



# 2017 Annual Meeting Montana Academy of Sciences

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## Abstracts for Poster Presentations (Alphabetical by Title)

### ANALYSIS OF BH3I-1 DERIVATIVE'S EFFECT ON CANDIDA SPECIES (POSTER)

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*Candida* species are the most common and arguably the most important causative agents of human fungal infections. Oropharyngeal, esophageal, vulvovaginal, and cutaneous candidiasis leads to significant morbidity while systemic infections in immunocompromised patients (patients with AIDS, tissue transplants, central venous catheters, or those undergoing chemotherapy) has a 35% mortality rate. During infection, it is essential that the dimorphic *Candida* species switch between different morphological states including transitions between budded or yeast-like cells and hyphal forms. The small molecule BH3I-1 has shown promising results at inhibiting hyphal formation in several *Candida* species. The goal of this study is to find a BH3I-1 derivative that inhibits hyphal formation in several *Candida* species at a lower minimum inhibitory concentration (MIC) than BH3I-1. A derivative with a low MIC that affects several *Candida* species may have a potential to be a broad-spectrum antifungal drug. The *Candida* species being tested against the BH3I-1 derivatives are: *C. albicans*, *C. glabrata*, *C. rugosa*, *C. krusei*, *C. tropicalis*, *C. lusitaniae*, *C. dubliniensis*, and *C. parapsilosis*. Currently, 36 BH3I-1 derivatives have been tested. Molecule 25 has an MIC about 4 times lower than BH3I-1 in *Candida albicans* and has also been shown to work in other *Candida* species at inhibiting hyphal formation. Other derivatives such as molecule #10 did not inhibit many of the tested *Candida* species, but showed a much lower MIC than molecule #25 in *C. rugosa*. Out of the 36 tested derivatives, molecule #25 has shown the promise for a broad-ranged antifungal drug.

## BEHAVIORAL ECOLOGY IN THE NORTHERN SCORPION (*PARUROCTONUS BOREUS*) (POSTER)

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The Northern Scorpion (*Paruroctonus boreus*) is a predatory arachnid. Although occurring at relatively high densities in local areas, conspecifics have seldom been observed sharing cover items. We investigated territoriality of scorpions by analyzing pairs of scorpions introduced into a habitat with a single, small cover item. We used mono- and bisexual pairs, similar and differently sized pairs, and pairs from the same or different populations. Scorpions were collected from two populations in south-central Montana.

Results indicate that scorpions do interact over cover items, though not to the extent that we had anticipated. When scorpions were housed singly, they spent 80% of their time under cover. When size-matched pairs were offered a single cover item, up to 60% of the time at least one scorpion was not under cover. Further, when differently-sized scorpions were paired, a similar result obtained with the larger scorpion excluding the smaller most often. Interestingly, these results all differed by population and sex. Scorpions from the naturally more-dense population excluded others more frequently than scorpions from the less-dense population. Additionally, males excluded other males more frequently than mixed-sex pairings excluded one sex or the other, or than females excluded other females. Finally, late in the experimental season (early Spring), there were six instances of cannibalism. In each case, females killed and consumed males. Though cannibalism has been previously documented in this genus, it has not been observed to be “seasonal” and has been attributed to size differential and not simply sex. In one of our cases, a smaller female killed and consumed a larger male. This pilot project provides several interesting questions to pursue regarding behavioral ecology in this species.

## CARDIAC AND PERCEPTUAL RESPONSES TO PERFORMING TANDEM CARDIOPULMONARY RESUSCITATION (POSTER)

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Introduction: Information regarding the physiological and perceptual response of the human body in the act of performing tandem cardiopulmonary resuscitation (CPR) relative to solo CPR is lacking. Purpose: The purpose of this investigation is to compare rescuer heart rate (HR), rating of perceived exertion (RPE), and CPR quality during Tandem-CPR and Solo-CPR. Methods: Thirteen healthy young adults (aged  $26.5 \pm 4.3$  yrs) were recruited from MSUB campus community. Participants completed two 6-minute bouts of CPR during a single session. Tandem and solo techniques were counterbalanced, with a 15-minute rest period separating the bouts. Values for HR and RPE were recorded using a Polar V800 HR monitor and Adult OMNI-RPE scale, respectively. A Laerdal Resusci-Anne CPR manikin was used to record compression score (0-100%), which is a value that incorporates compression rate and depth to illustrate CPR quality. Mean HR, peak RPE and CPR compression scores were examined with dependent

t-tests between CPR techniques. Statistical significance was accepted at  $p < 0.05$ . Results: Sample mean HR per bout was significantly lower in Tandem-CPR than in Solo-CPR ( $111.2 \pm 16.8$  vs.  $126.1 \pm 19.3$ ,  $p < 0.0001$ ). Peak RPE was significantly lower during Tandem-CPR compared to Solo-CPR ( $3.2 \pm 2.0$  vs.  $5.0 \pm 2.5$ ,  $p < 0.05$ ). Compression scores were significantly higher for Tandem-CPR when compared to Solo-CPR ( $96 \pm 3\%$  vs.  $94 \pm 5\%$ ,  $p < 0.05$ ). Discussion: Current findings call for a professional recommendation that tandem CPR be used when available, based on perception, performance, and physiological differences. This confirms professional guidelines. This study does not account for the anecdotally reported stress incited in CPR context; further research should examine this aspect.

#### CBU\_1932: A HYPOTHETICAL DNA-BINDING PROTEIN OF THE Q FEVER PATHOGEN *COXIELLA BURNETII* (POSTER)

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*Coxiella burnetii* is an obligate intracellular bacterial pathogen that resides within a lysosome-like acidic compartment of the eukaryotic host cell and may cause acute and chronic human infections. Our recent transcriptome analysis of *C. burnetii* demonstrated that the CBU\_1932 open reading frame displayed an exceptionally high transcript level at 11,481 transcripts per million (TPM), well above average transcript quantity for remaining ORFs in the genome. Due to its high transcript level we hypothesize the corresponding protein may play an important role for *Coxiella*. Analysis of the CBU\_1932 locus indicates that one of the adjacent ORFs, CBU\_1933 is a hypothetical DNA binding protein. The protein encoded by CBU\_1932 ORF consists of 66 amino acid residues with an unusually high percentage (42%) of residues being basic, including 20 lysines. Using BLAST algorithms we found CBU\_1932 had no similarity with currently defined proteins, but has orthologues in other human intracellular pathogens such as *Legionella* and *Chlamydia*. Due to the high number of basic residues in CBU\_1932, and linkage with a hypothetical DNA binding protein (CBU\_1933), we hypothesize that CBU\_1932 may also encode a protein involved with binding DNA or other negatively charged substrates. To address this hypothesis, we are in the process of cloning the 201-base pair CBU\_1932 ORF into pMAL-c5x expression plasmid and analyzing the recombinant protein using DNA-binding protocols including electrophoretic mobility-shift assay EMSA. We are confident that characterization of this high-level transcript/highly basic protein will lead to a better understanding of the unique metabolism of *Coxiella* and other intracellular pathogens.

#### CHARACTERISTICS OF THE EFFECT OF EXOGENOUS CAMP ON *C. ALBICANS* MORPHOGENESIS IN STRAINS LACKING NRG1P, RFG1P, OR TUP1P (POSTER)

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The opportunistic human pathogen *Candida albicans* causes both superficial and life threatening systemic infections and is a leading cause of fungal disease in immunocompromised individuals such as those with AIDS. *C. albicans* can grow in different cell shapes, also known as morphologies, including

yeast-like cells and a variety of filamentous forms, such as true hyphae and pseudohyphae. Yeast, hyphae and pseudohyphae, have been observed at the sites of *Candida* infection and there is strong evidence that morphogenesis, the transition between yeast and filamentous growth forms, is essential for its virulence. Many studies have implicated the second messenger molecule cAMP in the regulation of morphogenesis due to its role in activating filamentation. Our lab and others have previously characterized the impact of the negative regulators, Nrg1, Rfg1, and Tup1 on the expression of HWP1, a hyphal specific gene. The goal of this project is to characterize whether the addition of exogenous cAMP will increase the expression of HWP1 in the absence of each of the negative regulators as well as test a small molecule derivative of BH3I's effects in conjunction with the exogenous cAMP. This will help us better understand the signal transduction cascade that controls morphogenesis in *C. albicans*.

#### DETERMINATION OF SOLUBILITY LIMITS FOR PDTC AND CU[PDTC]BR (POSTER)

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Carbon tetrachloride ( $\text{CCl}_4$ ) is an organic compound that once widely used as an industrial solvent, degreaser, and grain fumigant. Improper disposal and ground water solubility issues led to  $\text{CCl}_4$  being listed as a priority pollutant by the U.S. EPA. We are currently investigating the use of 2,6-pyridinedithiocarboxylic (PDTC) and its copper coordination compound  $[\text{Cu}(\text{PDTC})\text{L}]^x$  as a potential  $\text{CCl}_4$  environmental remediation technology. PDTC and  $\text{Cu}[\text{PDTC}]\text{Cl}$  have a very low solubility in water, which decreases the effectiveness of the molecule as a remediation technique. Using a ligated iron UV-Vis spectroscopy assay PDTC's solubility was determined to be 39 mM in a pH 7.6 (check this number) buffer. The solubility of  $\text{Cu}(\text{PDTC})\text{Br}$  in pH 7.6 buffer was determined to be 50 mM via oversaturation UV-VIS assay. The results from both solubility studies were used to confirm a high throughput assay based on dynamic light scattering. The solubilities determined in this study will act as an initial benchmark for the comparison of future novel PDTC derivatives.

#### DEVELOPING A YEAST MODEL OF AMYOTROPHIC LATERAL SCLEROSIS INVOLVING THE SOD1 GENE (POSTER)

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Amyotrophic lateral sclerosis (ALS), one of the most common neuromuscular diseases in the world, is an unremittingly progressive disease that degenerates motor neurons in the brain and spinal cord. Roughly 10% of ALS cases are considered familial and can result from mutations in more than dozen different genes. The most common mutations in familial ALS occur in the SOD1 gene. SOD1 encodes a copper-zinc superoxide dismutase that detoxifies oxygen free radicals. To date, approximately 140 mutations in SOD1 (many of which are missense) have been linked to familial ALS. Evidence suggests that these mutations induce SOD1 protein misfolding and aggregation into cytotoxic structures. We are developing a yeast model of ALS based on the expression of mutant human SOD1 proteins. Such a yeast system will permit high throughput genetic screens to identify genes that enhance or suppress the toxic

phenotypes associated with mutant SOD1 expression (thereby identifying critical supporting or suppressing pathways), as well as chemical screens to identify compounds that inhibit mutant SOD1 toxicity.

## DOES THE MISREGULATION OF CODON-BIASED GENES IN THE ANTERIOR PITUITARY CONTRIBUTE TO FAMILIAL DYSAUTONOMIA? (POSTER)

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Familial Dysautonomia (FD) is a devastating neurodevelopmental and neurodegenerative childhood disease characterized by a diminished number of autonomic neurons. FD children suffer from a multitude of autonomic symptoms including cardiovascular instability, gastrointestinal incoordination, and respiratory dysfunction. FD patients also exhibit an abnormal autonomic stress response, tend to be small in stature, and have difficulty gaining and maintaining weight. FD results from a mutation in the *IKBKAP* gene and diminished levels of the corresponding protein IKAP, a scaffold that assembles the multi-subunit complex, Elongator. Elongator functions in the modification of tRNAs that mediate translation of AA- and AG-ending codons. IKAP is expressed throughout the autonomic nervous system and historically FD symptoms have been attributed to autonomic dysfunction. Here we show that IKAP is also robustly expressed in the pituitary gland, both during development and in the adult. We hypothesize that many FD symptoms may actually result from aberrant pituitary regulation of the autonomic nervous system. To test this hypothesis we are currently generating a conditional knockout mouse where *Ikbkap* will be selectively ablated in the anterior pituitary. While waiting for our mouse model, we have been optimizing techniques for quantifying pituitary specific genes that are likely candidates for Elongator regulation based on their content of AA- and AG-ending codons.

## ENDOPLASMIC RETICULUM-ACTIVATED C/EBP HOMOLOGOUS PROTEIN MEDIATES THE PALMITATE-ENRICHED DIET INDUCED INCREASE IN THE LIPOGENIC EXPRESSION IN THE LIVER (POSTER)

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Non-alcoholic fatty liver disease (NAFLD) is a wide spectrum pathophysiological disorder characterized by insulin resistance, hepatic steatosis, and inflammation. Diets rich in saturated fat are known to evoke insulin resistance, ER stress, and de novo lipogenesis and thereby contribute to the pathogenic mechanisms involved in NAFLD. Palmitic acid (palmitate) is the most abundant saturated fatty acid in the diet and palmitate-enriched diets are known to cause NAFLD in a multitude of rodent models of NAFLD. Palmitate-enriched diets are known to induce steatosis by inducing the expression of genes involved in de novo lipogenesis. However, the signaling mechanisms and the downstream molecular mediators involved have not been elucidated. In this study, we explored the role of palmitate-induced ER stress and subsequent induction of C/EBP Homologous Protein (CHOP) expression in the

modulation of expression and transcriptional activities of Liver X Receptor alpha (LXR $\alpha$ ) and Sterol Response Element Binding Protein 1c (SREBP1c), two indispensable transcription factors and master regulators of genes involved in de novo lipogenesis. We demonstrate, in exogenous palmitate-treated HepG2 cells and in the livers of palmitate-enriched diet-fed mice, that palmitate evokes ER stress leading to the induction of CHOP expression. We further show that CHOP mediates the up-regulation in expression levels and transcriptional activities of LXR $\alpha$  and SREBP1c. Our study identifies a unique ER stress-CHOP-LXR $\alpha$ /SREBP1c signaling pathway that mediates palmitate-induced up-regulation of lipogenic gene expression in the liver that may play a critical role in the etiopathogenesis of NAFLD.

## THE EFFECTS OF CHRONIC CORTISOL EXPOSURE ON THE INNATE IMMUNE RESPONSE OF LARVAL DANIO RERIO (POSTER)

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Chronic stress is known to cause a variety of health complications linked to a dysregulated immune response, which could be an outcome of chronically elevated stress signaling mediated by the glucocorticoid steroid hormone cortisol. Previous research has shown that zebrafish embryos treated with cortisol for the first 5 days of development matured into pro-inflammatory adults with atypical regulation of immune-related genes (Hartig et al., 10.1242/bio.020065, 2016). The purpose of this study was to determine how chronic exposure to cortisol affects the innate immune response in larval zebrafish. To that end, the migration of neutrophils and macrophages were monitored following tail fin amputation. Results from this study provide evidence that cortisol-treated larvae had an increased number of macrophages near the amputation site, while the number of neutrophils was not significantly affected by cortisol exposure. These results suggest that chronically elevated glucocorticoid signaling specifically up-regulates the macrophage response to injury.

## THE EFFECT OF THE CLUFFY WEDGE ON THE AVERAGE VERTICAL JUMP HEIGHT OF VOLLEYBALL ATHLETES (POSTER)

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The aim of this study was to determine if placing the big toe in a dorsiflexed position affects the average vertical jump height of volleyball players and whether the grade of functional hallux limitus (FHL) limits its effectiveness. A polyurethane wedge (Cluffy Wedge) was placed under the big toe of 30 volleyball athletes. Two sets of three vertical test jumps were completed for each individual and t-tests of average jump heights showed no difference with or without the Cluffy Wedge. The subjects were then tested for FHL. Results showed slight significance in average jump heights for FHL grade 0 ( $p=0.1401$ ), while showing no significance in FHL grade 1 ( $p=0.6949$ ) and 2 ( $p=0.9045$ ). In conclusion, using a Cluffy Wedge to induce dorsiflexion of the big toe did not affect the average jump height of volleyball athletes and the FHL grade had no influence.

## FACTORS INFLUENCING OWNERS' WILLING TO PAY FOR VETERINARY MEDICINE SERVICES FOR PET DOGS: A PILOT STUDY (POSTER)

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The field of veterinary medicine has been changing dramatically over recent decades, as society changes its perspective on animals. Throughout human history, people have seen animals as a resource; now we are seeing them as companions or friends. A way to measure this changing perspective is to look at the amount of money that people are willing to spend on their pets. This study looked at dogs, in particular, and utilized a survey method to analyze factors that could influence people’s willingness to pay for veterinary medicine services. Factor analyzed included the severity of the condition (life-saving aspect of surgery), likelihood of normal recovery, age of the dog, income level of owner, amount of owner’s dog experience, and whether the owner was a health professional of any kind. Results: People were more willing to pay when the surgery was life-saving and when there was high likelihood of recovery. People were willing to pay more for younger dogs. Not surprisingly, there was a positive correlation between income level and owners’ willingness to pay for veterinary services. Amount of experience with dogs had a varied effect on willingness to spend. It appeared that the owners’ connection with health care professions also had an impact, although it did not reach statistical significance: those that were connected to the healthcare fields seemed more likely to pay. This study has implications for the practice of veterinary medicine today.

## HOW AQP3B INFLUENCES CONVERGENT EXTENSION THROUGH NONCANONICAL WNT SIGNALING (POSTER)

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Aquaporin-3b, Aqp3b, is an aquaglyceroporin, a membrane water channel that is present during gastrulation and various other stages of development. Gastrulation organizes cells, via convergent extension, into germ layers, which will later form different body tissues. During gastrulation, cells fold into the embryo, then merge by convergent extension to form the long body axis. These cell movements are regulated by noncanonical Wnt signaling, an intercellular signaling pathway that controls the migration and polarity of tissues. When Aqp3b is inhibited using a morpholino oligonucleotide (MO), convergent extension does not occur properly, suggesting a link between Aqp3b and noncanonical Wnt signaling. To assay these defects, we use the Keller tissue explanting method to observe convergent extension. Our goal is to determine which parts of the Wnt signaling pathway are influenced by Aqp3b. We conducted rescue experiments by inhibiting Aqp3b and injecting an RNA or DNA construct of several proteins involved in Wnt signaling. Successful rescue with Dvl1 $\Delta$ Dix and Dvl2 $\Delta$ Dix constructs indicated that Aqp3b is involved in noncanonical Wnt signaling, since Dvl $\Delta$ Dix acts in all noncanonical Wnt signaling. Further, Aqp3b acts through the Wnt/Ca<sup>2+</sup> subpathway, indicated by rescue by PKC, and through a branch of the Wnt/PCP pathway, indicated by successful rescue with RhoA but not with Rac1. Aqp3b does not directly affect the Wnt/Ror2 pathway. In conclusion, I have demonstrated that the ability of Aqp3b to influence convergent extension is dependent on noncanonical Wnt signaling, specifically the Wnt/Ca<sup>2+</sup> pathway and the RhoA branch of Wnt/PCP pathway. I am collecting additional data to ensure statistical significance.

## MODELING THE DISTRIBUTION OF POLLUTANTS FOR A MINE (POSTER)

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The institution of new mining facilities is a constant threat to environmental persistence and abundance. This issue can be minimized with greater insight into how pollutants spread. Understanding the dispersal of pollutants allows cleanup efforts to be directed in an extremely efficient manner. To determine the spread of pollutants, the Belt Creek Drainage which has several mining facilities was chosen to be tested for specifically Arsenic, Selenium, and Lead. Using ICP (Inductively Coupled Plasma) the concentrations of each pollutant was determined at various points along the drainage system. The data retrieved was then synthesized in GIS (Geographic Information Systems) to create a gradient showing the concentration changes across distance within the river system. This type of analysis is extremely applicable to understanding how and where pollutants can be predicted to accumulate and can enhance the effectiveness of pollutant cleanup efforts.

## OXIDATION OF D-GLUCOSE AND D-MANNOSE (POSTER)

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The study of monosaccharides has been around and researched since the 1800's. In this experiment, D-glucose will undergo benchtop oxidation using nitric acid and heat to produce gluconic acid and glucaric acid. The same oxidation process will then be done using D-mannose. The resulting aldonic and aldaric acids of each compound will then be compared to determine if stereochemistry effects the ease at which an aldohexose can be oxidized at C-1 and C-6.

## PROXIMATE ANALYSIS OF FISH FEED (POSTER)

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An aquaponic system shows evidence of increasing economic efficiency and environmental sustainability. An efficient aquaponic system is heavily dependent on the quality of aquatic life in it. In order to ensure the quality of the aquatic life, the quality of its feed must be ensured as well. This research uses proximate analysis to analyze fish feed in terms of percent moisture, fat content, crude fiber extraction and crude protein extraction and correlate these with fish body composition as well.

## RIVERINE CARBON CYCLING AS A FUNCTION OF SEASONALITY (POSTER)

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Montana has one of the most dynamic climate regimes in all of the United States, with seasonal changes spanning a large range of temperatures. In Montana, we depend on water originating from snow and glacial melt. These freshwater ecosystems are considered to be some of the most vulnerable to climate change on Earth. Glacially fed ecosystems are unique habitats for a vast array of life and geochemical processes, including carbon cycling. In order to study carbon cycling in environments vulnerable to change, an interdisciplinary approach including biogeochemical analyses of river DOM production and external allochthonous inputs is necessary to evaluate the impacts of climate change. The overarching hypothesis for this work is: Seasonal changes in Montana rivers will cause shifts in carbon cycling as ecosystems respond to changes in temperature. Unlike our initial hypothesis that the amount of sunlight and temperature would play a bigger role in what was happening, the time of the year was much more significant. In Big Sky OC levels in June for the sunny and canopy covered reaches were similar, 1.24 and 1.23 mg C/L, respectively; whereas at the end of July OC in the sunny reach was 0.42 mg C/L and the canopy cover reach was 0.955 mg C/L. The same trend is seen for the urban location in Bozeman. Cell abundance in the reaches followed similar trends, which were not solely based on temperature.

## SYNTHESIS OF [Cu(PDTC)<sub>y</sub>L]<sub>x</sub> COORDINATION SERIES (POSTER)

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Carbon tetrachloride (CCl<sub>4</sub>) is a potential environmental contaminant in water, and soil. We are interested in studying potential CCl<sub>4</sub> environmental remediation technologies. Specifically we are interested in 2,6-pyridinedithiocarboxylic (PDTC) and the dechlorination properties of its copper based coordination compounds, [Cu(PDTC)<sub>y</sub>L]<sub>x</sub>. In the interest of creating better performing more soluble coordination compounds we seek to understand the link between coordination compound electronic structure and reactivity. With this ultimate goal in mind we present IR, UV-Vis and NMR evidence for the successful synthesis of a series of [Cu(PDTC)<sub>y</sub>L]<sub>x</sub> coordination compounds. Here L=Cl, Br, I, CN, PPh<sub>3</sub> as well as the dimer species [Cu(PDTC)<sub>2</sub>].

## THE USE OF TRICHOSTATIN A TO RESCUE TRKA+ NEURONS IN A MOUSE MODEL OF FAMILIAL DYSAUTONOMIA (POSTER)

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Familial dysautonomia is a severe, recessive disease that devastates the peripheral nervous system, culminating in death of most patients by age 40. Studies have shown that there is a reduced number of both TrkA+ neurons and acetylation in familial dysautonomia patients and our mouse model of familial dysautonomia. Another feature of familial dysautonomia is a decrease in histone acetylation. This study evaluated the ability of the histone deacetylase inhibitor, Trichostatin A, to rescue the reduced number of TrkA+ neurons in the dorsal root ganglia in our mouse model of familial dysautonomia. Pregnant dams were treated with either 1mg/kg of Trichostatin A (experimental) or vehicle alone (control), at E8.5, E10.5, and E12.5, a time frame corresponding to neurogenesis in the mouse dorsal root ganglia. Immunohistochemistry was used to quantify the number of TrkA+ neurons at E17.5. Trichostatin A-treated knockout embryos (n=3) showed a significant increase in the number of TrkA+ neurons over vehicle only knockout embryos (n=3) (132.9% increase;  $p < .00001$ ). Trichostatin A (1mg/kg) effectively rescues the number of TrkA+ neurons in our mouse model. Further studies will explore the cellular mechanisms via which histone deacetylase inhibition prevents neuronal cell death as well as the possible benefits of using these therapeutics for familial dysautonomia symptom management.

## ZEBRAFISH STRESS SYNAPSE (POSTER)

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Stress is a behavior characterized by uneasiness, nervousness, and fear that all organisms are faced with every day. Both environmental and chemical factors can trigger stress responses within the body. In this experiment, zebrafish (*Danio species*) will be stressed with environmental and chemical factors. Their cortisol levels, which is produced when stressed, will be tested by performing cortisol assays. The environmental factors that will be tested with light/dark apparatus that will investigate how environmental conditions stress the zebrafish. A beaker stressor will be used to investigate how the size of the environment effects stress on the zebrafish. Caffeine will be the chemical factor tested because it is addictive. Different concentrations of caffeine will be administered and the effects of these concentrations will use the cortisol assays. An ANOVA test will be done to quantify the data. The purpose of the experiment is to see how environmental and chemical factors, such as caffeine, induce different stress responses based off of zebrafish behavior and cortisol assay results.