

MICROBEADS FOR PERIPHERAL NERVE REGENERATION DRUG DELIVERY IN FREEZE-CAST SCAFFOLDS

Claire Adner

Freeze casting, the controlled unidirectional solidification of water-based solutions and slurries, is a new technique for the fabrication of tissue scaffolds with highly aligned porosity and guiding features for use as conduits for the regeneration of peripheral nerves. Growth factors, which are known to play a critical role in the regulation of regenerative cell growth, can be incorporated into these scaffolds during their manufacture, because freeze casting is a cold process. Polymeric microbeads, which can be frozen into the scaffold architecture without altering it, are a means for the controlled delivery of such growth factors. The focus of this study is the manufacture of alginate microbeads using microfluidics to determine whether release differs between scaffolds into which the growth factor is loaded directly and those into which the growth factor is incorporated using microcapsules. Presented will be effects of processing conditions on microbead size and geometry, their microstructural features before and after freezing and freeze drying, and patterns of microbead distribution within the freeze-cast tissue scaffolds as determined by optical, scanning electron, and confocal microscopy.

DEVELOPING AN ECONOMICAL IDENTIFICATION METHOD FOR PLASTICS OF UNKNOWN ORIGIN

Matthew Adner

Plastics have become a critical pollutant in the world's oceans and landfills. For example, it is estimated that more than 8 million tons of plastic enter the ocean every year. Given the prevalence of plastic, there is a need and pressure to do research on the effect of its presence in the environment. A challenge that accompanies research on plastic pollution is that there are many different types of plastics that are hard to distinguish from one another. Current methods of detection use Fourier Transform Infrared Spectroscopy. This, however, is very costly and presents a significant barrier to entry for any group seeking to study plastics. Using a basic Beckman Coulter DU®520 General Purpose UV/Vis spectrophotometer, I have developed a promising approach for plastic identification. In this research I report preliminary findings for distinguishing between Polyvinylchloride (PVC), which is commonly used in water piping, and Polyethylene Terephthalate (PETE), which is commonly used in disposable water bottles. To test the viability of this procedure, I started with known plastic materials that I then ground into a powder, so that the material could be suspended in cuvettes for spectrophotometric analysis. The plastic powder was filtered through a series of sieves to get a uniform size between 300 and 150 microns. Two different amounts – 0.08g and 0.12g – of each plastic were suspended in a solution of 32.5% sucrose to 67.5% distilled water. One milliliter of this solution was added to quartz cuvettes and scanned in the spectrophotometer, registering absorption and percent transmittance (%T) at wavelengths between 200 and 1100nm, in 100nm increments. Each sample was scanned six times, and results for each scan were recorded. The reference sample cuvette contained only the sugar solution. Although there was variance in readings across the scans of each sample, the PVC and PETE profiles were highly distinguishable, with the highest readings of the one plastic lower than the lowest reading of the other in almost every wavelength. Thus, using a standard spectrophotometer, I was able to successfully distinguish Polyvinylchloride (PVC) from Polyethylene Terephthalate (PETE) according to their differing absorption and %T profiles. These initial results are promising as they show that it may be possible to develop a cost-effective system for identifying a broad variety of plastics.

EFFECTS OF DIPHENHYDRAMINE ON NEUROTRANSMITTERS, NEURONS, AND MEMORY IN *C. ELEGANS*

Eden Anne Bauer

Caenorhabditis elegans is a transparent, microscopic nematode with an entirely mapped neurological system. This allows for studies on neuronal activity as a simplified model of the human neurological system. Antihistamines have recently been linked to memory loss, specifically dementia, in humans. Last year, we performed an investigation behaviorally training *C. elegans* and exposing “trained” nematodes to antihistamines, hypothesizing behavior change and memory dysfunction would occur when the nematodes were exposed to the antihistamines diphenhydramine (Benadryl) and cetirizine (Zyrtec). The results implied that *C. elegans* became behaviorally trained to cease reacting to vibrations applied to the side of their petri dishes in short taps, then recommenced reacting to vibrations after exposure to antihistamines, indicating a possible loss of the memory of that training. This year, our experiment was based off this prior research. Two tagged strains of *C. elegans*, EAW113 (*dop-1::gfp, dop-3::rfp*) and EAW115 (*unc-17::gfp, mod-1::mCherry*), were synchronized to the L1 larval stage. The *C. elegans* were then conditioned to stop reacting to vibrations applied to the side of the dish with an electric toothbrush. This process was repeated for a total of 180 minutes over two days, resulting in the *C. elegans* showing signs of becoming conditioned to the vibrating “taps”, as indicated by a decline in the percentage of *C. elegans* responding to the vibrations. After conditioning, diphenhydramine (Benadryl) or sterile water was added to ensure direct exposure of the *C. elegans*. Groups exposed to diphenhydramine exhibited a loss of conditioned response of the behavioral training, while the control groups retained the conditioned response to the vibration stimulus. The EAW113 and EAW115 strains were utilized because they had specific neurotransmitter receptors tagged with either green fluorescent protein (GFP) or red fluorescent protein (RFP or mCherry). These receptors are for dopamine (*dop-1* and *dop-3*), serotonin (*mod-1*) and acetylcholine (*unc-17*). This enabled us to directly observe specific neural receptor expression. Images taken using fluorescent microscopy indicated that *C. elegans* exposed to antihistamines had lower levels of the neurotransmitters than control *C. elegans*. These findings correlate with previous data gathered from a nearly identical training and antihistamine exposure experiment performed on wildtype *C. elegans*. Results from both experiments suggest a link between antihistamines and behavior change or memory dysfunction in the nematode *C. elegans*.

GENERATIONAL EFFECTS OF ROUNDUP AND ITS COMPONENTS ON WILD TYPE AND DAF-9 *C. ELEGANS*

Fiona Barthel and Emma Herndon

When exposed to high stress levels, organisms tend to pass on certain phenotypic traits or tolerance levels to their offspring, sometimes through epigenetics. This experiment explores the epigenetic tolerances that Roundup® and its component ingredients create in the model organism, *Caenorhabditis elegans*. If a significant percentage of the nematodes can survive an exposure to a low dilution of Roundup®, their progeny may have a higher tolerance, survival rate, and lessened stress response to Roundup® due to epigenetic changes caused by the experience of the parent. To expand upon our previous research, we treated both the wild type and a *daf-9* mutant strain, which has been shown to be more stress-resistant than the wild type strain. Rather than treating *C. elegans* with Roundup® alone, separate plates of nematodes were treated with equivalent concentrations of pure glyphosate (Roundup's listed active ingredient), polyethoxylated tallow amine (POEA, the second known ingredient of Roundup®, a surfactant) and a combination of glyphosate and POEA (to detect possible synergistic effects of the two ingredients and the possibility of separate unknown toxic ingredients in Roundup®). These strains were treated with Roundup® concentrations increasing from 2% to 5% in increments of 0.5% in order to test whether *C. elegans* could develop a tolerance to Roundup® over successive generations. In our previous research, the final generation in the control plates, which had not been exposed to Roundup® in earlier generations, were treated with equal dilutions (2.0% Roundup®) to the test plates, which had been subjected to increasing concentrations of Roundup®. In this final generation, the test plates had 100% survival rate while the control plates had 60% and 53% survival, respectively. This suggested that *C. elegans* have the ability to develop a tolerance to Roundup® over multiple generations, but nematodes that are not predisposed to tolerate Roundup® have a lower survival rate when exposed to Roundup®. Contrary to our previous results, the survival rates for both the generational controls and the experimental groups were all 100% throughout exposure to all of the dilutions of Roundup® and its components. This suggests that our previous results are inconclusive as to whether or not *C. elegans* developed a tolerance against Roundup® and further stresses the importance of repeating experiments. Because of the expanded nature of this study, differences in experimental methods may, in part, have created these contrary results.

THE EFFECT OF LIGHT EXPOSURE ON HOW DIFFERENT STRAINS OF *C. ELEGANS* LEARN

Morgan Biele

To investigate learning under stress, *Caenorhabditis elegans* were conditioned to an environmental threat of vibrations applied to their culture plates. *C. elegans* are transparent soil-dwelling organisms susceptible to DNA damage caused by ultraviolet light. Light exposure poses a survival threat that may influence learning in *C. elegans*. Wild type and neuroigin deficient (*nlg-1*) *C. elegans* were tested because the neuroigin deficiency decreases responsiveness to sensory stimuli and may play a role in learning. I hypothesized that *nlg-1* mutant and wild type *C. elegans* would show different rates of learning when exposed to ultraviolet versus visible light. Cultures were prepared with five worms of equal age per plate. One plate of wild type and one plate of mutant *C. elegans* were exposed to each of the three illumination conditions: ultraviolet light, visible light, and darkness. After 20 minutes of light exposure, the learning process commenced with the wild type plate being tapped at 120 beats per minute for 20 seconds with the bare tip of an electric toothbrush held against the rim of the culture plate. There were multiple tapping periods with gaps of non-tapping for 25 minutes. Each plate received about 1000 taps in order to desensitize/habituate the worms to the tapping. After digitally recording this process with a stereo microscope at 30-40x magnification, reversals, where worms reverse direction in response to a tapping, were measured in worm lengths to see how the worms adapted to the tapping stimulus. *C. elegans* often reverse direction when they sense vibration as a survival method. Both strains of worms successfully learned, as shown by reversal distances decreasing over time. As shown by reversal distances decreasing over time, both strains of worms did “learn” at different rates, with the wild types appearing to learn more effectively in all three types of light exposure. It is unclear whether the source of the decrease comes partly from neuronal damage in the light or is from learning only. While more data still needs to be analyzed, the data supports the notion that there is a distinct difference in the rates of learning with the different lights and between the two strains, which can be potentially applied to human learning.

EFFECTS OF RADIO-FREQUENCIES ON DIFFERENT STRAINS OF C. ELEGANS

Joseph Blackburn

With the rapid increase in cell phones and portable digital communication, exposure to radio-frequencies (RF) has become a health concern. This study examined the effects of radio-frequencies from a Wi-Fi router on five specific strains of *C. elegans*. These strains were wild type, *daf-9*, *nlg-1*, *cat-2*, and *tph-1*, which were chosen to get a range of different mutants related to stress and sensory defects. I hypothesized that *C. elegans* would demonstrate some avoidance or population decline when cultured in close proximity to 2.4 GHz RF. The basic materials of the experiment were 60mm seeded agar plates, five of each *C. elegans* strain mentioned above, a Wi-Fi router (2.4 GHz Wireless-G Broadband Router), and a Faraday bag that would block out all external radiation for control plates. Plates from the experimental groups were placed within 3.0-9.0 cm (based on the plate diameter) of the router and the control plates were placed in the Faraday bag. All plates and the router were stored in a metal drawer to block external RF sources. Population growth was documented by counting the number of worms per plate at given intervals. Avoidance was measured via migration away from the router by counting worms on the near side and far side of each 60 mm plate. Three strains (wild-type, *daf-9*, and *tph-1*) migrated away from the source. All stains showed population declines but none were significant with p-values less than 0.05. The results marginally support the hypothesis that radio-frequencies have a detrimental effect on the population growth. The hypothesis that the radio-frequencies generated by the router would cause an avoidance reaction by creating a migration away from the source of the RF was supported for three of the *C. elegans* strains. In this case, it is the general population of wild type, the serotonin deficient *tph-1* and the stress resistant *daf-9* that showed the greatest sensitivities to the radio-frequency as measured by migratory behavior in culture plates.

INVESTIGATING THE EFFICACY OF HERBAL MEDICINALS ON PARASITIC WORM INFECTIONS

Elizabeth Dixon

Herbal remedies have been used to treat parasitic worm infections. Lady's-slipper roots (LSR) as well as black walnut hull extract (juglone) are herbal remedies for roundworm infection. This study investigated the effectiveness of these herbal treatments in killing the nematode *Caenorhabditis elegans*. Three LSR extracts were prepared for testing from plants grown in our lab in sterile tissue culture: a dried-root water extract, a fresh-root water extract and a fresh-root alcohol extract. All three were prepared by grinding roots using a mortar and pestle. Because alcohol kills worms, the alcohol extract was evaporated and re-suspended in sterile tap water. All preparations were passed through a cell strainer to remove debris. We also tested a commercial Lady's slipper root water extract. Two juglone extracts were made: a strained decoction or tea of 500 mg of juglone powder heated and steeped in 50 ml of sterile tap water, and a strained tincture from 1 gram of juglone dissolved in 10 ml 70% ethanol. Additionally, a commercial Juglone water extract was tested. The nematocide ivermectin and 70% ethanol were used as positive controls and sterile tap water was used as a negative control. Two techniques were used to expose *C. elegans* to the extracts: flooding the culture plates with extracts and adding the extracts to the culture medium. Worm survival was monitored with a stereo microscope at 30-40x magnification. Chemotaxis experiments were also performed to explore avoidance or attraction to the extracts. No observable difference from controls in either survival or behavior was found for all LSR extracts. LSR chemotaxis showed no avoidance or attraction. With all preparations of juglone, flooding the plates with the extracts was not as effective at killing worms as mixing the extracts into the medium. The commercial preparation of juglone showed a dose-response pattern of death rate of 100% at the highest concentration, 92% at 1/2 strength, 25% at 1/4 strength, and 8.33% at 1/8th strength. Ivermectin was lethal to the worms at 0.005 g in 10 ml of ethanol. Seventy percent ethanol was also lethal to the worms. These results suggest that juglone might be an effective alternative treatment for roundworm infections to drugs such as ivermectin and a thorough examination of juglone's effectiveness and side effects in mammals should be investigated.

THE EFFECTS OF A DISRUPTED CIRCADIAN RHYTHM ON *C. ELEGANS* LONGEVITY AND EGG LAYING

Katherine Duan

Circadian rhythms exert broad control over the physiology and behavior of most organisms. These include intermediary metabolism, immune function, neuro-endocrine function, sleep-wake cycles, and optimal physical and mental productivity. Disruption of circadian rhythms is linked to abnormal behavior, increased disease risk, and reduced life span. The primary regulator, or *zeitgeber* (time-giver), for circadian rhythms is light. This investigation reports the effects of a disrupted circadian rhythm on the lifespan and egg-laying of the nematode *C. elegans*. Four groups were used: 1) a control group of wild type N2 worms exposed to 12h of light/12h of dark per day; 2) a long day (LD) group of wild type N2 worms exposed to 24h of light/0h of dark per day; 3) a long day *hif-1* mutant worm group (LD-h) exposed to 24h of light/0h of dark per day; and 4) a long day group of N2 worms with an artificial insulin cycle (LD-i). Mutant *hif-1* worms in the LD-h test group were used because *hif-1* is a homolog of a human circadian gene associated with lipid metabolism and diabetes. The secretion of insulin in humans follows a circadian rhythm with higher daylight levels. The artificial insulin cycle in the LD-i test group was employed to test the effects of providing an additional *zeitgeber*, which the worms could in-turn use to adjust their circadian clock. All worms were entrained by a 12-hour light, 12-hour dark cycle for 3 days. Following the entrainment period, test groups were exposed to a continuous 24-hour daylight period. During the entrainment period, egg-laying followed a circadian cycle with more eggs laid during light hours than dark hours. Following the natural life cycle of *C. elegans*, egg production stopped after the 3-day entrainment period. Circadian rhythm disruption decreased the median lifespan of wild type *C. elegans* by 20.0%. The addition of insulin in the LD-i group decreased the negative effects of a disrupted circadian rhythm by 7 percentage points, resulting in a decreased median lifespan of 13%. Down regulation of the *hif-1* gene increased the negative effects of a disrupted circadian rhythm by 13 percentage points, resulting in a decreased median lifespan of 33%. These results demonstrate that disruption of the circadian rhythm negatively affects health in *C. elegans*. These responses are exacerbated by changes in circadian gene expression and can be counteracted by additional circadian clock regulators, such as exogenous insulin.

EFFECTS OF EXOGENOUS GLUCOSE AND INSULIN ON *C. ELEGANS*

Emily Gaw

Glucose has been shown to shorten the lifespan of the nematode *C. elegans* that has an evolutionarily conserved insulin signaling pathway similar to that of humans. This study examined the effects of periodic high-glucose exposure on activity levels and survival rates in *C. elegans*. The purpose was to investigate whether there is a “tipping point” where exposure to excess glucose becomes irreversibly harmful. Two experimental groups – “single-shocked worms” (worms administered glucose on day 1 and 2 of adulthood) and “double-shocked worms” (worms administered glucose on day 1, 2, 5, and 6 of adulthood) – were compared to a control of unshocked worms (worms of the same age that received no glucose). The experiment confirmed the deleterious effects of glucose exposure to *C. elegans*. An unexpected increase in survival rates was observed in the double-shock groups as compared to the lower survival rates in single-shocked groups (the double-shock groups had similar survival rates when compared with the control groups). The results suggest a quick adaptation process to high glucose conditions. This is supported by a decrease in activity levels post glucose introduction, followed by a rise in activity. The results also suggest that the changes may be irreversible, as the single-shocked worm groups did not survive well when normal conditions were restored. Rather, the worms survived better when the glucose shock was reintroduced. In an additional experiment, insulin was administered in place of glucose to *C. elegans* under the same experimental conditions. Similar results were recorded, suggesting that the observed effects were based in the insulin signaling pathway.

THE EFFECTS OF ACETAMINOPHEN ON *C. ELEGANS* LONGEVITY AND EGG COUNT

Alisa Halchenko

The objective of this study was to understand the effects of acetaminophen on *Caenorhabditis elegans* lifespan and fertility. The experiment was based off a study that assessed the effect of aspirin on the lifespan of *C. elegans*. The original study showed that aspirin causes stress in *C. elegans* similar to caloric restriction, resulting in increased stress resilience and prolonged longevity. Assuming that acetaminophen treatment invokes a similar mechanism, the expectation was to observe prolonged lifespan and decreased egg production, one of the known stress outcomes in *C. elegans*. Wildtype and *daf-9* mutant *C. elegans* were provided with OP50 *Escherichia coli* seeded plates containing a diluted concentration of acetaminophen and water at 50, 100, and 200mM, each plate hosting 4 worms of the same type. The plates were assigned to 5 study groups: 3 treatment groups corresponding to each treatment concentration had 3 wildtype *C. elegans* plates each, 2 plates with untreated wildtype *C. elegans* were assigned as a control group, and 2 plates with *daf-9* treatment group with 100mM acetaminophen concentration. *Daf-9* mutants were included as they have been shown to be stress resistant compared to wildtype worms. The observed increased movement speed suggests that acetaminophen solution causes stress in *C. elegans* worms. Increased egg production in the wildtype *C. elegans* treatment groups was also observed when compared to the control groups. The highest average egg laying per worm was observed in the lowest concentration of acetaminophen treatment group and declined as the concentration of acetaminophen increased. The results of this study showed that treating *C. elegans* with acetaminophen increased both their egg laying (at low concentrations) and lifespan (at higher concentrations), similar to the findings of the aspirin study. Conducting the experiment only once and therefore needing to verify reproducibility and statistical significance of the results is the main limitation of the study.

THE IMPACT OF LEACHED WATER FROM PLASTIC BOTTLES ON DAPHNIA MAGNA

Alexander Kish

Plastic is now the most common container material for bottled water. Concerns have been raised that plastic may not be safe for storing water because the plastics or additives to the plastics are migrating into the water, particularly when heated. Here, we study the effects of water stored in plastic containers on the heart rate of *Daphnia magna*. *D. magna* is a model organism for toxicological studies because it is transparent and rapidly sensitive to substances in its fresh water environment. Two heating methods were used for water stored in 8 different containers: PETE, HDPE, Tritan (Nalgene), TPU, Polyurethane, Stainless steel, Glass, and disposable baby food containers. In both methods, non-heated controls were used in addition to the material controls of heated glass and stainless steel. In the first method, the containers were lined up along the rear south-facing window of a sedan in the sun with all windows closed for 8 hours on a hot summer day. In the second method, containers were placed in an incubator at 60°C for 5 hours in the dark. Water samples from the containers were placed in glass tubes and adult *D. magna* were added. After 1 hour, their heart rates (HR) for the following hour were captured using slow motion video and an Olympus stereo microscope. For sedan-stored samples from PETE bottles, the HR were significantly below the control, while samples from HDPE and Nalgene bottles had significantly higher HR than the control. *D. magna* exposed to samples from a polyurethane container showed drastically reduced heart rates compared to the control. The HR of *D. magna* in water from the stainless steel bottle were significantly higher than the control. For these experiments, the least significant p-value was found to be 1.5×10^{-4} . For samples from the incubator, the HR of *D. magna* exposed to water stored in HDPE containers were significantly higher than the control. A TPU container caused the daphnia's heart rate to fall below the control. For these experiments the least significant p-value recorded was 5.3×10^{-3} . These results suggest that, when a plastic bottle is left in a hot car, some sort of leaching occurs, since water from the plastic bottles had a greater impact on the heart rate of *D. magna* than water from the glass and stainless steel bottles. The results presented here also suggest that there is a difference between waters leached in the car and those leached in the controlled incubator environment, since waters stored in the incubator caused the daphnias' heart rates to deviate less from the control than waters stored in the sedan.

THE IMPACT OF JUUL E-LIQUID AND COMPONENTS ON CAENORHABDITIS ELEGANS

Leanna Kish

According to the US FDA, use of electronic nicotine delivery systems (ENDS) has reached “an epidemic proportion.” Although they are marketed as a healthier alternative to traditional cigarettes, the FDA issued a lengthy warning in 2018 of the risks of addiction and marketing of ENDS aimed at America’s youth. The FDA, in particular, addressed the popular JUUL products. With the rapid and continued rise in the use of ENDS, research on their safety is under scrutiny. This study examined the chemotaxis behavioral effects of JUUL e-cigarette liquids and their ingredients on stress resistant *daf-9* mutant *Caenorhabditis elegans*. Individual culture plates were divided into four concentric areas and the test solution was placed in the center area. Each of the four circles was assigned a number, 1 being the center area and 4 being the outermost area. The JUUL flavors used were Classic Menthol, Mango, Crème Brûlée, Cool Cucumber, and Fruit Medley. The concentrations tested were 1%, 25%, 50%, and 100% and were made by diluting the test substances in *E. coli* bacteria or tap water. Worms were transferred into the center area and the number of worms in each area on the plate was visually monitored for seven hours with an Olympus stereo microscope. For mango diluted in *E. coli*, the percent of worms in the center of the 1% concentration plates ranged from 60% to 70% and the percent of worms in the center of the 100% concentration plates ranged from 10% to 60%. The worms exposed to 25% and 50% concentrations fell in a dose response pattern. For mango diluted in water, the percent of worms in the center of the 1% concentration plates ranged from 32% to 58% and the percent of worms in the center of the 100% concentration plates ranged from 10% to 60%. The 25% and 50% concentrations fell into a similar dose response pattern to the Mango diluted in *E. coli*. Crème Brûlée had similar results to Mango, however, the other flavors did not show as clear of a dose response pattern. For all of the individual ingredients, as well as a mock JUUL solution of the listed ingredients, the percent of worms in the center never fell below 60% across all of the concentrations of *E. coli* dilutions and water dilutions. This data suggests that the JUUL E-liquid has more severe results on *C. elegans* behavior than the listed individual ingredients of the product. The results vary among flavors, suggesting that the flavorings may be an important variable.

IMPACT OF BLUE LIGHT ON THE SURVIVAL RATES OF *C. ELEGANS* AND THEIR OFFSPRING

Audrey Lewellen

Circadian rhythms play a regulatory role in animals and plants, and their circadian clocks are regulated by light with visible light playing varying roles depending on the time of day. Although the effects of different wavelengths of visible light have been studied for decades, its effect on invertebrates has only recently been researched. This experiment tests whether the exposure of blue light on a tiny invertebrate nematode *C. elegans* has life-shortening effects on its first and second generations of offspring. Since *C. elegans* lives in compost and rotting plant matter, it is unclear to what levels or wavelengths of light they are adapted to, in particular the higher frequency wavelengths. These nematodes may experience stress when exposed to blue light, and this form of stress may shorten their lives. To test this, three groups of worms were raised in different light conditions and the overall survival rates in each condition were compared. When the original worms generated offspring, the offspring were studied under a stereo microscope for two to three weeks to determine the rates of survival. Results suggest that exposure to blue light shortens the life span of *C. elegans* and that the effects carry on to the next generation, shortening the life spans of the offspring as well. Further research will be necessary to determine how strongly *C. elegans* is impacted by exposure to blue light.

THE PURSUIT OF HAPPINESS: THE EFFECT OF HERBS ON SEROTONIN-DEFICIENT *C. ELEGANS*

Shreya Nagri

Serotonin is a neurotransmitter that is thought to be responsible for mood in humans and other multicellular organisms. Many people take prescription drugs that are thought to increase levels of serotonin to treat symptoms of depression, but these drugs can also have debilitating side effects or be ineffective for some patients. Herbal remedies such as lavender flower, lavender leaf (*Lavandula stoechas*), St. John's Wort (*Hypericum perforatum*) and ashwagandha (*Withania somnifera*) are thought to be natural alternatives to these drugs, but may not have the same efficacy as prescription medication. A chemotaxis experiment was used to test the effects of each herbal remedy on *tph-1* mutant *C. elegans* that are serotonin-deficient. Decoctions of each were made by boiling an herb with 40 mL of sterile tap water for every gram of herb used. A dose response experiment with 100%, 10% and 1% dilutions of the original decoction was conducted to test the effects of the herbal remedies on the worms. Fecundity, behavior and mortality were monitored for five days for control and experimental conditions. None of the worms died through this experiment, so the concentrations of the decoctions were not lethal. The lavender flower was not used in the dose response portion of the experiment because of its similarity to the lavender leaf. The lavender leaf decoction and St. John's Wort decoction showed increases in *tph-1* mutant *C. elegans* lethargy and changes in fecundity when compared to the control, suggesting a possible increase in serotonin levels. The worms laid fewer eggs when treated with herbs. The lower the concentration of herbal remedy, the faster changes were observed in worm behavior, suggesting the lower concentration may be more effective in the worms. The ashwagandha decoction results suggested a similar trend but were inconclusive because of contaminated cultures. Future work will repeat these experiments to verify the results, investigate serotonin levels, and determine the effects of these treatments on wild type *C. elegans*.

MOTILITY OF DOPAMINE AND SEROTONIN-DEFICIENT *C. ELEGANS* IN FED AND STARVED CONDITIONS

Saia Patel

The neurotransmitters dopamine and serotonin play important roles in animal nervous system function. Abnormal dopamine and serotonin signaling results in a variety of diseases and disorders, including ADHD, addiction, and Parkinson's disease. The exact roles of dopamine and serotonin in the human brain, especially related to motor control, are not understood. Therefore, the goal of this research was to determine how defective dopamine and serotonin signaling in *Caenorhabditis elegans* affects locomotion rates. Locomotion enables *C. elegans* to seek out the bacteria they feed on to survive in the wild and in culture. In this experiment, wild-type, CBI 112 (*cat-2*) dopamine-deficient, and GRI 321 (*tph-1*) serotonin-deficient worms were chosen because these mutants have been seldom used in experiments relating to locomotion. Half of the worms in each strain were food-deprived for one hour, while the rest were fed a sufficient supply of their food, *Escherichia coli* OP50. After one hour, the worms were transferred to a plate that did or did not have *E. coli* OP50. The number of body bends of *C. elegans* were enumerated from 20 second digital recordings. Body bends were chosen as a more accurate representation of the worms' locomotion rate, rather than speed of travel. The results suggest that food-deprived *C. elegans* that were introduced to seeded plates had fewer body bends during the observed 20 second periods than the worms subjected to other conditions. In addition, the fed worms that were introduced to seeded plates had fewer body bends than the worms introduced to unseeded plates. This suggests that, in the presence of *E. coli*, the worms slow down in response to entering a seeded plate.

MICROBIAL REGULATION OF HEAT STRESS RESPONSE IN CAENORHABDITIS ELEGANS

Fiona Sweeney

There is an increasing amount of evidence showing that the microbiome is a contributor to both immune and stress responses in a variety of organisms. One of these organisms is the nematode *Caenorhabditis elegans*. Our 2017 study showed that a more diverse microbiome, which consisted of *E. coli* OP50, *E. coli* HB101, *Lactobacillus acidophilus*, and *Bifidobacterium infantis*, improved the lifespan of *C. elegans* that underwent heat stress. The question addressed in this study is whether *C. elegans* will have a longer lifespan under heat exposure when they have a microbiome that is more diverse and representative of their natural microbiome. This study used wild-type *C. elegans* that were grown on plates seeded with the following bacteria: 1) a control of *E. coli* OP50; 2) a mix of *Pseudomonas fluorescens* and *Pseudomonas putida*; and 3) a mix of OP50, *P. fluorescens*, *P. putida*, *Acetobacter aceti*, and *Enterobacter aerogenes*. These bacteria were chosen because they are more representative of *C. elegans*' microbiome in the wild than were the four strains chosen in our 2017 studies. The worms were incubated for 3 hours at 37°C and their survival rate was recorded. There was no significant difference in survival between the group that had one strain of bacteria and the group that had two strains of bacteria. There was a significant decrease in death rate for the five-strain mix compared to the control ($p = 0.006$) and the *Pseudomonas* mix ($p = 0.007$). The death rates of the control, *Pseudomonas* mix, and five strain mix were 91%, 86%, and 41%, respectively. This suggests that a higher and more representative level of microbiome diversity decreases the probability of death in response to heat stress.

IGF-1 SIGNALING PATHWAY AND THE EPIGENETIC MEMORY OF THERMOTOLERANCE IN *C. ELEGANS*

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The goal of this study was to see if the Insulin/IGF-1 Signaling pathway (IIS pathway) is involved in the epigenetic memory of thermotolerance in *Caenorhabditis elegans* (*C. elegans*). The IIS pathway mediates longevity, metabolism and stress-resistance, which includes thermotolerance. This pathway activates a family of small heat shock proteins (such as hsp16) that helps maintain protein homeostasis by preventing other proteins from unfolding during exposure to extreme heat. It has been shown that higher temperatures can induce a heat resistance that can be inherited in *C. elegans*. The two experiments presented in this report explore the connection between the IIS pathway and this inherited thermotolerance. The first experiment investigated the duration of the inherited thermotolerance by analyzing the death rates of *C. elegans* at extreme temperatures (37°C) for progeny of nematodes grown in warmer (25°C) and optimal (20°C) temperatures over 5 generations. The control group was the progeny of nematodes grown at 20°C and the experimental group was the progeny of nematodes grown at 25°C to induce thermotolerance. Wild type worms and two strains of *C. elegans* with mutations in the IIS pathway (*daf-16* and *age-1*) were compared to see if the number of generations that inherited thermotolerance in the experimental group would change. The *daf-16* was a null mutation (less thermotolerant) and the *age-1* was a down-regulated mutation (more thermotolerant). Wild type and *daf-16* mutants inherited the thermotolerance for 4 generations whereas *age-1* mutants inherited the thermotolerance for 5 generations. An ANOVA test among the three strains for each generation showed that there was no statistical difference in the rate at which the thermotolerance was lost among the three strains ($p > 0.05$). This suggests that this pathway is not responsible for how long the epigenetic memory of thermotolerance lasts. The second experiment compared the relative expression levels of heat shock protein 16 (*hsp-16.1* and *hsp-16.2*) after heat shock in the progeny of the control (20°C) and experimental (25°C) groups using quantitative real-time PCR (RT-PCR). There was no observed trend in reference to the association between heat shock protein and inherited thermotolerance. Although initial results are inconclusive, this experiment established a reliable RNA extraction and RT-PCR protocol for future use in our lab.