within select regions.

Presenter: Kareem Ismail
Poster Number: 30
Home Institution: Macalester College
Program: LSSURP
Faculty Mentor: Dr. Robert Kratzke
Poster Title: **Synergistic Chemotherapy And Viral Therapy Treatment Induces Immunogenic Cell Death In Non-Small Cell Lung Carcinoma**

Abstract: Non-small cell lung carcinoma (NSCLC) is a form of epithelial lung cancer and accounts for 85% of all lung cancers. Recently immunotherapy have been shown to be superior to chemotherapy when treating NSCLC. Previous studies have shown that VSV-IFN can enhance the body's immune response to NSCLC. Therefore it is hypothesized VSV-IFN could induce immunogenic cell death (ICD) which might be potentiated by combination with chemotherapy. Unlike normal apoptosis, ICD can induce an effective antitumor immune response through the secretion of DAMPs (danger associated molecular patterns). Once activated by the DAMPs the mature dendritic cell delivers the antigen to the t-cell in order to fight the cancer. H460 and H838 cell lines were treated on a 96 well plate with various concentrations of VSV-IFN and Pemetrexed to assess the efficacy of the combination. Cell viability was assayed using the Cell counting kit 8 (CCK8) at 24 hours, 48 hours, and 72 hours after treatment. The induction of ICD was assayed by secretion of ATP and HMGB1 using ELISA kits. VSV-IFN and pemetrexed combination therapy were strongly synergistic when analyzed by the Chou and Talalay Method for both H838 and H460 cells. VSV-IFN induced ATP and HMGB1 secretion after treatment, an effect that was slightly increased with combination therapy with pemetrexed. Therefore, we conclude that VSV-IFN and pemetrexed are synergistic. VSV-IFN induces ICD which is slightly enhanced by combination chemotherapy. Further study of the combination should be carried out in animal models of NSCLC.

Presenter: Jenna Kelly
Poster Number: 31
Home Institution: Coe College
Program: LSSURP
Faculty Mentor: Dr. Bin He
Research Advisor: Christopher Cline
Poster Title: **The Effects of Repetitive Transcranial Magnetic Stimulation to Multiple Motor Areas on BCI Performance**

Abstract: Brain-computer interfaces (BCI) are potentially useful for assisting disabled individuals in everyday tasks such as communication and mobility. However, an estimated 15-30% of the general population cannot efficiently use sensorimotor rhythm BCIs. While it is not currently understood why this occurs, certain networks are suspected to play a role in BCI aptitude, especially in the motor system. This research explores whether altering motor regions of interest through noninvasive neuromodulation affects BCI performance. Volunteers are instructed to perform a series of BCI tasks. Repetitive transcranial magnetic stimulation (rTMS) is then applied in between blocks of BCI tasks to induce temporary inhibition within the targeted area, comparing the subject's accuracy before and after rTMS disruption. EMG analysis was implemented in order to check for subject compliance with BCI task instructions. EEG analysis examined the power of oscillations in the alpha band during each task. These methods will be used to investigate whether altering activity in a particular region of the motor network can affect BCI performance, providing insight into the differences between levels of BCI aptitude.

Presenter: Andrew-Kemal Kirchmeier
Poster Number: 32
Home Institution: Macalester College
Program: LSSURP
Faculty Mentor: Dr. Mark Schleiss
Research Advisor: Kaitlyn M. Anderholm, Claudia Fernández-Alarcón
Poster Title: **Sequence Analysis of UL144 and UL128-131A in HCMV Clinical Isolates**

Abstract: Human cytomegalovirus (HCMV) is the most common congenital viral infection in the U.S. Sequelae include sensorineural hearing loss and mental disability. The UL144 open reading frame (ORF), a highly variable region, encodes a tumor necrosis factor receptor homologue. Sequence variation is thus useful in understanding the epidemiology of the infection. The pentameric complex (PC), composed of gH, gL, UL128, UL130, and UL131A, plays a major role in HCMV dissemination in vivo. Recently, the PC has received considerable attention as a potential vaccine target. Since strain characterization could provide insights into the molecular basis of HCMV disease, we analyzed variations of UL144 and UL128-131A genes in HCMV clinical isolates. Five clinical isolates and their cell culture adapted derivatives from known HCMV-positives at a Minnesota children's hospital were analyzed. UL144 and the UL128-131A region of the PC were amplified from these samples by conventional PCR using previously published primer pairs. Sanger sequencing was performed on the resulting products. Sequence variation was analyzed using bioinformatics tools. Phylogenetic analysis of the UL144 ORF indicated that the nucleotide and amino acid sequences diverge into three major groups: subgroup 1A, 1/5; subgroup 1C, 1/5 and group 3, 3/5. Sequence analysis of the 128-131A genes showed that PC genes were conserved among the samples as described in the literature. These results support previous work describing distinct subgroups of UL144. Further characterization and analysis of strain variation of UL144 and the PC could provide better understanding of the molecular epidemiology between strains of HCMV in Minnesota.

Presenter: Cristel Kpegba
Poster Number: 33
Home Institution: Savannah State University
Program: LSSURP
Faculty Mentor: Dr. Jeffrey Miller
Research Advisor: Martin Felices
Poster Title: **Enhancing NK Cells Function for Immunotherapy in Ovarian Cancer**
Abstract:

Ovarian cancer accounts for more deaths than all the gynecologic malignancies combined. The survival rate of the disease has only improved by 12% over the past four decades. This static survival rate denotes significant lack of advancement in the field. In this study, we investigated the potential of NK cell immunotherapy in ovarian cancer. We used an IL-15 super agonist, ALT803, to expand NK cells from healthy donor Peripheral Blood Mononuclear Cells (PBMCs) incubated with ascites fluid from ovarian cancer patients or ovarian cancer patient ascites NK cells. We hypothesized that ALT803 and a combination of ALT803 with Keytruda, a PD-1 blocking antibody, will help induce proliferation and rescue functionality of normal NK cells treated with the ovarian cancer soluble microenvironment or NK cells from ovarian cancer patient's ascites. We tested our hypothesis by assessing NK cell function and expansion via flow cytometry. The results showed that ALT803 significantly enhance NK cells expansion in ovarian cancer patient ascites. These results suggested that NK cells immunotherapy can help improve the ovarian cancer survival rate.

Presenter: Angela Liu
Poster Number: 34
Home Institution: Columbia University
Program: LSSURP
Faculty Mentor: Dr. Anna Lee
Poster Title: **The Role of PKCε in Regulating Liver Metabolism in Female Mice**
Abstract:

Nicotine serves as one of the most common substances of abuse in the United States. Protein kinase C epsilon (PKCε) plays an important role in nicotine consumption, since it regulates the expression of nicotinic acetylcholine receptors (nAChRs) in the brain, which are implicated in nicotine addiction mechanisms. Male PKCε knockout mice exhibit reduced nicotine self-administration in comparison to their wild type counterparts, illustrating the important effect of PKCε in regulating nicotine consumption. Male PKCε knockout and wild-type mice do not have differences in nicotine clearance, suggesting that the difference in consumption is not due to altered nicotine metabolism. When we used female mice, there was no significant genotype difference; both wild type and PKCε knockout mice consumed the same amount of nicotine. We sought to examine whether this sex-linked difference in nicotine consumption was influenced by a difference in nicotine metabolism. Thus, we measured the concentration of the primary nicotine digesting enzyme, CYP2A5, in liver samples from female mice using western blotting. We found that the concentrations of CYP2A5 were not significantly different between the two groups, suggesting that nicotine liver metabolism is not influenced by PKCε in female mice. Therefore, nicotine consumption is not impacted by liver metabolism and must instead be determined by other factors. Ultimately, through examining the molecular basis behind nicotine addiction, we can better understand how it functions and identify potential treatments for the future.

Presenter: Frances Lugo-Jiménez
Poster Number: 35
Home Institution: University of Puerto Rico-Arecibo
Program: LSSURP
Faculty Mentor: Dr. Nevin Young
Research Advisor: Roxanne Denny, Shaun Curtin , Diana Trujillo
Poster Title: **The Role Of A Dicer-Like Protein In Symbiotic Nodulation In *M. Truncatula***
Abstract:

Mutated legume plants were analyzed at both the genotypic and phenotypic level to determine if a candidate gene (a dicer-like protein) was critical for nodulation. During nodulation, atmospheric nitrogen is fixed by rhizobial bacteria in legume nodules. *Medicago truncatula* is a model for the study of legume genomics because of its small and well-characterized genome and resemblance to economically cultivated legumes, such as alfalfa. Candidate genes responsible for nodulation were identified in earlier investigations by genome-wide association studies (GWAS). One line of mutants was made using TALENs, which caused a double strand break in the DNA sequence that codes for a candidate gene. These mutants were genotyped to determine if the mutagenesis was successful. Another line of mutants was generated using Tnt1 retrotransposons, which are segments of DNA inserted in the DNA sequence of the candidate gene. Plants were screened for mutations using selected PCR primers to amplify within the gene region and thereby identify insertions into the DNA sequence. To quantify phenotypic variation, seeds of mutated plants were planted and examined after four weeks. Based on statistical t-test analysis, mutants and wild-types differed significantly in nodule number in the root region five centimeters below the crown. Mutated plants averaged a higher value of nodules than the wildtype.

Presenter: Joyce M. Maldonado-Rivera
Poster Number: 36
Home Institution: University of Puerto Rico-Ponce
Program: LSSURP
Faculty Mentor: Dr. Theoden Netoff
Poster Title: **Measuring Neural Activity in Cerebral Organoids**
Abstract:

Neurological conditions affect nearly 1/6 of the population worldwide, out of the which, approximately 54 million suffer from epilepsy. Development of effective patient specific therapies emphasizes the need of in vitro models for further comprehension of neurological disorders. To cope with limitations of conventional two-dimensional (2D) cultures, three-dimensional (3D) cultures derived from induced pluripotent stem cells (iPSCs) have enabled the in vitro development of miniature organs, known as organoids. Cerebral organoids (cOrg) are iPSC derived 3D culture systems which possess features of the human brain. We employed the use of Multi-Electrode arrays in order to measure significant neurological activity within cOrgs derived from healthy and epilepsy patients. By electrically stimulating the organoids, manipulating extracellular potassium concentration and addition of kynurenic acid (KYNA) we examined alterations in neuronal activity within the organoids. Our aim for this project is finding whether activity from organoids derived from controls is different from those derived from patients with epilepsy.

Presenter: Delfina Mancebo
Poster Number: 37
Home Institution: Providence College
Program: LSSURP
Faculty Mentor: Dr. Mark Thomas
Research Advisor: Brian Sweis
Poster Title: **The Effects Of Morphine And Cocaine On Impulsive Reward-Seeking Behavior In A Novel Neuroeconomic Decision-Making Task**

Abstract: Opiate (e.g., morphine) addiction has been driving the drug overdose epidemic in recent years. Studies looking at impulsivity in reward-seeking behavior have found that human opiate addicts are not willing to wait for delayed rewards. Similarly, studies using rodent models found that rodents treated with opiates devalued delayed rewards. While previous work in our lab has characterized changes in brain circuit connectivity over time with chronic exposure to different drugs of abuse, little is known about how these underlying neural changes drive impulsive decision-making behavior. Our lab developed a novel decision-making task in mice that models human-like neuroeconomic behavior. In this "Restaurant Row (RRow)," task, mice forage for food in a maze with 4 reward zones. Upon entry into a reward zone, tones are played and tone-pitch signals the delay length that must be waited to earn a flavored pellet. Each reward zone has uniquely flavored pellets as a means to capture subjective valuation decision-making processes between individuals. Delays are random at each entry and mice have 1 hour to get their food for the day. This means they have a budget of time to spend at each restaurant while making stay or skip decisions. At baseline, animals displayed individual preferences for each restaurant as a function of willingness to wait for food. This will set the stage for further experiments where neural modulation approaches can be taken to ameliorate drug induced changes in neural circuitry in efforts to normalize subjective valuation and decision-making behavior, identifying potential targets for more precise therapeutic interventions.

Presenter: Sofia Martir-Gonzalez
Poster Number: 38
Home Institution: University of Puerto Rico-Mayaguez
Program: LSSURP
Faculty Mentor: Dr. Kaylee Schwertfeger
Research Advisor: Emily Irey
Poster Title: **Identifying Secreted Factors In Triple Negative Breast Cancer Cells Activating JAK/STAT Signaling Pathway In Macrophages**

Abstract: Breast cancer is the second most common cancer in women, with a 3% mortality rate. Previous studies have shown that JAK/STAT signaling plays an important role in growth and proliferation in breast cancer cells. Our lab has previously shown that secreted factors from tumor cell conditioned media activate STAT3 and STAT5 signaling in THP-1 human macrophage cells. New therapies aim to target JAK/STAT signaling in breast cancer, but little is understood about how this treatment route may affect the surrounding immune cells. Therefore, our lab is interested in understanding the tumor-macrophage interactions in the breast cancer tumor microenvironment. Within the triple negative cancer subtype, preliminary data revealed variation in the ability of each cell line to activate JAK/STAT signaling in macrophages. We looked at gene expression of known STAT3 and STAT5-activating cytokines and growth factors via qPCR to identify patterns responsible for this signaling variation. Our results show expression of certain cytokines correlates to robustness of STAT3/STAT5 activation, especially IL-6 (STAT3 activator) and GM-CSF (STAT5 activator). In addition to gene expression, we also validated by western blot with protein isolated from concentrated conditioned media from each cell line and saw similar results. We are in the process of blocking these factors and assessing the impact on STAT activation in THP-1 cells. Identifying the factors secreted by triple negative breast cancer cells that are responsible for altering signaling in macrophages will help us understand the consequences of eliminating JAK/STAT signaling in non-tumor cells within the tumor microenvironment, as well as, potentially reveal other therapeutic targets.

Presenter: Jena' Mazique
Poster Number: 39
Home Institution: William Carey University
Program: LSSURP
Faculty Mentor: Dr. Robert Meisel
Poster Title: **Reward Circuitry: Oh the DREADD**
Abstract:

Sexual behavior in female hamsters is an effective model to examine reward circuitry in the brain. Two key areas that process sexual reward are the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAc). Our lab had previously shown increased NAc and mPFC activity following sexual behavior. Here, we used Designer Receptors Exclusively Activated by Designer Drugs, or DREADDs, to selectively inhibit the mPFC during sexual behavior to determine if it was the source of the increased NAc activity during sex. To decode this circuitry, hamsters were stereotaxically injected with a human synapsin h4Dmi DREADD construct and allowed to recover for three weeks while the DREADD was expressed. Animals were then either given sex experience for ten minutes with a male or remained in their home cage with a male present in the room. Half of each treatment condition was given CNO, the selective activator for the DREADD. Finally, brain tissue was collected and sliced for immunohistological processing of cFos, a marker of neuronal activity, as well as for a DREADD specific tag. If the mPFC was indeed responsible for increased NAc activity from sexual reward, then animals with inhibited mPFC activity should have reduced activation in the NAc compared with animals not given the CNO. Understanding the reward circuitry could lead to therapies for sexual disorders.

Presenter: Janayah McClellan
Poster Number: 40
Home Institution: University of Illinois-Urbana
Program: LSSURP
Faculty Mentor: Dr. Rita Perlingeiro
Poster Title: **Characterization of -Dystroglycan Hypoglycosylation in FKRP Dystrophic Mice**
Abstract:

Dystroglycanopathies refer to a common and heterogeneous subset of muscular dystrophies associated with aberrant O-glycosylation of alpha-dystroglycan (□□DG), which results from mutations in several genes, including the Fukutin-Related Protein (FKRP) gene. FKRP encodes a putative glycosyltransferase involved in □-DG glycosylation. Impairment of this critical component of the dystrophin glycoprotein complex is correlated with □-DG hypoglycosylation. Patients suffering from Limb-Girdle Muscular Dystrophy (LGMD2I), a type of Dystroglycanopathy, display significant and progressive muscle wasting, which can begin at early childhood (severe phenotype) or at adulthood (usually milder phenotype). There is no cure or treatment for these diseases. A mouse model, FKRPP448L, designed to resemble patients with LGMD2I emphasizes the importance of FKRP in maintaining proper functional glycosylation of □-dystroglycan. The FKRPP448L strain is a homozygous knock-in mouse carrying a proline to leucine missense mutation that recapitulates the clinical phenotypes of LGMD2I. Although the FKRP mouse model is a valuable tool to study the pathogenesis of FKRP-defective muscle; a comprehensive characterization of these mice has not been described. Specifically, is is not clear whether all muscles are equally affected. This is required information when considering using this mouse model for developing therapies for LGMD2I. Therefore, the goal of this project is to perform a thorough characterization of FKRP-mutated skeletal muscles in order to gain extensive comprehension of the existing model. For this purpose, I extracted protein from several skeletal musdes from FKRP mice, including Tibialis Anterior (TA), Quadriceps, Diaphragm, Abdominal muscles, Acromiotrapezius, Spinodeltoideus, Biceps Brachii, and Extensor Carpi Ulnaris, in addition to cardiac muscle and performed a Wheat Germ Agglutin (WGA) precipitation to enrich for glycosylated proteins. Purified protein was analyzed by western blot and incubated with IIH6, an antibody that recognizes functional glycosylated □-DG. My findings show that several muscles, including Tibialis Anterior (TA), Quadriceps, Diaphragm, and Abdominal FKRPP448L skeletal musdes as well as FKRPP448L cardiac muscle demonstrate reduced positivity for IIH6. However, Acromiotrapezius, Spinodeltoideus, Biceps Brachii, and Extensor Carpi Ulnaris FKRPP448L muscles stained positive for IIH6, meaning functional glycosylated □-DG. The results show that not all muscles are equally affected by the FKRPP448L missense mutation. Taken together, these findings indicate the TA, Quadriceps, Diaphragm, Abdominal muscles and cardiac muscle should be targeted when utilizing this mouse model for testing therapeutic strategies, such as stem cell transplantation.

Presenter: Robert Mendez
Poster Number: 41
Home Institution: California State University
Program: LSSURP
Faculty Mentor: Dr. Timothy Starr
Poster Title: **Defining WAC as a Tumor Suppressor in Colorectal Cancer**

Abstract: Current standards of Colorectal Cancer (CRC) therapy rely on surgery and chemotherapy, which have proven to be largely ineffective in the later stages of CRC. As CRC ascends to the top of the list of commonly diagnosed cancers and ranks second in lethality worldwide, the demand for better treatments grows as well. In order to better understand CRC tumor genetics a transposon-based forward genetic screen was performed in mice. Results from this screen uncovered 77 candidate CRC genes, 78% of which are mutated or dysregulated in human CRC, making these genes primary candidates as drivers of tumorigenesis. One gene uncovered during the screening known as WW domain containing adapter with coiled-coil (WAC) has been identified as a possible tumor suppressor based on both previous literature and recent observations. Young Adult Mouse Colon (YAMC) cells, which are immortalized via a temperature sensitive SV40 antigen, were used in an orthotopic tumor model. Given that the YAMC cells are non-tumorigenic, unexpected tumor formation was observed during dissection 8-14 days post injection. PCR analysis confirmed the recovered polyps were not a result of injected YAMC cells as the expected SV40 band was absent. Future direction involves manipulating the expression of WAC using either shRNA or CRISPR/Cas9 in YAMC cells to further evaluate the function of WAC in an orthotopic tumor model.

Presenter: Maria Camila Merino-Franco
Poster Number: 42
Home Institution: University of Minnesota-Twin Cities
Program: LSSURP
Faculty Mentor: Dr. Chun Wang
Poster Title: **Mucoadhesive Wafers for Sublingual Delivery of Vaccines: the Effect of Material Configuration on Delivery Performance in vitro**

Abstract: World-wide distribution of many traditional vaccines as subcutaneous or intramuscular injections is afflicted with a variety of challenges. Some of these challenges are: poor patient compliance, waste management, the need to maintain cold-chain conditions, and inability to elicit mucosal immune response against certain pathogens such as HIV. Engineering better biomaterials to enable sublingual (under the tongue) delivery of vaccines could be a solution to these challenges. Recently our lab has developed a wafer made of biocompatible and mucoadhesive polymers and shown to maintain protein activity even at 75oC and elicit antigen-specific mucosal immune response in mice. The goal of this project is to improve the mucoadhesion and penetration of protein antigen into sublingual tissue by designing and testing different configurations of the polymer materials. The different configurations include bilayer versus single-layer, and wafers (made by lyophilization) versus tablets (made by compression). We developed protocols of material processing and determined the release rate, mucoadhesion and penetration of a model antigen using pig tongue tissue. Results from these experiments will help us identify the best material configuration with the most potential for sublingual delivery. In the future, these materials will not only be useful for vaccine delivery, but may also be applied to delivering therapeutic agents for treating diseases such as head and neck cancer.

Presenter: Nahom Mossazghi
Poster Number: 43
Home Institution: Normandale Community College
Program: LSSURP
Faculty Mentor: Dr. Timothy Ebner
Poster Title: **Optogenetic Manipulation Of The Cerebellum In A Mouse Model Of Episodic Ataxia Type 2**

Abstract: Episodic ataxia type 2 (EA2) is a neurodegenerative disease caused by a mutation in the CACNA1A gene that encodes for the pore-forming Cav2.1 (P/Q type) Ca2+ voltage-gated channels. These mutations greatly affect Purkinje Cells (PCs), which are expressed at high levels in the cerebellar cortex, resulting in 30-40% reduction in function. Individuals with EA2 exhibit several characteristics such as absence seizures, mild ataxia, and dystonic attacks. Stress, caffeine, and alcohol trigger dystonic attacks in EA2 patients. The tottering mice (tg/tg) are used as a model animal to study EA2 because of a missense mutation in the Cacna1a gene and exhibit similar phenotypes. Flavoprotein autofluorescence optical imaging has revealed transient, episodic low-frequency oscillations (LFOs) occurring before dystonic attacks. Previous research has shown that LFOs play a significant role in the dystonic attacks. These oscillations occur both in the cerebellar cortex and motor cortex. The link between the cerebellum and the motor cortex in performing motor functions is well documented both anatomically and physiologically in rodent and non-human primates. Therefore, the question we asked was whether we could manipulate LFOs in the motor cortex by optogenetically stimulating the cerebellar cortex that we injected with channelrhodopsin (ChR2). Our preliminary result showed an increase in the power of oscillations in the motor cortex of the ChR2 injected tg/tg mice as compared with WT and non-injected tg/tg mice. These results will give us some insight into how LFOs interact and influence dystonic attacks and help us develop better therapeutic options for EA2 patients.

Presenter: Riken Nathu
Poster Number: 44
Home Institution: University of Florida
Program: LSSURP
Faculty Mentor: Dr. Harry Orr
Research Advisor: Tyler Tschumperlin
Poster Title: **Absence of Disease in Mice Expressing ATXN1[82Q] that No Longer Interacts with Capicua - A Study in Gene Expression.**

Abstract: Spinocerebellar Ataxia Type 1 is a progressive neurodegenerative disorder that primarily affects the cerebellum and brainstem. SCA1 is characterized by a loss Purkinje cells in the cerebellar cortex. SCA1 is caused by a trinucleotide CAG expansion in the gene ATXN1 which encodes glutamine-expanded ATXN1 protein. Transgenic mice expressing mutant ATXN1 [82Q] in Purkinje cells have long been used as a tool to model cerebellar aspects of disease. A region within the ATXN1 protein known as the AXH domain mediates interactions with other nuclear proteins, including the transcriptional repressor Capicua (Cic). Previous studies in mouse models of SCA1 have shown that reduction in Cic levels are linked to an improvement in SCA1 pathology. Furthermore, mutating the ATXN1 [82Q] AXH domain to abolish the interaction with Cic results in amelioration of disease. Recent studies also identified a select group of genes designated as the Magenta Module that are linked to SCA1 pathology. Most Magenta Module genes decrease over time in SCA1 mouse models. In this experiment we questioned whether AXH mutated mice would exhibit similar levels of gene expression to that of disease mice and how expression of these genes may change with disease progression. Quantitative PCR was used to measure relative expression of these genes in the cerebellum. Preliminary studies found that some genes in AXH mutated mice expressed similarly to wild type mice. However, as mice aged, the levels of expression for AXH mutated mice significantly decreased, similar to the disease model. These findings suggest that the AXH mutation corrects for the expression of Magenta genes early in life, but it does not rescue gene expression later on. We have outlined a gene expression timeline in these mice to be used in future studies.

Presenter: Jennifer Neufeld
Poster Number: 45
Home Institution: Bethel College
Program: LSSURP
Faculty Mentor: Dr. Larry Wackett
Research Advisor: James Christenson
Poster Title: **Biochemical Mechanisms in Bacterial Olefin Biosynthesis**
Abstract:

Hydrocarbons extracted from crude oil have diverse applications for everyday products such as gasoline, cosmetics, and plastics. Recently, Ole enzymes have been found in over 300 bacteria and are proven to catalyze the conversion of fatty acid derived acyl-CoA molecules into alkene hydrocarbons or olefins. There are four ole genes: oleA, oleB, oleC, and oleD that are found in bacteria genome, however, enzymatic functions of the proteins are only known for OleA, OleD, and OleC. OleA initiates the biosynthetic pathway and condenses two acyl-CoA molecules to make a long chain β-keto acid. OleD catalyzes a reduction of that intermediate to a β-hydroxy acid that is converted by OleC to the final olefin product. Recently, another laboratory that has worked on these enzymes reported in the scientific literature that OleC exclusively produces cis-olefins from the penultimate intermediate, the β-hydroxy acids; however, our data suggests otherwise. All four diastereomers of the β-hydroxy acid were chemically synthesized and separated by HPLC into their syn and anti pairs. Upon reaction with OleC, the syn and anti β-hydroxy acids were taken to cis- and trans-olefins, respectively. Furthermore, we demonstrated that OleB engenders a preference for cis-olefin formation during the final OleC reaction. This is the first reported function for the OleB enzyme.

Presenter: Stephanie Oliveras-Santos
Poster Number: 46
Home Institution: University of Puerto Rico-Ponce
Program: LSSURP
Faculty Mentor: Dr. Alfonso Araque
Poster Title: **Spatial Properties Of Astrocytic Networks In Different Brain Regions**
Abstract:

Astrocytes are active glial cells of the central nervous system that not only support neuronal activity, but also have the potential to regulate synaptic activity by reciprocally communicating with neurons as suggested by the tripartite synapse. Traditionally, they have been viewed as a homogeneous population, with similar characteristics and functions throughout the brain such as metabolic and nutrient support to neurons. However, recent data indicates that astrocytic morphology and function may differ between brain regions. In many different brain areas, astrocytes are connected through gap-junctions, forming a syncytium. The spatial extension of these astrocytic networks has been suggested to be relevant for the astrocyte-mediated synaptic support and regulation. We aimed to investigate the spatial extension of the astrocytic network in the hippocampus and in the nucleus accumbens. The experimental protocol consisted in whole-cell patch clamp recording of identified astrocytes in the hippocampus and the nucleus accumbens core of acute brain slices. Cellular identification was based on morphological (relatively small soma and numerous and short cell processes) and electrophysiological criteria (e.g., resting membrane potential, resting conductance and absence of action potentials). Those cells identified as astrocytes were then filled with biocytin (a gap-junction permeable dye) via the recording patch pipette. Biocytin was later visualized using immunofluorescence imaging techniques. Our preliminary results indicate differences in the spatial extension of the intercellular diffusion of biocytin in hippocampal and nucleus accumbens astrocytes, suggesting phenotypic differences in the astrocytic networks of the hippocampus and the nucleus accumbens. Future studies will investigate the potential alterations of astrocytic networks under pathological conditions.

Presenter: Erik Olson
Poster Number: 47
Home Institution: Loyola University of Maryland
Program: LSSURP
Faculty Mentor: Dr. Matthew Johnson
Research Advisor: Julia Slopsema
Poster Title: **Imaging of Peripheral Nerve Stimulation using Fast Voltage-Sensitive Dyes**
Abstract:

Introduction: Computational neuron models have been a useful tool to advance our understanding of complex circuits in the brain. One function used in many simulations of electrical stimulation within the brain is the so-called 'activating function', which is a second order spatial derivative relating extracellular voltage along the membrane of an axon to the depolarization and hyperpolarization along the axon. However, this model likely over-simplifies current flow through the membrane, leading to truncation errors. The long term goal of this project is to visualize axonal activity from an external voltage source in order to validate and/or improve the activating function's representation of axonal activation. Methods: The ventral nerve cord and its nerve offshoot roots were dissected from large crayfish and soaked in a voltage sensitive dye solution (1 mL of Ringer solution, 30 µM Di-8-Anepps) for an hour at room temperature to allow the dye to integrate to the lipid membrane of axons within the cord and nerve roots. Fluorescence microscopy was used to image axons in the nerve roots using a Nikon A1RMP Confocal Microscope at the University of Minnesota Imaging Center. Several imaging techniques were employed including single photon excitation, ratiometric dual excitation/dual emission, and averaging to reduce noise in the signal. Electrical stimulation (3 Hz pulses, 100 µs monophasic pulse width, 0.5-5 volts) was applied through a tungsten microelectrode positioned adjacent to the nerve root undergoing imaging. Results: An analysis protocol written in Fiji and MATLAB was developed to align and average the membrane voltage dynamics for a given stimulation trial (20-40 sec). Initial results show that there is activation of the cord roots, but additional refinement of the protocol is needed to improve the signal to noise ratio. Future directions for this project will include testing multiple stimulation modalities such as bipolar configurations and developing experimentally informed computational models to assess the ability of the activating function to accurately predict axonal activation.

Presenter: Christian J. Ortiz-Hernandez
Poster Number: 48
Home Institution: University of Puerto Rico-Mayaguez
Program: LSSURP
Faculty Mentor: Dr. Satoshi Ishii
Poster Title: **High-Temperature Adaptation of Arcobacter Pathogens**
Abstract:

The members of the genus Arcobacter are Gram-negative spiral bacteria closely related to Campylobacter spp. that are important foodborne pathogens. Some Arcobacter can also infect humans, but their significance is underestimated due to inadequate detection protocols currently used in hospitals and health departments. Unlike Campylobacter, Arcobacter strains cannot grow at 42ºC, which limits them to replicate inside the birds intestines. If Arcobacter can adapt to high temperature environment, they can infect birds (e.g., chickens) and eventually humans (via consumption of contaminated foods). The main focus of the project was to study the impact of high-temperature adapted bacteria on human health by understanding the adaptive mechanisms of these pathogens in response to temperature increase. The preliminary experiment was to confirm no growth of Arcobacter strains at elevated temperatures: 39ºC, 40ºC, and 42ºC. Temperature adaptation was then performed on A. butzleri strain ATCC 49616 (clinical isolate) and A. butzleri strain A096 (environmental isolate) by incubating cell cultures in a liquid medium at 38ºC and increasing the incubation temperature every ten generations by 0.2ºC. The high-temperature adapted bacteria were cryopreserved for future genetic/genomic analysis.

Presenter: Stephanie Ortiz-Valle
Poster Number: 49
Home Institution: University of Puerto Rico-Humacao
Program: LSSURP
Faculty Mentor: Dr. Nicholas Levinson
Research Advisor: Emily Ruff
Poster Title: **FRET-based Assay of AurA Kinase Allostery for High Throughput Screening**
Abstract:
ATP competitive kinase inhibitors have been known to be important cancer drug targets. The nature of the kinase ATP binding site leads to poor selectivity which can cause toxicity and develop clinical resistance to these inhibitors. Allosteric kinase inhibitors have great potential to overcome these problems but are challenging to identify since the structural movements that mediate allostery are on a scale of nanometer or less. An assay was developed to distinguish these structural movements using Foster Resonance Energy Transfer (FRET) with the goal of performing a high throughput screening. AurA kinase was the protein studied, where protein samples were prepared with a double labeling of FRET dyes, the donor fluorophore (Alexa 488) and the acceptor fluorophore (Alexa 568). To test and validate FRET assay, TpX2 and ADP were used where a change in FRET confirms structural changes which are seen in the crystal structures. A high throughput screening was performed using this method and preliminary data indicates MLN8054, MLN8237, and TAE684 as possible targets.

Presenter: Christian Pérez-Torres
Poster Number: 50
Home Institution: University of Puerto Rico-Aguadilla
Program: LSSURP
Faculty Mentor: Dr. Erik Peterson
Research Advisor: Juliet Crabtree
Poster Title: **The Effect Of PTPN22 On The Response To Fungal Infections**
Abstract:
PTPN22 is a gene that encodes for the protein Pep in mice and Lyp in humans. One of its functions is to promote pattern-recognition receptor (PRR) signaling in myeloid cells such as dendritic cells. A coding variant of this gene, PTPN22 R620W, has been implicated in the increased susceptibility to autoimmune diseases like Rheumatoid Arthritis and Systemic Lupus Erythematosus. This variant has also been implicated in an increased risk of infection. An increased frequency of the coding variant has been reported amongst patients suffering from a skin fungal infection. However, the mechanism by which PTPN22 regulates the anti-fungal response is not completely understood. Dectin-1 is a PRR that is able to induce anti-fungal responses by the immune system through the recognition of □□glucans. One of the ways this PRR achieves this is by the induction of type 1 IFNs. Since our laboratory previously reported that PTPN22 modulates type 1 IFN production, we hypothesized that PTPN22 controls the expression of genes downstream of fungal pattern-recognition receptors. The goal of this study is to find out the effect of PTPN22 on this response. Preliminary results show that PTPN22 KO mice are unable to clear C. albicans as well as WT mice. This indicates that PTPN22 has a role in the response against fungal infections. Further work will assess whether PTPN22 is required for efficient production of type 1 IFNs by Bone Marrow-derived Dendritic Cells (BMDC) upon stimulation of Dectin-1. A possible role of PTPN22 in the activation of Syk (a signaling protein crucial for the induction of type 1 IFNs by Dectin-1) will be investigated.

Presenter: Jessica Phan
Poster Number: 51
Home Institution: Pomona College
Program: LSSURP
Faculty Mentor: Dr. Paul Mermelstein
Research Advisor: Katie Tonn, Kellie Gross
Poster Title: **Caveolin-1-Mediated Signaling Highlights Sex Differences in Cocaine Drug Addiction**
Abstract:

Caveolin-1 (Cav1) is an integral membrane protein that regulates intracellular signal transduction. Recent studies suggest that Cav1 overexpression in the hippocampus increases structural plasticity underlying learning and memory. Because this plasticity parallels that seen in the nucleus accumbens (NAc) during addiction to drugs of abuse, we were interested in whether Cav1 overexpression in this brain region would affect corresponding behavioral output. Specifically, we hypothesized that virally-mediated overexpression of Cav1 in the NAc of rats would increase psychostimulant-induced behavioral sensitization. To test this, we measured the progressive amplification of locomotor activity in response to repeated low-dose cocaine administration following intracranial injections of either neuron-specific Cav1 or an RFP control virus. In females, Cav1 overexpression promoted sensitization, as evidenced by an increase in locomotor activity from the first to last day of treatment, when compared to control animals. Conversely, this effect of Cav1 was absent in males. Considered together, these data indicate that sex plays an important role in how Cav1 mediates cellular organization and signaling pathways within the functional output region of the NAc. Such results emphasize the importance of studying both sexes in biomedical research.

Presenter: Phillip Prince
Poster Number: 52
Home Institution: Savannah State University
Program: LSSURP
Faculty Mentor: Dr. Nathan Springer
Poster Title: **Triggering an Abiotic Cold-Stress Response in Ohio43 Strain of Maize**
Abstract:

Maize is an essential crop in the agricultural industry through its production of food and fuel. By improving the yield in maize, farmers are able to keep pace with a changing climate in their local environments. One way to improve these yields is through improved cold tolerance. Cold tolerant maize varieties exhibit stronger resistance to unexpected cold weather during their initial stages of development. In order to assess the impact of cold temperatures, maize seedlings were grown under controlled, uniform conditions. Next, individual groups were exposed to mild ($2^{o}C$) and severe ($0^{o}C$) cold stress environments for increments of 16 and 24 hours. The Ohio43 strain of maize was used in these experiments because it is particularly sensitive to cold stress. Leaf coloration was the initial determining factor for evaluations. Furthermore, image processing through Matlab software permitted quantitative analysis of characteristics such as biomass, leaf diameter, longest path, plant height, and stem width to provide justification to the proposed procedure. Recommendations for future use include applying this procedure to other varieties of maize to assess their strength or susceptibility to cold stress, stressing Ohio43 for longer time periods or with more severe temperatures, and crossing the Ohio 43 strain with a more cold-tolerant strain to produce a more durable hybrid.

Presenter: Olivia Prosak
Poster Number: 53
Home Institution: Brown University
Program: LSSURP
Faculty Mentor: Dr. Marija Cventanovic
Poster Title: **Analyzing the Role of Astrocytic NF-kB in Early Stage Spinocerebellar Ataxia 1 Pathology**

Abstract: Spinocerebellar Ataxia 1 (SCA 1) is an autosomal dominant neurodegenerative disease symptomatically characterized by difficulty with coordination and balance. Caused by an unstable expansion of a CAG trinucelotide repeat in the ATAXIN-1 (ATXN1) gene, SCA1 results in the selective degeneration of cerebellar Purkinje cells. SCA1 pathology is not fully understood, however, including the potential active role that glial cells could have in disease progression. Astrocytes, which are responsible for repairing tissue, maintaining synaptic homeostasis, and aiding in neuroprotection, are of particular interest as past research has shown that astrogliosis and neuroinflammation occur at a pre-symptomatic stage of SCA1 in mouse models. Studies also showed that the activation of nuclear factor-κB (NF-κB), an important inflammatory regulator, is enhanced in astrocytes from SCA1 transgenic mice. We genetically engineered the SCA1 mouse model, ATXN1 [82Q] mice, to inhibit the activation of NF-κB specifically in astrocytes once exposed to the inducible promoter tamoxifen (TMX) in order to investigate the role astrocytic NF-κB may play during early stages of SCA1 pathogenesis. Four-month-old mice in the early stage of SCA1 were assessed for behavioral and pathological changes. ATXN1 [82Q] mice in which NF-kB was inhibited in astrocytes (ATXN1[82Q] TMX-treated mice) performed significantly worse than oil-treated control ATXN1 [82Q] mice on rotarod, a test of motor behavior. Consistent with a worse behavioral phenotype, immunohistochemical analysis showed that ATXN1 [82Q] TMX mice had significantly exacerbated degeneration of Purkinje neurons along with trends towards a decrease in reactive proliferation of cerebellar astrocytes and a decrease in the expression of glial fibrillary acidic protein (GFAP), a marker of astrocytic activation. These results suggest that astrocytic NF-kB may be neuroprotective during the early stage SCA1. In the future, more pathology and behavior studies could be conducted to expand this study and to explore the effect that NF-kB could have at later stages of SCA1.

Presenter: Mariela Rivera-De Jesús
Poster Number: 54
Home Institution: University of Puerto Rico-Mayaguez
Program: LSSURP
Faculty Mentor: Dr. Michael Smanski
Poster Title: **Production Optimization Of Ent-Atiserenoic Acid, A Serofendic Acid Precursor, In Streptomyces Using Multivariate Design**

Abstract: Serofendic acid, a novel sulfur-containing atisane-type diterpenoid, has been found to protect epithelial cells, neurons, and cardiomyocytes from nitric oxide cytotoxicity and glutamate neurotoxicity, becoming a potential treatment for strokes and epilepsy. This molecule was first discovered in trace amounts in fetal calf serum, thus a more reliable and sustainable source is needed for preclinical studies. A synthetic metabolic pathway of a late stage intermediate - ent-atiserenoic acid (eAA) - of this molecule was engineered in a recombinant Streptomyces strain. To further increase production levels in our synthetic system, we are applying multivariate experimental design to optimize both the genetic regulation of the engineered pathway and the fermentation conditions. For genetic regulation optimization, we aimed to optimize isoprenoid precursor production with synthetic gene clusters of eight genes encoding for the methylerithritol phosphate (MEP) pathway. The expression levels of the individual genes were permutated according to a five-level Plackett-Burmann model. We designed a library consisting of 125 full synthetic gene clusters, requiring a total of 295 constructs to be built, including intermediate plasmids. Approximately 40% of these constructs have been completed. The fermentation optimization project focused on the components of the PCNM fermentation medium, including carbon and nitrogen source, pH, and trace elements present in medium. Seven components of the medium were distributed using a two-level Plackett-Burmann model to optimize production conditions. The Plackett-Burman medium optimization identified variables with a significant impact on eAA production, some combinations which resulted in a two-fold increase in final titers.

Presenter: Melissa Rivi
Poster Number: 55
Home Institution: University of Minnesota-Twin Cities
Program: LSSURP
Faculty Mentor: Dr. Daniel Bond
Research Advisor: Rebecca Calvo
Poster Title: **A Game of Clones: Determining the Regulation of Extracellular Components Important for Electron Transfer in *Geobacter sulfurreducens***

Abstract: *Geobacter sulfurreducens* is a Gram negative bacterium that uses extracellular electron acceptors for respiration. *G. sulfurreducens* electrophysiology has garnered interest because of its ability to use a graphite electrode as an electron acceptor and generate electricity when grown in a fuel cell. However, the mechanism by which it transports electrons to different acceptors is poorly understood; by understanding and manipulating these mechanisms, we can potentially engineer a more powerful bacteria capable of generating larger amounts of electricity. We think *G. sulfurreducens* physically connects to its acceptor and transfers electrons through a combination of outer membrane cytochromes and pili termed nanowires. To determine nanowire expression dynamics and regulation, I built luciferase reporter constructs to monitor transcription of nanowire forming genes (omcS, pilA) and will use transposon mutagenesis to identify genes that regulate expression of nanowire forming genes when given different electron acceptors. omcS and pilA encode the cytochrome and major pilin subunit that form the nanowire. By uncovering the regulation and expression of these genes, we will be better equipped to engineer a higher current-producing Geobacter as a potential fuel source.

Presenter: Nelson Rodriguez-Merced
Poster Number: 56
Home Institution: University of Puerto Rico-Mayaguez
Program: LSSURP
Faculty Mentor: Dr. Masato Yamamoto
Poster Title: **Oncolytic Adenovirus Expressing IFN-α in Combination with Chemoradiation for the Treatment of Pancreatic Cancer**

Abstract: Pancreatic adenocarcinoma (PDAC) is the 4th leading cause of cancer related deaths in the US. Unless curative resection can be performed, there is no effective treatment against the disease. As most patients are diagnosed in the late stage of PDAC, only 20% are electable for surgery resulting in low short and long term survival of the disease. Despite that, previous clinical trials have shown that administration of systemic IFN-α (IFN) combined with Gemcitabine (GEM) and radiation in an adjuvant setting improved 2 year overall survival of patients in 20 to 35%. Although effective, some of the trials drawbacks included IFN systematic toxicity and low IFN intra-tumor concentration. These resulted in high patient dropout, and as IFN is known to be a chemo-radio sensitizer and cytotoxic to cancer cells, low IFN concentrations at the tumor site might have hampered therapy's full potential. In the attempt to improve the downsides of this promising therapy, we have developed an oncolytic adenovirus expressing human IFN (OAd-IFN). To improve infectivity and oncolysis, vector has Ad5/3 fiber modification and overexpresses Adenoviral Death Protein. Since Cox-2 is up-regulated in PDAC cells, the Cox-2 promotor was induded upstream of the Adenovirus E1 region, which is responsible for viral replication. Replication controlled by cox-2 promoter is proven to restrict viral replication to PDAC cells. IFN was placed in the Adenovirus E3 region and is overexpressed in a replication dependent manner. By designing the vector to specifically target PDAC, OAd-IFN will concentrate IFN in pancreatic cancer cells increasing its therapeutic effect while lowering systematic toxicity. In vitro Crystal violet assays demonstrated that the OAd-IFN specifically targeted PDAC cells. Colony formation assay using OAd-IFN in combination with 0.93nM and 1.87nM of GEM and 1 and 2 Gy of radiation therapy showed that combinations of OAd-IFN with GEM, radiation, or chemo-radiation increased cell killing of MiaPaca-2 and S2013 PDAC cells. In summary, our data indicates that OAd-IFN was effective when combined with chemo-radiation. Effectiveness of OAd-IFN in combination with chemo-radiation, in a set up mimicking IFN clinical, suggests OAd-IFN holds great potential in the development of a better IFN therapy improving treatment for PDAC.

Presenter: Paul Shafer
Poster Number: 57
Home Institution: University of Minnesota-Twin Cities
Program: LSSURP
Faculty Mentor: Dr. Romas Kazlauskas
Poster Title: **Characterizing A Model Pathway For The Divergence Of Hydroxynitrile Lyases From Esterases Within The Hydrolase Fold Superfamily Of Enzymes**

Abstract: Enzyme engineering improves enzymes for use in commercial products and industrial processes. Engineering new catalytic function into enzymes is difficult, so it is useful to study divergent evolution of enzymatic function. However, the molecular mechanisms underlying the natural evolution of related enzymes with different functions are poorly understood. Here, we examine hydroxynitrile lyases (HNL's) divergence from esterases within the hydrolase fold superfamily of enzymes. Specifically, we constructed a model evolutionary pathway from the tobacco plant esterase SABP2 to a triple mutant SABP2 variant with HNL function, and have characterized esterase and HNL activities for many of the 47 possible variants within this pathway. By observing molecular mechanisms underlying the emergence of new catalytic function in nature, we have gained insights which will allow engineers to more effectively guide the rational design of enzymes for new catalytic function.

Presenter: Chris Shin
Poster Number: 58
Home Institution: Baylor University
Program: LSSURP
Faculty Mentor: Dr. Ran Blekhman
Poster Title: **Immune and Microbiome-related Gene Expression Across Cancers**

Abstract: Certain diseases, such as cancer, can induce changes in the genome, which causes DNA to act abnormally. Studies have shown that these effects on the genome are especially noticeable in the immune system and more recently, the microbiome. By analyzing and comparing the genomes of normal and cancerous cells, we can observe which specific genes in the DNA are affected by cancer and figure out new ways of reversing the negative effects of cancer in those parts of the genome. The Cancer Genome Atlas, TCGA, is a publically accessible data portal that provides genomic sequencing data for both tumor and normal samples. We downloaded TCGA data that had been run through RNA-Seq, a sequencing approach used to quantify the relative quantities of RNA, from the National Cancer for Biotechnology Information's Gene Expression Omnibus a database of gene expression data. The goal of the project was to find patterns in differential expression of genes that are related to the human immune system as well as related to microbiome composition. This project compared the differential expression of six different lists of immunity related genes from InnateDB as well as a list of genes known to be associated with microbiome composition. We used R, a statistical computing language, to organize this data and create graphs. Overall, the gene expression for 23,685 genes in 1,400 total samples among 15 types of cancer were calculated. Analysis of this data showed that there was significant (alpha = 0.05) differential gene expression in immune related genes in the following types of cancer: cervical squamous cell carcinoma and endocervical adenocarcinoma, kidney renal papillary cell carcinoma, kidney renal clear cell carcinoma, liver hepatocellular carcinoma, lung squamous cell carcinoma, and uterine corpus endometrial carcinoma.

Presenter: Yeidymar Sierra-Moya
Poster Number: 59
Home Institution: University of Puerto Rico-Arecibo
Program: LSSURP
Faculty Mentor: Dr. Michael Sadowsky
Poster Title: **Identifying *Sinorhizobium* Repressor Genes Involved in Symbiosis**

Abstract: In agriculture, biologically useable nitrogen is often a limiting resource, and added to the soil through the application of fertilizer. Legumes, such as soybean, are able to obtain biologically useable nitrogen through symbiosis with rhizobia, a soil bacterium. The bacteria and the legume form a nodule on the plant root, which allows nutrient exchange through direct contact. The legume, *Medicago truncatula*, and nitrogen-fixing bacterium, *Sinorhizobium meliloti*, are used as a model system to understand the symbiosis in legumes. The amount of nodules formed is highly variable depending on which genotype of *Medicago* is inoculated with which strain of *Sinorhizobium*. To identify the genes involved in preventing nodule formation a random transposon library of *Sinorhizobium* T027 was generated. Three candidate genes were identified as potential repressor genes, preventing the formation of nodules. This study was done to validate genes through targeted gene deletions. Our goal is to improve our understanding of *Medicago/Sinorhizobium* symbiosis and the complex signal exchange needed for nodule formation.

Presenter: Nicholas Sorenson
Poster Number: 60
Home Institution: Macalester College
Program: LSSURP
Faculty Mentor: Dr. Mark Schleiss
Research Advisor: Mary Pat Osborne, Erin Osterholm
Poster Title: **Mother to Baby: An Evaluation of Cytomegalovirus (CMV) Transmission via Breast Milk and Protective Maternal CMV Antibodies in Very Low Birth Weight (VLBW) Infants**

Abstract: CMV can be transmitted from mother to infant via breast milk. For healthy immunocompetent infants the infection is asymptomatic; however premature and VLBW infants are at risk for development of symptomatic CMV. Moreover, the long-term neurodevelopmental consequences for CMV infections acquired by this route are not well characterized in VLBW infants. This study examined the prevalence of CMV infection by examining breast milk for CMV antibodies in breast-fed mother-infant dyads, toward the goal of testing the hypothesis that passive antibodies may play a role in protecting these infants. Samples were collected from 36 VLBW infants who were admitted to the Neonatal Intensive Care Unit (NICU) at the University of Minnesota Masonic Children's Hospital. Serial collection of breast milk and infant blood and serum samples were obtained from birth to 90 days of age. Viral load from breast milk and blood was determined using quantitative polymerase chain reaction (qPCR). We were able to identify 10 out of 29 mothers shedding CMV in their breast milk by qPCR, with a median viral load ranging from 271 to 103,000 copies/ml of milk. Two of the 11 infants that were exposed to CMV in breast milk had demonstrable DNAemia with a mean of 276 copies/ml of blood. Enzyme-linked immunosorbent assays (ELISA) were performed on select serum samples to evaluate CMV IgG antibody levels. We found CMV IgG antibodies in infant serum as early as one day after birth and 11 out of 36 infants were found to be positive for CMV IgG within a week of birth. This suggests passive immunity from mother to infant. Further qualitative analysis of the maternal and infant antibody response could elucidate how protective the mechanism of protective passive immunity is in uninfected infants.

Presenter: Mario Soto-Soto
Poster Number: 61
Home Institution: University of Puerto Rico
Program: LSSURP
Faculty Mentor: Dr. Paul Iaizzo
Poster Title: **Method for the Determination of Adipose Distribution on the Epicardial Surface of Human Hearts**
Abstract: Human epicardial adipose tissue (EAT) is a cardiovascular factor of growing interest. It is known that epicardial fat is generated from brown adipose tissue during embryogenesis. Furthermore, autopsy observations propose that their EAT distribution varies with age and may be altered by cardiovascular disease. The EAT distribution varies regionally across the heart. As such, a deep knowledge of anatomical distribution of the EAT on the heart is of vital importance to clinicians and the medical device industry. For this reason many efforts have attempted to provide a deeper insight into the EAT fat distribution of the human heart. For this experimentation human hearts, non-viable for transplant, (n=112) were obtained from Life Source (Minneapolis, MN), a local organ procurement agency. The hearts were placed under pressurized fixation using a solution of 10% formalin. Post-fixation, each heart was imaged in three different planes using the following areas as reference: the left ventricular margin, the diaphragmatic surface, and anterior right ventricle. All 2D surface images were analyzed by a computational algorithm which measured the EAT and the existent bare patches in each hearts. With the use of this data, 2D masks were created allowing precise construction of the average EAT distribution of the human heart. Previous literature suggests that the measurements taken in this study were accurate. This work allows precise measurement in a 2D imaging plane. Further work will move towards creating a model that can be implemented in 3D, so to quantify the thickness of the EAT on the human heart.

Presenter: Mallory Thomas
Poster Number: 62
Home Institution: University of Minnesota-Twin Cities
Program: LSSURP
Faculty Mentor: Dr. Michael Sadowsky
Poster Title: **Optimizing DNA Extraction From Woodchip Bioreactor Microbial Community**
Abstract: Agricultural runoff carries excess nutrients, such as nitrogen, to surface water (lakes and rivers) via subsurface drainage. These excess nutrients result in ecosystem overload, inducing eutrophication of aquatic ecosystems and human health concern. Passive woodchip bioreactors utilize denitrifying bacteria to intercept the flow of drainage water and reduce amount of nitrate that will reach surface waters. This study optimized methods of extracting DNA from inlet/outlet water, port water, and woodchip samples. We will use these methods to quantify baseline microbial community present in water and woodchip samples. Determination of background community will be used to analyze the effect of bioaugmentation and biostimulation treatments aimed at increasing the reduction of nitrate concentrations in subsurface drainage.

Presenter: Jenna Thomforde
Poster Number: 63
Home Institution: College of St. Scholastica
Program: LSSURP
Faculty Mentor: Dr. Lisa Peterson
Poster Title: **Synthesizing Furan Metabolite Assays for Extinction Coefficient Quantization**
Abstract:
Furan is found in tobacco smoke, food, and other air pollutants. Previous studies have shown that furan, a carcinogen in rodents, can be converted into a reactive metabolite in humans. The metabolite cis-2-butene-1,4-dial then further forms reaction products that can be measured in human urine. These metabolites are derived from either the direct reaction of BDA with lysine or the reaction of BDA with cysteine then lysine. Standards for these metabolites were synthesized and used to determine their extinction coefficient by quantitative NMR and HPLC methods. Stable isotopically labeled standards were synthesized and will be used to quantify the concentrations of these metabolites in human urine using LC-MS/MS methods.

Presenter: Claire Tolan
Poster Number: 64
Home Institution: University of San Diego
Program: LSSURP
Faculty Mentor: Dr. Deepali Sachdev
Poster Title: **IGF-I Regulates Metastasis Of Triple Negative Breast Cancer By Upregulation CTGF And VEGF**
Abstract:
Breast cancer cells are stimulated by insulin-like growth factor I (IGF-1), which signals via the type I IGF receptor (IGF1R) to promote proliferation, survival and metastasis of triple negative breast cancer (TNBC). Previously, we have shown that in a model of high risk metastatic TNBC with the MDA435A/LCC6 metastatic cancer cells, inhibition of IGF signaling using a C-terminally truncated IGF1R blocks metastasis. LCC6 cells with truncated IGF1R (LCC6-DN) which behaves in a dominant negative manner to inhibit signaling failed to form metastases in mouse models compared to LCC6 cells with wild type functional IGF1R (LCC6-WT). The Sachdev lab has recently identified an IGF1R regulated metastasis signature comprising 53 genes by expression profiling of LCC6-WT and LCC6-DN cells. Herein we hypothesize that activation of IGF1R regulates metastasis by affecting levels of vascular endothelial growth factor (VEGF) and connective tissue growth factor (CTGF), two of the genes in the signature, which have been implicated in the progression and angiogenesis of TNBC. In this project, regulation of the expression of these two genes by IGF-1 was validated in the LCC6-WT and LCC6-DN cell lines. Activation of signaling in LCC6-WT and inhibition of signaling in LCC6-DN cells by IGF-I was verified by determining phosphorylation of downstream PI3K by western blotting. Transcript levels of these genes were quantified with qPCR, using cDNA synthesized from LCC6-WT and LCC6-DN cells after IGF-1 stimulation for 4 or 24 hours. Our data indicate an increase in expression levels of VEGF and CTGF following IGF-1 stimulation for 4 hours in the LCC6-WT cell line. IGF-1 treatment did not increase these genes in LCC6-DN cell lines, which was expected due to the truncated IGF-1 receptor. Additionally, inhibition of IGF1R with an inhibitory antibody blocked IGF-1 mediated upregulation of VEGF and angiogenesis in LCC6-WT tumors. These results suggest that IGF-1 signaling promotes tumor growth and metastasis by regulating VEGF and CTGF.

Presenter: Zachariah Tritz
Poster Number: 65
Home Institution: St. Olaf College
Program: LSSURP
Faculty Mentor: Dr. Subbaya Subramanian
Poster Title: **Significance Of ACKR4-Maintained Chemokine Gradients In T Cell Activation And Migration In CRC**

Abstract: Atypical Chemokine Receptor 4 (ACKR4) is a decoy receptor for homeostatic chemokines CCL19, CCL21, and CCL25, which play vital roles in T cell activation and trafficking. By reducing the bioavailability of these chemotaxic molecules, ACKR4 can affect regular T cell function. Disrupted T cell migration and activation are hallmarks of colorectal cancer (CRC), the third leading cause of cancer mortality in the US, and we hypothesized that ACKR4 might play a role in dampening patient immune responses. Through immunofluorescent imaging of patient tumor slides, we found that high levels of ACKR4 expression were correlated with high T cell tumor infiltration and high levels of T cell activation in the Lymph Node (LN) ($p<0.05$). No correlation was found between the poorly immunogenic microsatellite stable (MSS) colon cancer subtype and higher ACKR4 expression. These data suggest that ACKR4 sharpens, rather than destroys, the signaling gradients of its ligands and thereby increases the ability of T cells to effectively navigate between the LN and the CRC. ACKR4's role in T cell trafficking could lend it clinical relevance, making the selective destruction of ACKR4-targeting miRNAs a CRC treatment option worth further investigation.

Presenter: Sunny Trivedi
Poster Number: 66
Home Institution: University of California-Los Angeles
Program: LSSURP
Faculty Mentor: Dr. Ameeta Kelekar
Research Advisor: Eric Hanse
Poster Title: **Characterizing a Novel Short Isoform of a Gluconeogenesis enzyme, FBP1, in Activated T cells**

Abstract: The enzyme Fructose 1,6 Bisphosphatase 1 (FBP1) is a rate limiting enzyme that catalyzes the conversion of Fructose 1,6 Bisphosphate to Fructose 6 Phosphate during gluconeogenesis. The ability to synthesize glucose through gluconeogenesis is primarily a property of liver and pancreatic cells. However, the Kelekar laboratory recently discovered that FBP1 expression is induced in primary human T cells during activation. Moreover, the FBP1 protein expressed in T cells is 10 kilodaltons (kD) shorter than the 37 kD form reported in other tissues. We hypothesize that the shorter form of FBP1 is generated by the selective utilization of an alternative internal translational start site. The purpose of this study is to identify and describe the characteristics of this shorter form and test its functionality. This will be the first description of a 27 kD FBP1 protein isoform. To test our hypothesis, truncation mutants targeting two potential alternative start sites were generated, and FBP1 protein expression verified by in vitro translation and by transfection into mammalian cells. The enzymatic activity of FBP1 synthesized from the mutated cDNA sequence and the effect of an established FBP1 regulatory molecule will be evaluated. This study is significant in revealing a unique expression pattern for FBP1 during T cell activation and has the potential to lead to the identification of a novel physiological function for this gluconeogenic enzyme.

Presenter: Stacy Uchendu
Poster Number: 67
Home Institution: Wesleyan University
Program: LSSURP
Faculty Mentor: Dr. Greg Vercellotti
Poster Title: **Heme-Binding Site on the TLR4/MD-2 Complex and its Potential Role in Sickle Cell Disease**

Abstract: In sickle cell disease (SCD), intravascular hemolysis releases free heme into the bloodstream that activates innate immune MD-2/TLR4 signaling leading to NF-κB activation, inflammation, vaso-occlusion, ischemia-reperfusion physiology, and tissue injury. The canonical MD-2/TLR4 agonist, lipopolysaccharide (LPS), is a constituent of the outer membrane of gram-negative bacteria and binds to MD-2 to initiate MD-2/TLR4 signaling. We hypothesize that heme binds to MD-2 at a site independent of the LPS binding site. This project aims to identify the heme binding site on the MD-2/TLR4 complex with the intention of inhibiting heme-mediated MD-2/TLR4 responses while retaining LPS-mediated responses. Using an online HemeBind algorithm, seven potential heme-binding amino acids were found clustered in close proximity at two sites on MD-2. In order to identify essential MD-2 amino acids required for heme-mediated MD-2/TLR4 signaling, site-directed missense (ms) point mutations of MD-2 were cloned that changed wild-type (wt) MD-2 amino acids to alanine. HEK293 cells were transfected with DNA plasmids coding for MD-2 (wt or ms), TLR4, CD14, an NF-κB luciferase reporter, and a renilla luciferase control to measure NF-κB activation in response to LPS and/or heme stimulation. Initial experiments demonstrated that when the cells were transfected with plasmids containing wt-MD-2 and treated with 10 ng/ml LPS and/or 10 μM hemin, there was NF-κB activation with LPS and no response with hemin. Additional experiments will determine the optimal concentration of hemin to activate MD-2/TLR4 signaling. Future experiments will be conducted using 10 ng/ml LPS and optimized heme concentrations to identify ms-MD-2 mutants that interrupt heme-mediated, but not LPS-mediated MD-2/TLR4 NF-κB activation.

Presenter: Abbigael VanDusen
Poster Number: 68
Home Institution: Ferris State University
Program: LSSURP
Faculty Mentor: Dr. Victor Barocas
Research Advisor: Julia Quindlen
Poster Title: **Discrimination of Vibrotactile Stimuli**

Abstract: Vibrotactile perception is useful for the design of vibrotactile applications in haptic devices. The study of the Pacinian corpuscle and its extreme sensitivity to high-frequency vibrations can help us further understand vibrotactile perception. The goal of this project was to determine the extent of Pacinian corpuscle discriminability in the fingertips via vibrational stimuli. A waveform generator was controlled via MATLAB code to transmit the signal to a vibrating disk. Experiments used same-different and oddity models to determine if the number of stimuli affected discriminability. Stimuli differed by (1) amplification of the threshold at a single frequency, (2) by phase shift of the higher frequency component of complex sinusoidal or square waveforms, and (3) by complex waveforms that differed in the high-frequency component. We found that amplification of four times the threshold is most discriminable against two times the threshold. Complex sinusoidal waveforms of 10+30 Hz and 100+300 Hz did not show significant discriminability between phase shifts or between each other; however, the square waves of 100+300 Hz were more discriminable than the 100+300 Hz sine waves. Preliminary results of complex signals differing in the high frequency component suggest that complex waveform discriminability is dependent on the ratio of the high frequency to low frequency component.

Presenter: Jenna Weber
Poster Number: 69
Home Institution: University of Pennsylvania
Program: LSSURP
Faculty Mentor: Dr. Troy Lund
Poster Title: **Do Traditional African Remedies Induce Hemolysis In A G6PD-Deficient Zebrafish Model?**

Abstract: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common genetic defect and enzymopathy worldwide, affecting an estimated 400 million people. G6PD deficiency causes acute hemolysis in children exposed to pro-oxidants and can ultimately result in life-long neurologic damage due to kernicterus. Common pro-oxidants include menthol, used as a therapeutic ointment in West Africa; naphthalene, an active ingredient in mothballs; fava beans, a popular staple in the Middle East, Mediterranean, and North Africa; and common antimalarial drugs such as primaquine. The present study investigates traditional remedies that were identified as potential pro-oxidants by Dr. Lund at a clinic in southwestern Nigeria. These various compounds and natural remedies were tested using a previously established zebrafish (Danio rerio) model for G6PD deficiency. Zebrafish embryos were injected with morpholinos to zebrafish g6pd that reduces gene expression. When exposed to pro-oxidants, G6PD-deficient embryos develop hemolysis and pericardial edema within 48-72 hours. Our results suggest that certain traditional remedies may also trigger this phenotype in the zebrafish and therefore may pose significant health risks to infants and children with G6PD deficiency. More research needs to be done to investigate the use of these potentially dangerous traditional remedies with the goal of increasing awareness and reducing the occurrence of severe hemolytic crises in children with G6PD deficiency.

Presenter: Austin Whitted
Poster Number: 70
Home Institution: Michigan State University
Program: LSSURP
Faculty Mentor: Dr. Colin Campbell
Research Advisor: Lisa Chesner
Poster Title: **Repair of DNA Plasmids Containing DNA-Protein Crosslinks via Nucleotide Excision Repair**

Abstract: The largest class of cancer chemotherapeutics are DNA alkylating agents. These drugs induce multiple types of DNA damage including inter and intra-strand crosslinks, DNA strand breaks, and DNA-Protein crosslinks. Failure to repair these lesions is associated with mutagenesis and cell death. We want to understand mechanisms of DNA repair, so we can make cancer treatment more effective. In this study we focused on DNA-Protein crosslinks (DPC's). We hypothesized that the Nucleotide Excision Repair (NER) pathway is involved in the repair of DPC's. To create a DPC repair substrate we used an oligonucleotide containing an 8oxo guanine residue and crosslinked it to human 8oxo-guanine DNA glycosylase (hOGG1) using NaCNBH3. This plasmid was transfected into cells that were deficient in the NER pathway and compared to cells that were not deficient in the NER pathway. A polymerase chain reaction based assay was used to quantify DPC repair. Over an eight hour time course we saw that cells proficient in NER repaired 57% of the DPC substrate and NER deficient cells repaired 33% of the DPC substrate. This tells us that NER does play a role in DPC repair. With this new knowledge we can now test for the involvement of other DNA repair pathways.

Presenter: Lucius Wilmerding
Poster Number: 71
Home Institution: Macalester College
Program: LSSURP
Faculty Mentor: Dr. Stephen Engel
Poster Title: **Combining Virtual Reality and EEG to Measure Visual Neuroplasticity**
Abstract:

Combining traditional EEG systems with Virtual Reality headsets allows researchers to study effects of neuroplasticity in V1 neuronal populations of healthy adults. Despite successful use measuring changes from contrast adaptation and long term sensory deprivation, the system setup is time consuming and potentially uncomfortable for participants. To address these problems, a prototype headset was designed and built using 3D modelling software and 3D printers. The system comprises a long strap which attaches to the VR goggles and several movable clips to which mobile EEG electrodes may be fastened. The system is simple and adjustable to allow precise control of electrode placement for different head phenotypes. Data from a study on neuroplasticity in the visual cortex was collected using the apparatus as a test of signal quality. In conclusion, the flexible headset combines mobile EEG recording and VR goggles allowing for more efficient setup and easier data acquisition.

Presenter: Emily Woska
Poster Number: 72
Home Institution: Duke University
Program: LSSURP
Faculty Mentor: Dr. Jakub Tolar
Research Advisor: Kirk Twaroski
Poster Title: **Mechanisms of Hematopoietic Cell Transplantation (HCT) in Recessive Dystrophic Epidermolysis Bullosa (RDEB) Patients**
Abstract:

RDEB is a heritable skin disease characterized by loss of function mutations in the collagen VII A1 (COL7A1) gene. Type VII collagen forms anchoring fibrils within the epidermal basement membrane zone, connecting the epidermal and dermal layers of the skin. A breakdown of this fundamental structure results in skin friability, blister development, and, ultimately, an elevated risk of aggressive squamous cell carcinoma, among other complications. In the past decade, HCT has proved an improved treatment for RDEB over the traditional course of palliative care, but our understanding of the underlying mechanisms of transplant success remains limited. Therefore, we have employed a multifaceted approach involving various technologies to explore and evaluate the mechanisms behind this phenomenon: [1] By Real-Time Quantitative PCR (RT-qPCR), we have shown an increase in overall COL7A1 expression after a HCT. This method has been validated by verifying a ~2.4 fold change in COL7A1 expression from tissue taken from an RDEB patient and donor pair. [2] Total RNA sequencing has identified other affected pathways and off-target effects that can be utilized in quantifying transplant effectiveness and providing secondary therapeutic targets. [3] Targeted RNA-Sequencing detects the relative proportion of expression of COL7A1 alleles, providing a view of HCT efficiency at the molecular level. Combined, these technologies will be used to identify and evaluate the underlying mechanisms of HCT in RDEB patients. Further, they will provide opportunities to enhance existing therapies, and to develop new therapeutic approaches.