Selegiline: The Effects of Monoamine Oxidase Inhibitors on the Behavior and Physiology of *Caenorhabditis Elegans*

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ABSTRACT

Research detailing the mechanisms behind antidepressants, such as monoamine oxidase inhibitors, is lacking. This study investigates the effects of selegiline on the behavior and metabolic pathways of the model organism *C. elegans* through egg-laying, thrashing, and lipid composition assays. A survey was conducted to contextualize these results in terms of broader impact by demonstrating depression prevalence. Our results showed that selegiline impacts the egg-laying timeline and thrashing frequency of *C. elegans*. Analyses of lipid assay data show marked differences in fatty acid composition. On a survey measuring depressive symptoms, WPI students averaged a score of 24.3, which is considered at risk for depression. These results highlight the prevalence of depression and the importance of understanding the physiology while providing insight into potential biological mechanisms implicated in depression.

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INTRODUCTION

Depression affects 264 million people and its prevalence is on the rise globally. Nearly 800,000 people die from suicide every year, making this the 2nd leading cause of death for people ages 15-29 (Culpepper, 2013; World Heath Organization, 2020). Yet the science behind how certain pharmacological treatments work to combat this disease is lacking. These staggering statistics coupled with this gap in knowledge warrants a deeper investigation into the mechanisms behind depression and antidepressants. Although SSRIs are the most commonly prescribed antidepressant with the least reported side effects, researchers' opinions on the effectiveness of this drug class vary (Vince, 2020). This may be due to research showing that other antidepressants are effective despite a tendency to have more side effects. In order to curb the depression pandemic, research first needs to advance scientific knowledge to understand mechanistically how a wider variety of antidepressant classes work.

This research will lay groundwork evidence of the mechanisms and effects of one class of antidepressant. Extrapolated from the mechanisms of antidepressants, this deeper understanding of the physiology of depression will answer a basic science question of how psychopharmacological treatments engage physiology to affect symptomatology. This interdisciplinary, convergent research will also integrate a neuroscience perspective for understanding psychophysiological processes and disorders.

Currently, little is known about the brain and its complicated and abundant neuronal pathways. This research will focus on experimentation using *C. elegans* to determine the effects of one antidepressant, selegiline. This research will lay the groundwork for more specified work

involving drug interactions at the molecular level. Given the similarities between human and *C. elegans* neurotransmitters and neural pathways, these results will give insight as to how these mechanisms function in humans. Determining how antidepressants work is pertinent in order to gain a better understanding of the physiology of depression.

This foundational research can support the development of a more effective antidepressant in humans. Increasing the knowledge base regarding depression could aid in new or improved therapies which has the potential to help millions of people who are suffering. Any piece of knowledge added to the puzzle that is the human brain will help understand hundreds of pathways, connections, and neurological disorders. Finally, given the truly convergent science approach that this research takes, this work could have a broad impact across various fields for a pressing societal issue.

BACKGROUND

This section provides an overview of the biology of depression, current state of antidepressant therapies, and information regarding the physiology of model organism *Caenorhabditis elegans (C. elegans)*. This section will depict the need for this research and relevant information for understanding the procedures that follow.

2.1 Depression

Working to find an effective treatment for depression would provide relief to the millions of people afflicted with the disease. In order to synthesize an effective antidepressant, the mechanisms underlying the disease need to be better understood. This research could also further general knowledge of how neurotransmitter function affects the body, which is pertinent to treating any neurological condition. A large body of research has investigated the biology behind depression. Structural and chemical abnormalities underlying depression have been identified (Delgado, 2000).

Current research shows that depression is caused by a variety of interactions between biological and psychosocial factors. By studying those suffering from depression, researchers have shown that some of the major neurotransmitters used by our body are implicated in depression (Syvälahti, 1994). The interaction of antidepressants has been essential when discovering the pathophysiology of depression. Research has shown a clear link between deviations, namely deficiencies, in neurotransmitter functioning and susceptibility to depression. However, the exact mechanism by which these deficiencies result in depression is still largely unknown.

2.2 Neurochemistry of Depression

Neurotransmitters are molecules that perform cell-to-cell signaling through chemical reactions. There are several classes of neurotransmitters in humans including: monoamines, amino acids, peptides, and catecholamines (Brody, 1999). Neurotransmitters allow cells to communicate by transmitting signals across synapses, which are junctions between neurons. These chemical signals travel from the presynaptic neuron across the synapse, bind to receptors on the postsynaptic neuron inducing an activity. Neurotransmitters are extremely important for normal neurological functioning; however, the complex nature of their systems and interactions leaves disease caused by their malfunction difficult to understand and treat. Neurotransmitter functioning is implicated in several major neurological disorders such as: Alzheimer's Disease, where acetylcholine dysfunction occurs; Parkinson's Disease, where dopamine levels are low; and major depressive disorder (MDD), where multiple neurotransmitters are implicated in the pathology (Kondziella, 2017).

Following action on the postsynaptic neuron, a neurotransmitter is typically degraded in the synapse or returns into the presynaptic neuron through a process called reuptake. These processes are imperative for neuromodulation and proper functioning of neurotransmitter systems. When these neurotransmitter systems are dysregulated, neurological disorders may occur as a result. For example, the majority of biological theories of depression focus on the release and reuptake of neurotransmitters specifically.

2.3 Monoamines (MAs)

One class of neurotransmitters especially relevant to proposed mechanisms of depression is monoamines (MAs). The central monoamines found in humans include serotonin, dopamine, epinephrine, and norepinephrine (NE) (Sheffler & Pillarisetty, 2019). Monoamines are released into the synapse between neurons and are important for cell signaling via binding to their respective receptors. Following action upon a receptor, reuptake or degradation of MAs occurs. MAs are important for a wide range of biological functions including regulation of mood, appetite, sleep, and different cognitive functions (Velasco & Tan, 2014). Considering the role of monoamines in neuroendocrine regulation, the depletion and dysfunction of these systems has been a major target in studies of depression physiology.

The monoamine hypothesis of depression posits that depressive symptomatology is a result of depleted synaptic levels of serotonin, norepinephrine, and dopamine. This theory has been supported by the mechanism of antidepressants, which commonly increase availability of the above-mentioned neurotransmitters. One study observing the effects of an antihypertensive medication called Reserpine showed that it causes depression in some patients. Mechanistically, Reserpine depletes monoamines from presynaptic terminals of neurons (Gillespie & Mackenna, 1961). Ample evidence has implicated monoamine deficiencies in patients with MDD. However, research has yet to identify the dysfunction of a specific monoamine pathway in patients with MDD. This suggests that the physiology underlying depression is much more complex than failure of a neurotransmitter pathway (Delgado, 2000; Heninger et al., 1996).

While the pathophysiology of depression is more complicated than monoamine levels alone, NE, serotonin, and dopamine pathways and functions play a role in the disorder. It is difficult to investigate how one monoamine system impacts the pathophysiology of depression without considering others. Several studies have shown that serotonin, norepinephrine, and dopamine systems interact extensively.

Norepinephrine, which is synthesized from dopamine, acts on the sympathetic nervous system by binding to adrenergic receptors (Juárez Olguín et al., 2016). NE is important for regulation of the nervous system and stress responses in the body. This specific branch of the nervous system, which is often referred to as the "fight or flight" branch, is responsible for mediating arousal effects in the body such as increased heart rate, vasoconstriction, and decreased digestion. Dysregulation of NE system has been identified in the pathology of depressive disorders; especially NE interactions with the serotonin system.

Within the central nervous system, serotonin is synthesized and stored in presynaptic neurons (Mohammad Zadeh et al., 2008). When a neuron is depolarized, serotonin is released into the synaptic cleft and is then able to bind to serotonin receptors. The serotonin transporter located on the presynaptic vesicle is responsible for the reuptake of the neurotransmitter where it is then degraded or converted into other molecules such as melatonin (Mohammah-Zadeh et al., 2008). Serotonin has a role in modulating various actions such as eating, locomotion, reproduction, mood, and cognition (Charnay & Leger, 2010).

The dopamine pathway is another monoamine pathway that has been studied for its role in depression. Dopamine is important for its role in modulating normal cognitive functioning, reproductive pathways and behavior, and even motor control. Dopamine receptors are widely expressed in the body as dopamine is produced in the central and peripheral nervous system (Juárez Olguín et al., 2016). Dopamine pathway dysfunction has been implicated in other neurological disorders as well such as Parkinson's Disease, Huntington's Disease, and Attention Deficit and Hyperactivity Disorder (Klein et al., 2019). The wide range of functions and widespread production of dopamine makes it difficult to study due to the interactions with other neurotransmitter pathways. However, this makes it even more important to fully understand the mechanism of action of dopamine, especially in the context of depression.

2.4 Pharmacological Treatments: Monoamine Oxidase Inhibitors (MAOIs)

Neurotransmitters are the primary target of antidepressant therapies. The majority of antidepressants modulate neurotransmitter levels in order to manage depressive symptoms. One such class of antidepressants called monoamine oxidase inhibitors (MAOI) targets monoamines, the neurotransmitter class previously discussed in detail. (MA). MAOIs were the first type of antidepressant used and have been revisited as a topic of research recently for their potential as a treatment for depression along with other neurological disorders, such as Parkinson's. MAOIs have proven effective and have frequently been found effective for cases of depression in which other antidepressant treatments fail (Cole & Bodkin, 2002).

MAOIs target monoamine oxidase (MAO), an enzyme responsible for the degradation of the above-mentioned monoamines. MAOs catalyze the oxidative deamination of monoamine

neurotransmitters (Bortolato et al., 2013). Recent research has shown that MAOIs do not only affect MA degradation, but directly target MA receptors (Vince, 2020). In humans, there are two classes of monoamine oxidases, MAO-A and MAO-B. Accordingly, MAOIs have been developed to selectively target one class or both classes or MAO. One MAOI, selegiline, selectively inhibits MAO-B at low concentrations but can act on MAO-A and MAO-B at higher concentrations. Selegiline is an irreversible inhibitor making it a good candidate for drug therapies (H et al., 1989; Moore & Saadabadi, 2021). Selegiline is typically administered as a transdermal patch where absorption into the blood occurs. This is the preferred method of administration for treating MDD because it results in higher concentration levels which are needed to inhibit MAO-A and MAO-B molecules. The figure below depicts the active site of MAO-B with notable amino acid residues labeled.

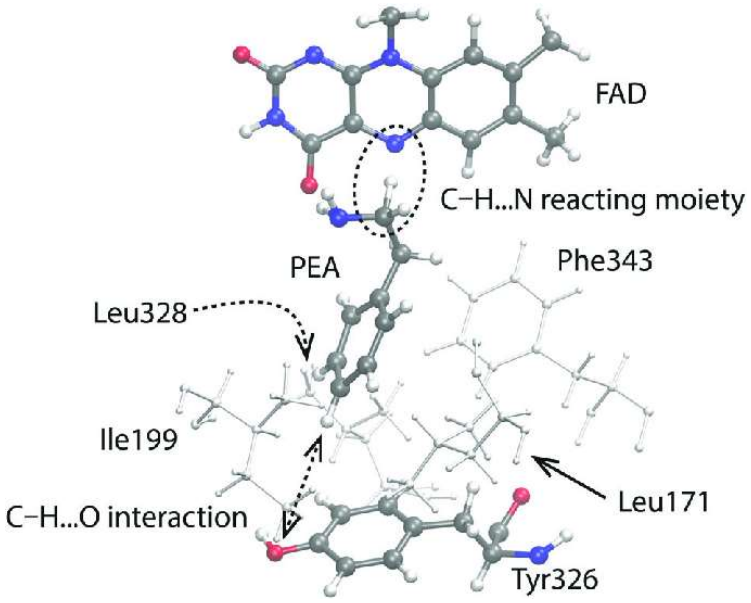

Figure 1*: Structure of the MAO-B active site,* (Finberg, 2016).

Selegiline is thought to irreversible inhibit MAO-B by binding to the active site. Irreversible inhibitors are thought to covalently bind to the N5 atom of the active site, this is referred to as the

reacting moiety in the figure. While the formation of the selegiline-enzyme complex has been detailed, the mechanisms by which selegiline dissociates are largely unknown (Finberg & Rabey, 2016). This indicates a need for further research into the molecular pathways behind selegiline treatments. Additionally, there are other competing theories that may elucidate the mechanism of selegiline.

While the theory that selegiline directly inhibits MAO is widely accepted, recent research has shown that other mechanisms may aid selegiline's therapeutic efficacy. One such theory states that selegiline's metabolites, such as amphetamine, directly causes increased neurotransmitter secretion (Moore & Saadabadi, 2021). The figure below depicts the conversion of selegiline to amphetamine.

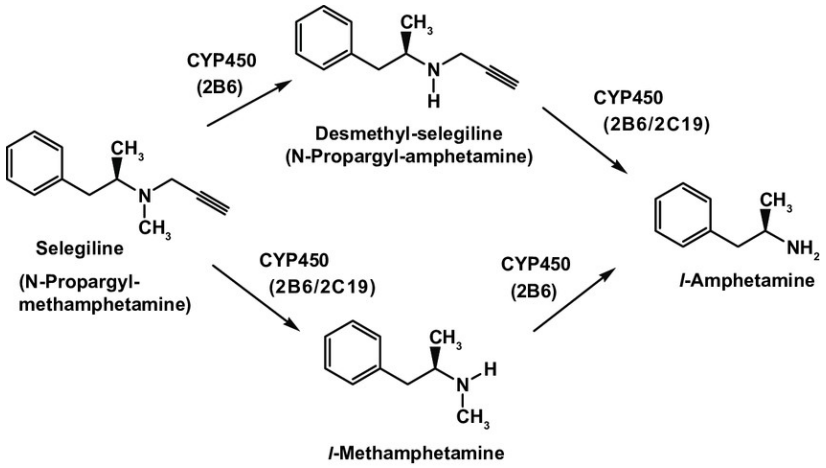

Figure 2: *Reaction pathway of selegiline being metabolized to amphetamine,* (Patkar, 2006).

As seen in the diagram, CYP450 are the enzymes responsible for catalyzing this conversion pathway. One study revealed that amphetamines can act directly on synaptic vesicles to induce neurotransmitter release. Amphetamines achieve this by diminishing the vesicle pH-gradient that is responsible for the retention of neurotransmitter in the vesicles. This decrease in vesicle retention of neurotransmitters leads to increased secretion (Freyberg et al., 2016). The evidence

supporting this alternate mechanism of action indicates the need for further research investigating whether selegiline increases availability of neurotransmitters by acting directly on MAO and neurotransmitter receptors or whether it's metabolites function to increase availability as well.

2.5 Model Organism: *Caenorhabditis elegans (C. elegans)*

Model organisms are utilized in research of human diseases and disorders. Model organisms are used to manipulate expression of genes and other experimental conditions to observe the phenotypic and external effects which would not be ethical to subject humans to. They are also used in experimental research, such as drug development, to give preliminary data on effects. *C. elegans* is an especially good model organism for neurological disorders. *C. elegans,* depicted in *Figure 3* below, are a 1mm long nematode with a lifespan lasting up to 2 weeks (Blaxter, 1998). Almost all *C. elegans* are self-fertilizing hermaphrodites, producing about 200-250 eggs in their lifetime. These animals have very primitive biology, comprising about 960 somatic cells, 300 neurons, and 17,800 distinct genes total (Bargmann, 1998; Bargmann & Horvitz, 1991). Due to their simplistic genome and biological nature, *C. elegans* have many functional counterparts in humans making it a reliable model in studying disease. *Escherichia coli* (*E. coli*) is their main source of diet, which is naturally found in soil, and the media used to grow and culture the animals.

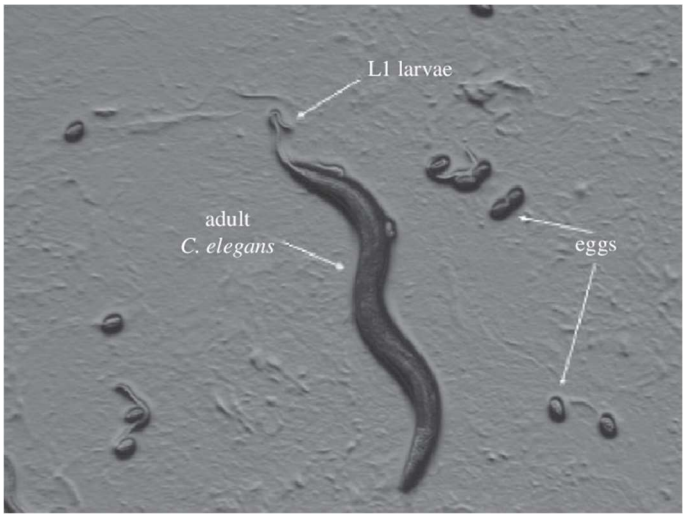

Figure 3: *C. elegans, a 1mm nematode worm,* (Chuang Lab).

Hermaphroditic *C. elegans* were the first model organism to have its entire neural network, consisting of 302 neurons, described and mapped (Cook et al., 2019). *C. elegans* are an especially good model for human neurological disorders given that the nematode and humans use homologous receptors in chemical signaling. The common neurotransmitters in humans such as serotonin, dopamine, and octopamine, a norepinephrine homolog, are also present in *C. elegans* (Pandey & Harbinder, 2012). Similarly, monoamine receptors in *C. elegans* are structurally and functionally similar to human receptors. For example, the dopamine receptors in *C. elegans,* D1-like and D2-like receptors, have consistent effects in many mammals, including humans (Wang et al., 2014). These similarities allow researchers to understand which neuroendocrine systems may be involved in human neurological disorders. This creates the possibility for more targeted therapeutics.

The following research aims to determine the biological and behavioral effects of Selegiline in the model organism *C. elegans*, namely, the functions that are analogous to those in humans. Locomotion is modulated by neurotransmitters such as acetylcholine, dopamine and serotonin, in

both humans and c. elegans. Egg-laying behavior is important to investigate because this reproductive pathway is stimulated by serotonin. Lipid composition is critical for efficient neurotransmission and is implicated in many neurological disorders in humans. Further understanding the effects of MAOIs in *C. elegans* will lay groundwork for a specific mechanism of action for the drug Selegiline by indicating the biological processes that the drug moderates.

2.6 Behavioral Assays

Behavioral assays are used in research to observe the behavioral changes of an organism. When conducting a behavioral assay, the animal's natural habitat must be taken in consideration. It is imperative that the experimental conditions do not interfere with the animal's natural way of living. Exposing the organism to excessive stress by over handling can cause discomfort which may lead to unintended behavioral or physiological changes rendering results inconclusive results (Võikar et al., 2004). Some of the more obvious considerations may be natural competition for food, the animal's life cycle, and innate behaviors, but as a researcher it's important keeping in mind the uniqueness of particular animal models as most animals do not share the same senses as humans. For example the Bombus terrestris bumblebee uses mechanosensory hairs to detect electric fields when searching for flowers to pollinate and the Zebra finch has tetrachromatic vision (Lind, 2016). Identifying these alternative variables is critical when determining experimental conditions for the model organism.

Studying the behavior of *C. elegans* can be used as a proxy measure of neuron functioning given that neuromodulated behaviors are sensitive to environmental alterations (Chen et al., 2013). Although causation of a behavior cannot be tied directly to the activation of a specific neuron,

they may indicate the pathways afflicted by certain conditions or treatments. Studying the actions and behaviors of model organisms also allows for the exposure of potential alternate effects which may have not been controlled or even noticed by the researcher.

Through egg-laying assays, observation of reproductive behavior and potentially identify neuronal pathways being affected by a particular treatment, in this case, selegiline. Locomotion assays can be used to observe the effects of a particular treatment on the mobility rate and ability to contract the muscles of the animals. The present research utilizes a thrashing assay to observe the rate of body bending in *C. elegans* to determine selegiline's effect on neuronal pathways associated with locomotion.

2.6.1 Egg-Laying Behavior.

C. elegans lay a set number of eggs, about 250, over their lifespan. Through self-fertilization, these animals store about 10-15 fertilized eggs in the uterus before expulsion through the vulva and subsequent new egg formation (Schafer, 2005). Egg-laying behavior is regulated by multiple environmental cues due to connections with sensory neurons . Vibration and mechanical stimulations of the NGM culture can cause inhibition of egg laying. Conversely, an overabundance of food causes an increase in egg laying (Desai & Horvitz, 1989). Because this specific egg-laying behavior is common among the 99.9% of hermaphroditic nematodes, observation rate of egg-laying can identify changes in behavioral timing of egg release.

2.6.2 Locomotion

The act of self-propulsion, otherwise known as locomotion, is an essential behavior for all animals to survive. Mobility of the *C. elegans* is dependent on several other behaviors such as navigation, foraging, and egg-laying . The animals move in an undulatory fashion, by generating thrusts of dorsal body bends to propel themselves forward or backward (Gjorgjieva et al., 2014). *C. elegans* are commonly studied in a crawling state on NGM agar plates, but when placed in a liquid medium buffer, the animals will shift to a swimming behavior. Research has shown that that locomotion behavior for swimming *C. elegans* is faster than crawling such that the density of a *C. elegans'* environment is inversely proportional to its movement (Suzuki et al., 2005). In the context of this research, forward-directed locomotion and thrashing behavior of *C. elegans* was investigated.

2.7 Egg-Laying and Locomotion Neuronal Circuit

Along with sensory neurons, egg-laying has been found to be temporally correlated with locomotion as well. Locomotor velocity is increased prior to an egg-laying event and interestingly reverse movements are inhibited. The connections between egg-laying and locomotion are due to the activity of the hermaphroditic specific motor-neurons (HSNs) to AVF interneurons. Synapses from the HSNs to the AVF result in a forward command synapsed to the AVB interneuron (Brewer et al., 2019). Research has shown that interfering with serotonin neurotransmission between the two neuron circuits results in a loss of this increased velocity prior to egg-laying events. These results indicate that during active egg-laying, HSNs may also modulate locomotion due to synaptic connections between the AVFs (Hardaker et al., 2001).

Egg-laying and locomotion are essential and primal behaviors of *C. elegans* and understanding the circuits underlying these mechanisms could help identify how antidepressants impact the serotonin pathway. Egg-laying behavior is controlled by a small circuit of six neurons; two serotonergic hermaphroditic specific neurons (HSNs), and six cholinergic ventral c neurons (VCs). These neurons are only seen in the hermaphroditic species of *C. elegans* and in the small percentage of male nematodes that exist these neurons undergo a programmed cell death (Collins et al., 2016).

2.7.1 Hermaphroditic Specific Neurons (HSNs)

The HSNs release serotonin and NLP-3 neuropeptides to induce and regulate egg-laying behavior in *C. elegans.* Research has shown that ablation of the HSNs results in significant reduction of egg-laying rate (Moresco & Koelle, 2004). Observing egg-laying rates for HSN-ablated animals indicates that these neurons are essential in onset of egg-laying, and therefore the egg-laying timeline. In HSN ablated animals, exogenous serotonin will rescue this defect causing regular egg-laying rates. This evidence supports the claim that HSNs promote egg-laying by releasing serotonin as a neuromodulator. It is interesting to note that serotonin deficient *C. elegans* are found to have a less severe effect on egg-laying rate compared to those animals with ablated HSNs; indicating that HSNs may also release other neurotransmitters along with serotonin to aid in egg-laying. Paradoxically, chronic exposure to high concentrations of serotonin directly to HSNs has been shown to have an inhibitory effect on egg-laying (Schafer et al., 1996).

2.7.2 Ventral C (VC) Neurons

The VCs are essential for vulval muscle contraction and vulval opening during egg-laying events of *C. elegans.* These neurons are cholinergic and express many neuropeptides. There is some evidence that VCs may also release serotonin. Because of the expression of a vesicular monoamine transporter, researchers believe these neurons also function in releasing a monoamine, most likely serotonin (Duerr et al., 2001) Although these neurons are involved in egg-laying, ablation of these neurons had little effect on egg laying. Evidence has shown that VCs may play an inhibitory role on egg-laying, such that when the VC4/VC5 neurons are ablated, there are fewer unlaid eggs suggesting a higher egg-laying success rate but potentially less eggs overall (Kopchock et al., 2020). Another VC neurotransmitter, acetylcholine, may have a fundamental role in egg-laying given that nicotinic agonists strongly stimulate egg-laying. This characteristic is only seen in functioning nicotinic receptors of the vulval muscles.

The way in which HSNs and VCs work together on a molecular and neurochemical level has yet to be understood. In summary, VCs release acetylcholine in order to stimulate vulval muscular contraction through the nicotinic receptors while feedback inhibits the HSNs through muscarinic receptors. It is possible that neurohormonal signaling may play a key role in egg-laying processes but simplicity this research will focus on the effects of monoamines, such as serotonin, on this process. Although this circuit is anatomically simple, evidence has shown that multiple neurotransmitters and signaling molecules are implicated in generating complex and temporal patterns of egg-laying behavior. Uncovering these mechanisms and effects on behavior are crucial steps towards future investigation.

2.8 Lipids Metabolism

Lipids are a class of insoluble macromolecules that have important roles in major biological processes. Lipids are made up of long hydrocarbon chains. One specific type of lipid is a fatty acid, which is the primary target of this research, which is characterized by a carboxylic acid group (~COOH) and a methyl group (~CH3) at either end. Hydrocarbon chains are nonpolar molecules and as a result lipid are hydrophobic, which means they repel water. There are two main types of fatty acids: saturated fatty acids and unsaturated fatty acids. Saturated fatty acids contain no double bonds as the carbons are completely saturated with hydrogen atom. This results in straight hydrocarbon chains that can pack tightly together. Unlike saturated fatty acids, unsaturated fatty acids can not achieve this tight packing. Unsaturated fatty acids contain one or more double bonds between carbon atoms. These double bonds result in bends and kinks in the hydrocarbon chain. These characteristics of fatty acids are depicted in the figure below.

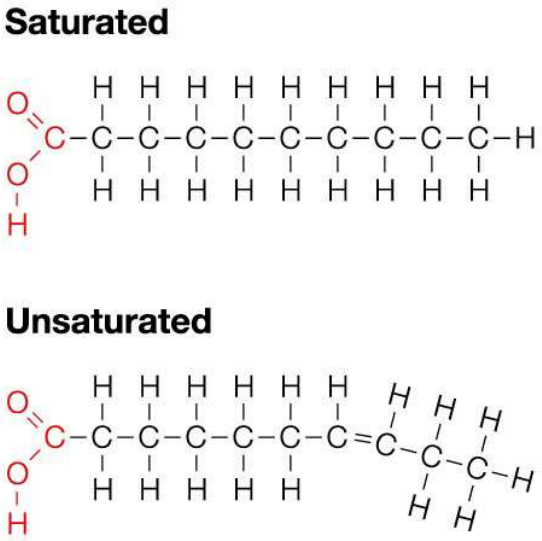

Figure 4: *Structure of saturated and unsaturated fatty acids,* (Carla, 2018)

These bends prevent the tight packing that is seen with saturated fatty acids. Lipids can be separated into various classes that each have unique structures and functions. The two main lipids involved in this study are phospholipids and triacylglycerols (or triglycerides).

Phospholipids contain two fatty acid tails and a hydrophilic phosphate head. Phospholipids form lipid bilayers due to their hydrophobic and hydrophilic parts. Phospholipid bilayers make up the structural component of cell membranes and their precise regulation is pertinent to survival. The other target lipid of this research is neutral lipids. These are uncharged lipids, typically triglycerides in *C. elegans*, that primarily serve as fat stores because they can be readily metabolized into glucose molecules for energy (Vrablik et al., 2015; Watts, 2009). In an analysis of *C. elegans* lipid droplets, researchers found that they were composed of neutral triacylglycerols surrounded by a monolayer of phospholipids. These lipid droplets function in fat homeostasis and growth in the nematode (Vrablik et al., 2015).

The nature of saturated and unsaturated fatty acids mentioned above is especially important to consider in the context of phospholipids. As previously stated, phospholipids make up cellular membranes, so the ability to tightly pack is important for membrane structure and fluidity. The differences in fluidity is depicted in *Figure 5* below.

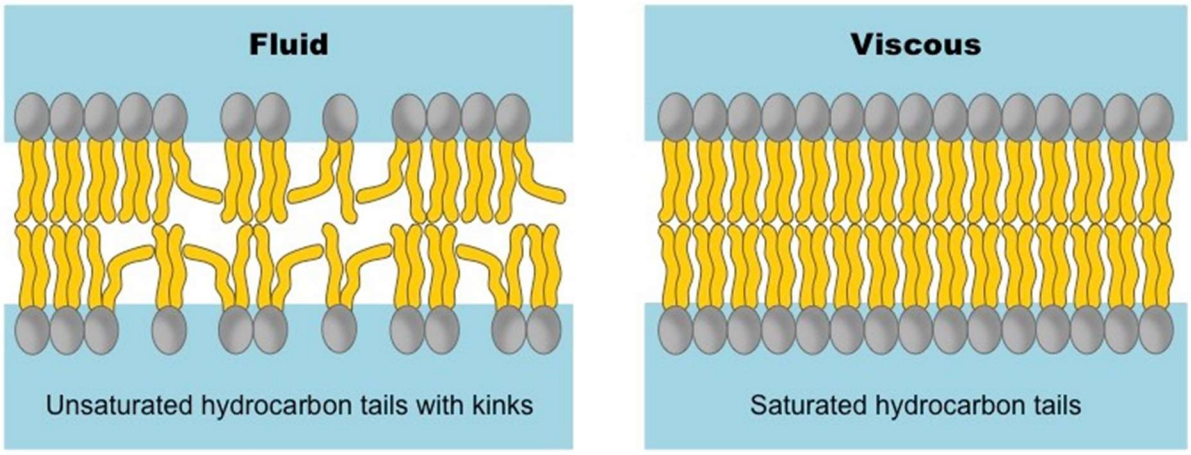

Figure 5: *A fluid (left) versus viscous (right) cell membrane due to saturated and unsaturated fatty acid composition,* (Urry, Lisa).

Membrane fluidity, which is crucial for cellular functioning, is dependent on the unsaturated and saturated fatty acid composition (Hac-Wydro & Wydro, 2007). Varying lipid composition can affect the function of proteins that sit in the membrane, which can lead to changes in cell signaling and therefore neurotransmission (Carta et al., 2014; Laganowsky et al., 2014). This is especially important to consider in terms of neurobiology of depression given that recent research has indicated that membrane lipids may play a crucial role in the pathogenesis (Burkhart et al., 1999; Carta et al., 2014; Postila et al., 2016). Membrane lipids regulate the function of membrane proteins, including those that are responsible for the transportation and signaling of neurotransmitters. Given that neurotransmission is implicated in depression, this research aims to investigate potential changes in lipid composition that may lead to altered membrane fluidity.

METHODOLOGY

The following section explains the procedures followed throughout the duration of this research. The methods conducted include: general maintenance of *C. elegans;* egg-laying and thrashing assays; lipid composition assay; and finally a psychological survey.

3.1 Strains, Maintenance, Synchronization and Counting

The N2 wild type strain of *C. elegans* was used for all experiments conducted. For the behavioral assays, *C. elegans* populations were maintained on 6cm Nematode Growth Medium (NGM) plates. These plates were prepared by pipetting 2-3mL of OP50 on and were left at room temperature until dried. *C. elegans* were transferred to new plates using a sterile titanium pick at least twice weekly. For the lipid assay procedure, *C. elegans* were maintained on 10cm High Growth Medium plates (HG) that were seeded with 50µL of OP50 *E. coli*. Sterile glass beads were used to spread the OP50 around the plate. The HG plates were left overnight in a 20℃ refrigerator. Worms were maintained by transferring between HG plates using an M91X buffer.

To synchronize a sample of *C. elegans*, adult worms were pipetted from a plate into a properly labeled centrifuge tube. The centrifuge tube was filled with M91X buffer and centrifuged at 2000rpm for 1 minute. The supernatant was then aspirated leaving the pellet of worms behind. A 12.5mL solution consisting of 2.5mL of bleach, 1.25mL of KOH, and 8.75mL of deionized H_2O was added to the centrifuge tube. The tube was then vortexed, and the worms were left in this solution for 6 minutes. The worms were washed by centrifugation at 2000rpm for 1 minute. The supernatant was discarded and M91X buffer was added to the tube. The washing step was

repeated twice more. Finally, M91X buffer was added to the tube and allowed to rotate in a 20C refrigerator for 12-24 hours to allow all the eggs to hatch.

The incubating eggs hatched into L1 larvae and were then counted before plating onto seeded HG plates. First, the L1 larvae were centrifuged at 2000rpm for 1 minute. The supernatant was then aspirated out to 1mL. Next, 1mL of M91X buffer was added to a properly labeled microcentrifuge tube. The centrifuge tube with L1 larvae pellet was vortexed and 10µL was pipetted out and into the microcentrifuge tube. The microcentrifuge tube was vortexed. The counting procedure was performed such that a total of 30µL of the microcentrifuge tube solution was pipetted onto a small unseeded plate, this was performed in 3 replicates of 10µL each. The number of *C. elegans* in each drop was counted, using a click counter, and recorded. The average number of *C. elegans* was then calculated. The result was used to calculate the volume needed to plate 5000 *C. elegans* onto a 10cm HG seeded plate.

3.2 Behavioral Assays

The following behavioral assays were set up the same way before observing different variables. To begin both experiments, the N2 worms were age synchronized using the previous bleaching and synchronization procedure above. The synchronized eggs were transferred to NGM agar plates seeded with OP50. The animals were grown to the end of L4 stage (~36 hours of growth) before transferring via titanium pick to the experimental conditions. Worms chosen for the assays were those displaying normal *C. elegans* behaviors, such as stimulation in response to touching. The amount of treatment given was determined based on the amount of *C. elegans* on the plate, which 2.5µL of the treatments were given. The selegiline treatment was diluted in

order to account for the animal's size. The daily dose of an adult male weighing 65kg was used to calculate the amount needed for a 1µg *C. elegans.* As a control, ethanol replaced selegiline instead of water because the sample of selegiline was prepared in an ethanol solution. This was done to remain consistent with the selegiline's chemical composition. Additionally, a 'no treatment' condition was also tested to observe the possibility of altering effects due to exposure to ethanol.

3.2.1 Egg-Laying Assay

The following section details the methods and execution of the egg-laying assay. After the animals were age synchronized and grown to adulthood, NGM agar plates were prepared with 5µL of treatment and 45µL of OP50. Three treatments, selegiline, control, and no treatment, were tested for each trial. Two adult age synchronized worms were transferred to a specific treated plate and left for 24 hours. The following day, treatment plates were prepared again the same way. The two worms were transferred to corresponding fresh treatment plates to be left for another 24 hours. The egg count for each conditional plate was recorded after transferring. The pattern was repeated for a total of 5 monitored days of egg laying. Once the data was obtained the eggs laid per *C. elegans* was calculated. The data for each treatment was plotted against each other to view the effects of each condition.

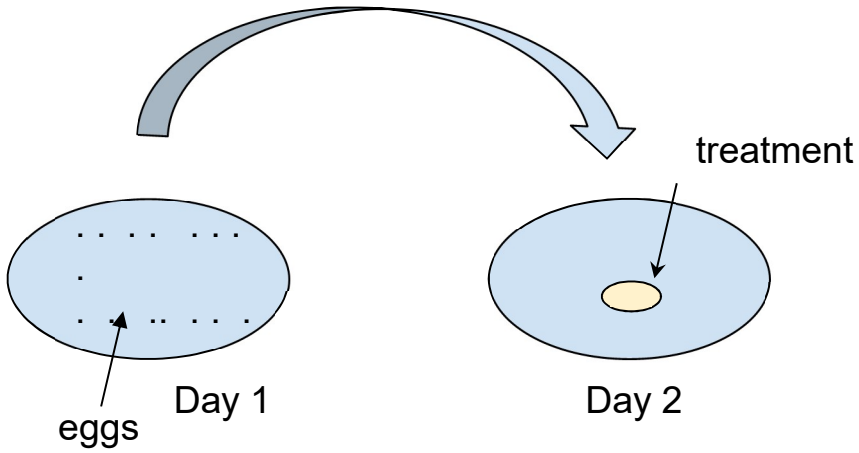

Figure 6: *Egg-laying transfer.*

3.2.2 Thrashing Assay

To observe the effects of selegiline on locomotion and the neuro-muscular system, a thrashing assay will be used. For this assay, individual worms were observed per plate and thrashing count was taken. A thrash is the movement of the head from the midline to the outside of the body. The individual nematodes were plated to treatment plates containing 2.5µL of treatment and 22.5µL OP50. The animals were left for 15 minutes before recording thrashing count per minute. When taking thrashing count, the *C. elegans* were transferred using a titanium pick to a sterile and blank NGM plate and allowed to crawl around for a minute. This was done to remove any remaining OP50 residue left on the body. This is important as residue can conglomerate on the surface of the body and inhibit movement. Once the thrashing count was recorded, the *C. elegans* were transferred back to their original plate and left for another 15 minutes before another count was taken. After 30 minutes count was taken, the *C. elegans* were transferred back to their corresponding plates and left for 24 hours. Thrashing count was recorded 24 hours post-exposure which concluded each trial.

3.3 Lipid Composition Assay

Given that lipid composition in the membrane is essential for neurotransmitter signaling, a lipid assay was performed to determine whether Selegiline alters lipid content. Lipids are especially important for the transporters that enable monoamines to be released and reuptaken making this assay pertinent to determining the mechanisms that Selegiline impacts. It is thought that Selegiline prevents degradation of monoamines in the synapse, however, this data may indicate whether there is neurotransmitter transporter involvement.

3.3.1 Lipid Extraction

The lipid extraction was performed on *C. elegans* at hour 68 in their life cycle. In glass vials, one for each sample, 4mL of chloroform/methanol (2:1) solution was added and the vials were sealed with PFTE-caps. All glass vials were labeled with the date, strain, and treatment condition. The *C. elegans* were washed off of HG plates using an M91X buffer and were collected into plastic centrifuge tubes. The tubes were centrifuged at 2000rpm for 1 minute. Following the collection of the worm pellet, the chloroform/methanol solution was used to transfer worm samples from centrifuge tube to labeled glass vial. Next, 20µL of a standard stock solution (containing 1 µg/µL of C11 phospholipid standard and 1 µg/µL of C13 triacylglycerol standard) was added to each glass vial. The vials were then vortexed to mix and left to shake for 1.5 hours at room temperature allowing the chloroform/methanol solution to extract all lipids.

Following the extraction, 800µL of 0,9% NaCL was used to clean the sample of *C. elegans* carcasses. Once the NaCl was added, each vial was vortexed and centrifuged at 2000rpm for 2 minutes separating the sample into two phases. The bottom phase containing extracted lipids was

moved into a fresh tube using a Pasteur Pipette. This solution underwent a complete dry down under nitrogen stream and was then resuspended in 1mL of chloroform. Following the resuspension, each sample continued to the separation protocol or, if necessary, was purged with nitrogen and stored in a -80℃ freezer overnight.

3.3.2 Lipid Separation

The lipid separation was performed using a 1mL Silica Column to isolate neutral lipids, glycolipids, and phospholipids. Three new glass vials were labeled for each sample to collect the flow through of each type of lipid. First, the column was pre-equilibrated with 3mL of chloroform that was allowed to flow through using gravity. The lipid sample suspended in chloroform was added to the column using a Pasteur Pipette and was eluted. The flow through was discarded. For the separation, neutral lipids for each sample were eluted first using 3mL of chloroform. These samples were collected in properly labeled vials. Glycolipids were eluted with 4mL of acetone/methanol (9:1) solution and were collected in properly labeled vials. Lastly, phospholipids were eluted from each sample with 3mL of methanol. Each vial now containing a specific type of lipid from each sample was exposed to a nitrogen stream until completely dried down.

3.3.3 Transmethylation and Mass Spectrometry

To perform transmethylation of the sample, 975µL of methanol and 25µL of Sulfuric Acid were added to the tube. The tube was then baked at 80℃ for 1 hour, vortexing every 15 minutes. When vortexed, the volume was checked, and methanol was added if volume was too little. Following the baking step, 1.5mL of HPLC-graded water and 200µL of hexane were added to

the tube. The tube was then vortexed and centrifuged for 2 minutes at 2000 rpm. The sample was snap freezed in dry ice for about 15 minutes leaving a layer of hexane containing the lipid sample. The samples were poured into labeled screw cap vials with glass inserts. These vials were placed in the GC/MS machine where 1μL was run of each sample.

3.4 Depressive Symptomatology Survey

In order to contextualize the results from the biological assays performed on *C. elegans*, a survey investigating depressive symptomatology at WPI was conducted. This survey assessed the prevalence of depressive symptoms, perceived stress, and socioeconomic status (SES) of students.

3.4.1 Participants

Participants included (n = 67) individuals who were college students from Worcester Polytechnic Institute (WPI). These participants were recruited through the WPI subject pool.

3.4.2 Procedure

The university's Institutional Review Board approved all procedures. Each participant completed a survey through Qualtrics, an online survey platform. Qualtrics has secure data centers that are in accordance with data privacy directives. Prior to answering the survey, participants read an electronic informed consent and indicated their agreement to participate by checking a box "Yes, I agree to participate." Participants who agreed answered a series of questionnaires. Data were collected from February to April of 2021 and all recorded responses were stored in Qualtrics until the end of the data collection period. Data were subsequently removed from the survey

platform and stored on a secure server at WPI. All data were stripped of any identifying information in order to maintain anonymity of each participant.

3.4.3 Measures

Demographic Questions. Participants reported their age, race/ethnicity, sex, current academic year, gender, and socioeconomic status (SES). Participants SES was evaluated objectively and subjectively. Participants self-reported the estimated income of their household, prior to tax deduction, and answered questions like "Do your parents own their home" and "How many bedrooms are there in your parents' house?" Additionally, participants were shown a picture of a ladder, where each rung was numbered 1-10 in ascending order, with a prompt stating the top of the ladder represents the people in society who are best off, have the most resources, highest level of education, and so on, while the bottom rung represents those who are the worst off, having the least amount of money and least education. The participant then selected the number they felt best represents where they are on this ladder.

Perceived Stress. Participants completed the 4-item version of the Perceived Stress Scale (PSS) (Cohen, Kamarck & Mermelstein, 1983). They reported their feelings and thoughts during the last month responding to questions that gauge the perceived stress of the participant. For example, one item asked, "In the last month, how often have you felt that you were unable to control the important things in your life." Participants ranked their feelings on a 5-point likert scale that ranged from "never" to "very often." A higher score of all 4-items indicated higher perceived stress levels.

Depressive Symptomatology. Participants responded to the Center for Epidemiological Studies Depression (CES-D) Scale, which assesses presence of depressive symptoms. Participants reported their feelings or behaviors during the past week according to a 4-point likert scale that ranged from "Rarely or none of the time (less than 1 day)" to "Most or all of the time (5-7 days)." The survey included negative items such as "I talked less than usual" or "I felt that I could not shake off the blues even with help from my family or friends" in addition to positively framed items such as "I enjoyed life." A higher total score (i.e. adding up the score for every question), where the range is from 0 to 60, indicated more depressive symptomatology.

3.4.4 Data Analytic Plan

Descriptive statistics were calculated to determine the sample characteristics of the demographics. Correlations analyses were performed to examine relationships between perceived stress levels, socioeconomic status, and depressive symptoms. A one-way ANOVA tested for differences in CES-D score by school year.

RESULTS

4.1 Egg-Laying Findings

A total of seven control trials and seven selegiline trials were performed, containing *two C. elegans* per trial. The egg-laying count was recorded for a total of four days per trial. For each of the treatments, the average number of eggs laid per *C. elegans* for each day was calculated. Next the average number of eggs laid on a given day under a specific treatment was calculated and plotted for comparison. A repeated measures ANOVA was conducted to test significance of this data.

The data is shown in *Figure 7*. The control is indicated by a grey color, with an overall slope of -6.6 between days 1 and 4. The selegiline treatment is indicated by the blue line, with a relative slope of -26.7 between days 1 and 4. The control trial begins day one with an average of 56 eggs laid per *C. elegans*, while the selegiline trial recorded an average of 92 eggs laid per day. The number of eggs laid for both treatments increased slightly, but a large decrease in egg count was observed from the selegiline group at day three; only 38 eggs laid per *C. elegans* was found, while the control averaged 53 eggs. The rate of egg laying was much quicker between days 1 and 2 of the selegiline treatment with a recorded slope of , but then suddenly declined by days 3 and 4. There was a larger variability in eggs laid per day over the course of four days when the *C. elegans* were treated with selegiline. Redistribution of egg-laying observed through egg count per day can be seen in those *C. elegans* treated with selegiline $f(3, 36) = 3.4$, $p = 0.028$, $\boldsymbol{\eta}^2 = 0.22$.

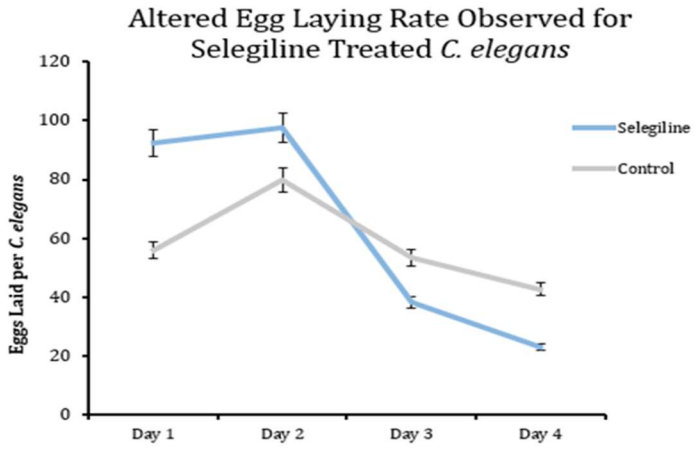

Figure 7: *Egg-laying in C. elegans in control (grey) and selegiline (blue) trials shows redistribution in treatment condition.*

4.2 Thrashing Findings

A total of nine control trails and nine selegiline trials were completed to measure the number of thrashes per minute for each *C. elegans*. These observations were recorded exactly 30 minutes and 24 hours post treatment exposure. The average number of thrashes for the control and selegiline treatment was calculated from all the trials run. These averages were plotted in a bar graph for comparison and a repeated measures ANOVA test was run to test for significance.

A repeated measures ANOVA showed selegiline significantly altered the thrashing count for both 30 minutes post exposure and 24 hours post exposure. The average thrashing count 30 minutes and 24 hours post exposure for the control group was about 38 thrashes per minute and about 38.8 thrashes per minute, respectively. Comparatively, the selegiline thrashing count was found to be 21.6 and 26.6 thrashes per minute, respectively. A decrease in mobility observed through thrashing count can be seen in those *C. elegans* treated with selegiline $f(2, 36) = 15.80$, $p < 0.001$, $\eta^2 = 0.53$. These results can be found in **Appendix A.**

4.3 Lipid Assay Findings

Relative analyses of lipid composition were performed for phospholipid and neutral lipid data. A total of eight control trials and seven drug trials were completed. The average percent of total fatty acid was calculated for each phospholipid and neutral lipid in the control condition and selegiline condition. The averages were plotted along with scatter dots showing each individual trial. Two trials from the drug condition were removed from the data set due to high variability. This may have been caused by an experimental error or death of *C. elegans* from the drug treatment.

Relative analyses of phospholipids and neutral lipids showed that selegiline treatment trended toward altered lipid composition. The results indicate that selegiline may increase the percent composition of saturated fatty acids, such as C18:0. Moreover, a small decrease in percent composition of unsaturated fatty acids, such as C20:5n3, was observed. This trend of increased saturated FAs and decreased unsaturated FAs was seen in both phospholipid and neutral lipid samples. The observed trends are shown in *Figure 8* below. The neutral lipid data can be found in **Appendix A.**

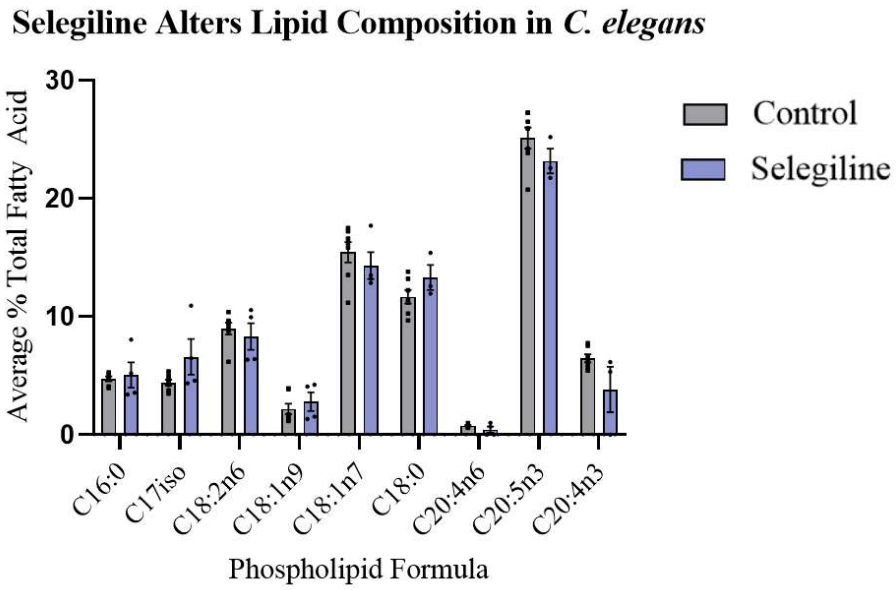

Figure 8: *Relative analysis of phospholipid data revealed increase in saturated FAs while decreasing polyunsaturated FAs.*

4.4 Depressive Symptomatology Findings

4.4.1 Sample Characteristics.

The sample (n = 67) was majority female (n = 55, 82.1%) and majority white (n = 41, 61.2%). Academic year was broken down into freshman, sophomore, junior, and senior with the following frequencies: 28.4%, 26.9%, 23.9%, and 20.9%, respectively. The majority of participants (n = 47, 74.6%) indicated that their estimated household income was $85,000 or above. Depression prevalence and perceived stress did not differ between any demographic groups. The average CES-D score was 24.3/60 and the PSS score was 7.75/15. A breakdown of the full sample characteristic can be found in **Table 1** of **Appendix A.**

4.4.2 Depression, Perceived Stress, SES, and Academic Year

A bivariate correlation revealed a positive association between PSS score and CES-D score $r(67) = 0.504$, $p < .001$. This relationship exists such that as perceived stress increases so does

the presence of depressive symptoms. This positive correlation is depicted in *Figure 9* below. Correlational analyses revealed no relationship between any SES and depression symptoms or perceived stress.

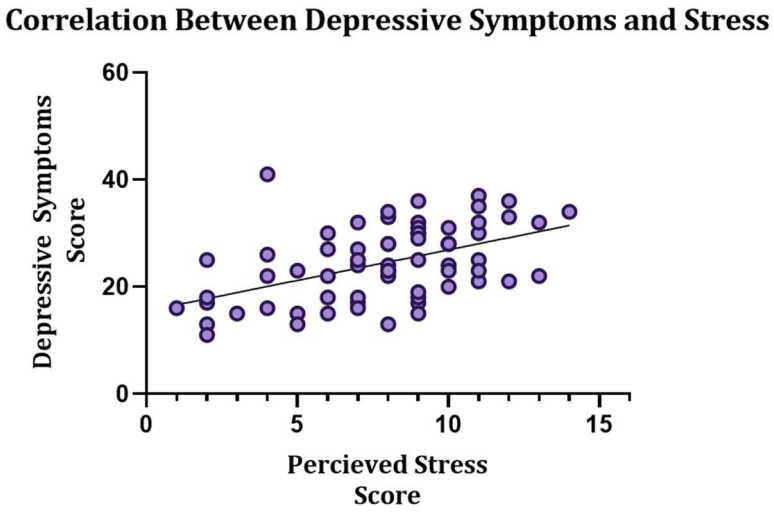

Figure 9: *Shows a positive correlation between PSS and CES-D score.*

A one-way ANOVA revealed no overall significant differences in mean CES-D score by academic year $f(1,3) = 2.55$, $p = 0.063$. However, Post-Hoc analyses revealed a significant difference in CES-D score exists between freshmen and juniors at WPI, where freshman

experience significantly more depressive symptoms $p = .037$. The trends of CES-D score by academic year are depicted in *Figure 10* below.

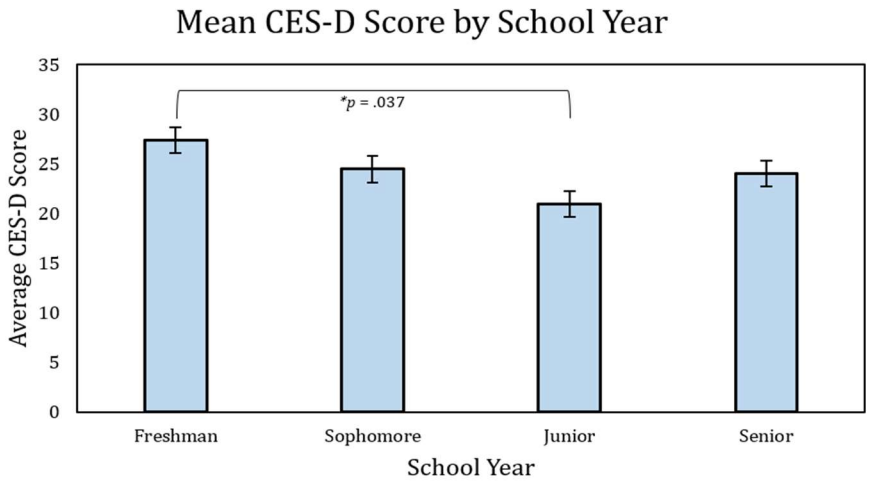

Figure 10: *One-way ANOVA post hoc analyses revealed a significant difference between freshman CES-D score and junior CES-D score.*

DISCUSSION

The results presented in this paper are preliminary steps to unveiling the molecular pathways implicated by the monoamine oxidase inhibitor, selegiline. As selegiline is a monoamine oxidase inhibitor, this drug will inhibit monoamine oxidase, thus increasing the availability of monoamines such as serotonin, norepinephrine, and dopamine. The previously discussed data shows that inhibition of monoamine oxidase increases egg-laying rate, decreases thrashing rate, and alters lipid composition of *C. elegans.* A survey of college students confirmed the need for further physiological research regarding depression and antidepressants.

5.1 Egg-Laying

In all the trials run, selegiline increased the initial egg-laying rate of *C. elegans*. Interestingly, by days 3 and 4 the egg-laying rate of selegiline treated animals had declined drastically compared to the control. Because these animals only lay a set number of eggs in their lifetime, there is a possibility that the amount of producible eggs had run out from this increased rate. Running out of eggs is a reasonable explanation as many female animals have a fertility window before entering ‘menopause’ where the individual becomes infertile due to a lack of eggs stored in the uterus. This can rule out a possibility of increased MAs inhibiting the egg-laying rate.

With this egg-laying timeline and information about the biology of the *C. elegans* reproductive system, it seems as though an increased availability of MAs is causing an increase in egg-laying rate. The question of which MA is mostly responsible for this has yet to be answered but previous research does show that serotonin increases egg-laying in *C. elegans* by stimulating the HSNs. Because serotonin is a MA and selegiline works to inhibit the enzyme that breaks down

MAs such as serotonin, this research shows that that selegiline is increasing the availability of serotonin thus potentially increasing the activation of HSNs.

Another interesting finding is the possibility of other MAs involved in egg-laying behavior. In HSN ablated animals, a serious decline of egg-laying rate was seen proving these neurons essential for egg-laying. Yet, in serotonin deficient animals, only a slight decrease in egg-laying rate was observed, implying that serotonin was not the only neuromodulator of egg-laying events. Paradoxically, high concentrations of serotonin have been shown to inhibit egg-laying rates in *C. elegans*. Because MAOIs will increase the availability of serotonin, an inhibition of egg-laying would be expected if serotonin were the only neuromodulator involved in egg-laying. Yet, the results show an increase of egg-laying which indicates other MAs could be involved in the induction of an egg-laying event.

One limitation occurred on day 5 where worm death was observed in all treatment trials. As *C. elegans* are fragile, microscopic nematodes it is certain that over-handling from transferring the worms to a different plate each day could have caused the animals to become too stressed. To counteract this, a different method could be used for transferring in which M91X buffer would be used to pipette the nematodes to new treatment plates. Contamination was also seen in some of the trials, although this is normal when handling individual worms for multiple days. Over exposure to microbes in the air during transfer or recording could've caused the contamination.

5.2 Thrashing

Thrashing assay results revealed that selegiline decreased the mobility of *C. elegans* which was observed by number of body bends or thrashes per *C. elegans* for 30 minutes or 24 hours exposure times. Body bends are connected to locomotion since this is the preliminary motion to propelling the animal in a forward or reverse motion. A higher thrashing number would indicate higher locomotive activity whereas lower thrashing count would indicate lower locomotive activity.

As stated, selegiline is an MAOI therefore increasing the availability of MAs available VCs have monoamine transporters present on their cell surface. Although the MA used by this transporter is unclear, the presence of this transporter gives reason to believe that interference with MA availability would induce changes to these cells. From the significant decline in thrashes for the selegiline treated groups, it can be inferred that the increased availability of MAs are in fact causing changes to the motor neurons. Whether or not these MAs are inhibitory or excitatory are yet to be explored.

Recent research has debated whether the VC neurons are inhibited or stimulated by serotonin. Two possible theories behind this mechanism were identified based on these findings. First is that serotonin is an inhibitory neurotransmitter to the VC neurons. Ablation of VCs has shown a slight increase in egg-laying rate indicating that these neurons may normally function in an inhibitory role egg-laying. Additionally, VCs are responsible for vulval contraction, an essential factor in egg-laying. With these two facts there is a possibility that stimulation of VCs causes contraction in a way which the *C. elegans* withholds the eggs, and once the VC is inhibited the

eggs are released. With an increase in egg-laying rate observed for cases of increased availability of serotonin, there is a possibility that serotonin is acting as an inhibitory neurotransmitter.

These results also support the theory that connections between the HSNs and AVF are interrupted due to increased serotonin, causing a decrease in the mobility of the *C. elegans*. In a normal egg-laying event, an increase in locomotor velocity is seen prior to the egg-laying. This happens when the HSNs signal to the AVF to induce a forward movement. Other research shows that increased amounts of serotonin will inhibit the forward velocity before an egg-laying event. A decrease in overall locomotor velocity was observed compared to the control. This is preliminary evidence that excessive serotonin could interfere with the communication between HSNs and AFV.

5.3 Lipid Composition

Lipid assay results revealed that selegiline impacts lipid metabolism in *C. elegans.* There was an increase in saturated fatty acids, namely C16:0 and C18:0, and a decrease in unsaturated lipids for both phospholipids and neutral lipids. This pattern of upregulation and downregulation indicate potential involvement of the enzymes that are responsible for the synthesis or degradation of specific fatty acids. Lipid synthesis is a process that is highly conserved in *C. elegans.* This metabolic process is shown in *Figure 11* below helps target what specific pathways may be impacted by selegiline.

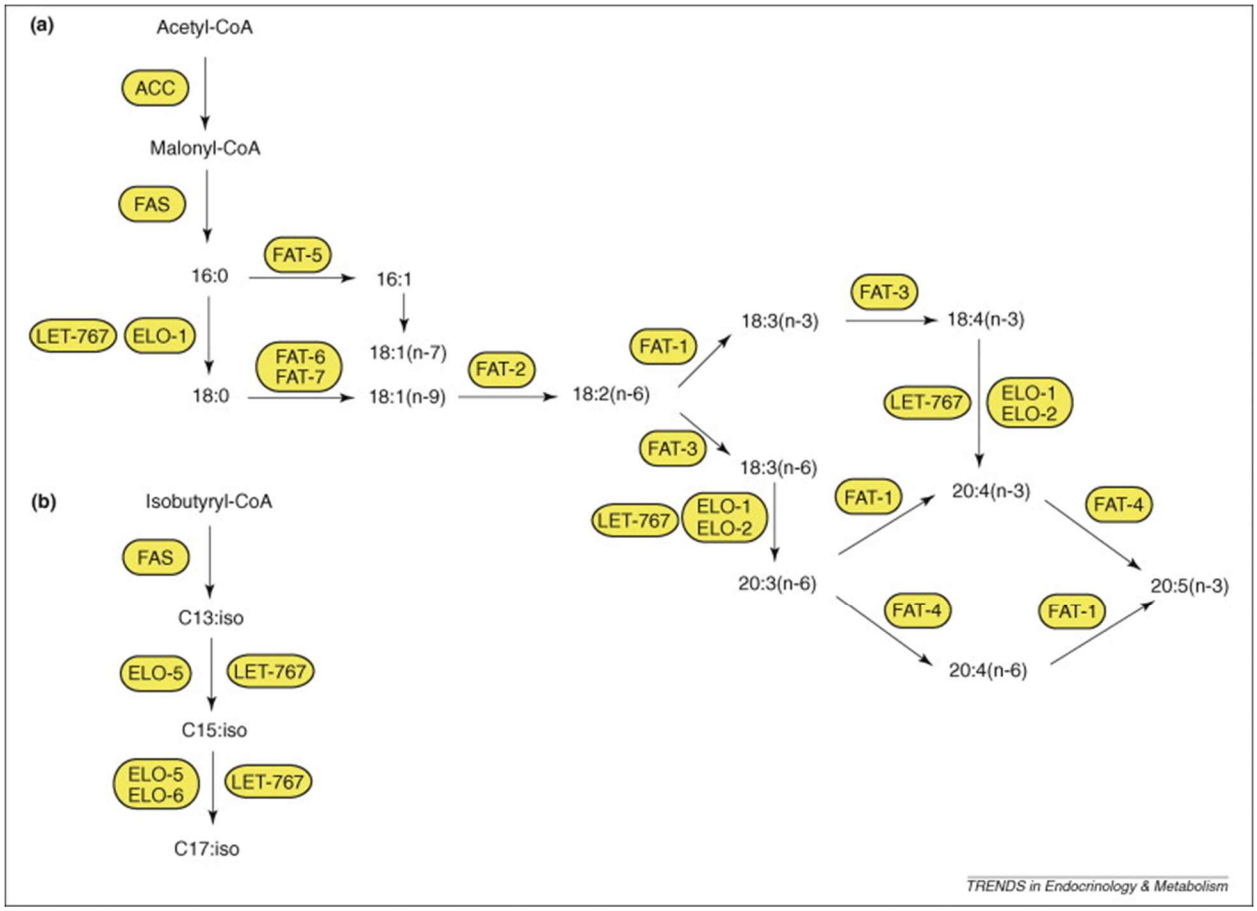

Figure 11: *Depicts the fatty acid synthesis pathways of C. elegans. Beginning with Acetyl-CoA. Enzymes are shown in yellow ovals,* (Watts, 2009).

The buildup saturated fatty acids may be caused by decreased activity of the enzymes that desaturate these molecules, such enzyme as FAT-5, FAT-6, or FAT-7 Dysregulation or inhibition of this enzyme could cause increased composition of C16:0 and C18:0. Lastly, the decrease unsaturated fatty acid composition, namely of C20:5n3, may be caused by dysregulation of the FAT-2 enzyme. This enzyme is the most likely candidate to explain the simultaneous increase in C18:1n9 and decrease in C20:5n3 and C20:4n3. The abovementioned enzymes are all desaturases, which function by removing hydrogens from carbon chains and double bonds are formed as a result (Lee et al., 2016). Desaturases are important for regulation

of converting saturated fatty acids to unsaturated fatty acids, which impacts membrane fluidity and cellular functioning.

The importance of the impact selegiline has on the composition of fatty acid composition extends beyond changes in the metabolic pathway. The clear increase in saturated fatty acids and concurrent decrease in unsaturated fatty acids has implications for membrane fluidity. Typically, *C. elegans* have low levels of saturated fatty acids in their membranes. Because phospholipids are the most abundant type of lipid in cell membranes, the increase in saturated phospholipids indicates an increase in saturation of *C. elegans* membranes. As mentioned earlier, this has implications for membrane fluidity. Higher levels of saturated fat decreases fluidity of the membrane. These changes in lipid composition link antidepressant functions to membrane fluidity alterations, which impacts neurotransmission. Because membrane proteins functioning is dependent on surrounding lipid composition, these changes may impact the release and reuptake of neurotransmitters, such as those implicated in depression.

One limitation with the lipid assay was high variability between the selegiline trials, while there was little variability between control trials. The variability was isolated to several trials that appeared to contain outliers when the data points were compared to the rest of the data set. This variability may have been caused by small sample sizes that did not meet the required threshold content for the GS-MS to function properly. As a result, background readings of other molecules in the sample would be picked up and identified as lipids when they were not. Conversely, a sample that saturated the machine may have produced this same effect. The decrease in sample size could be explained by *C. elegans* death on the plate during the one-hour exposure period.

Given the trends in lipid composition, it is possible some worms in the sample were dying off with exposure to the drug. In order to counteract this, phenotypic analysis to gauge the health of the worms could be conducted prior to collection of the sample from the plate. If the worms were dying off from the drug, a shorter exposure time might be able to counteract this limitation.

Future research should investigate the specific metabolic pathways involved in the specific lipid changes that were observed. A more quantitative method should be used in order to conduct a more precise analysis of how lipid content is changing, rather than the relative analysis that was conducted in this research. Research regarding the ideal lipid membrane composition for effective transmission of neurotransmitters implicated in depression could provide direction for more targeted therapies. If an ideal membrane composition were identified, perhaps a medication or specific diet could be used to achieve this membrane composition and treat depressive symptoms.

5.4 Depressive Symptoms at WPI

The findings of the survey indicate that a significant correlation exists between stress and depressive symptoms among WPI students. This relationship is important to investigate especially given that research has shown college aged students tend to have higher stress levels compared to the general population. This was confirmed by the average stress score of WPI students. Additionally, the average CES-D score of 24.3 out of a possible 60 is concerning since a score of 16 and above on the CES-D scale is considered at risk for clinical depression. Moreover, the increased CES-D score for freshman indicates social connections may be a predictor of depressive symptoms. Those new to college, in a changing environment, with few

social connections, are the most depressed. During freshman year, students begin to make connections and continue to build meaningful relationships until junior year, where their social capital is at an all-time high. This is when depressive symptoms are least prominent in WPI students. However, a trend upward in depressive symptoms was seen for seniors. This could be explained by increased stress due to job searching, loss of social capital (i.e. many friends may have graduated), and uncertainty about what the future holds.

While these results are alarming, it is important to consider that they may not be an accurate indication of the typical stress and depression levels of WPI students. The data was collected during the COVID-19 pandemic, a time of prolonged stress, uncertainty, and grief along with all the mental health consequences that accompany these negative mental states. Research has highlighted negative psychological impact on college students specifically. One survey of nearly 33,000 college students found that 39% of students screened positive for either major or moderate depressive disorder. The same survey found that 34% screened positive for anxiety disorder (Eisenberg, 2020). Another study found that out of 195 students, 71% indicated they had increased stress and anxiety due to the COVID-19 pandemic. In one study, depression symptoms in a sample of adults were measured and compared to pre-COVID-19 depression levels. This study found that 8.5% of adults had moderate to severe depressive symptoms pre-pandemic, compared to 27.8% during the pandemic.

While this limitation does need to be kept in mind, these findings do confirm previous research showing higher prevalence of depression, anxiety, and stress in college students. Research conducted during the pandemic confirms that exposure to stress is associated with a greater risk

of depression. Given that college was already identified as a stressful time period for most, these findings demonstrate that the risk factors of depression in this key population need to be identified. Depression rates have increased in this population, but in order to curb this pressing health issue, the contributing factors need to be identified so that college students can be better equipped to deal with the stress levels associated with college. The COVID-19 pandemic has exploited previously existing gaps in depression and stress levels that college students face. Given the considerable percentage of college students that face depression, future research should investigate how students view mental health and how they are equipped to deal with stressors. Additionally, research should investigate whether social connections function as a mitigating factor of depressive symptoms in this age group.

CONCLUSION

The multiple biological effects observed in this research highlight the complicated nature of antidepressants. Selegiline impacts egg-laying, thrashing, and lipid metabolic pathways in *C. elegans.* The results support the hypothesis that MAOIs increase neurotransmitter availability, namely serotonin. The results found from the egg-laying and thrashing assay indicates selegiline alters the availability of neurotransmitters implicated in these neuronal pathways, thus affecting the behavior of *C. elegans*. The changes in lipid composition suggest that antidepressants may treat depression by altering membrane fluidity and therefore providing a favorable environment for increased release or production of neurotransmitters through membrane proteins. The survey exemplifies the need for this further biological research. The large proportion of students who are struggling with depressive symptoms could be aided by therapeutic treatments that target the true pathophysiology of depression.

REFERENCES

Bargmann, C. I. (1998). Neurobiology of the Caenorhabditis elegans genome. In *Science* (Vol. 282, Issue 5396, pp. 2028–2033). American Association for the Advancement of Science. https://doi.org/10.1126/science.282.5396.2028

Bargmann, C. I., & Horvitz, H. R. (1991). Control of larval development by chemosensory neurons in Caenorhabditis elegans. *Science*, *251*(4998), 1243–1246. https://doi.org/10.1126/science.2006412

Blaxter, M. (1998). Caenorhabditis elegans is a nematode. In *Science* (Vol. 282, Issue 5396, pp. 2041–2046). American Association for the Advancement of Science. https://doi.org/10.1126/science.282.5396.2041

Bortolato, M., Pivac, N., Muck Seler, D., Nikolac Perkovic, M., Pessia, M., & Di Giovanni, G. (2013). The role of the serotonergic system at the interface of aggression and suicide. In *Neuroscience* (Vol. 236, pp. 160–185). NIH Public Access. https://doi.org/10.1016/j.neuroscience.2013.01.015

Brewer, J. C., Olson, A. C., Collins, K. M., & Koelle, M. R. (2019). Serotonin and neuropeptides are both released by the HSN command neuron to initiate C. elegans egg laying. *PLoS Genetics*, *15*(1). https://doi.org/10.1371/journal.pgen.1007896

Brody, T. (1999). Nutritional Biochemistry. In *Nutritional Biochemistry*. Elsevier. https://doi.org/10.1016/b978-0-12-134836-6.x5000-8

Burkhart, B. M., Gassman, R. M., Langs, D. A., Pangborn, W. A., Duax, W. L., & Pletnev, V. (1999). Gramicidin D conformation, dynamics and membrane ion transport. *Biopolymers - Peptide Science Section*, *51*(2), 129–144. https://doi.org/10.1002/(SICI)1097-0282(1999)51:2<129::AID-BIP3>3.0.CO;2-Y

Carta, M., Lanore, F., Rebola, N., Szabo, Z., Da Silva, S. V., Lourenço, J., Verraes, A., Nadler, A., Schultz, C., Blanchet, C., & Mulle, C. (2014). Membrane lipids tune synaptic transmission by direct modulation of presynaptic potassium channels. *Neuron*, *81*(4), 787–799. https://doi.org/10.1016/j.neuron.2013.12.028

Charnay, Y., & Leger, L. (2010). Brain serotonergic circuitries. *Dialogues in Clinical Neuroscience*, *12*(4), 471–487. https://doi.org/10.31887/dcns.2010.12.4/ycharnay

Chen, P., Martinez-Finley, E. J., Bornhorst, J., Chakraborty, S., & Aschner, M. (2013). Metal-induced neurodegeneration in C. elegans. In *Frontiers in Aging Neuroscience* (Vol. 5, Issue MAY). Frontiers Media SA. https://doi.org/10.3389/fnagi.2013.00018

Cole, O. J., & Bodkin, J. A. (2002). MAO inhibitors: An option worth trying in treatment-resistant cases | MDedge Psychiatry. *Current Psychiatry* , *1*(6), 40–47. https://www.mdedge.com/psychiatry/article/66132/depression/mao-inhibitors-option-worth-trying-treatment-resistant-cases

Collins, K. M., Bode, A., Fernandez, R. W., Tanis, J. E., Brewer, J. C., Creamer, M. S., & Koelle, M. R. (2016). Activity of the C. elegans egg-laying behavior circuit is controlled by competing activation and feedback inhibition. *ELife*, *5*(NOVEMBER2016). https://doi.org/10.7554/eLife.21126

Cook, S. J., Jarrell, T. A., Brittin, C. A., Wang, Y., Bloniarz, A. E., Yakovlev, M. A., Nguyen, K. C. Q., Tang, L. T. H., Bayer, E. A., Duerr, J. S., Bülow, H. E., Hobert, O., Hall, D. H., & Emmons, S. W. (2019). Whole-animal connectomes of both Caenorhabditis elegans sexes. *Nature*, *571*(7763), 63–71. https://doi.org/10.1038/s41586-019-1352-7

Culpepper, L. (2013). Reducing the burden of difficult-to-treat major depressive disorder: Revisiting monoamine oxidase inhibitor therapy. *Primary Care Companion to the Journal*

of Clinical Psychiatry, *15*(5). https://doi.org/10.4088/PCC.13r01515

Delgado, P. L. (2000). Depression: The Case for a Monoamine Deficiency. , *61*. https://www.psychiatrist.com/jcp/depression/depression-case-monoamine-deficiency

Desai, C., & Horvitz, H. R. (1989). Caenorhabditis elegans mutants defective in the functioning of the motor neurons responsible for egg laying. *Genetics*, *121*(4), 703–721.

Duerr, J. S., Gaskin, J., & Rand, J. B. (2001). Identified neurons in C. elegans coexpress vesicular transporters for acetylcholine and monoamines. *American Journal of Physiology-Cell Physiology*, *280*(6), C1616–C1622. https://doi.org/10.1152/ajpcell.2001.280.6.C1616

Finberg, J. P. M., & Rabey, J. M. (2016). Inhibitors of MAO-A and MAO-B in psychiatry and neurology. In *Frontiers in Pharmacology* (Vol. 7, Issue OCT, p. 340). Frontiers Media S.A. https://doi.org/10.3389/fphar.2016.00340

Freyberg, Z., Sonders, M. S., Aguilar, J. I., Hiranita, T., Karam, C. S., Flores, J., Pizzo, A. B., Zhang, Y., Farino, Z. J., Chen, A., Martin, C. A., Kopajtic, T. A., Fei, H., Hu, G., Lin, Y. Y., Mosharov, E. V., McCabe, B. D., Freyberg, R., Wimalasena, K., … Javitch, J. A. (2016). Mechanisms of amphetamine action illuminated through optical monitoring of dopamine synaptic vesicles in Drosophila brain. *Nature Communications*, *7*. https://doi.org/10.1038/ncomms10652

Gillespie, J. S., & Mackenna, B. R. (1961). The inhibitory action of the sympathetic nerves on the smooth muscle of the rabbit gut, its reversal by reserpine and restoration by catechol amines and by DOPA. *The Journal of Physiology*, *156*(1), 17–34. https://doi.org/10.1113/jphysiol.1961.sp006655

Gjorgjieva, J., Biron, D., & Haspel, G. (2014). Neurobiology of caenorhabditis elegans locomotion: Where do we stand? In *BioScience* (Vol. 64, Issue 6, pp. 476–486). Oxford

University Press. https://doi.org/10.1093/biosci/biu058

H, H. E., V, M., K, S., R, L., S, S. J., M, A., M, S., M, K., & K, R. U. (1989). Pharmacokinetics and metabolism of selegiline. *Acta Neurologica Scandinavica*, *80*, 93–99. https://doi.org/10.1111/j.1600-0404.1989.tb01788.x

Hac-Wydro, K., & Wydro, P. (2007). The influence of fatty acids on model cholesterol/phospholipid membranes. *Chemistry and Physics of Lipids*, *150*(1), 66–81. https://doi.org/10.1016/j.chemphyslip.2007.06.213

Hardaker, L. A., Singer, E., Kerr, R., Zhou, G., & Schafer, W. R. (2001). Serotonin modulates locomotory behavior and coordinates egg-laying and movement in Caenorhabditis elegans. *Journal of Neurobiology*, *49*(4), 303–313. https://doi.org/10.1002/neu.10014

Heninger, G. R., Delgado, P. L., & Charney, D. S. (1996). The revised monoamine theory of depression: A modulatory role for monoamines, based on new findings from monoamine depletion experiments in humans. In *Pharmacopsychiatry* (Vol. 29, Issue 1, pp. 2–11). Georg Thieme Verlag. https://doi.org/10.1055/s-2007-979535

Juárez Olguín, H., Calderón Guzmán, D., Hernández García, E., & Barragán Mejía, G. (2016). The role of dopamine and its dysfunction as a consequence of oxidative stress. In *Oxidative Medicine and Cellular Longevity* (Vol. 2016). Hindawi Publishing Corporation. https://doi.org/10.1155/2016/9730467

Klein, M. O., Battagello, D. S., Cardoso, A. R., Hauser, D. N., Bittencourt, J. C., & Correa, R. G. (2019). Dopamine: Functions, Signaling, and Association with Neurological Diseases. In *Cellular and Molecular Neurobiology* (Vol. 39, Issue 1, pp. 31–59). Springer New York LLC. https://doi.org/10.1007/s10571-018-0632-3

Kondziella, D. (2017). The Top 5 Neurotransmitters from a Clinical Neurologist's Perspective.

Neurochemical Research, *42*(6), 1767–1771. https://doi.org/10.1007/s11064-016-2101-z

Kopchock, R. J., Ravi, B., Bode, A., & Collins, K. M. (2020). The female-specific VC neurons are mechanically activated, feed-forward motor neurons that facilitate serotonin-induced egg laying in C. elegans. In *bioRxiv* (p. 2020.08.11.246942). bioRxiv. https://doi.org/10.1101/2020.08.11.246942

Laganowsky, A., Reading, E., Allison, T. M., Ulmschneider, M. B., Degiacomi, M. T., Baldwin, A. J., & Robinson, C. V. (2014). Membrane proteins bind lipids selectively to modulate their structure and function. *Nature*, *510*(7503), 172–175. https://doi.org/10.1038/nature13419

Lee, J. M., Lee, H., Kang, S. B., & Park, W. J. (2016). Fatty acid desaturases, polyunsaturated fatty acid regulation, and biotechnological advances. In *Nutrients* (Vol. 8, Issue 1). MDPI AG. https://doi.org/10.3390/nu8010023

Lind, O. (2016). Colour vision and background adaptation in a passerine bird, the zebra finch (Taeniopygia guttata). *Royal Society Open Science*, *3*(9). https://doi.org/10.1098/rsos.160383

MOHAMMAD-ZADEH, L. F., MOSES, L., & GWALTNEY-BRANT, S. M. (2008). Serotonin: a review. *Journal of Veterinary Pharmacology and Therapeutics*, *31*(3), 187–199. https://doi.org/10.1111/j.1365-2885.2008.00944.x

Moore, J. J., & Saadabadi, A. (2021). Selegiline. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/pubmed/30252350

Moresco, J. J., & Koelle, M. R. (2004). Activation of EGL-47, a Gαo-coupled receptor, inhibits function of hermaphrodite-specific motor neurons to regulate Caenorhabditis elegans egg-laying behavior. *Journal of Neuroscience*, *24*(39), 8522–8530.

https://doi.org/10.1523/JNEUROSCI.1915-04.2004

Pandey, P., & Harbinder, S. (2012). The Caenorhabditis elegans D2-like dopamine receptor DOP-2 physically interacts with GPA-14, a Gαisubunit. *Journal of Molecular Signaling*, *7*, 3. https://doi.org/10.1186/1750-2187-7-3

Postila, P. A., Vattulainen, I., & Róg, T. (2016). Selective effect of cell membrane on synaptic neurotransmission. *Scientific Reports*, *6*. https://doi.org/10.1038/srep19345

Schafer, W. R. (2005). Egg-laying. In *WormBook : the online review of C. elegans biology* (pp. 1–7). https://doi.org/10.1895/wormbook.1.38.1

Schafer, W. R., Sanchez, B. M., & Kenyon, C. J. (1996). Genes affecting sensitivity to serotonin in Caenorhabditis elegans. *Genetics*, *143*(3), 1219–1230. https://doi.org/10.1093/genetics/143.3.1219

Sheffler, Z. M., & Pillarisetty, L. S. (2019). Physiology, Neurotransmitters. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/pubmed/30969716

Suzuki, M., Tsuji, T., & Ohtake, H. (2005). A model of motor control of the nematode C. elegans with neuronal circuits. *Artificial Intelligence in Medicine*, *35*(1–2), 75–86. https://doi.org/10.1016/j.artmed.2005.01.008

Syvälahti, E. K. G. (1994). Biological aspects of depression. *Acta Psychiatrica Scandinavica*, *89*, 11–15. https://doi.org/10.1111/j.1600-0447.1994.tb05795.x

Velasco, A., & Tan, Z. (2014). Fatty Acids and the Aging Brain. *Omega-3 Fatty Acids in Brain and Neurological Health*.

Võikar, V., Vasar, E., & Rauvala, H. (2004). Behavioral alterations induced by repeated testing in C57BL/6J and 129S2/ Sv mice: Implications for phenotyping screens. *Genes, Brain and Behavior*, *3*(1), 27–38. https://doi.org/10.1046/j.1601-183X.2003.0044.x

Vrablik, T. L., Petyuk, V. A., Larson, E. M., Smith, R. D., & Watts, J. L. (2015). Lipidomic and proteomic analysis of Caenorhabditis elegans lipid droplets and identification of ACS-4 as a lipid droplet-associated protein. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, *1851*(10), 1337–1345. https://doi.org/10.1016/j.bbalip.2015.06.004

Wang, D., Yu, Y., Li, Y., Wang, Y., & Wang, D. (2014). Dopamine receptors antagonistically regulate behavioral choice between conflicting alternatives in c. elegans e115985. *PLoS ONE*, *9*(12). https://doi.org/10.1371/journal.pone.0115985

Watts, J. L. (2009). Fat synthesis and adiposity regulation in Caenorhabditis elegans. In *Trends in Endocrinology and Metabolism* (Vol. 20, Issue 2, pp. 58–65). Elsevier. https://doi.org/10.1016/j.tem.2008.11.002

APPENDIX A: Supplemental Figures and Tables

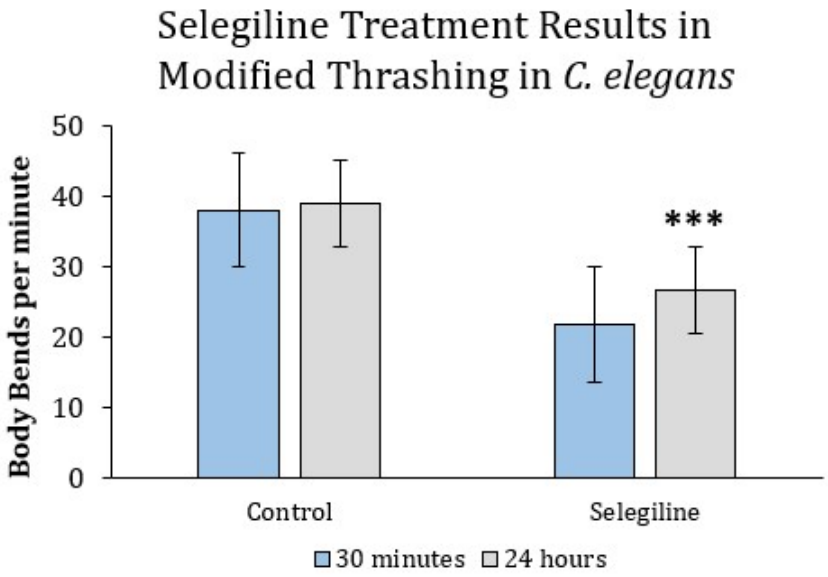

Figure 1. Thrashing assay results indicate that selegiline significantly decreased frequency of thrashes following acute exposure and normal thrashing was not regained for at least 24 hours, $f\,(2,30) = 15.80$, $p < 0.001$, $\eta^2 = 0.53$.

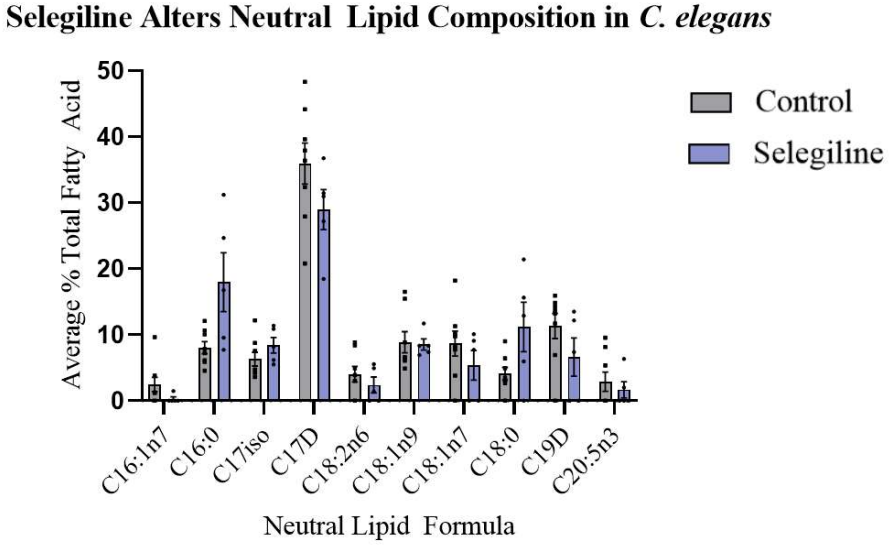

Figure 2. Thrashing assay results indicate that selegiline significantly decreased frequency of thrashes following acute exposure and normal thrashing was not regained for at least 24 hours, $f\,(2,30) = 15.80$, $p < 0.001$, $\eta^2 = 0.53$.

Table 1.
Characteristics of the full sample

Variable	Overall (n = 67)
Sex	
Male	17.9%
Female	82.1%
Age (years)	
Race/Ethnicity	
White	61.2%
Black	7.5%
Latina	7.5%
Asian/Pacific Islander	17.9%
Other or multiracial	6.0%
Annual Household Income	
$85,001-$100,000	12.7%
$100,001-$150,000	25.4%
$150,001-$200,000	15.9%
More than $200,000	20.6%
Academic Year	
Freshman	28.4%
Sophomore	26.9%
Junior	23.9%
Senior	20.9%
CES-D Score	24.3 (7.11)
Perceived Stress Scale Score	7.75 (3.14)
SES Ladder	6.84 (1.36)

Note. Numbers in parentheses represent standard deviations.