Understanding the Impact of SSRIs on Avoidance Behaviors in *Caenorhabditis elegans*

A Major Qualifying Project Submitted to the Faculty of Worcester Polytechnic Institute in partial fulfillment of the Bachelor of Science in Biology and Biotechnology by:

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Abstract

Selective Serotonin Reuptake Inhibitors (SSRIs) have been a commonly prescribed treatment for mood disorders such as anxiety and depression since their introduction in 1987. However, very little research shows that we fully understand how these drugs work biochemically and how they affect downstream communication within the nervous system. Using *C. elegans* as a model organism, the impacts of SSRIs can be measured through communication and avoidance behaviors and the results can be applied to humans. Experiments using sertraline hydrochloride showed that SSRIs inhibited avoidance behaviors which reduces survivability of the worm when put in the context predator/prey cues. This is related to the effects that SSRIs have on humans by connecting the avoidance behaviors of *C. elegans* to the side effects that are the result of long-term SSRI usage.

Introduction & Background

Mood disorders are some of the most prevalent conditions that affect society today. They are characterized by distorted and inconsistent emotional states that interfere with an individual's ability to function in day to day life. These disorders include major depressive disorder, anxiety disorders, bipolar disorder and even seasonal affective disorder (SAD), among others. The most common mood disorders are major depressive disorder and anxiety disorders. Depression affects 7.1% of all U.S. adults, which translates to approximately 17.3 million adults (Major Depression, 2018). Anxiety disorders encapsulate a wide range of conditions, including generalized anxiety disorder, social anxiety disorder, and separation anxiety disorder, to list the most common in this category of mood disorder. This wide range of disorders affect 19.1% of U.S. adults and 31.9% of U.S. adolescents (Anxiety Disorders, 2018).

Disorders are characterized by the fact that they interfere with daily activities and prevent an individual from completing tasks unhindered. Depression and anxiety are thus a significant issue in American society, as it impacts the lives of a large portion of the population, and the symptoms can be debilitating and long-lasting. As an individual that lives with an anxiety disorder, I can speak from personal experience how exhausting it can be to live and work with a mood disorder. The illogical fear it leaves an individual with can paralyze them from making a decision, or leave their thoughts spiraling for hours. In addition, anxiety disorders specifically can manifest in physical symptoms such as stomach irritation and pain, headaches, dizziness and nausea, as well as shortness of breath. With a large percentage of the population suffering these symptoms at younger ages, and an even larger percentage suffering from additional mood disorders, it becomes clear how critical research into mood disorders has become.

The nervous system communicates through neurons, a cell type that composes the brain, spinal cord, and peripheral nervous system. A neuron is activated through an influx of sodium or calcium and an efflux of potassium. This change in ions depolarizes the cell, which allows the soma of the neuron to reach a certain threshold to fire an action potential (Feher, 2017). These action potentials communicate within a neuron before release chemical or electrical signals to neighboring neurons, which eventually results in both voluntary movement and involuntary control of organs. Neurons communicate with each other through neurotransmitter, which travel from one neuron to another by crossing the synaptic cleft (Feher, 2017). One of these neurotransmitters is serotonin. Serotonin is a monoamine neurotransmitter used for communication within the brain and peripheral nervous system in humans. Additionally, in humans, serotonin has been indicated to be involved in physiological responses associated with behavioral arousals and feeding (McIntosh, 2018). This neurotransmitter is therefore implicated in many bodily functions and regulation, as well as voluntary and involuntary movements.

One of the most common treatments for mood disorders such as depression and anxiety target the reuptake of this neurotransmitters at the synaptic cleft. Selective Serotonin Reuptake Inhibitors (SSRIs) limit the removal of serotonin from the synaptic cleft. These drugs allow serotonin to remain in the synaptic cleft, causing the effects of the neurotransmitter to be prolonged. An individual will experience mood stabilization, and some relief from the symptoms they are experiencing as a result of depression and anxiety (Major Depression, 2018) However, it is unclear what the long-term effects of these SSRIs are. As of 2013, 34.3 million people in the US use SSRIs every day to regulate mood disorders and while some benefit, scientists are observing additions to SSRIs forming, and drastic mood swings occurring when an individual misses a dosage of medication (Carey & Gebeloff, 2018). With more prescriptions being written

every year, it is critical that more information is collected. The SSRI chosen for this project was Sertraline. Sertraline is the active ingredient in an SSRI more commonly known as Zoloft. More than 37 million Americans were prescribed Zoloft in 2016 (Top 300 Drugs, 2020), making it the most prescribed psychiatric medication. This highlights the need of a greater understanding of how Sertraline impacts behaviors at a deeper level.

This project is concerned with narrowing the scope of the molecular interactions of which serotonin might play a roll downstream of the synaptic cleft, by illuminating the interactions between neurons that rely on serotonin to communicate. There are multiple questions at the heart of this project. What are the long-term effects of changing serotonin communication throughout the nervous system? How does changing the ability of using serotonin as a neurotransmitter for communication change behavior? These questions were investigated using *Caenorhabditis elegans*, or *C. elegans*, as a model organism. *C. elegans* are a common species of worm that live in rotting fruit. Visible to the naked eye, one might find a colony of *C. elegans* in an apple on the ground of an orchard. These small worms might seem simple, but they are a host to many traits that allow them to serve as model organisms.

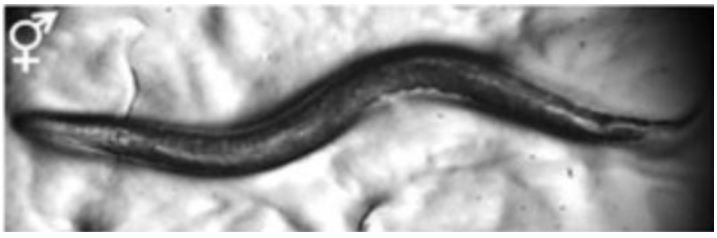

Figure 1: Hermaphroditic Adult *C. elegans at adult stage. Photograph was taken on a petri dish with bacterial lawn at 0.1 mm* (Chalfi, 2015)

C. elegans are either hermaphrodites or males. Hermaphroditic worms can self-fertilize, allowing the organism to have a fast reproduction rate and reach upwards of 300 progenies,

1,000 if fertilized by a male *C. elegans (Chalfie, 2015)*. The worms reach their young adult stage within 3 days. *C. elegans* have a simple nervous system, however it is useful for understanding basic neuronal interactions that can be applied to human nervous systems. *C. elegans* demonstrate facets of nervous system as complex as synapse formation, glial function and neural regeneration (Chalfie, 2015). In addition, *C. elegans* can be used as a model organism to understand behaviors, including chemotaxis and avoidance behaviors.

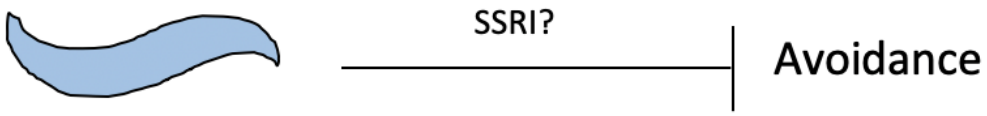

Figure 2: Can SSRIs inhibit avoidance? Worms have natural avoidance tactics that are characterized by specific movements. In response to negative stimulus, a worm will reverse movement with two backwards bends, or it will stop movement and perform an omega bend, in which the worm takes the shape of an omega. SSRIs have the potential to inhibit avoidance by lowering the organism's awareness of their surrounding and thus limiting response to negative stimuli, which can range from pheromones to predator cues.

The importance of this project lies within the numbers of people which suffer greatly from mood disorders and suffer from the lack of understanding surrounding SSRIs. The unintended side effects that accompany SSRIs, including daily nausea and vomiting, weight gain, mood swings, and erectile dysfunction further complicate the experience of an individual with a mood disorder (Selective Serotonin Reuptake Inhibitors, 2017). Furthermore, an individual desiring to stop an SSRI treatment must be carefully weaned off the treatment plan by

a medical doctor as the side effects of suddenly stopping an SSRI regimen include suicidal ideation (Carey, 2018). As the stigma of discussing mental health issues begins to decrease, and more people seek treatment for mood disorders, it is critical that we increase our understanding of how we are treating individuals suffering from these afflictions. This understanding starts at a molecular level, and this MQP seeks to elucidate how a common SSRI, Sertraline, changes the avoidance behaviors of *C. elegans.*

Methodology

osas#9 Experiments

Wild type N2 worms were used for the osas#9 experiments. These worms were washed off their plates using M9 and moved to 5 plates for testing. Once on their testing plates, the worms were starved for up to 24 hours to achieve necessary conditions for osas#9. This is due to this chemical being a pheromone released by starving worms. After these worms are starved, they are tested using a mouth pipette to administer drops of osas#9 and solvent control, which in this case is ethanol. The osas#9 is prepared using a 10 mM stock of osas#9 in ethanol and diluting it with 90 uL of water to create a 1 mM working stock. The ethanol control is created by combining 10 uL of pure ethanol and 90 uL of water to create a 10% ethanol working stock. During osas#9 avoidance assays, twelve young adult worms were exposed to osas#9 or control agent drops using a mouth pipette and results were recorded to achieve an avoidance index. Avoidance assays are performed by exposing worms to varying substances counting the number of worms that perform an avoidance behavior. This number is divided by the total number of worms that were exposed to the substance and this gives a value known as the avoidance index.

Sertraline Exposure

Primarily, the project consisted of experiments focused on measuring avoidance behaviors of wild-type *C. elegans* exposed to sertraline. The worms were washed off plates, indiscriminate of age, with M9, a type of ionized water. After being washed into a micro-centrifuge tube with M9, the worms were allowed to pellet to the bottom. The excess M9 was removed, and the worms were exposed to 50 uL of sertraline (0.0034 mg/mL) for exactly three minutes (Millapore Sigma, 2019).

10 mL M9 water + 34.269 mg stock Sertraline = 0.034269 mg/mL dissolved Sertraline

1 mL of 0.034269 mg/mL solution + 9 mL M9 water = 0.0034269 mg/mL working Sertraline solution

Initial Sertraline Calculation

The sertraline was then removed, the worms washed with 50 uL of M9, and then plated onto five plates. Once the worms were on the plate, an avoidance assay was performed using 1M glycerol and a solvent control of water to compare reactions of worms exposed and not exposed to sertraline. The control worms not exposed to sertraline underwent the same process of plating but were only exposed to M9. During the avoidance assays, twelve young adult worms were exposed to glycerol or control agent drops using a mouth pipette and results were recorded to achieve an avoidance index.

Worm Selection

Both sertraline exposure testing and osas#9 experiments were performed on N2 and mod-1 worms. N2 worms are wildtype, self-fertilizing hermaphroditic worms (WormBase, 2020). These worms acted as a control for the experiments in the sense that they have no mutations within

their genome that results in a phenotypic change. mod-1 worms are named after the *mod-1* gene, which encodes a serotonin gated ion channel. This channel is responsible for passage of chloride ions and is indicated in a worm's behavioral responses to starvation (WormBase, 2020). These worms were selected due to this gate controlling ion transport specifically in a serotonin receptor signaling pathway. This mutation is important in elucidating the interaction of serotonin in avoidance behavior and how the behaviors are changed by SSRIs. The question was, how will the exposure to SSRIs change the behaviors of mod-1 worms specifically. However, very limited testing was able to be done on mod-1 worms. The initial plate of mod-1 worms received was contaminated. This led to time being dedicated to raising clean stocks of mod-1 worms. Due to the COVID-19 pandemic reducing access to the lab, only two trials of data for mod-1 worms was able to be collected with inconsistent results. For this reason, it has been excluded from the focus of this project. The effects of and SSRI such as sertraline on mod-1 worms would be an interesting focus of a future MQP.

Results

The results of the experiments testing sertraline exposure of N2 wildtype worms were completed from October through March of the 2019-2020 academic year. The trials were completed on the main bench of the Srinivasan lab in Gateway Labs or in the humidity-controlled room of Gateway Labs, depending on availability. Trials were not completed on the main bench if humidity exceeded 35%.

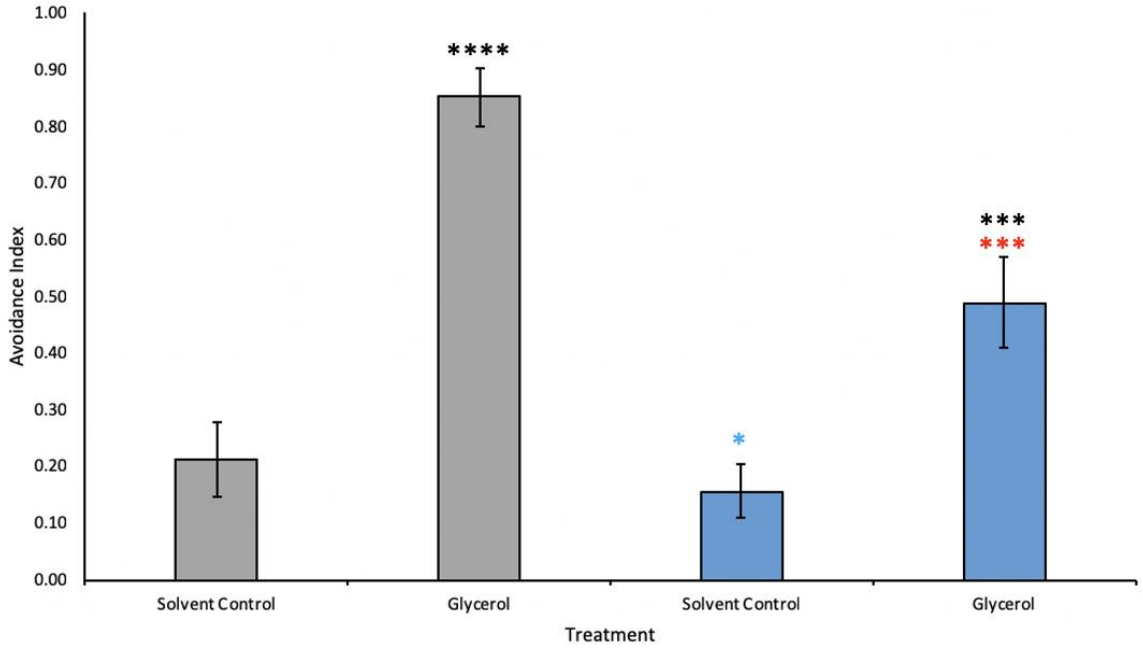

Figure 3: Avoidance index of worms exposed to Sertraline. Error bars are SEM and n = 35 for each condition. Grey bars indicate worms that did not receive Sertraline treatment. Blue bars indicate Sertraline treatment. Black asterisks indicate statistical significance between solvent control and glycerol in both cases of untreated vs. treated worms. Red asterisks indicate statistical significance between untreated and treated worms exposed to glycerol. Blue asterisks indicate statistical significance between untreated v. treated worms exposed to the solvent control, M9. This significance is defined in Table 1.

Avoidance index refers to the number of worms displaying avoidance behavior over the number of worms exposed to a substance. As a refresher, an avoidance behavior is classified as an omega bend, or two full turns backwards away from the direction the worm was originally traveling in (WormBase, 2020). The worms were given a four-second window to complete an avoidance behavior because the sertraline resulted in a slower reaction time to both solvent control (M9

water) and glycerol. As seen in Figure 3 the worms exposed to sertraline in blue, had a lower avoidance index when exposed to both the solvent control and the glycerol.

Table 1: P-Value Comparisons of Figure 3A

Samples Compared	P-Value
Untreated Solvent Control v. Untreated Glycerol	0.00001
Treated Solvent Control v. Treated Glycerol	0.00039
Untreated Glycerol v. Treated Glycerol	0.00012
Untreated Solvent Control v. Treated Solvent control	0.01714

As can be seen in the tables above, the comparison of worms tested for avoidance behavior have p-values that strongly support statistical significance. When compared to each other, the solvent control data sets have a very high p-value. Upon realizing this early in the year, I repeatedly tried to bring this number down by replacing the solvent control with a fresh stock. This seemed to briefly remedy the data in smaller batches of data.

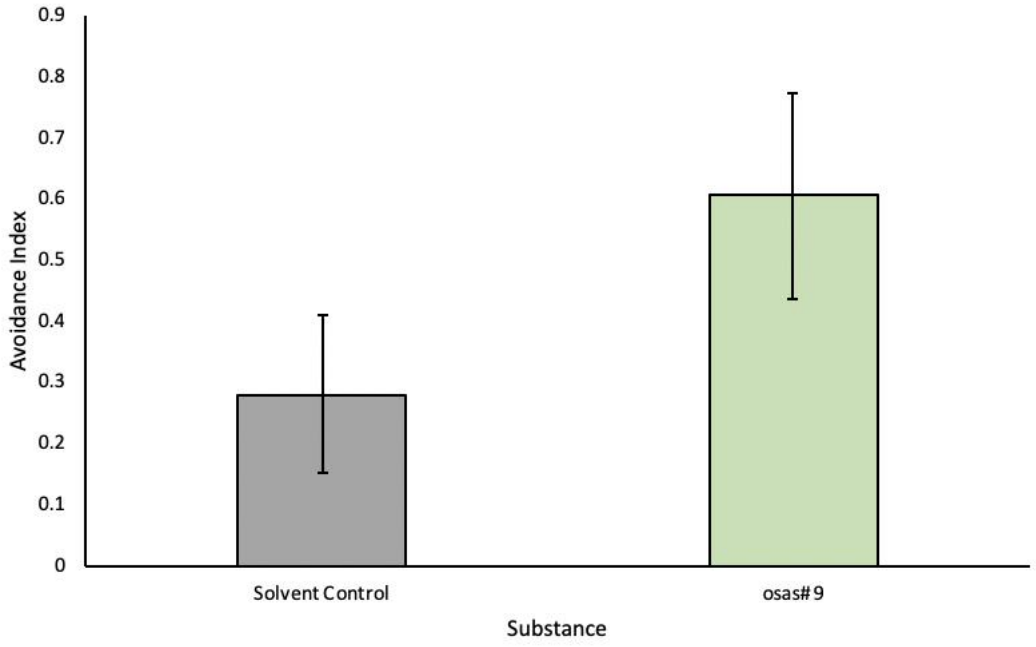

Figure 4: Avoidance index of worms exposed to osas#9. 10% ethanol Solvent Control, gray, compared to 1uM osas#9 stimulation, green. Error bars are SEM and n = 30 for each group. No statistical significance calculated between differing substances; p = 0.1106.

Figure 4 shows that although the worms exposed to osas#9 had a higher avoidance index than worms exposed to ethanol, but not significantly so. The error bars overlap and show that although worms are expected to avoid osas#9 consistently, as it's a chemical emitted by starving worms, the average avoidance index was approximately 0.6.

Discussion & Significance of Findings

The Impact of Sertraline on Avoidance Behaviors

As shown in Figure 3 of the results section, Sertraline significantly lowers the number of avoidance behaviors of worms exposed to both the solvent control, M9 and glycerol. Why is this? Sertraline is an SSRI, meaning that it inhibits the removal of sertraline from the nervous

systems of the worms. The results shown in this project suggest that limiting the removal of sertraline from the synaptic cleft in *C. elegans* lengthens the reaction time and reduces reaction completion to substances that typically result in highly consistent avoidance. The worms exposed to the SSRI showed a delayed response to glycerol, or no response at all. In addition, the treated worms consistently showed a reduced avoidance behavior in response to the glycerol. By reduced behavior, I mean that the worms would engage in approximately half of the omega bend or would only reverse and back up with one turn, instead of two. After this, the worm would stop moving and remain unresponsive. This is unusual behavior due to *C. elegans* strong avoidance to glycerol when untreated with Sertraline; these worms had an avoidance index of 0.90. The reduction in avoidance behaviors can be accounted to many biochemical interactions that could be occurring within the nervous system of the *C. elegans.* Serotonin is a multifaceted neurotransmitter with many roles as a communicator. This makes it hard to theorize exactly why the abundance of serotonin due to the SSRI exposure reduces avoidance behaviors. However, these results allow us to speculate how this relates to typical communication behaviors with *C. elegans.*

The behavioral assays performed while exposed to sertraline seem to reduce the recognition to the chemical the worms are supposed to be avoiding. A lethal application of these results is the inability to respond to predator cues. Predator cues are an aspect of chemical communication that is essential for survival. Senses allow animals to communicate with each other, even when it is unintentional. Chemosensory cues in animals can be in the air, the water, or along the ground, and can be perceived by other animals through smell or ingestion (Rosier, 2011). These cues, once recognized by prey, are utilized as information to avoid the threat. In some cases, prey can even perceive the concentration of these chemical tracks to understand how

long ago the predator was present, and which direction the animal was traveling in, to further avoid a confrontation (Rosier, 2011). *C. elegans,* despite their size and simplification, are no different than other animals when it comes to utilizing chemical communication to increase their odds of survivability. *P. pacificus,* a common predator of *C. elegans*, excretes small molecules called sulfolipids. When *C. elegans* perceive and process these molecules, they avoid the chemical through reversal, and exhibit stress behaviors such as not laying eggs (Liu, 2019). In the context of this study, untreated worms would respond to sulfolipids from predators with avoidance, as they should. However, as seen in Figure 3, avoidance behaviors are significantly reduced by Sertraline. This calls into question, would a treated worm ignore the cue, and continue towards the predator, or would it perform a limited avoidance behavior as witnessed in this study? This question opens this study to the next steps that can be considered for the direction of the project. How would treated worms respond to sulfolipids, and would it be different than how they responded to glycerol, which already has a high avoidance rate in untreated worms? These questions can be further explored and expanded on in future MQPs.

The overarching goal of this study was to gain a greater understanding of how SSRIs impact avoidance behaviors in *C. elegans,* and how that can be applied to humans. As previously explained, some common side effects of SSRIs in humans include loss of appetite, decrease in libido, feeling irritated, and somnolence, also known as excessive sleepiness. These can be connected to this study depending on how one categorizes an avoidance behavior in a human. In the simplest definition, an avoidance behavior occurs when an organism perceives stimulus and rejects it. In that context, over-sleeping, as well as appetite and libido loss could be classified as an avoidance behavior. Because humans have a larger and more complicated nervous system, the avoidance behaviors exhibited will be multi-faceted, and might not be as singular as simply

turning away from a stimulus we don't like. Some individuals that take SSRIs as a treatment for depression report lack of specific feelings towards anything; a sort of numbness that, while it removes the sadness one might feel while experiencing depression, it does not boost levels of happiness. They simply feel nothing. This could also be an avoidance behavior exasperated by SSRIs in the sense that it removes the proper perception of the experiences an individual is experiencing. The issue with SSRIs remains in the lack of understanding. It is unclear from this study if SSRIs are affecting the worms' ability to perceive the glycerol as a negative stimulus to avoid or affecting their decision in trying to avoid it. The best indicator that the SSRIs are affecting their decision-making behaviors are the worms that initiate an avoidance behavior and then stop. In addition, testing worms' response to osas#9 after exposure to sertraline would be an interesting study. Because osas#9 is a starvation signal, it would be a possible connection to SSRIs resulting in loss of appetite if the worms were undeterred by the signal indicating a lack of food. These are two examples of avenues that can be expanded upon further in a future MQP that would elucidate the functions of SSRIs and allow us to understand their method of action in humans.

Conclusions & Next Steps

This project was exciting to work on as a major qualifying project. Having spent the year researching SSRIS, mood disorders, and *C. elegans*, as well as designing and performing experiments, I have gained a greater appreciation of the mental health status of the United States. This situation cannot be classified as anything short of a crisis. The statistics are staggering, and some of the personal anecdotes of individuals who have suffered from a mood disorder are heartbreaking, however, there is hope in research. SSRIs have great potential to be a successful

line of treatment of a wide variety of mood disorders, and when paired with cognitive or dialectical behavioral therapy (DBT), people who struggle with mood disorders can thrive and be happy and healthy. With that being said, there is much progress to be made in terms of understanding SSRIs and their method of action. From this study, we can conclude that SSRIs affect the avoidance behaviors of worms significantly by reducing the number of avoidances exhibited when exposed to glycerol, a substance that typically has a very high avoidance index in *C. elegans.* This can be connected to the experience humans have when prescribed SSRIs for mood disorders, as it results in behaviors such as excessive sleep, lack of interest in life, and decreased libido and appetite. This project began with the question of, can we use *C. elegans* as a model to understand how communication and avoidance behaviors change when exposed to SSRIs? Can we use achieve a better understanding of the value of serotonin as a neurotransmitter within the nervous system? The questions have been answered through this project. *C. elegans* prove to be a great model for experiments concerning SSRIs and have great potential in terms of expanding this project in different directions. In addition, this project showed that serotonin, and the limitation of removing serotonin from the synaptic cleft, can be life and death. In terms of communication such as predator and prey cues, SSRIs are hazardous in that the limit the organisms ability to remove itself from the dangerous situation.

There are many directions this project can continue in. Before the pandemic of COVID-19, this project originally planned to encompass utilizing a strain of *C. elegans* called *mod-1*. The gene *mod-1* encodes a serotonin gated ion channel, which controls passage for chloride. This gate controls ion transport in a serotonin receptor signaling pathway (Wormbase, 2019). How this strain of worm interacts with an SSRI would be interesting to explore because they have shown to be resistant to paralysis or locomotion impairment when exposed to serotonin

treatments. This is a future direction that could assist in helping us understand how serotonin is involved in different communication behaviors of *C. elegans.* In addition, there is a genetic portion of this project that could be explored. Are the progeny of worms exposed to an SSRI affected as well? Is there an epigenetics portion of this treatment that changes the avoidance behavior in the future generation, and if so, how many generations are impacted? This is important because as more people are prescribed SSRIs, there will be more people having children while actively taking this treatment. This project has potential to be expanded in many different directions because there is so much to learn about SSRIs and how they impact communication in the nervous system. There is much progress to be made, but SSRIs have the potential to be an effective and safe treatment for a myriad of mood disorders and improve the lives of many.

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References

Any Anxiety Disorder. (2017, November). Retrieved September 15, 2019, from https://www.nimh.nih.gov/health/statistics/any-anxiety-disorder.shtml.

Anxiety Disorders. (2018, July). Retrieved September 15, 2019, from https://www.nimh.nih.gov/health/statistics/any-anxiety-disorder.shtml.

Carey, B., & Gebeloff, R. (2018, April 7). Many People Taking Antidepressants Discover They Cannot Quit. Retrieved October 4, 2019, from https://www.nytimes.com/2018/04/07/health/antidepressants-withdrawal-prozac-cymbalta.html.

Chalfie, M., Corsi A., Wightman, B. (2015). A Transparent window into biology: A primer on *Caenorhabditis elegans.*

Facts & Statistics. (n.d.). Retrieved October 8, 2019, from https://adaa.org/about-adaa/press-room/facts-statistics.

Feher, J. (2017, February 24). Propagation of the Action Potential. Retrieved September 18, 2019, from https://www.sciencedirect.com/science/article/pii/B9780128008836000252

Ferguson J. M. (2001). SSRI Antidepressant Medications: Adverse Effects and Tolerability. *Primary care companion to the Journal of clinical psychiatry*, *3*(1), 22–27. https://doi.org/10.4088/pcc.v03n0105l;oiuymjntrbfgrbgngtv

Liu, Z., Kariya, M., Chute, C. (2019, March). The Chemistry of Fear. Natural History. Retrieved March 24, 2020, from https://www.wpi.edu/sites/default/files/inline-image/News/News-Articles/Natural%20History.pdf

Major Depression. (2018, February). Retrieved September 15, 2019, from https://www.nimh.nih.gov/health/statistics/any-anxiety-disorder.shtml.

McIntosh, J., Wilson, D. (2018, February 2). What is serotonin and what does it do? Retrieved October 1, 2018, from https://www.medicalnewstoday.com/articles/232248

Mellem, J., Brockie, P., Madsen, D. *et al.* Action potentials contribute to neuronal signaling in *C. elegans*. *Nat Neurosci* **11,** 865–867 (2008). https://doi.org/10.1038/nn.2131

Millipore Sigma. (2020). Sertraline Hydrochloride. Retrieved on October 21, 2019, from https://www.sigmaaldrich.com/catalog/product/sigma/s6319?lang=en&region=US

Mood disorders. (2018, December 20). Retrieved September 11, 2019, from https://www.mayoclinic.org/diseases-conditions/mood-disorders/symptoms-causes/syc-20365057.

Rosier, R. L. & Langkilde, T. (2011) Behavior Under Risk: How Animals Avoid Becoming Dinner. *Nature Education Knowledge* 2(11):8

Selective Serotonin Reuptake Inhibitors. (2017, September 17). Retrieved September 24, 2019, from https://www.mayoclinic.org/diseases-conditions/depression/in-depth/ssris/art-20044825

The Top 300 Drugs of 2020. (2020). Retrieved January 20, 2020 from https://clincalc.com/DrugStats/Top300Drugs.aspx