# Summer Programs Symposium

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**Abstract Booklet**

# Summer Program for Undergraduate Research (SPUR)

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**Alcohol-Induced Developmental Brain Injury in Human 3D Cerebral Organoids**

**Background:** Nearly 1 in 10 women around the world consume alcohol while pregnant, putting children at risk for fetal alcohol spectrum disorder (FASD). One significant consequence of FASD is alcohol-induced developmental neurotoxicity manifesting as intellectual difficulties and behavioral problems throughout life[1]. Despite its prevalence, the minimum amount of ethanol that could trigger neurotoxicity in human brains is unknown as are the mechanisms behind how alcohol negatively affects the fetal brain. This gap in knowledge is largely due to the lack of human tissue to study detailed pathways. To bridge this gap, this study proposes to use human induced pluripotent stem cells (iPSCs)-derived three-dimensional (3D) human cerebral organoids, i.e. mini brains, to study alcohol-induced developmental brain injury. We and others have shown that 3D mini brains resemble the fetal brain in their complex structure, gene profiles, and function[2]. The main hypothesis is that 3D mini brains exposed to ethanol will have decreased cell viability in a dose-, duration-, and frequency-dependent manner.

**Methods:** Mini brains were generated following a previously established protocol[3]. Briefly, human iPSCs were allowed to aggregate to form embryoid bodies and further neuroepithelium. The aggregates were then embedded in Matrigel droplets and then cultured in a rotating chamber for 2 months. Mini brains were exposed to increasing doses of ethanol (0-50 mM), as well as increasing time (0-24 h) and frequencies (1 vs 6 exposures of 6 hours per day for 6 consecutive days). Neuroapoptosis was quantified from the expression of cleaved caspase-3, a known marker for apoptotic cell death, measured by Western Blot.

**Results:** Two-month old 3D mini brains showed different brain cell types such as functional neurons, astrocytes, oligodendrocytes, and vascular cells. Following ethanol exposure, cleaved caspase-3 expression was increased in a dose-dependent manner, with 50 mM being the highest (500% of control without ethanol exposure). The lowest ethanol concentration to induce neuroapoptosis was 25 mM.

**Conclusion:** This study demonstrated that ethanol exposure induces neuroapoptosis in 3D human mini brains in a dose-, duration-, and frequency-dependent manner. Specifically, we were the first to identify that ethanol at 25mM (equivalent to 3 standard drinks) triggered neuroapoptosis in human brain cells, suggesting which minimum doses of ethanol might become toxic and be related to fetal risk. This provides valuable insights into the modeling potential of 3D mini brains on alcohol neurotoxicity. Additionally, this novel human model will allow further research into the mechanisms behind alcohol-induced developmental brain injury, potentially finding neuroprotective and therapeutic strategies for FASD-related neurodegeneration.

**Keywords:** FASD, cerebral organoids, induced pluripotent stem cells (iPSCs), apoptosis, Western Blot

**References:**
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## Potential Neuromodulation Treatment to Address Peripheral Nerve Injuries

**Background**

Peripheral nerve injury in combat soldiers has long recovery process and causes long-term disability. Even with optimal nerve repair after injury, there is an initial loss of communication between the peripheral and central nervous systems. As a preliminary study funded by the United States Department of Veterans Affairs, we are testing if loss of peripheral/central connectivity is a cause of poor outcomes. Accordingly, we are testing a therapy that could maintain connectivity by generating impulse activity proximal to the site of the nerve injury while regeneration occurs, using behavioral and functional magnetic resonance imaging (fMRI) to evaluate the efficacy of this treatment.

**Methods**

Sensorimotor function was analyzed by a behavioral test (MotoTrak system) that automatically measures of forelimb function in rats. Specifically, rats with suitable baseline motivation are trained to use their right forepaw to pull lever to get food. Outcomes to evaluate median nerve function include the force that the rat uses to pull the lever, and the percent of pulls that are above a set threshold force (hit rate). Additionally, rats were scanned (9.4 tesla) during sedation (dexmedetomidine) before nerve injury and 1, 4, 8 and 12 weeks after surgery. During the fMRI scan, a non-noxious stimulation was applied to right front paw to identify connectivity to the somatosensory cortex. After the initial behavioral test and fMRI scanning, rats had their right median nerve transected and immediately reapposed, and a cuff electrode placed proximal to the injury. Half of the subjects were given treatment with continuous electrical stimulation from a battery-powered stimulator carried in a rat backpack, while the others had the same treatment, but the stimulator remained off (Sham stimulation). Testing was performed in a blinded manner.

**Results**

Two rats received sham stimulation and 11 rats received treatment stimulation. Rats with sham stimulation showed a significant drop in hit rate and increase in median force from the pre-surgery conditions compared to those rats with treatment stimulation. Imaging data show that rats with sham stimulation had a significant drop in fMRI activity compared to those rats with stimulation.

**Conclusion**

The stimulation treatment shows promising effects in expediting the process of healing and restoring better function.

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**Continuous enzyme kinetics analysis of nNOS and Calmodulin fused to an Avidity Tag demonstrate no effect on electron transport and nitric oxide synthase activity compared to wild type**

Nitric oxide (NO) is a critical signaling molecule in the cardiovascular and nervous systems and also acts as a toxic chemical component in the host response to infection. In mammals, NO is produced by three NO synthase (NOS) isoforms: inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS), which regulate immune and inflammatory responses, vasodilation, and neuronal activity, respectively. A structure of the entire NOS enzyme is not yet available, but crystal structures of isolated domains have revealed that an *N*-terminal oxidase domain binds heme and tetrahydrobiopterin and the arginine substrate and that a *C*-terminal domain binds the electron carriers FAD and FMN and the cosubstrate NADPH. NOS activation requires binding of the calcium-dependent signaling protein calmodulin (CaM), and NOS function is regulated through a series of tightly controlled conformational equilibria. While several studies have identified multiple NOS conformations, the sequence of conformational states and timing of interconversion between these states is unknown, furthermore it is unclear how each conformational change correlates with the catalytic cycle of the enzyme.

Site-directed mutations of nNOS results in the disruption of specific subdomain interactions which can then be assayed in single-molecule fluorescence studies. To enable single-molecule studies and kinetic analysis of nNOS conformational states, an Avidity Tag (AviTag) was fused to both CaM and NOS, allowing for attachment of nNOS to avidin-coated slides for single-molecule fluorescence analysis of conformational.

Here, we cloned, expressed, and purified nNOS and CaM fused to an AviTag for binding to streptavidin-coated slides. To determine if addition of an AviTag to nNOS or CaM interfered with binding and activation of nNOS, the rates of electron transfer were determined measuring cytochrome *c*, ferricyanide, and DCIP reduction. In addition, NO synthase activity was assayed by monitoring the NO-dependent conversion of oxyhemoglobin to methemoglobin. These activity assays demonstrated that the addition of an AviTag to nNOS or CaM does not significantly impair electron transfer or NO synthase activity. These results indicate that nNOS or CaM fused with an AviTag can be used in single-molecule experiments. These single-molecule experiments will allow detailed kinetic analysis of the pathway of electron transfer from the NOS oxidase domain to the reductase domain.

Although NO has many significant and beneficial physiological functions, it can also lead to the development of neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, and ischemic brain injury. This study into determining the efficacy of the addition of an AviTag to nNOS and CaM opens the door to more efficient single molecule fluorescence studies, allowing for greater understanding of the NO-related neurotoxicity.

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## Determining the Interplay Between $O$-GlcNAcylation and Phosphorylation on the Homeobox Protein OTX2.

**Background**

$O$-GlcNAcylation is a dynamic and nutrient-sensing post-translational modification (PTM). This modification of proteins with one residue of $N$-acetylglucosamine is very similar to phosphorylation and often competes with it. Furthermore, phosphorylation and $O$-GlcNAcylation are two essential PTMs involved in physiological and pathological processes including type 2 diabetes, Alzheimer's disease, cancer, and obesity. Orthodenticle homeobox 2 protein (OTX2) is an $O$-GlcNAcylated protein involved in eye and brain development and a key oncogene in medulloblastoma. Although we already know that OTX2 is both phosphorylated and $O$-GlcNAcylated, little is known about the role of these PTMs in regulating OTX2's localization, stability or functions. Thus, we hypothesize that $O$-GlcNAcylation and phosphorylation compete on the homeobox protein OTX2 to regulate its stability.

**Methods**

HeLa cells were transfected with 6 different OTX2 constructs encoding for partial or full myc-tagged version of OTX2. Cells were treated with 100nM of Thiamet-G, which prevents the removal of $O$-GlcNAc residues from proteins. Twenty-four hours after transfection, cells were lysed in RIPA buffer supplemented with protease and phosphatase inhibitors. The soluble protein fraction was conserved (TE) and OTX2-myc was enriched by immunoprecipitation (IP) using a myc antibody. TE and IP were then run on SDS Page, and western blotted with phosphorylation, $O$-GlcNAcylation, and myc-tag antibodies. Some TE were loaded on a SDS-PAGE gel supplemented with Phos-tag, which specifically retains phosphorylated proteins during migration and allows their visualization by a mass shift.

**Results**

After transfection and IP of the full length OTX2, we were able to successfully enrich for OTX2-myc and observed its O-GlcNAc modification by immunoblot. Incubation with phospho-serine and phospho-threonine antibodies did not show phosphorylated bands for OTX2 constructs. However, when a Phostag gel was used, upshifted bands were observed for various OTX2 constructs including the full-length, and the homeodomain, retention domain and OTX2-dimerization domain proteins. Surprisingly, Thiamet-G treatment has little to no effect on the phosphorylated bands of OTX2.

**Conclusion**

A better understanding of OTX2's PTM is necessary to improve the diagnostic and treatment of medulloblastoma tumors as well as other chronic diseases. In this study, OTX2's $O$-GlcNAcylation and phosphorylation were investigated. Although we observed both PTMs on OTX2 in separate experiments, we were not able to observe a competition between them. In the future, improving the detection of both PTMs simultaneously seems necessary to assess this interplay.

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**The Effects of HDL on the MAPK and PI3K Signaling Pathways in Peritoneal Macrophages**

**Background**
Atherosclerosis is a chronic inflammatory disease characterized by the retention of macrophage foam cell-derived plaque in the artery wall. Scavenger receptor B1 (SR-BI) and cluster of differentiation 36 (CD36) play critical roles in regulating cholesterol homeostasis in macrophages and mediating the development of atherosclerosis. Pro-atherosclerotic scavenger receptor CD36 binds to oxidized low-density lipoprotein (oxLDL) and stimulates macrophage uptake of cholesterol. On the other hand, athero-protective SR-BI binds to high-density lipoprotein (HDL) and regulates cholesterol removal from macrophage foam cells. Previous literature has demonstrated that HDL restores migration in RAW 264.7 macrophage foam cells by initiation of the MAPK (i.e. Erk1/2) and PI3K (i.e. Akt1) signaling pathways. However, despite the extensive studies conducted on each scavenger receptor, the influence SR-BI and CD36 have on each other is unknown and is a long-term goal of the lab. As a first step, our study aims to determine the effect of HDL-mediated activation of signaling pathways in wild-type (WT) peritoneal macrophages. We hypothesize that HDL-stimulated WT peritoneal macrophages will exhibit greater phospho-Akt (pAkt) and phospho-Erk1/2 (pErk1/2) expression over time.

**Methods**
Male and female WT mice [11-13 weeks of age] were injected in the peritoneal cavity with 4% thioglycollate. Four days post-injection, peritoneal macrophages were collected and plated in complete media. Macrophages were serum-starved overnight and then treated with serum-free media (SFM) or SFM containing 50 μg protein/mL HDL for 0, 5, 10, and 30 minutes. Macrophages were lysed and proteins were separated by 8% SDS-PAGE. Levels of pErk/total Erk and pAkt/total Akt were assessed by immunoblot analysis. β-actin and α-tubulin were used as loading controls.

**Results**
The ratio of pAkt/Akt and pErk/Erk expression decreased over time in the presence of HDL in WT macrophages. One-way ANOVA revealed statistically significant differences between the 5 min ($p \leq 0.01$), 10 min ($p \leq 0.01$), and 30 min ($p \leq 0.001$) time points versus 0 min for pErk expression in the presence of HDL.

**Conclusion**
We demonstrate a reduced expression of signaling proteins (i.e. Erk1/2, Akt1) associated with macrophage migration when treated with HDL. These results are contrary to our hypothesis and suggest possible discrepancies between signaling pathways in different macrophage models, thus holding important implications for the selection of macrophage models to study scavenger receptors and signaling pathways. Further investigation is required to test the effects of thioglycollate, an inflammatory agent, and SFM, and whether pre-stimulated pathways accurately represent physiological conditions. Furthermore, these experiments will be repeated in macrophages isolated from SR-BI and CD36 knock-out mice to determine how signaling is altered in their absence.

**Keywords:** Atherosclerosis, peritoneal macrophage, SR-BI, CD36, HDL, MAPK, PI3K

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Single nucleotide polymorphisms (SNPs) are the most common genetic variation found in the human genome, with 4-5 million occurring in each individual. While many of these variations are non-phenotypic a small assortment of SNPs have been associated with human disease through genome wide association studies (GWAS).  How a SNP can impact the genome depends on whether it is in a coding or non-coding region. While coding SNPs alter the genetic sequence and thus the amino acid sequence of proteins, the functional impact(s) of non-coding SNPs are not as well understood. We hypothesize that some non-coding SNPs may alter local gene function by perturbing local chromosome interactions and disrupting local gene expression. The focus of this study was on a non-coding SNP commonly associated with blood pressure regulation in humans. This SNP is found in a genomic region distal to the *Npr3* gene, which encodes a receptor for atrial natriuretic peptide (ANP). Using CRISPR-Cas9 gene editing, heterozygous deletion of this region in human embryonic kidney cells (HEK293) led to a marked increase in *NPR3* mRNA expression but other local genes were unaffected, suggesting this region normally plays an inhibitory role in regulating *NPR3* expression. We hypothesized that deleting the orthologous region in an animal model may lead to similar dysregulation of *Npr3* expression. In a similar approach, we deleted the SNP-orthologous region using the CRISPR-Cas9 system in the Dahl salt-sensitive (SS) rat background, which is an experimental animal model of human hypertension. Quantitative real time PCR (qRT-PCR) was then used to examine relative expression of genes between the mutant rats and wild-type rats. qRT-PCR was run with *Gapdh*, *Npr3*, *Sub1*, and *Tars* where *Gapdh* was used as the housekeeping gene to normalize target gene expression. *Sub1* and *Tars* were chosen in accordance with the previous study as they are situated locally to the deletion region and were unaffected in the previous study completed in HEK293 cells. Kidney cortex as well as the mesenteric artery of these rats were chosen for quantitative analysis as these are known sites of *Npr3* expression. The long-term goal of this study will be to determine how this distal region of *Npr3* impacts its expression, ANP/NPR3 signaling, and the salt sensitive hypertensive phenotype of SS rats.

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## 3D Bioprinting Cardiomyocyte Cells Derived from Induced Pluripotent Stem Cells

**Background:**
Cardiovascular disease is the leading cause of death worldwide. Often, in cases of severe heart failure, the only treatment options are mechanical circulatory support or heart transplantation. However, a scarcity of available donor hearts results in patients dying before treatment. Three-dimensional (3D) printing of human cardiac cells has emerged as an area of promising research to address this problem. These cells not only can create a functional readout needed for optimal development/testing of combination drug therapy, but have the potential to be utilized in surgical repair and regenerative therapy. This study aimed to develop an effective protocol for 3D-bioprinting cardiomyocytes (CMs) derived from induced pluripotent stem cells (iPSCs), to create a realistic bioengineered cardiac tissue model.

**Methods:**
The survival and growth of CMs was tested against various bioinks and the stability of printed structures was evaluated by different cell crosslinking means and monitored for 9-12 days after they were printed. An alginate/gelatin-based bioink was first used to 3D-print the cells. The alginate (3%) and gelatin (7%) were sterilized as powder under UV radiation and mixed in cell culture media at $37°C$. CMs were harvested with trypsin and recovered in media with ROCK inhibitor (ROCKi), which promotes cell survival. CMs and bioink were placed in two separate syringes. Using a sterile connector, both solutions were mixed gently and homogeneously. The CM/bioink mixture was then placed in the bioprinter dispenser, which bioprinted at $30°C$. A second bioink (GelXA laminink: Cellink—Gothenburg, Sweden) was a pre-prepared solution of gelatin, xanthan gum, alginate, and laminin 521. The preparation of cells as well as printing used the same procedure as the alginate/gelatin bioink. Upon heating this bioink to $37°C$, the same procedure as the alginate/gelatin-based bioink was used to print CMs. Hydrogels were crosslinked using either UV at 365 nm during bioprinting, 100mM $CaCl_2$ after bioprinting, or UV followed immediately by 100mM $CaCl_2$. Culture media was either added or replaced the $CaCl_2$ following the crosslinking.

**Results:**
The CM cells were successfully printed using the alginate/gelatin-based bioink. No difference was noted in the growth of CMs or their printed structure from day 0 to day 9. CMs were also successfully printed using the GelXA laminink. While there was no difference in the printed structure of the CMs from day 0 to day 12, there were more CMs clumping together into small clusters. Lastly, the CM structures were successfully crosslinked with all three of the procedures above. There was no difference between the printed structures or CMs in the three variances.

**Conclusion:**
As CM morphology did not change when printed with the alginate/gelatin-based bioink or GelXA laminink, there appears to be no significant difference in the 3D environment created by either bioink. 3D printed CM growth and survival did not change with either bioink. However, the GelXA laminink appeared to be the preferred condition for CM clustering; CM cells tend to clump together when they are living in a healthy environment. However, even this condition was not optimal as shown by the small cluster sizes. There also did not appear to be any significant difference in the environments that the three crosslinking techniques created. CM growth and survival, as well as 3D printed structure, did not differ with any of the crosslinking procedures. Future directions include testing more bioinks, crosslinking techniques, and printed shapes of CMs for better CM growth and survival.

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**TNF-alpha improves retention of endothelial cells on fibronectin-coated substrate for improved blood compatibility of cardiovascular devices**

**Background:** Coronary interventions including vascular grafts and stents are used on over 1 million Americans each year. Despite their widespread use, post-implantation complications including thrombosis, vessel re-narrowing, and infection are still prevalent. Development of a healthy endothelium on the surface of these devices would help lower these risks and improve patient outcomes. Therefore, there is great interest in developing a method to improve endothelial cell (EC) adhesion on all blood-contacting devices. Previously, we determined the inflammatory cytokine tumor necrosis factor (TNF)-$\alpha$ shows promise in improving EC adhesion. Here, we definitively test the effect of TNF-$\alpha$ on EC adhesion including a dose response.

**Method:** Porcine blood outgrowth endothelial cells (BOECs) were cultured onto fibronectin coated 35 mm dishes overnight and subsequently exposed to TNF-$\alpha$ at a concentration of 0 ng/$\mu$l (control), 0.4 ng/$\mu$l, 4 ng/$\mu$l, or 40 ng/$\mu$l for 1 hour. The plates were then placed into a previously validated parallel plate flow chamber apparatus for exposure to physiological shear stress conditions (15 dyn/cm$^2$) for 30 mins. Nuclear staining (NucBlue) was used to image cells located within the flow path before and after shear stress exposure. The average percentage cell retention was used to assess EC adhesion.

**Results:** Control samples (n=5) had an average cell retention of 32.4±11.35%, whereas TNF-$\alpha$ exposed samples showed 59.21±11.79%, 72.70±17.39%, and 71.25±10.80% cell retention in the 0.4 ng/$\mu$l (n=6), 4 ng/$\mu$l (n=6), and 40 ng/$\mu$l (n=5) samples, respectively. A one-way analysis of variance (ANOVA) demonstrated a statistically significant difference between the means of the samples (F=8.571, p=0.0009) and a subsequent Tukey's pairwise analysis showed a statistically significant difference when comparing each treatment group to the control using p<0.05 (0.4 ng/$\mu$l: p=0.036, 4 ng/$\mu$l: p=0.001, 40 ng/$\mu$l: p=0.002), but no statistically significant difference when comparing each treatment to one another.

**Conclusion:** TNF-$\alpha$ is effective at enhancing EC adhesion, and a concentration of 0.4 ng/$\mu$l was optimal in this study. As a result, TNF-$\alpha$ shows utility in optimizing cardiovascular devices, including stents and grafts. Additional research is required to understand the influence of TNF-$\alpha$ on cells seeded to a substrate other than fibronectin-coated plastic (e.g. fibronectin-coated graft biomaterials) and its effects on EC adhesion over the course of long-term shear stress exposure time.

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**Identification of novel cardiovascular disease genes using integrated data resources at Rat Genome Database**

## Background

Cardiovascular disease is the leading cause of death worldwide. Comprehensive characterization of cardiovascular disease-related genes and loci has become essential to aiding cardiovascular research. The human *AGTRAP-PLOD1* locus is highly associated with cardiovascular diseases. A 2013 study by Michael J. Flister suggested the orthologous *Agtrap-Plod1* rat locus is associated with cardiovascular related-phenotypes. Integrated disease resources at the Rat Genome Database (RGD) provide data and tools to further study the human chromosomal region. Expanding this region might identify additional cardiovascular disease-related genes and be useful to suggest further experimentation on these genes.

## Methods

Gene and Ortholog Location Finder (GOLF) displays the human orthologs to the six genes investigated in the previous study. In order to find novel cardiovascular disease-related genes, we expanded the chromosomal region using JBrowse. The Multi Ontology Enrichment Tool (MOET) was used to select the best cardiovascular disease enriched region. For additional genes of interest, a Pubmed search was conducted to investigate and annotate such genes to cardiovascular diseases.

## Results

The involvement of the rat *Agtrap-Plod1* locus in cardiovascular diseases is further confirmed by hypertensive phenotypes in the aged *Nppb* knockout rat. To find more disease genes in the human locus, three expanded regions were delineated using JBrowse. The *AGTRAP-PLOD1* locus is further enriched for cardiovascular disease annotations with the inclusion of the human *MFN2* gene. *MFN2* is located directly after *PLOD1*, thus the expansion adds no other genes. The *MFN2* gene report page lists cardiovascular related-diseases and phenotypes to make it a good candidate gene for a knockout study. Literature research was performed on other genes that do not have cardiovascular disease association. Among them, *CLCN6* and *C1orf167* are found to be associated with cardiovascular diseases. New cardiovascular disease annotations and related phenotypes were added to the database.

## Conclusions

Upon analyzing cardiovascular disease annotations in the expanded *AGTRAP-PLOD1* locus, *Mfn2* was found to be a strong candidate for a mutant phenotype study similar to the 2013 paper. *CLCN6* and *C1orf167* were curated with cardiovascular disease and related phenotype annotations. This investigation suggests that research might be done to explore cardiovascular phenotype associations with the rat gene *Mfn2*. In addition, it suggests that more investigation could be done for candidate genes like *C1orf167* for cardiovascular diseases.

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**Longitudinal Reproducibility of MR Perfusion Using the 3D pCASL with Multiple Postlabeling Delay**

## Background
While 3D pseudocontinuous arterial spin labeling (pCASL) is the currently recommended non-invasive MR perfusion technique, it can be confounded by varying arterial transit times (ATT) across the brain and with disease. In this study, a Hadamard encoding scheme was used to acquire multiple post-labeling delays (PLD), which were used to estimate ATT and thus correct CBF. The goal of this study was to assess longitudinal reproducibility of a Hadamard encoded 3D pCASL sequence in a set of normal volunteers.

## Method
Imaging was performed on 52 healthy male young adults at 4 time points (TPs) over 45 days. An "enhanced" 3D pCASL (eASL) sequence was acquired with seven PLD (1.0-3.7s) using Hadamard encoding. A long (3.5s) labeling block was divided into 7 sub-boluses with control and label sub-boluses corresponding to the Hadamard matrix. Images for each delay were extracted using a linear combination of the eight images. Additional T1-weighted anatomical images were also acquired for registration. ATT and corrected flow (cCBF) were computed using the one-compartment model. Uncorrected flow (unCBF) was obtained by averaging the delay images with PLD > 1.5s. The longitudinal reproducibility was analyzed across four-time intervals: 7, 14, 30, and 45 days. Several reproducibility metrics, including within and between subject coefficient of variation (wCV and bCV respectively), the repeatability coefficient (RC), and intraclass correlation coefficient (ICC), were analyzed in gray matter (GM) and 17 different ROIs of functional networks across GM.

## Results
The GM ATT wCV (3.9%) showed a longitudinal stability for ATT across the four sessions, compared to both unCBF and cCBF. For each time interval, the wCV was lower for cCBF compared to unCBF for all ROIs and the whole GM. For most ROIs, the ICC was higher for cCBF compared to unCBF. In addition, the bCV values were always higher than the wCV for each time interval and ROI.
A power analysis yielded power curves for every session that followed a near identical trend. Specifically, for GM for every time interval, a higher sample size was needed for unCBF than cCBF.

## Conclusion
Our results suggest that eASL with multiple PLD using Hadamard encoding is a robust tool for measuring changes in CBF and ATT over time. This MRI perfusion technique can be acquired in the same amount of time as standard pCASL scans and can be adopted to monitor hemodynamic information for patients with disease intervention.

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Using Machine Learning to Predict Individual Motor Function in Patients with Brain Tumors

Background:
Pre-surgical mapping of motor function using functional MRI data is critical to patients with brain tumors occupying the central motor region, as the successful identification of activation in this region is crucial for brain tumor resections and post-surgery outcome. Currently, task-based fMRI is routinely utilized for pre-surgical mapping but has obvious limitations. Alternatively, resting-state fMRI offers advantages that offers solutions to the limitations of task-based fMRI and permits the scans to be utilized for a broader range of patients. Currently, there is no standard approach to analyze resting-state fMRI data for pre-surgical mapping.

Purpose:
The purpose of our study is to determine if the Machine Learning Neural Network (NN) approach can successfully predict activation from resting-state fMRI data in patients with space-occupying tumor in the central region. Additionally, the NN approach will be compared to a conventional method of Independent Component Analysis (ICA), to determine which method has better correlation to motor activation assessed using task-based fMRI.

Materials and Methods:
This study included 109 patients with centrally located brain tumors, ranging from ages 23 to 79. Additionally, there were 47 controls ranging from ages 23 to 66. Motion activations in the patients and healthy controls were evaluated utilizing task-based fMRI data and the ICA and NN approaches on resting-state fMRI data. Dice coefficients were calculated to determine the similarity between the different approaches of resting-state data and task-based data and then compared utilizing the Wilcoxon U-test and Spearman Correlation Coefficients.

Results:
Localization of the hand motor activation was more effective utilizing the NN approach than the ICA approach as the Dice Coefficient between the NN predicted map and active task activation was significantly higher than that of the Dice Coefficient of the ICA derived map and the active task activation for the tumor side, non-tumor side and both sides combined. Furthermore, significantly more head motion was detected during active task-based fMRI than the resting-fMRI.

Conclusion:
The Machine Learning: Neural Network approach of analyzing resting-state fMRI data yielded more success in the prediction of hand motor activation in comparison to the active and passive task-based data than the ICA approach in patients possessing a brain tumor within the central motor network despite possible space-occupying effects. Furthermore, resting-state fMRI data contained less head-motion than that of the task-based fMRIs further adding to the clinical significance of the method for presurgical mapping.

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**Cardiac Magnetic Resonance Imaging for Non-Invasive Cardiac Function Assessment**
## Background:
Magnetic Resonance Imaging (MRI) is a noninvasive imaging modality that utilizes magnetism and radiofrequency to create images of the inside of the body. MRI's are competitive with other imaging modalities because they do not require radiation to obtain images and provide high resolution to produce images with high tissue contrast. Cardiac magnetic resonance (CMR) utilizes special MRI scanning techniques and applies them to the cardiovascular system to produce images which are analyzed by commercial software's to show regional function (RF), global function (GF), and other parameters of the heart. CMR is used as a diagnostic tool for cardiac disease because image analysis is simple and can prevent diagnosis by invasive procedures. The purpose of this work is to create a specialized, user-friendly MATLAB program to provide GF parameters and perform more specialized functions than current commercial software's. This program will be used to create masks of cine CMR images to be used in future training of Artificial Intelligence (AI) for advanced cardiac analysis. The in-house created program and an open software will be used to analyze GF and RF of rats treated with radiotherapy (RT) and a control set of rats to see the effect of RT on cardiac function.

## Method
A MATLAB graphical user interface (GUI) was developed for a user to draw contours around areas of cine CMR images of the heart and create masks of the contoured blood pool and myocardium. The program was then enhanced to extract the area of the blood pool during end diastole and end systole to calculate GF parameters– ejection fraction (EF), end diastolic volume (EDV), and myocardial mass (MM). Masks generated in this program will be saved to train for (AI) that will eventually analyze cine CMR image on its own. The GUI was then used to analyze 8 different rats (4 treated with RT and 4 controls) to determine the effect of RT on cardiac function. Additionally, RF values—radial strain ($E_{rr}$), circumferential strain ($E_{cc}$), longitudinal strain ($E_{LL}$), and motion magnitude—were obtained for the anterior, interior, septal, lateral, basal, mid-ventricular, and apical sections of the heart using programs called Horos and Intag to further analyze the effects of RT. The GF and RF parameters were compared between RT-rats and controls with unpaired, two-sided t-tests. In order to determine the validity of the results from the in-house created software and RF analysis, inter-observer and inter-software agreements were tested with Bland Altman plots.

## Results
The mask-creating GUI was used to create over 2,000 myocardial masks that will be used for AI training. The GF results showed RT-rat versus control to show little sensitivity of RT: EF (83.2%±5.47% vs 67.7%±3.98%), EDV (0.246 ml ± 0.035 ml vs 0.347 ml± 0.031 ml), and MM (0.565 g ± 0.125 g vs 0.465 g ± 0.038 g). Given that normal EF is >50% the values indicate that there are no significant differences overall GF, but hypertrophy is seen due to an increased MM. RF results show that specific regions of the RT-rats versus control differed significantly due to a $p$-value less than 0.05 amongst $E_{cc}$ for the heart regions: septal (-10.52±1.73% vs -15.0±2.78%), mid-ventricular (-10.6%±3.78 vs -14.1%±3.46%), apical (-7.46±3.51 vs -11.9±2.25%), and overall(-9.81±2.17% vs -13.0±2.70%) as well as tissue motion for the anterior (4.73±0.75 vs 2.70±0.108) and mid-ventricular (7.92±1.79 vs 5.43±2.15) sections. $E_{rr}$ and $E_{LL}$ showed no significant differences between the two groups. Finally, Bland Altman plots showed inter-observer and inter-software differences that were within 2SD agreement limit.

## Conclusion
The GUI produced in this work has more specialized functions and produces accurate results than commercial software's. The masks produced from the GUI will be used to train AI to create faster, more advanced cine CMR image analysis. Additionally, the results showed that GF parameters do not show significant differences between RT-rats and controls whereas RF parameters do. This indicates that RF analysis of CMR images is more sensitive to RT treatment and can be useful in early detection of RT induced dysfunction of the heart. This study is limited by sample size; however, more subjects are being studied to allow for statistically significant findings in the future.

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**A dual-acting Farnesoid X Receptor (FXR) agonist and Soluble Epoxide Hydrolase (sEH) inhibitor treats kidney fibrosis in a mouse model.**

## Background
An estimated 33 million Americans, or every 1 in 6, suffer from Chronic Kidney Disease (CKD). It is a prevalent issue that often results in loss of kidney function which requires dialysis or renal transplant. Lack of viable treatments for CKD necessitates the search for new solutions. This project investigates the ability of a novel drug, DM509, to treat kidney fibrosis, an inherent indicator of CKD.

## Method
To test the anti-fibrotic action of DM509, C57BL/6J mice models with unilateral ureteral obstruction (UUO) induced kidney fibrosis were used. The mice were separated into 3 groups: group 1 contained 5 healthy control mice, group 2 contained 8 mice that had UUO surgery, and group 3 contained 8 mice that had UUO surgery and were treated with DM509 (10 mg/kg/day in drinking water). Treatments were started on day 3 of post UUO surgery and continued for 7 days, after which the blood and kidney tissue were collected. Biochemical assays were performed in combination with histological analysis to determine the renal action of DM509. Kidney function was assessed from blood urea nitrogen (BUN) levels, renal fibrosis was assessed from renal hydroxyproline content and histological analysis using Picrosirius red (PSR). PSR staining determined collagen positive renal fibrotic area in different experimental groups. Renal inflammation was determined from monocyte chemoattractant protein-1 (MCP-1) levels in the kidney tissue.

## Results

| | BUN [mg/dL] | Hydroxyproline [μg/mg total protein] | Renal fibrotic area [%] | MCP-1 [pg/mg total protein] |
|---|---|---|---|---|
| *Healthy Control (n=5)* | 44.5±15.2 | 1.89±0.90 | 0.7±0.06 | 2.8±1.4 |
| *UUO Control (n=8)* | 76.5±10.4 | 5.25±2.33 | 4.3±1.2 | 40.8±10.7 |
| *UUO + DM509 (n=8)* | 50.3±12.5 | 3.11±0.60 | 1.5±.09 | 31.2±7.6 |

## Conclusion
DM509 improves renal function and reduces renal tubular injury and inflammation in mouse UUO models. Most importantly, DM509 highlights an ability to reduce kidney fibrosis, which is a hallmark pathophysiological event in any form of CKD.

**Keywords** Renal fibrosis, unilateral ureteral obstruction (UUO), chronic kidney disease (CKD), blood urea nitrogen (BUN), dual-acting drug

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**The effect of lysosomal and GSK3β inhibitors on the PRR7-induced reduction of GPR177 protein levels in Cos-7 cells**

**Background**
Secreted Wnt molecules are integral for both the synaptogenesis and maintenance of synapses. With these Wnt molecules in mind, our specific research focus was on the protein Proline-rich 7 (PRR7), a recently identified Wnt inhibitor that blocks Wnt secretion to ultimately promote synapse loss. In order to understand the mechanism by which PRR7 induces synapse loss, we are investigating the role of GPR177, a protein that is involved in Wnt secretion. To be more specific, our working hypothesis is that PRR7 leads to the lysosomal degradation of GPR177 via activation of the protein kinase GSK3β which alters the endosomal trafficking of GPR177. To test this proposed mechanism, either lysosomal or GSK3β inhibitors were administered to Cos-7 cells that were co-expressing both GPR177 and PRR7 in order to see whether GPR177 protein levels changed as a result of the pharmacological treatments.

**Method**
Cos-7 cells, derived from monkey kidney tissue, were utilized for all experiments and cells were seeded into a 6-well plate at the start of each week. Once the cells were ~80% confluent, a total of 3 µg of plasmid was added for transfection. Drug treatments occurred 6 hours post transfection and harvesting of cells took place 18 hours after drug treatments. Cells were treated with either lysosomal inhibitors such as leupeptin (50 mg/mL), lactacystin (10 µM), chloroquine (100 µM), and bafilomycin A (100 nM and 1µM) or GSK3β inhibitors such as BIO (250 nM), SB 415286 (10 µM), and TDZD 8 (10 µM). Positive controls were transfected with GPR177 alone and left untreated while negative controls were transfected with both PRR7 and GPR177 and treated with DMSO. Total cell lysates were prepared utilizing 2x SDS sample buffer and equal amounts of each lysate were loaded into SDS-PAGE gels for Western blot analysis. The resulting Western images were quantified and drug treated GPR177 levels were compared to the positive control.

**Results**
Cos-7 cells co-expressing both PRR7 and GPR177 showed a reduction in GPR177 protein levels to 35-40% of the positive control. In contrast, the treatment of the lysosomal inhibitor chloroquine, a drug that prevents the alkalization of lysosomes and ultimately inhibits lysosomal acidic hydrolases, restored GPR177 protein levels to levels similar to the positive control (~113%). In addition, leupeptin, a drug that directly inhibits lysosomal proteases, had GPR177 protein levels around 55% of the positive control. Other inhibitors such as lactacystin, (a proteasome inhibitor), and Bafilomycin A, (an $H^+$ ATPase inhibitor), had no effect on GPR177 levels. To test the effect of GSK3β inhibitors on PRR7 induced reduction of GPR177 protein levels, BIO and SB 415286, which bind to the ATP binding pocket of the GSK3 enzyme to prevent its activity, and TDZD 8, which induces a conformational shift in the GSK3 enzyme to prevent its activity, were used. Although BIO restored GPR177 levels to 60% of the positive control, both SB 415286 and TDZD 8 showed no such effect.

**Conclusion**
These results indicate that the inhibition of the lysosomal degradation pathway as well as GSK3β may counteract the detrimental effects of PRR7 on GPR177, supporting the hypothesis that PRR7 promotes lysosomal degradation of GPR177 via GSK3β activation. However, some drugs also failed to increase GPR177 protein levels which may be due to their reduced efficacy. Therefore, further experiments utilizing fresh batches of these pharmacological agents and testing other pharmacological approaches are necessary in order to confirm our present findings.

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**Environmental Enrichment and Drug-Seeking Behavior in Rodent Models**

**Background**

Addiction is modeled in a laboratory setting through the use of animals learning to self-administrate drugs of abuse. The persistence of the drug-seeking behavior after abstinence can be used as an indicator of addictive behaviors. Addiction goes beyond just substances of abuse, with such factors as stress and context playing a role. Animals that are stressed have been shown to have increased drug-seeking behaviors and animals with environmental enrichment (EE) show a decrease in these behaviors.

The medial prefrontal cortex (mPFC) provides emotional input to the nucleus accumbens (NAc) and can increase drug-seeking behavior. Activation of the mPFC is shown in both humans and rodents to be involved with addiction, cravings, and recollection of situations involving substances of abuse.

**Method**

Coordinates of the NAc core were determined using a brain atlas, then confirmed through stereotaxic infusion of tetraspeck beads. The animal was then immediately sacked and perfused with PBS, and the brain sectioned fresh at a thickness of 10µm for imaging.

Using the NAc coordinates, 1µL of AAVrg-CAG-tdTomato was bilaterally injected into the NAc core by stereotaxic surgery. After recovery, animals were kept in EE cages for three weeks with toys changed out three days a week. The animals were then euthanized and perfused, and brains were harvested for passive CLARITY. Immunohistochemistry was performed on brain slices taken from EE studies of cocaine-seeking (CS) rats using Rabbit Sy-Sy (cfos), Goat Anti-Rabbit Alexa Fluor 633, and DAPI to visualize the activation of the mPFC.

**Results**

CS rats that received environmental enrichment during a month-long abstinence showed a greater decrease in continued drug-seeking when compared to rats kept in their home cage during abstinence. This change is not observed when using other animal models such as mice.

The tracks from the tetraspeck bead infusions confirmed accurate coordinates for the NAc core, and the same coordinates were used for the AAVrg-CAG-tdTomato infusion.

**Conclusion**

Environmental enrichment can play a role in the reinstatement of drug-seeking behaviors, though the applicability of this to human addiction is not straight-forward. Using a retrograde viral tracer paired with another viral tracer would give insight into the neuronal projections within the reward pathway. Expanding further, it may be interesting to look at inputs from both the prefrontal cortex and the ventral tegmental area in the nucleus accumbens.

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## Analysis of lung cancer cell death caused by the loss of Rac1 and Rac1b GTPases

Lung cancers typically arise in the cells lining the bronchi and parts of the lung such as bronchioles or alveoli. Non-small cell lung carcinoma (NSCLC) is the most common type of lung cancer with 80-85% of lung cancers being NSCLC. Rac1 and Rac1b are found in normal healthy cells and are over expressed in certain types of cancers like NSCLC. Rac1 and Rac1b are small GTPase proteins that are involved in regulating a variety of cellular processes such as cell signaling, cell proliferation, migration, and transcription. We have developed small interfering RNA (siRNAs) to target Rac1 specifically, Rac1b specifically, or both Rac1 and Rac1b. When we treat lung cancer cells with these siRNAs we see a decrease in cell proliferation. The goal of this study is to determine if knocking down Rac1 and/or Rac1b causes apoptosis in NSCLC. Therefore, we hypothesize there will be greater cell death in NSCLC cells treated with the siRNAs to the GTPases compared to the mock control or siRNA control treatment. Sytox Green assays were used to evaluate cell death after treatment with two siRNAs for Rac1, Rac1b, and both Rac1 and Rac1b on human NSCLC H1703. The Sytox Green stain was used because it is a nucleic acid stain that can easily penetrate cells with compromised membranes and will not cross membranes of live cells. After incubation with the Styox Green nucleic acid stain, the nucleic acids of dead cells fluoresce bright green. Following the incubation with Sytox green, we measured the fluorescence of each well on a plate reader to quantify the cell death in each case. Interestingly, we did not see significant cell death after treating with siRNAs as compared to the mock control or siRNA control treatment. This means the siRNAs are not killing the cells, but rather they are slowing down cell growth. In the future we would like to follow up these experiments with flow cytometry as another way to confirm that we are not seeing apoptosis as a result of treatment with these siRNAs.

Key words: Non-small cell lung carcinoma (NSCLC), Rac1, Rac1b, siRNAs, H1703- cell line, cell apoptosis

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**Epac2 in Ventral Tegmental Area Regulates Dopamine Release in Nucleus Accumbens**

**Background**

Addictive drugs such as cocaine can bring rapid and intense satisfaction that evokes feelings of reward and pleasure, which results in high possibilities of drug abuse and addiction. The mechanism of cocaine addiction is by promoting the release of a neurotransmitter in the brain called dopamine (DA) that originates from the Ventral Tegmental Area (VTA) of the brain. DA is then transported by long neuron axons and released to another brain region: nucleus accumbens (NAc). In summary, the satisfaction brought by cocaine is caused by the increased amount of dopamine release in the NAc. But is there any alternative to reach the same level of DA without the intake of cocaine?

Cyclic adenosine monophosphate (cAMP) is a second messenger that plays important roles in many biological processes. Among its various functions, cAMP can activate Epac (exchange protein directly activated by cAMP) that regulates a wide array of physiological functions, while its detailed effects are awaiting to be explored. Pilot study indicates that natural DA responses were decreased in Epac2 knockout mice compared with the wild type mice. Based on this result, the goal of this project is to test whether the specific Epac2 agonist would modulate DA release in wild type mice and Epac2 knockout mice.

**Method**

We measured DA release in the NAc in slices prepared from wild type and Epac2 knockout mice using fast-scan cyclic voltammetry (FSCV), a highly sensitive method for monitoring real-time DA release with excellent spatiotemporal resolution. FSCV was performed blind to mouse genotype, and the DA release is evoked by single pulses with two-minute intervals in a fixed intensity. After a stable baseline of DA release was detected, Epac2 agonist Sp-8-BnT-cAMPS (S-220) was added. The evoked DA signals are compared before and after drug perfusion. Different concentrations of S-220 were perfused to the slices. The signals from the knockout mice were compared with the wild type mice.

**Expected Outcomes**

We anticipate that the Epac2 agonist S-220 will enhance DA response in wild type mice but not Epac2 knockout mice.

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**Sequestration of Proteins by Lipid Droplets in *Mycobacterium***

Tuberculosis (TB) is a leading cause of death in the world due to an infectious agent. In 2017, WHO reported 10 million cases and 1.3 million deaths worldwide, and in the U.S. there were over 9000 new cases of TB reported in 2018. This disease is caused by the bacterium *Mycobacterium tuberculosis*. The high death rate associated with *M. tuberculosis* infections is due in part to the ability of this organism to establish a latent infection and re-activate decades later following suppression of the host's immune response. During latency, bacteria enter into a state of non-replicating persistence and use neutral fatty acids stored in lipid droplets as their primary energy source. To determine if lipid droplet production and entrance into non-replicating persistence are linked, the complete proteome of lipid droplets was determined by mass spectroscopy in a non-pathogenic surrogate for *M. tuberculosis*, *Mycobacterium smegmatis*. Interestingly, numerous proteins that are associated with cellular physiology, and that are normally present in the cytoplasm or at the plasma membrane, were identified on lipid droplets. One of these proteins, HupB, is normally cytoplasmic and regulates the process of DNA replication. Based on this and other published information, we hypothesized that lipid droplets may sequester HupB as they form, and that this relocalization event may inhibit DNA replication and promote entrance of *Mycobacterium* into a state of non-replicating persistence during latency. To test this possibility, the determinant encoding HupB was modified by cloning to incorporate a 3XFlag epitope tag at the N-terminus to facilitate tracking. The tagged allele was then exchanged with the wild-type allele in the chromosome of *M. smegmatis* by homologous recombination. Current efforts are underway to track 3xFlag-HupB localization before and after lipid droplets form to determine when the protein localizes to lipid droplets, and to monitor DNA replication under these conditions to assess how HupB relocalization impacts replication of the bacterium. Ultimately, understanding the mechanisms by which *M. tuberculosis* establishes latency will be critical to developing effective therapeutics that are better able to control infection by this organism.

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## Phenotypical characterizations of regulatory T cells that undergo proliferation upon interaction with $BD_L$

Multiple sclerosis (MS) is a chronic autoimmune disorder, in which the failure of self-immune tolerance leads to autoreactive CD4+ T cells attacking the central nervous system. $CD4^+$ T cells are immune cells that play an essential role in protecting the body from harmful microorganisms. $CD4^+FoxP3^+$ T regulatory cells (Treg) are a T cell subset essential for self-tolerance that suppress autoimmunity by negatively regulating autoreactive $CD4^+$ T cell expansion and migration. Promoting Treg proliferation/expansion is one promising therapy for treating autoimmune disorders. B cells are an additional immune cell subset important for normal immune responses. Recently the Dittel laboratory discovered a new B cell subset ($BD_L$), that exhibits an IgD low phenotype and promotes Treg homeostasis/proliferation, thereby maintaining self-tolerance. To learn more about the relationship between Treg and $BD_L$, specific markers for the Treg that interact with $BD_L$ need to be identified. Thus, this project aimed to identify the phenotype of Treg that interact with $BD_L$. To accomplish this, we searched the literature to identify important cell surface proteins important for Treg function that can be used for phenotyping by flow cytometry. From this search we chose GITR, CD39, LAG-3, PD-1 and CTLA-4 as potential Treg markers. The expression of each protein was evaluated by staining Treg with fluorochrome-conjugated antibodies followed by flow cytometry. We found that the mean fluorescent intensity (MFI) levels of GITR and CD39 on Treg were high, indicating a high level of protein expression. In addition, we found that LAG-3 was expressed at a median level, while PD-1 and CTLA-4 were low. When we examined each marker for the percent positive Treg we found 99.8% $\pm$ 0.12 $GITR^+$, 86.9% $\pm$ 0.6 $CD39^+$, 10.2% $\pm$ 2.5 $PD-1^+$, 3.1% $\pm$ 0.1 $LAG-3^+$ and 2.26% $\pm$ 0.34 $CTLA-4^+$. These data demonstrate that while GITR and CD39 are expressed by the majority of Treg; PD-1, LAG-3 and CTLA-4 represent unique subsets. Since we know that $BD_L$ induce Treg proliferation, we also evaluated the markers on proliferating and nonproliferating Treg. This was accomplished by examining the expression of Ki67- an intracellular marker for cellular proliferation. All specific Treg subsets exhibited higher Ki67 expression than the total Treg population, which was 51.5% $\pm$ 3.05. Comparing Treg subsets shows that $PD-1^+LAG-3^+$ Treg exhibited higher level of proliferation (74.87% $\pm$ 4.086) than $PD-1^-LAG-3^-$ Treg (52.3% $\pm$1.082). Similarly, $CTLA-4^+CD39^+$ Treg (68.07% $\pm$ 2.367) exhibited significantly higher Ki67 expression than $CTLA-4^-CD39^-$ Treg (20.63% $\pm$ 0.7234). Taken collectively, we were able to identify unique Treg subsets using the identified marker combinations. The markers along with Ki67 staining will be used in future experiments to assess whether Treg that interact with $BD_L$ are a unique subset. Understanding the Treg phenotype expanded by $BD_L$ will advance our understanding of Treg-induced tolerance and will support the development of therapies targeting $BD_L$.

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## DOUBLE TROUBLE: COINFECTION OF MHV68 AND K. PNEUMONIAE

### Background
Gammaherpesviruses are oncogenic double-stranded DNA viruses whose infections are species specific and persist for the lifetime of the host. Infection drives a robust germinal center (GC) response resulting in the production of memory B cells in which the virus establishes latency. Murine gammaherpesvirus 68 (MHV68) is specific to rodents and is used as an animal model to study human gammaherpesviruses *in vivo*. The Tarakanova lab recently discovered that the proinflammatory cytokine interleukin-17 (IL-17) is selectively pro-viral for MHV68 by promoting MHV68 driven GC response. Infection with *Klebsiella pneumoniae (KPn)* is known to induce a robust IL-17 immune response. With this in mind, we asked whether naturally increasing IL-17 by coinfecting mice with MHV68 and *KPn* would have a pro-viral effect.

### Method
C57BL6/J mice were infected with MHV68. 9 days post infection when the GC is beginning to develop, the same mice were infected with *KPn.* At peak GC response, 16 days after initial infection, mice were euthanized and the lungs, spleen, liver, and peritoneal cells were harvested. Organs were homogenized, then serial dilutions were plated on LB agar plates to determine the CFU/mL of *KPn.* The GC response in the spleen was determined via flow cytometry. ELISAs were used to measure serum levels of total IgG, total IgM, and MHV68 specific antibodies. Viral assays are currently on going to determine the frequency of cells harboring viral DNA as well as the frequency of viral reactivation in the spleen and peritoneal cells.

### Results
Determining the CFU/mL of *KPn* indicated that one coinfected mouse cleared the bacterial infection prior to the harvest. Surprisingly, the GC response was significantly attenuated in coinfected mice still harboring bacteria and mice infected with *KPn* alone when compared to the GC response typically observed in MHV68 infected mice. Interestingly, there was no significant difference in serum levels of total IgG, total IgM, or MHV68 specific antibodies between coinfected mice and mice infected with MHV68 alone despite the difference in GC response.

### Conclusion
Contrary to our initial hypothesis, coinfection of mice with MHV68 and *KPn* attenuated the GC response. It is possible that bacterial infection stimulates or inhibits immune response elements leading to the attenuation of the GC response.

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**Neuroepithelial Bodies (NEBs) in a rat model of Bronchopulmonary Dysplasia (BPD)**

## Background
Infants born < 28 weeks gestation have immature lungs and neural control of breathing mechanisms necessitating respiratory therapies like supplemental oxygen (hyperoxia). Although necessary, hyperoxia increases the risk for developing lung disease called bronchopulmonary dysplasia (BPD). With time, infants are able to regulate breathing independently despite sustained lung disease. The mechanisms contributing to this adaptation are unknown. Pulmonary neuroepithelial bodies (NEBs) are clusters of pulmonary neuroendocrine cells (PNECs) located in the conducting pulmonary airways that may elicit chemosensory information to the respiratory control network. Moreover, they are hypertrophied and hyperplastic in human BPD. Thus, it was hypothesized that NEBs would be hypertrophied and hyperplastic in a rat model of BPD.

## Methods
Sprague Dawley rat pups were raised in either normoxic (n=3) (21% $O_2$) or hyperoxic (n=2-3) (95% $O_2$) conditions for the first 10 postnatal (P) days of life and then returned (kept) in room air. Ventilation was measured from 10 to 21 days of life. Groups of rats were euthanized at P10 and P21, and lung tissue was flushed and fixed for histologic analyses of NEBs. For each animal, a total of four lung tissue sections (4µm) were immunofluorescently stained for calcitonin gene related peptide (CGRP), a marker of NEBs. Stained tissues were imaged using fluoroscopy. NEB hypertrophy and hyperplasia were measured using *Metamorph Software*.

## Results
P10 hyperoxic animals tended to have hypertrophic and hyperplastic NEBs compared to normoxic animals. The average size of the NEBs and PNECs were larger in hyperoxic rats (397.47µm ±208.79, 43.26µm ±2.43, respectively) versus normoxic rats (299.01µm ±2.68, 36.89µm ±1.17, respectively). Hyperoxic rats had more PNECs (9.62 ±4.18) than normoxic rats (8.42 ±0.65). At P21, there were no changes in size or number of NEBs and PNECs between groups. P10 normoxic rats had a greater number of NEBS (15 ±6.24) than hyperoxic rats (9.5 ±0.5) which was reversed in P21 normoxic (21 ±5.29) and hyperoxic (25.67 ±4.98) rats.

## Conclusion
Due to low sample sizes it is difficult to make firm conclusions. Additional samples are needed. Trends suggest NEB hyperplasia and hypertrophy in P10 hyperoxic rats. Lower average number of NEBs in P10 hyperoxic rats may be a consequence of sampling error, requiring normalization to area of airways assessed.

**Keywords:** bronchopulmonary dysplasia (BPD), neuroepithelial bodies (NEB), hyperplasia, hypertrophy, pulmonary neuroendocrine cells

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**Superoxide Dismutase Activity in Wild Type and Mas1 Receptor Knockout Rats.**

**Background:** Past experiments have shown that high salt diets cause endothelial dysfunction (an important predictor of cardiovascular disease and adverse cardiovascular events). High salt diets also reduce the antioxide enzyme activity of superoxide dismutase (SOD) in normotensive Sprague-Dawley rats. Subsequent studies demonstrated that the detrimental effects of high salt diet on endothelial funnction can be prevented by direct activation of the crucially important transcription factor NFR2, which binds to antioxidant response elements in the promoter region of hundreds of antioxidant and cell protective genes. One target of for the beneficial effects of NRF2 is superoxide dismutase (SOD).

There are three types of superoxide dismutases (SODs): copper/zinc, manganese, and iron. Organs such as heart, liver, brain, red blood cells, and kidney contain high concentrations of SODs. In humans, there are three forms of SOD: cytosolic SOD (Cu/Zn SOD), mitochondrial SOD (MnSOD), and extracellular SOD. Superoxide dismutases play a part in antioxidant defense by catalyzing the dismutation of superoxide radicals. This form of protection helps prevent the development of diseases linked to oxidative stress and the development of endothelial dysfunction—a major contributing factor to cardiovascular diseases. Endothelial function is affected by specific hormones and receptors including ANGII (via the $AT_1$ receptor) and ANG-(1-7) (via the Mas1 receptor). Binding of ANG-(1-7) to the Mas1R can activate NRF2 defenses and restore impaired endothelial function. In this study, the enzymatic activity of SOD was measured in male and female rats lacking the Mas1R in the Dahl salt-sensitive (SS) rat genetic background and in their wild type littermates. We hypothesized that the Mas1R KO rats would have lower SOD activity than their littermates despite gender.

**Methods and Results:** Cayman superoxide dismutase kits were used to determine the SOD activity in liver samples of male and female Mas1R KO rats and wild type littermates. The cytosolic and mitochondrial SOD activity was determined by separating the supernatant (cytosolic) from the pellet (mitochondrial). In these experiments, the Mas1R KO female rats had higher cytosolic SOD activity than the males. By contrast, male Mas1R KO rats and their wild type littermates had higher mitochondrial SOD activity than the females despite diet. Male Mas1R KO rats had higher mitochondrial SOD activity than their wild type littermates despite diet.

**Conclusion**: The data collected did not agree with our original hypothesis. There is more information to be discovered about how other factors within the body can affect SOD activity and how loss of the Mas1 receptor can affect blood pressure and vascular function.

**Keywords**: ANGII, ANG –(1-7), Mas1 receptor, NRF2 transcription factor, superoxide dismutase

# Effect of Fructose-Induced Changes in Renal Microvascular Function & Blood Pressure

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Background. A drastic increase in fructose consumption over recent decades has been paralleled by a growing prevalence and incidence of hypertension along with some of its comorbidities, such as diabetes, renal failure, and cardiovascular disease. High fructose corn syrup is a commonly found ingredient in the western diet. The use of fructose as a sweetener does not come without consequences, as fructose has been shown to promote salt-sensitive hypertension.[1] The mechanisms by which fructose induces an increase in blood pressure and its effect on renal microvascular function are not yet completely understood. The goal of this study is to understand the mechanisms that lead to fructose-induced impairment in renal small resistant microvessel function and its relation to the development of hypertension.

Methods. Four groups of salt resistant Sprague Dawley rats (8 weeks old) were kept on low salt (LS; 0.4% NaCl) or high salt (HS; 8% NaCl) diet for two weeks, with or without an addition of 20% fructose (FT) to a drinking water provided ad libitum. Blood pressure was monitored with surgically implanted telemetry devices. Electrolyte balance and indicators of renal function were analyzed among experimental groups using urinary (24 hours collections) and plasma samples. At the end animals were euthanized, and renal vessels were isolated by the method described previously for the assessment of renal microvascular smooth muscle excitability by the two photon microscopy.[2]

Results. We found that after 28 days, animals experienced elevated systolic blood pressure when given a high salt and fructose diet ($107 \pm 3$, $113 \pm 3$, $114 \pm 4$ and $131 \pm 5$, mmHg, LS, LS+FT, HS and HS +FT correspondingly). Furthermore, these changes correlated with shift in electrolyte balance ($123 \pm 70$ vs $284 \pm 91$ Na/Cre ratio, $14 \pm 10$ vs $36 \pm 12$ K/Cre ratio, $1.4 \pm 0.3$ vs $0.7 \pm 0.4$ Ca/Cre ratio, HS+FT vs HS correspondingly; $p < 0.001$ ANOVA). Two photon microscopy showed a decrease in frequency of SMC spontaneous excitability ($34.38 \pm 3.23$, $51.00 \pm 6.91$, $43.68 \pm 6.70$, $52.14 \pm 11.66$ sec, LS, LS+FT, HS and HS +FT correspondingly), as well as a decrease in amplitude of the spontaneous $Ca^{2+}$ oscillations in SMC ($269 \pm 24$, $192 \pm 25$, $248 \pm 33$, $185 \pm 21$ a.u., LS, LS+FT, HS and HS +FT correspondingly)

Conclusions. Increased sodium and potassium reabsorption in the kidney were significant contributors to the development of FT induced hypertension. Renal microvessels exposed to FT revealed a significant decrease in spontaneous vasomotion in both LS and HS diets. Microvessel results indicate impaired microvascular function in response to endogenous release of endothelin. These results indicate the negative health effects of FT on blood pressure and microvascular function.

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**Encapsulating IL-12 and HPPH in Liposomes for Laser-Triggered Release**

**Background:** Immunotherapy has been very effective in the treatment of melanoma cancer. Cytokines such as interleukin 12 (IL-12) have been extensively evaluated for its potential to enhance antitumor immune response. IL-12 antitumor activities are mediated by the activation of T and natural killer (NK) lymphocytes to produce interferon-gamma (IFNγ). However, systemic bolus injections of IL-12 causes serious toxicities. Ongoing research involves delivering the maximum dose of IL-12 into tumors. In this study, the red light activatable anticancer photodynamic therapy drug 2-(1-Hexyloxyethyl)-2-devinyl pyropheophorbide-a (HPPH) was loaded in liposomes together with the immuno-stimulating cytokine IL-12 for immunotherapy of cancer. We hypothesized that encapsulating a combination of IL-12 and HPPH in liposomes with laser-triggered release will result in a larger quantity of IL-12 released into the tumor as compared to IL-12 loaded liposomes without photodynamic triggering.

**Method:** The method used to prepare the liposomal formulations was the thin-film hydration followed by extrusion. Two different samples of liposomes were prepared; IL-12 loaded liposomes and IL-12 and HPPH loaded liposomes. The liposome size and zeta potential were determined by dynamic light scattering (DLS) using the NANO ZS (Malvern Instruments, CA, USA). Lastly, the total amount of lipid in the samples was determined by inorganic phosphorus analysis. Both samples of liposomes were treated for 2 minutes with a 660 nm diode laser at room temperature using the power output at 52 mW. The amount of IL-12 released from the liposomes was measured by ELISA[1].

**Results:** The table below illustrates the characterization of liposomes containing the immuno-stimulating cytokine IL-12.

| Type of loaded liposomes | Size (nm) | Zeta (mV) | Amount of IL-12 released (ng/mL) |
|---|---|---|---|
| IL-12 and HPPH with laser | 142 | +7 | 441.51 |
| IL-12 and HPPH without laser | 142 | +7 | 73.01 |
| IL-12 with laser | 140 | +4 | 241.16 |

**Conclusion:** The combination of IL-12 and HPPH in liposomes with laser-triggered release allows almost double the amount of IL-12 released compared to IL-12 loaded liposomes without photodynamic triggering. Future experiments with immuno-stimulating cytokines in liposomes will be useful for projected applications for cancer treatment.

**Keywords:** immunotherapy, cytokine, photodynamic therapy, laser-triggered release

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[1] Sine J et al. Photo activation of HPPH encapsulated in "Pocket" liposomes triggers multiple drug release and tumor cell killing in mouse breast cancer xenografts. *Int J Nanomedicine*. 2015;10:125-154.

Aysiah Jaeke – St. Norbert College
Brian Hoffmann, PhD
MCW Department of Biomedical Engineering

**Hyperglycemia-Induced Inhibition of the Angiotensin 1 Receptor Pathway in the Vascular Endothelium**

## Background

According to the Center for Disease Control and Prevention's, heart disease and diabetes are two of the leading causes of death in the United States. It is commonly known that hyperglycemia, associated with diabetes, can damage blood vessels to contribute to cardiovascular disease. However, the etiology behind the negative outcomes of prolonged elevated glucose on endothelial cells is not yet fully understood. Our laboratories' previous study identified changes in endothelial cell surface glycosylation resulting from hyperglycemia. One such modified protein was the Angiotensin 1 Receptor ($AT_1R$), which was glycosylated adjacent to the active site. The aim of this study was to further investigate the effects of this hyperglycemia induced modification of $AT_1R$. Activation of the $AT_1R$ by angiotensin II (AngII) at subpressor doses is associated with positive endothelial cell growth, proliferation, and tube formation. However, AngII binding to the Angiotensin 2 Receptor ($AT_2R$), has been shown to elicit opposing effects.

## Method

A two-week high glucose treatment of rat cardiovascular endothelial cells (RCMVECs) impaired function in a tube formation analysis (TFA), an effect further exacerbated by AngII possibly through $AT_2R$. Therefore, we hypothesized that hyperglycemia-induced glycosylation of $AT_1R$ inhibits AngII binding, allowing for a higher rate of binding in the $AT_2R$ associated with poor tube formation and cell proliferation. In order to test this prediction, we used the PNGasef enzyme for N-glycosylation removal at the cell surface, opening the $AT_1R$ active site, in combination with endothelial functional assays and AngII treatment of endothelial cells under varying glycemic conditions. These conditions were tested through TFAs and protein analysis of ERK1/2 activations through phosphorylation, a known contributor to $AT_1R$ signaling.

## Results

We observed a decrease in tube length, tube area, and number of branch points in a high glucose environment plus Ang-II but recovered functionality with the addition of PNGase-f to this group. While normal glucose treated cells had shown ERK1/2 activation, no observable increases in ERK1/2 were observed in response to AngII in the high glucose conditions.

## Conclusion

Further analysis is currently underway to further validate this mechanism of $AT_1R$ impairment, but the data we have so far lead us to believe that hyperglycemia-induced glycosylation of the normal endothelial function by blocking the $AT_1R$ active site.

Janelle Lanham – Milwaukee School of Engineering
Dr. Ranjan Dash & Dr. Brian Hoffmann
MCW Department of Biomedical Engineering

**Hyperglycemic Cellular Respiration Experiments and Isolated Mitochondrial Modeling**

**Background:** Endothelial cells (ECs) rely mostly on glycolysis to meet their energy demands. In diabetic ECs, this critical process may be altered, forcing the cell to rely more heavily on mitochondrial respiration (1). This respiration can be specifically examined by using different drugs to block respiratory complexes on the mitochondria. Oligomycin (OMN) blocks Complex V, thus revealing the cells 'leak' respiration. FCCP acts as an uncoupler, which determines the cells maximal respiration. Rotenone (ROT) and Anitmycin A (AA) block Complexes I and III, respectively (2), which provide the cells non-mitochondrial respiration. Computational modeling is also widely used in our lab, specifically programming in MATLAB is used to model isolated mitochondrial and cellular respiration under different conditions. Enzymes and transporters that govern mitochondrial function are described mathematically with different flux expressions, which are then used to develop dynamic mass balance equations for metabolites, $O_2$ and cations that are solved to simulate and predict mitochondrial and cellular functions.

**Method:** Rat cardiac microvascular endothelial cells (RCMVECs) from GK and WKY rat models, where GK is more likely to develop type 2 diabetes mellitus (T2DM) and WKY is a control, were grown under normal and hyperglycemic conditions. Four groups of cells were grown under their respective conditions for the same amount of time with all other variables being constant. Cells were then isolated, counted, and placed in an Oroboros oxygraph (O2k) respirometer. Once a stable baseline respiration was achieved, OMN was added, and approximately two minutes later, FCCP was added in small doses until the respiration stopped increasing. At that point ROT and AA were added to stop all mitochondrial respiration. Data collected from the O2k system was smoothed to reduce noise as well as normalized for cell number. In areas of interest, a stable section of the data was averaged, and bar graphs were made for easy comparison. In modeling, MATLAB code that was originally developed for modeling temperature variation in isolated mitochondria (3) was modified to predict changes in isolated mitochondrial respiration with different ADP concentrations. The predictions from this model did not match the experimental data (4). Therefore, model parameters, specifically the Michaelis-Menton constant ($K_m$ of ADP for adenine nucleotide translocase (ANT)) was changed until the modeling results fit more closely to experimental data.

**Results:** The FCCP-induced maximal respiration in both GK and WKY cells did not exceed the cells basal respiration under both normal and hyperglycemic conditions, in contradiction with expectations. The WKY high glucose cells had the lowest response of the four groups in every category except non-mitochondrial respiration. In GK cells, the hyperglycemic environment improved basal respiration, but impaired leak, max, and non-mitochondrial respirations. WKY grown in normal glucose had consistently higher respiration than GK normal glucose. In the isolated mitochondrial modeling studies, all the simulation results from each $K_m$ of ADP for ANT trial was gathered and compared to the experimental data (4). Five times the original codes' $K_m$ of ADP value was found to produce the most accurate model simulation results.

**Conclusion**

While there were differences in respirations between different groups, none were statistically significant. Having more trials would be a key advantage in this area. Data from the O2k respirometer was often noisy, and hence more time spent optimizing the protocol specifically for these cells would be beneficial. The modeling codes could be further modified to even more accurately predict mitochondrial respiration and increased in complexity to accommodate for cytosolic processes, to model the whole cell experiments.

*(numbers) correspond to references in the accompanying presentation

Cassandra Gliszinski - Milwaukee School of Engineering
Peter S. LaViolette, PhD
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**Quantification of Anti-Angiogenic Properties of Bevacizumab in CD31 Stained Autopsy Samples of Glioblastoma Multiforme**

## Background

Glioblastoma multiforme (GBM) is the most aggressive brain tumor with a mean overall survival of about 15 months, thus finding ways to prolong survival is imperative. Bevacizumab (BV), a vascular endothelial growth factor inhibitor, has been developed to slow angiogenesis. This study aims to characterize the pathological differences in vascularity between patients who were treated with BV and those who were not using digitized immunohistochemistry slides taken at autopsy. We hypothesized that patients treated with BV would have a quantifiably different vasculature compared to treatment naive patients.

## Method

Brain samples from 9 patients were obtained at autopsy, stained with CD31 immunohistochemistry (IHC), and digitized using a slide scanner. From these slides, 50 tiles were taken from patients who had been given BV, as well as 50 tiles from patients who had not. These tiles were analyzed using custom code created in MATLAB to generate a binary vessel segmentation and analyze vessel morphology. Wall thickness, area, circularity, major and minor axis length, perimeter, and vessel subtype were measured on each identified vessel. The vessel subtypes were categorized as Type 1 (capillaries and microvessels), Type 2 (mother vessels), or Type 3 (glomeruloid microvascular proliferations and bridged mother vessels). A one-way analysis of variance (ANOVA) was used to quantify vessel type and feature differences between the groups.

## Results

From all 100 tiles, there were more Type 1 and Type 2 vessels in BV patients. Statistical significance was found between several of the features when comparing vessel subtypes of patients with BV and patients without. The average area of Type 3 vessels of BV and non-BV patients showed a significant difference ($p < 0.0001$), as well as the average circularity of Type 1 vessels ($p < 0.001$). The average minor axis length of all 3 vessel subtypes also showed a significant difference [(Type 1, $p < 0.0001$), (Type 2, $p < 0.0001$), (Type 3, $p < 0.0001$)].

## Conclusion

The pathological characteristics of tumor vasculature are measurably different between patients treated with BV when compared to a group of control patients. Our findings suggest that BV has a role in changing tumor vascularity and may explain differences in survival between these two groups of patients. Future studies should aim to quantify differences in outcomes dependent on the vascular features.

Anna Wilczynski – Marquette University
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MCW Department of Radiology

**A quantitative analysis of tumor aggression using IHC staining on glioblastoma multiforme at autopsy**

## Background

Glioblastoma mutliforme (GBM) is the most common primary brain tumor in adults and is considered a grade IV tumor. Following biopsy or surgery, staining tissue samples using Ki67 and Epidermal Growth Factor Receptor (EGFR) allows pathologists to analyze GBM, specifically aggression of the tumor. Ki67 highlights mitotically active cells allowing the visualization of proliferation and active tumor invasion. Slides stained with Ki67 will have more abnormal positive cells in areas of tumor. The cell surface protein EGFR is overexpressed in about 60% of GBM. It promotes cell growth and division as well as overall cell survival. Tumors with abnormal EGFR often show excessive growth of cells. Slides stained with EGFR will have a higher percentage of brown in areas of tumor.

## Method

Patients were prospectively recruited for an ongoing project in the LaViolette Brain Bank. At autopsy, whole brain samples were sliced axially to match the contrast enhanced T1 MRI scan acquired prior to death. Specimens suspected to contain tumor were sectioned at autopsy; embedded on histology slides; H&E, Ki67, and EGFR stained; then digitized. Due to large file size, the digitized slides were tiled using a MATLAB script to allow for processing. Custom MATLAB scripts were run on 25 tumor and 25 non-tumor tiles stained with Ki67 and EGFR. Both scripts masked the tiles to only view positive staining. Metrics were measured on each positive cell on the Ki67 stained tiles. The overall percentage of EGFR was calculated on each EGFR stained tile. Additionally, the intensity of the brown color was separated into weak, moderate, and strong categories and the percentage of each category was then calculated. A t-test assuming unequal variances was performed on each metric calculated on the Ki67 tiles.

## Results

There was a significant difference found in eccentricity, orientation, and distance to nearest cell ($p<0.0001$). The percent EGFR overall along with weak, moderate, and strong had a higher average in tumor versus no tumor. Results show that there was a significant difference found between the overall percent EGFR with a $p< 0.005$

## Conclusion

Ki67 positive cells in tiles containing tumor had more abnormal metrics supporting the claim that tumor cells are more abnormal when compared to non-tumor cells. The higher percentages of EGFR in tumor slides confirm the hypothesis that EGFR mutations help GBM cells proliferate. These findings can assist in identifying tumors and gaining a deeper understanding into the mechanisms used to promote growth.

Andrew Yetka
Neurogranin Abstract

Neurogranin Regulation

Neurogranin (Ng) is a postsynaptic protein that enhances synaptic function and is abundant in the hippocampus, a brain region that is important in learning and memory. Many lines of evidence show that increasing neurogranin level in the hippocampal neurons enhances synaptic plasticity, which is the cellular model for learning and memory. Moreover, neurogranin transgenic mice show enhanced learning and memory. Thus, increasing neurogranin levels may have beneficial effects on learning and memory. In search of modulators that may enhance neurogranin levels, we have tested the effect of Norepinephrine (NE) on neurogranin levels in hippocampal neurons. To do this hippocampal organotypic slice cultures were made using P6 or P7 rat pups and these were treated with NE for various amounts of time. These treated slice cultures were then fractionated, and protein quantification was performed. Homogenate and synaptosomal samples were then created and run through a gel using an SDS page. Once the SDS page was finished the gels were transferred to a membrane and visualized with fluorescent antibodies. This method and process allowed for quantification of Ng. Due to the small sample size and some technical variability, data were inconclusive.

Key Words: Neurogranin, Norepinephrine, Synaptic Plasticity

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MCW Institute for Health and Equity

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**Exploring Conditions Which Have Facilitated the Transition from Prescription Opioid Misuse to Heroin Drug Use**

**Background**
The current opioid epidemic has resulted in varying public health responses. The goal of this research is to determine whether laws and policies put in place by the state of Wisconsin to reduce opioid misuse and diversion have had adverse effects on illegal opioid use. Our hypothesis is that Wisconsin laws regarding the prescription drug and monitoring plan (PDMP) have contributed to an increase in heroin use through decreasing the availability and increasing the price of prescription opioids.

**Methods**
Non-drug using key informants (e.g. first responders, substance abuse treatment providers, pharmacists, etc.) were interviewed to collect qualitative data on the opioid crisis in Wisconsin. Questions regarded key informants' knowledge and perceptions of laws put in place in Wisconsin, their effectiveness at combating opioid misuse, and unintended consequences of these laws. Interviews will be coded using MAXQDA software to allow for qualitative analysis of the collected data.

**Results**
The results of this research show a rise in heroin use and a decrease in prescription opioid misuse in WIs. Key informants agreed that current Wisconsin policies have worked for decreasing accessibility of prescription opioids. They also agreed that heroin is on the rise and is more prevalent on the streets than prescription opioids.

**Conclusion**
The findings of this project support the original hypothesis. As stricter laws and policies were enacted to combat prescription opioid misuse, drug users shifted to the more easily accessible and cheaper drug of choice, heroin. These findings are important for contributing to further research on the current drug crisis and potential laws and policies that will successfully confront and prevent the progression of this epidemic.

**Keywords**
Opioid Epidemic
Prescription Opioids
Heroin
Public Health
Wisconsin

Courtney Stephens – Marymount University

William Hughes, Ph.D. and Andreas Beyer, Ph.D.

MCW Cardiovascular Center-Department of Medicine

**Interaction Between Autophagy and Telomerase within the Cardiovascular System**

**Background:** Autophagy is a cellular recycling process that maintains homeostasis through clearance of damaged organelles that impede normal physiological function. Specifically, accumulation of damaged mitochondria is linked to initiation and progression of chronic diseases, and increasing autophagy may mitigate these physiological defects. Previous evidence from our lab, as well as others have demonstrated that autophagy and telomerase (specifically the catalytic subunit of telomerase, telomerase reverse transcriptase, TERT) are critical in maintaining the physiological microvascular endothelial function via cross-talk between TERT and autophagy within other tissues and cell types. Utilizing a genetically modified rat model in which TERT function is lost the goal of this project was to determine how TERT regulates autophagy within the heart. We expected that the loss of TERT results in decreased autophagy, which could explain the increased risk for cardiovascular defects. We also expected that an initiation of autophagy, independent of TERT would restore the physiological function.

**Method**: Wild-type (WT) and TERT knockout (KO) rats (12 wk old) were treated with the autophagy activator trehalose (2% in drinking water for 4 weeks) or vehicle. Hearts were collected at the time of sacrifice, snap frozen and prepared for western blot analysis. Western blots were utilized to examine levels of protein related to autophagy, microtubule-associated protein light chain (LC3B) and p62, while GAPDH was used as loading control.

**Results**: LC3B levels were not different between WT and KO ($p = 0.99$), and did not increase in response to trehalose supplementation (WT vs. WT + trehalose: 0.81; KO vs. KO + trehalose: 0.17). Similarly, p62 was not different between WT and KO ($p = 0.21$), did not change in WT with trehalose supplementation (WT vs. WT + trehalose $p = 0.14$), but increased in KO with trehalose supplementation ($p < 0.05$) with no differences in treated groups ($p = 0.24$).

**Conclusion**: In contrast to our hypothesis, there were no differences in markers of autophagosome formation (LC3B expression) between groups or in response to trehalose supplementation. In addition, there were no differences between groups in expression of p62 indicating equal levels of autophagosome clearance prior to supplementation. The increase in p62 in the KO group in response to trehalose was unexpected and could indicate a hyper-activation of autophagy and an aggregation of the p62 protein.

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Department of Medicine

**Effect of Enalapril on Preventing Doxorubicin-Induced Cardiotoxicity in H9C2 Cells**

**Background**

Anthracyclines are a class of chemotherapy drugs with cardiotoxic effects, in which oxidative stress has been established as the primary cause. Angiotensin-converting enzyme (ACE) inhibitors have been investigated as a potential counteractant of anthracycline induced cardiotoxicity. The aim of this study was to examine whether an ACE inhibitor, Enalapril, reduces oxidative stress *in vitro* when given as a pretreatment to Doxorubicin (Dox), a commonly used anthracycline. This was tested by observing the expression levels and locations of oxidative stress responsive proteins, nuclear factor erythroid 2-related factor 2 (Nrf2) and kelch-like ECH-associated protein 1 (Keap1), in Enalapril and Dox treated H9C2 cells.

**Method**

H9C2 cells were treated with 500nM Enalapril, Dox, Enalapril & Dox, or Media containing 0.1% DMSO. In all cases, Enalapril pretreated Dox by 3 hours. Cells were fixed 6 hours after initial drug exposure. Immunocytochemistry was used to determine Nrf2/Keap1 abundance and location. MitoSOX was used to determine the rate of reaction oxygen species (ROS) formation. For both experiments, images of the cells were taken with NIS-Elements Microscope Imaging Software and analyzed for Corrected Total Cell Fluorescence. A Western Blot was used to determine Nrf2/Keap1 protein expression level. Protein expression bands were visualized with Chemiluminescence Imaging and analyzed for Normalized Signal Intensity.

**Results**

H9C2 cells treated with Dox showed an increase in oxidative stress as indicated by elevated Nrf2 protein expression levels along with Nrf2 translocation into the nucleus. Pretreatment of Enalapril helped to mitigate the effects of oxidative stress as evidenced by a relative decrease in Nrf2 expression level and translocation. The rate of ROS formation also decreased when cells were pretreated with Enalapril.

**Conclusion**

These patterns suggest that pretreatment of Enalapril may reduce oxidative stress and protect against doxorubicin induced cardiotoxicity *in vitro*. The results also provide a promising foundation for expanding the scope of significance to *in vivo* studies and for clinical trials in humans.

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## The Role of Gut Microbiome in Sickle Cell Disease Pain

### Background
The gut microbiome plays an important role in human health. Shifts in gut microbiota have been linked to changes in anxiety, depression, anhedonia, and pain-like behaviors in rodents. All these symptoms have also been reported by patients with sickle cell disease (SCD), the most common inherited blood disorder. To date, nobody has examined what effect gut microbiome manipulation has on SCD symptom severity.

### Methods
To explore this question, we fed transgenic SCD mice a probiotic or antibiotic cocktail through their drinking water for 4 weeks. The Townes mouse model of SCD, which carries the same hemoglobin mutation as patients, recapitulates SCD phenotypes and exhibits hypersensitivity to mechanical and cold stimuli. We hypothesized that altering the gut microbiome would alter the cold and mechanical hypersensitivity exhibited by Townes SCD mice. Townes mice received one of two treatments: (1) VSL#3, a commercially available probiotic mixture that contains 3 strains of *Bifidobacterium*, 4 strains of *Lactobacillus*, and 1 strain of *Streptococcus* or (2) an antibiotic cocktail consisting of Ampicillin, Vancomycin, Neomycin and Metronidazole. During the four weeks of treatment, hind paw sensitivity to mechanical stimuli was measured using calibrated von Frey filaments and cold sensitivity was evaluated by measuring the withdrawal time after applying dry ice under a glass platform. Weight and treatment consumption were also measured across time.

### Results
Administration of the antibiotic cocktail was detrimental to SCD mice so was therefore terminated after one week of treatment. Data gathered suggests that ingestion of the probiotic VSL#3 decreased mechanical hypersensitivity in SCD mice after 1 week of treatment. This response to mechanical stimuli was consistent through the next 3 weeks of testing. Probiotic treatment had no effect on cold hypersensitivity of SCD mice.

### Conclusion
Altering the gut microbiome with a probiotic lessened mechanical pain in SCD mice. This shift in the gut microbiome did not ameliorate the cold hypersensitivity of SCD mice.

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**Optic nerve regeneration in YAP mutant zebrafish post optic nerve crush surgery.**

## Background

Yes Associated Protein (YAP), a transcriptional coactivator highly conserved throughout the animal kingdom, promotes organ growth and cell proliferation, and is frequently considered an oncogene, as it can overcome contact inhibition. YAP is inactivated by the Hippo pathway through phosphorylation, which sequesters the protein in the cell's cytoplasm. Due to this inactivation, the Hippo pathway promotes apoptosis, especially in organ growth, and is frequently studied in its applications as a tumor suppressor.

Zebrafish are model organisms and ideal for use in regeneration studies, as these fish can regrow almost all tissues in their bodies, including nerves, something which mammals cannot do. Zebrafish can regrow axons in their central nervous system, and when significantly damaged, their optic tract regenerates and the fish recover full vision within two to four weeks. Knowing that YAP promotes cell growth, we hypothesized that YAP signaling was involved in successful nerve regeneration in zebrafish. To test this hypothesis we examined optic nerve regeneration in YAP mutant fish with the expectation that regeneration would be compromised.

## Methods

YAP mutant fish were genotyped by PCR and Restriction Fragment Length Polymorphism. Optic nerve crush surgeries were performed on anesthetized adult GAP-43:GFP /YAP wildtypes, heterozygotes, or homozygous mutants by gently pulling the eye from the socket enough to pinch the optic nerve with a #5 forceps. Optic tract tissue was harvested four days post-injury, fixed in paraformaldehyde, and cleared using the passive clarity technique. Finally, the tissue samples were imaged via confocal microscopy and activation of the GAP was measured from the initial crush site.

## Results and Conclusion

Wildtype, heterozygous, and homozygous YAP mutant fish were successfully genotyped and underwent optic nerve injuries. The visual system from each animal was fixed and cleared to visualize nerve regeneration. However, the cleared tissue was exceedingly fragile and only one sample was left intact for imaging. Imaged under a florescent dissecting scope, florescence could be seen throughout the right optic nerve and into the optic tectum. This demonstrates the feasibility of using this method to monitor optic nerve regeneration. Future work may involve handling tissues with greater care so that confocal data can be obtained and regenerative growth can be quantified in YAP mutants versus controls.

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**Hyperoxia impairs angiogenesis function of fetal pulmonary artery endothelial cells by altered balance of angiopoietins 1 and 2**

The use of supplemental oxygen offers life-saving benefits for neonates in respiratory failure. However, excess or prolonged exposure to oxygen can also damage the lungs. The specific effects of oxygen on the lung endothelial cells and angiogenesis are relatively unclear. In this study, fetal lamb pulmonary artery endothelial cells (PAECs) were used to test the effects of hyperoxic conditions. To determine the mechanisms of decreased angiogenesis, we assessed the angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2) ligands and their respective biological pathway receptors under normoxic and hyperoxic environments. Western blotting was the method that was used to determine the levels of Ang1, Ang2, and downstream signaling molecule, Tie2. Additionally, we used Ang1 peptide and Ang2 blocking antibody treatments to determine whether they improve the tube formation function of PAEC in vitro in Matrigel. Tube formation is a measure of angiogenesis function of PAEC and was assessed in a quantitative manner with the angiogenesis tool of imageJ. Our results show that under hyperoxia conditions, PAEC angiogenesis pathway was downregulated. Adding the Ang1 peptide treatment during hyperoxia showed a trend for improving angiogenesis. The Ang2 antibody treatment to hyperoxia cells improved the tube formation function. Hyperoxia reduced Ang1 and Tie2 protein levels. We conclude that hyperoxia impairs angiogenesis function of endothelial cells by downregulation of Ang1-Tie2 pathway. Increased activity of Ang2 contributes to impaired angiogenesis under hyperoxia. Whether the use of Ang1 peptide or blocking Ang2 with antibody will protect the pulmonary vessels during oxygen use requires additional studies.

Key words: Ang1 peptide, Ang2 antibody, hyperoxic, normoxic

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**Human immune reconstitution in transgenic knock-in (KI) mice**

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Immunotherapy harnesses the bodies' immune system and is an emerging treatment to fight cancer. Murine xenograft models (human tumors grown in immunodeficient mice) have been used to study cancer treatments for sarcoma but require a human immune system to develop novel immunotherapies. Natural killer (NK) cells, an innate immune system cell, are known to attack tumor cells but have been inadequately studied as immunotherapy. Transplanting human immune cells (humanizing) can be performed in immunodeficient mice. NOD.Cg-Prkdc$^{scid}$ IL2Rgamma$^{null}$ (NSG) mice are profoundly immunodeficient and allow engraftment of human cells but there is poor maturation of NK cells. By using transgenic knock-in mice that are mutated to produce human cytokines, this may allow for better maturation of NK cells and better understanding of immunotherapy effects. We proposed utilizing three different murine models to study human NK cell engraftment for cancer immunotherapy development: NSG, NSG-SGM3 (producing human stem cell factor, GM-CSF, and IL-3), and NSG-IL-15 (producing human IL-15). We hypothesized that the NSG-IL15 mice will be superior in developing mature NK cells due to the role of IL-15 in promoting NK cell development.

Human hematopoietic stem (CD34$^+$) cells were injected intravenously into sub-lethally irradiated mice of NSG (n=4), NSG-SGM3 (n=4), and NSG-IL-15 (n=4) backgrounds and we monitored the reconstitution via flow cytometry analysis of peripheral blood. NSG without human CD34+ administration and human peripheral blood were utilized as controls. At 6 weeks, 100% of all mice strains acquired a ratio of human CD45$^+$/ mouse CD45$^+$ of 25% or greater, meeting the definition of humanized. B (CD19$^+$), T (CD3$^+$NKp46$^-$), NK (CD3$^-$NKp46$^+$), and myeloid (CD33$^+$) cells were present in all backgrounds. The NK cell population comprises a higher frequency of human lymphocytes for NSG-IL15 (22.2%) when compared to NSG (6.7%; p=0.12) and NSG-SGM3(8.3%; p=0.11). Further, the mature NK cell population (CD56$^{dim}$/CD16$^+$) was more prevalent in the NSG-IL15 mice (84.1%) compared to NSG (31.6%; p=0.06) and NSG-SGM3 (34.4%; p=0.02). In summary, our results demonstrate a successful humanization in NSG, NSG-IL15, and NSG-SMG3 murine models. Further, we identify the NSG-IL-15 transgenic mouse as an ideal candidate to further classify the effector functions of mature NK cells to study NK cell mediated immunotherapy *in vivo*. Our humanized murine models currently await tumor implantations for assessment of the differential maturation effect on human sarcoma tumor clearance.

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**Development of Immunotherapy for Pediatric Acute Myeloid Leukemia**

### Background and Significance

Immunotherapy is an emerging treatment that can be applied to many different types of cancer. One form of immunotherapy consists of patient T cells that have been engineered *ex vivo* to express a chimeric antigen receptor (CAR). CARs are synthetic receptors composed of an intracellular domain that is responsible for triggering cytotoxicity, and an extracellular domain that binds to an antigen expressed on the surface of a cancer cell. Acute Myeloid Leukemia (AML) is a type of blood cancer, commonly seen in pediatric patients, against which immunotherapies may be effective. Our objective is to construct a CAR that will recognize an AML cell-surface antigen, and induce cytotoxicity against AML cells.

### Methods

An anti-AML target antigen was identified, expressed, and purified by the lab. Post translational modification was assessed by using a deglycosylation kit and Coomassie Blue staining of SDS-PAGE. We panned an antibody phage display library for single chain variable fragments (scFv) that specifically bind to this target. Promising antibody clones were identified and their specificity for the antigen was assessed by ELISA. The DNA sequence of each clone was determined. Further assessment will be carried out by examining binding to AML antigen-expressing cell lines. OCI AML2 cells have been selected as one cell line for these studies. These cells do not naturally express the antigen, so they were transduced with lentivirus carrying a transgene for the AML protein. Transduction was analyzed with flow cytometry. From the pool of transduced cells, clonal cell lines are being developed by limited dilution and will be assessed for level of antigen expression.

### Results

The purified anti-AML protein exhibited a higher molecular weight than expected. Deglycosylation and Coomassie Blue staining of SDS-PAGE revealed that this was due to post translational glycosylations. The antibody phage display library was panned with this protein and 10 clones were selected for further analysis. ELISA results demonstrated that each clone tested was specific for the AML-antigen. DNA sequencing of the clones revealed that they share the same sequence, thus there is one candidate scFv. OCI AML2 cells that were transduced with lentivirus showed 30% transduction with the AML antigen. The clonal cell lines are growing, but will require more time before evaluation.

### Summary and Conclusion

One scFv specific for the anti-AML antigen was identified from the antibody phage display library. Once the clonal cell lines expressing the AML antigen have been expanded and verified they will be used to further validate the binding and specificity of the scFv. The scFv will then be developed into a CAR. The cytotoxic ability of this CAR will be tested *in vitro* and *in vivo* using mouse models of AML. Ultimately we aim to assess this CAR in clinical trials with the goal of improving AML patient outcomes and overall quality of life.

*Keywords: AML, Immunotherapy, Antibody, CAR*

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**Effects of gluten free diet and probiotic on survival and chemokine expression in the Biobreeding rat model for Type 1 Diabetes.**

**Background**

Type 1 diabetes (T1D) is an autoimmune disease which destroys insulin-producing β-cells (found in islet clusters) of the pancreas needed for glucose regulation. Environmental factors such as diet and microbiome are linked to diabetes incidence. Dietary wheat activates innate inflammation in disorders such as Celiac disease, and innate inflammation is likewise present in T1D families and rodent models of T1D. In rat models, a gluten-free hydrolyzed casein diet (HCD) increases survival and reduces inflammation. Recent studies have shown that the probiotic *Lactobacillus plantarum* 299v can lower inflammation in human subjects. This study examined whether *Lactobacillus plantarum* 299v supplementation could enhance the benefits of a gluten-free diet in spontaneously diabetic rats.

**Method**

The Diabetes Prone Bio Breeding Rat (BBDP) possesses the T1D risk loci IDMM1 and IDMM2, and spontaneously develops diabetes by about 63 days of age. Rat survival and β-cell production of the proinflammatory chemokine CXCL10 was examined in rats provided a gluten free diet with *L. plantarum* supplementation. *L. plantarum* at 5 x 10^6 cfu/µl PBS was delivered by gavage at a daily dose of 50 x 10^6 cfu/g of bodyweight. Control groups included BBDP provided HCD, BBDP on normal chow, BBDP on normal chow plus *L. plantarum,* and Fisher F++ rats (possess the IDDM1 locus but do not develop diabetes) on both normal chow and HCD. Survival studies were carried out to 130 days of age. Fasted blood glucose was measured every other day beginning at 45 days of age. Diabetes was defined as the first date of two consecutive fasted readings of ≥250mg/dl. Pancreas from rats aged 40±2 days were stained with fluorescent anti-CXCL10 and anti-insulin. CXCL10 protein expression was quantified in 14-28 islets per rat, using three rats per cohort.

**Results**

T1D onset in BBDP rats provided normal chow was not significantly affected by administration of *L. plantarum*, although one of the 12 rats in this group survived. Gluten-free hydrolyzed casein diet significantly delayed diabetes, with HCD/*L. plantarum* providing the greatest overall survival; 25% of the rats reached 130 days disease-free, with onset delayed to an average of 84.9 days as compared to 72.2 days for HCD alone. Results show a decrease in β-cell CXCL10 expression in rats provided HCD and *L. plantarum* compared to rats fed HCD alone. Expectedly, the Fisher F++ rats showed the lowest levels of CXCL10.

**Conclusion**

Rat survival shows the benefit of the probiotic *L. plantarum* when combined with a gluten free diet. Although one rat on normal diet with probiotic lived to 130 days, survival of this group was not statistically significant from rats on normal chow. The reduction in CXCL10 islet expression further suggests that probiotic supplementation with a gluten-free diet ameliorates T1D pathogenesis.

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**Pulmonary arteriovenous malformations: Apoptotic protein levels in capillary endothelial cells**

**Background**

Patients born with single ventricle congenital heart disease are treated with three palliative surgeries: Norwood, Glenn, and Fontan. After the Glenn procedure, 60-100% of patients develop pulmonary arteriovenous malformations (PAVMs). With the Glenn, effluent from the hepatic vein (HV) is excluded from pulmonary circulation. After the Fontan, HV blood flow returns to pulmonary circulation and PAVMs regress. This suggests that an unknown hepatic factor is essential in preserving the normal integrity of the pulmonary vasculature. We investigated the role of the hepatic factor in the formation of PAVMs through signaling pathways such as apoptosis. Our preliminary data suggests blood from the HV decreases capillary endothelial cell apoptosis. The objective of our study was to analyze apoptotic protein levels in endothelial cells treated with patient serum.

**Method**

We collected blood from the HV and SVC from pediatric patients undergoing palliative cardiac surgery. We treated capillary endothelial cells with patient serum and performed western blot assays to analyze apoptotic protein levels. Our study focused on pro-apoptotic proteins Bax, p53, cleaved caspase-3, and cleaved PARP-1. We also analyzed anti-apoptotic protein Mcl-1. We used beta-actin to normalize the protein sample sizes and Image J to quantify relative protein expression. Paired t-tests were used to compare protein levels.

**Results**

We found no significant difference in Bax levels (HV: $1.01 \pm 0.22$, SVC: $1.03 \pm 0.25$, n=9 (p=0.81)). With limited patient samples, there was a non-significant upward trend of p53 in SVC treatment (HV: $1.30 \pm 0.16$, SVC: $1.37 \pm 0.11$, n=4 (p=0.09)). No differences were observed in pro-apoptotic proteins (cleaved caspase-3 and cleaved PARP-1) or anti-apoptotic protein (Mcl-1) (n=4).

**Conclusion**

There were no significant differences in pro-apoptotic and anti-apoptotic protein levels in endothelial cells after serum treatments. A non-significant upward trend of p53 for the SVC treatment supports our hypothesis formed from preliminary data that blood from the HV decreases apoptosis. A limitation to these results is the low number of patient samples. Further analysis with more patient samples is needed to confirm any differences in apoptotic protein levels with HV and SVC treatments.

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**Antifungal activity of chemokines in treating *Candida* species**

**Background**

*Candida* species are the most common pathogen known for inducing fungal mucosal infections. *Candida* normally lives as a commensal organism in healthy individuals. However, it can cause serious, life-threatening infections in the immunocompromised, those who have had invasive surgery, and those in the intensive care unit (ICU) for significant amount of time[2]. The two most common strains of *Candida* are *C. albicans* and *C. glabrata*, with *C. albicans* being the most common strain. Though antifungal drugs are available, they are not always capable of treating both these common strains of *Candida*. Specifically, *C. glabrata* is known to be more resistant to treatment than *C. albicans*[3]. Antimicrobial peptides (AMPs) are found in many multicellular organisms and are known to be directly cytotoxic to bacteria, fungi, parasites, and viruses[4][5].

**Method**

In this study, the antifungal properties of SEA CCL28 and WT XCL1 were tested[*]. 100 µL of $5 \times 10^4$ cells/mL of *C. albicans* (strain Caf2-1) and *C. glabrata* (ATCC stain No: 2001) were incubated with 100 µL of serials dilutions of the chemokines at 25ºC for two hours. 50 µL of the mixtures were then spread onto agar plates to culture at 30ºC for 48 hours. Each treatment was done in triplicate. Colonies were counted after 48 hours to determine the anti-fungal potency of SEA CCL28 and WT XCL1. Two clinical isolates of *C. albicans* from human oral candidiasis (gifted by Dr. Michaeil Lionakis, NIH) were tested under the same conditions with the chemokine CCL28 Δ3.

**Results**

Results show that both SEA CCL28 and WT XCL1 are potent AMPs to *C. albicans* exhibiting complete killing at a concentration of 1µm. Conversely, *C. glabrata* only attains ~79% killing when treated with SEA CCL28 and WT XCL1. Increasing concentration of chemokine did not result in more killing. Both proteins lose killing activity to *C. glabrata* when the concentration is higher than 2µM. CCL28 Δ3 exhibited complete killing of the two clinical isolates tested.

**Conclusion**

Chemokines SEA CCL28 and WT XCL1 are highly cytotoxic to *C. albicans* but less potent to *C. glabrata*; these findings will help develop more effective drugs to fight fungal infections.

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[*] Chemokines isolated by Brian Volkman's lab

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**The Effects of High-Rate Pulse Trains on Speech Perception in Cochlear Implant Users**

**Background:** Cochlear implantation is a successful method to correct sensorineural hearing loss. Although cochlear implant (CI) research has made incredible advancements in technology over the years, there are still many areas to improve. Speech perception remains challenging for CI users in noisy conditions, due to abnormally high neural synchrony, leading to a decreased dynamic range and poor electrode discrimination. A proposed solution for this is presenting high-rate pulse trains (HRPs), which is a form of constant-amplitude pulsatile electrical stimulation. It is theorized that the presence of HRPs drive auditory nerve fibers into a relative-refractory state causing a larger number of desynchronized neurons, and therefore more closely modeling a normal cochlea.

**Methods:** MED-EL CI users who had an AzBio score of at least 30% and a narrow pulse duration (μs) were eligible for this study. Demographics of the participants were also reviewed beforehand (age at implantation, age at test, length of deafness, etiology, internal device & electrode, etc.) To account for the variability in most-comfortable levels (MCLs) across participants, the HRP stimuli was designed to fit each participant individually. However, high-rate (HR) levels were kept constant across the groups, consisting of five different levels (0, 20, 36, 52, 68μA) including a baseline with no HRPs. Speech perception testing stimuli consisted of the Institute of Electrical and Electronics Engineers (IEEE) sentence lists and were presented in both quiet and noise conditions. In addition, subjective measures were taken after each condition, in which the participants rated how the sentences sounded in terms of clearness, word distortion, and easiness to understand.

**Results:** Participants' maximum speech perception scores with a given HRP stimulus was indicated as "max-HRP.' There were both decrements and improvements in performance while using HRPs, but the majority of participants showed similar or increased percentage correct in their max-HRP condition in either quiet or noise. Regarding the calculations of seven participants, improvements in speech perception with max-HRP in quiet ranged from 0.2-15.7%, and 2.4-18.0% in noise. According to the subjective measures, participants were consistent in marking their max-HRP level as their level of preference – rating high in clarity, low in word distortion, and the easiest condition to understand. Further statistical calculations will be performed once the testing portion is complete.

**Conclusion:** Our results thus far indicate that the addition of HRPs can be beneficial to MED-EL CI users in speech perception. It is possible that the HRPs presented in this study improved electrode discrimination and allowed for an increased dynamic range in participants, although this was not directly measured. The majority of the participants improved in speech perception performance in the addition of HRP stimuli, with the exception of one individual who scored very well at the baseline. This indicates that HRP stimuli may be more beneficial to individuals who have lower baseline speech perception scores. Further analyses will be conducted in the future since there are more participants scheduled for this experiment. This study was valuable in our understanding of the usage of high-rate pulse trains with speech perception and contribute to our experience with cochlear implant technology.

**Keywords:** Dynamic Range, Electrode Discrimination, Neural Desynchronization, Speech Perception, Constant-Amplitude Pulsatile Electrical Stimulation, Stochastic

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## Transcriptional Role of Stra6 In Zebrafish RPE

Vitamin A (all-*trans*-retinol) is vital in maintaining visual function. Fetuses will not develop their eyes fully if there is a mutation on both copies of the *stra6* gene, causing disorders such as anophthalmia and Matthew-Wood syndrome. Vitamin A is needed in one's diet throughout their life, and deficiencies can lead to concerns such as blindness, retinal degeneration, and immune disorders. The STRA6-retinol binding protein 4 (RBP4) axis is important in providing the retina vitamin A which is rapidly turned over in the eyes. STRA6 is a plasma membrane-bound receptor that binds holo-RBP4, which binds and carries vitamin A circulating in the bloodstream and cerebrospinal fluid, allowing it to be soluble in extracellular and intracellular aqueous solution. If any mutations occur on the RBP4-binding site of STRA6 holo-RBP4 cannot bind. Holo-RBP4 binding to STRA6activates the Janus kinase (JAK)/STAT cascade that induces the transcription of STAT3- and STAT5-regulated genes. The JAK/STAT pathway is highly pleiotropic and is associated with developmental and homeostatic processes, including cell proliferation, differentiation, cell migration, and apoptosis. The first goal was to use *stra6 -/-* zebrafish to rescue photoreceptor degeneration, through the use of dietary supplementation with retinyl palmitate or beta-carotene. The second goal was to clone and use various interleukin cytokines as activators of the JAK/STAT pathway, while STAT3:mCherry and STAT5:eGFP reporter constructs were used as readouts. Our hypothesis is that STRA6regulates the transcriptional process vital for RPE regulation and function. A total of 5 mutant zebrafish were provided dietary supplementation of retinyl palmitate and beta carotene. Vitamin A supplements were delivered by absorbing them onto fish food before monitored feeding. Zebrafish were provided beta-carotene, retinyl palmitate, or vehicle food alone. Retinal images were taken on the right eye of each zebrafish, using optical coherence tomography (OCT) once a week to note changes to each retina. Eye metrics were acquired using the OCT's InVivoVue software and the results were analyzed using SPSS. Zebrafish were sacrificed and cryosectioned for histology to examine photoreceptor morphology. Interleukins used in this study were amplified from zebrafish cDNA and cloned into the Gateway system. The computer program ApE was used to design primers for each activator and used Multisite Gateway to BP clone IL2, and IL15, microinjecting the plasmid into zebrafish embryos.

After controlling for the effect of pre-treatment measurements, treatment type showed significant differences in the post-treatment measurement results. The retinyl palmitate treatment was found to have significantly greater increases in retinal thickness compared to the control and beta carotene treatments, whereas no significant difference was found between control and beta carotene.

In *stra6 -/-* zebrafish, mutations to the RBP4-binding site prevent transportation of vitamin A to the eyes, leading to visual deficiencies. Retinyl palmitate facilitates the vitamin A recovery, through alternative uptake routes. These findings could provide information for alternative treatments that allow eyes to develop properly and reduce the possibility of visual disorders and diseases.

While working at my internship, I performed additional work on a variety of projects with Dr. Ross Collery, with many involving immunohistochemistry. For one of these projects, I performed immunohistochemistry of ablated ultraviolet photoreceptors, where we tracked the progression of macrophages and Muller glia morphology. In another project, I performed immunohistochemistry of *lrp2* mutant and wild-type zebrafish in order to determine the extent of macrophage activation, in the choroid plexuses. I also conducted

immunohistochemistry to visualize cones, rods, and nuclei, as well as to compare a variety of macrophage markers, (4C4 antibody, anti-Lcp1 antibody, anti-MPEG-1 antibody, and GS-IB4 solectin.

In addition to immunohistochemistry, I performed genotyping on other projects. As part of Dr. Collery's research into the function of the *serpine3* gene during eye development, I genotyped *serpine3* mutants, wildtype, and heterozygotes, and then calculated their specific eye measurements. I also genotyped dopamine receptor d3 Zebrafish to help with research into the role of dopamine signaling on axial length during eye development. Other tasks I performed included learning transgenic techniques, such as microinjections, to work on zebrafish embryos.

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**Developing a Deep Learning Program to Determine Cancerous Regions on MRI**

**Background**
Although many MRI scans are done per year, the best way to determine if a conventional scan shows cancer or pseudo-progression is by a clinician's best estimation. Over the years, more advanced imaging techniques have been developed to provide more confidence in the diagnoses. Using these methods in combination with specialized software programs, this lab is in the process of developing a deep learning network that can sort through images and determine if a region of contrast enhancement shows tumor or pseudo-progression.

**Method**
A Deep Learning program was designed that is fed a training set of images, allowing it to learn what would be classified as either tumor or non-tumor regions of an image. This training set was made using patients' MRIs collected before surgery. This provided images that might show areas of treatment effect or tumor progression inside the contrast-enhancing area. The images were then manually processed, placing regions of interest in known tumorous or non-tumorous areas. This training set was then used to allow the Deep Learning program to learn whether specific regions should be classified as tumor or non-tumor.

**Results**
Using the training set of images, the program was trained to determine regions of tumor and non-tumor within the lesion. This trained program was then tested with a different set of images, the testing set. The program correctly classified specific regions about 93% of the time. These preliminary results are very promising, but there is still room for further refinement and improvement.

**Conclusion**
The main purpose of this project was to develop a Deep Learning network that could take in medical images from MRIs and determine if the contrast-enhancing regions contained tumor or non-tumor. It is hoped that this will help solve clinical questions, for example if some of the tumor was missed after a surgery. It could also help distinguish regions of contrast enhancement as treatment effect or tumor progression.