# The Effects of Monoamine Oxidase Inhibitors (Selegiline and Phenelzine) on the Egg Laying Behavior and Lipid Composition of *Caenorhabditis Elegans*

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*In partial fulfillment of the requirements for the*
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# Abstract

While previous research has hypothesized that there is a correlation between depression and a depleted level of these monoamines in the synapse, proof is lacking. Studies show that inhibitor drugs such as selegiline increase the amount of monoamines available by inhibiting the levels of monoamine oxidase, but there is no data on selegiline's specific impact (6). These studies demonstrate that monoamine oxidase antidepressant drugs increase the egg-laying rate and change the lipid composition of the *C. elegans*. These results show that these drugs are causing changes that induce increased activity of neurological stimulants such as elongase I and elongase II, enzymes key in lipid metabolism regulation. Additionally, there is a shift to polyunsaturated fatty acids from saturated fatty acids - molecules that serve as precursors in the lipid membrane. The different drugs induced different changes to the lipid composition, likely because of the various different pathways that impact depressive behavior, showcasing that each patient needs specialized care when being treated for depression.

## Acknowledgements

The success of this project would not have been possible without the help of many WPI faculty and students. First I would like to thank the chemistry and biochemistry department for funding this research and providing me with the background knowledge that helped me succeed in this project.

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# Introduction

Antidepressant drugs are critical in treating and relieving symptoms for depression patients. They serve to treat the illness as well as help balance emotions and reduce symptoms such as anxiety, restlessness, and thoughts of suicide (1). Monoamines, such as epinephrine, norepinephrine, dopamine, and serotonin, are neurotransmitters found in the body (3). It is hypothesized that there is a correlation between depression and a depleted level of these monoamines in the synapse, however research is lacking. Monoamine oxidase inhibitors are a class of antidepressants that look to target monoamine receptors and affect its degradation. There are many monoamine oxidase inhibitor drugs that are used to treat depression, however the mechanisms as to how these drugs interact with the neuronal pathways at the molecular level is largely unknown. This drug increases the amount of monoamines available by inhibiting the levels of monoamine oxidase (2).

The antidepressants focused on in this research are selegiline and phenelzine, which are both monoamine oxidase inhibitors. By studying the metabolic pathways and behavior of *C. elegans* with these drugs, the impact of monoamine oxidase inhibitors on the nervous system can be observed. This research provides researchers with a better understanding of how these antidepressants work on various neurological pathways in the body and allows for the further development of these antidepressants to create more effective drug treatment options that are developed on a patient to patient basis.

These studies have critical significance in its relation to the function and effectiveness of antidepressant drugs. Previous research done by a group out of Howard Hughes Medical Institute looked at the impact of Miansenin (a tricyclic-related antidepressant) and found that this drug increased the nematode *C. elegans* lifespan by ~30% (31). This team was able to prove that by the drug blocking the serotonin receptors (a monoamine that is involved in modulating mood, sensory perception, and appetite), the worms' egg laying rate increased as well as the lifespan of the worms (31). However, the direct action mechanism that was affected could not be concluded, proving the need for continued research on this topic.

Previous studies performed by a group of students at Worcester Polytechnic Institute show that by inhibiting the monoamine oxidase, there was an increase in the worms' egg-laying rate and the lipid composition of the *C. elegans* is altered (4). They showed through lipid composition assay experiments that there is a correlation between the alterations in membrane

fluidity and how the lipid composition changes. It has been proven that the lipid metabolism of the *C. elegans* is impacted by selegiline. This drug causes a decrease in the unsaturated lipids of the phospholipids and neutral lipids, and an increase in the saturated fatty acids (3).

This research piggybacks off of these studies and provides further understanding of selegiline's impact, as well as that of another monoamine oxidase inhibitor antidepressant drug, phenelzine. One way the impact of these antidepressant drugs were explored was by observing the egg-laying pattern of the nematode worms. It is proven that an increase in monoamines causes an increase in the rate of egg-laying (with the reasoning of this largely unknown). Seeing as these antidepressants work to inhibit the breakdown of the inhibitory enzyme of monoamines (such as serotonin), it was demonstrated that the drug increases serotonin's availability and thus neuron activity (4). This research also explores how changes to the lipid composition impact the worm's behavior. Previous results present a possible pathway as to how antidepressant drugs treat depression - they change the membrane fluidity and allow for the membrane proteins to release additional neurotransmitters (4). These studies look to confirm this prediction and show the impact of making changes to the drug concentration. This research has critical significance in its relation to antidepressant drugs, as monoamines are molecules that are especially important due to regulating mood, sleep, appetite, and other cognitive functions (5).

First, the egg laying and lipid pruficiation assay experiments were repeated with varying concentrations of selegiline to determine if there is a correlation between the concentration of drug used and its impact. Next, experiments were performed to combine selegiline with another monoamine inhibitor, phenelzine, to observe the effects of combined drug therapy. Both sets of experiments study the metabolic pathways and behavior of *C. elegans* with selegiline to show the impact of monoamine oxidase inhibitors on the nervous system. Exploring how changes to the composition of the lipid impact the worm's behavior provides more insight on the pathways that antidepressant drugs impact to treat depression. Understanding this pathway better allows for the future manipulation of antidepressant drugs that will have improved ability to target symptoms and treat depression. This information aims to provide scientists and drug makers with increased knowledge on how they can modify selegiline and other drugs of its class to be more effective and have less harmful side effects.

# Background

The following section provides an informative overview of the key concepts and methods critical for the research.

## *Science of Depression*

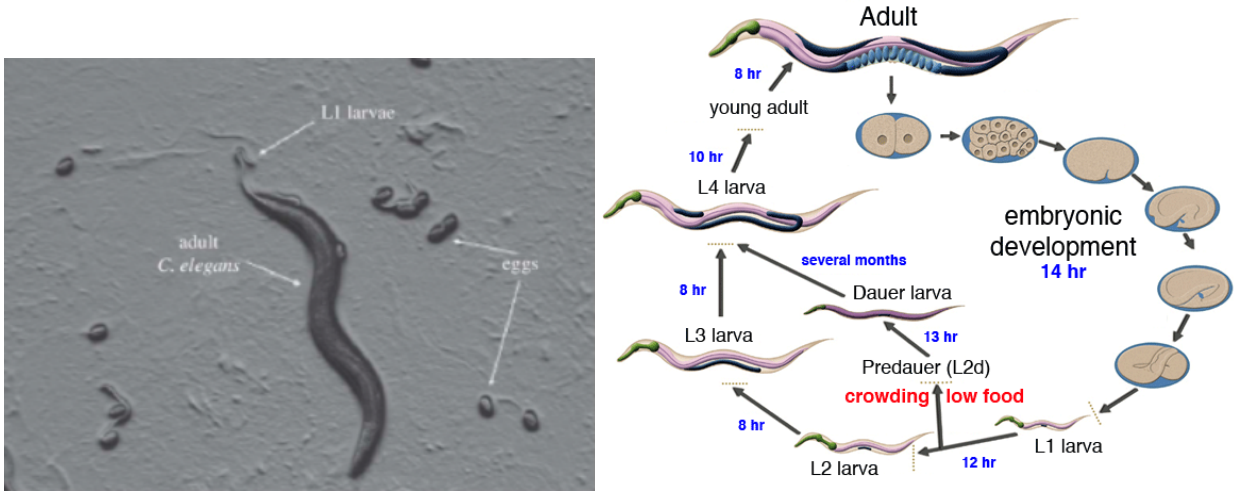
Depression is a medical condition that is correlated with many symptoms including loss of energy, melancholy, trouble concentrating, loss of pleasure, and suicidal thoughts. People struggling with this disease struggle to complete day-to-day activities and in severe cases start to think that life isn't worth living. One in every ten adults are affected by depression each year in the United States (32), and depression is the leading cause of disability worldwide (33). There is a large range of symptoms of depression, with the most common ones including hopelessness, sadness, and emptiness. Many people struggling with depression have issues thinking and concentrating and are constantly very tired (33).

In order to provide relief to the millions of people who are affected by depression, there has been an increased push to find effective treatments. This is a hard task as it is hard to determine the root issue for each individual, and it requires better understanding of the underlying mechanisms of the disease, and how they impact the body's neurotransmitters. While it used to be that brain chemistry was blamed for depression, a Harvard Medical School report concluded that the most important aspect ("more important than levels of specific brain chemicals") to figuring out how the brain regulates mood is "nerve cell connections, nerve cell growth, and the functioning of nerve circuits (33)" Additional research that looked at the correlation between biological and psychosocial factors shows that neurotransmitters are implicated with depression. These studies show that when there are deficiencies and other deviations in the functionality of the neurotransmitters, there is a high likelihood that someone will exhibit depressive symptoms (34).

## *Caenorhabdits elegans* (*C. elegans*)

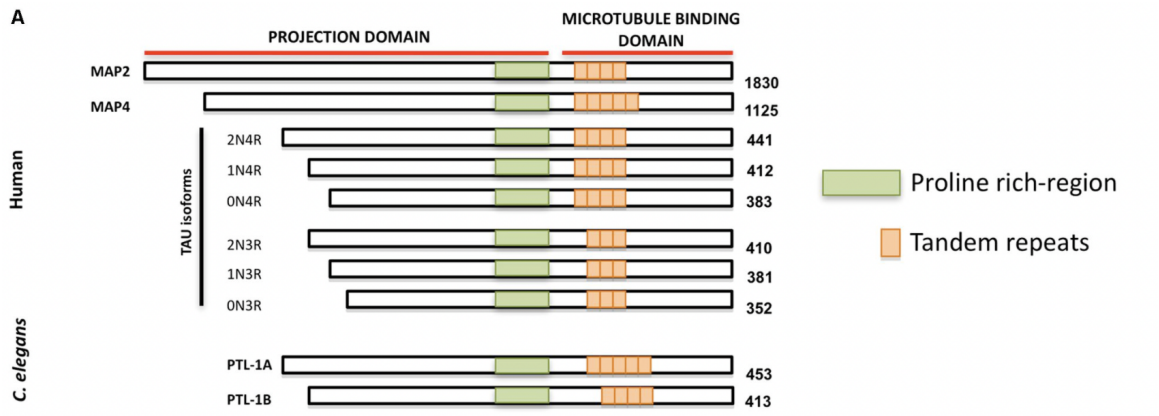
For these experiments, *C. elegans* will be used as the model organism. For both the egg laying and lipid composition experiments, the N2 wildtype was observed. They have a lifetime

of around 2 weeks and are around 1mm long as a nematode (7). The average adult produces 200-250 eggs in their lifetime (8). Their primary diet includes consuming *Escherichia coli* (*E. coli*), which can be found in the soil naturally. For experimental use, the media used contains *E. coli* so that the worm has an adequate food supply (9). The entire neural network of these organisms have been mapped out and described in previous studies. This system contains 302 neurons (9).



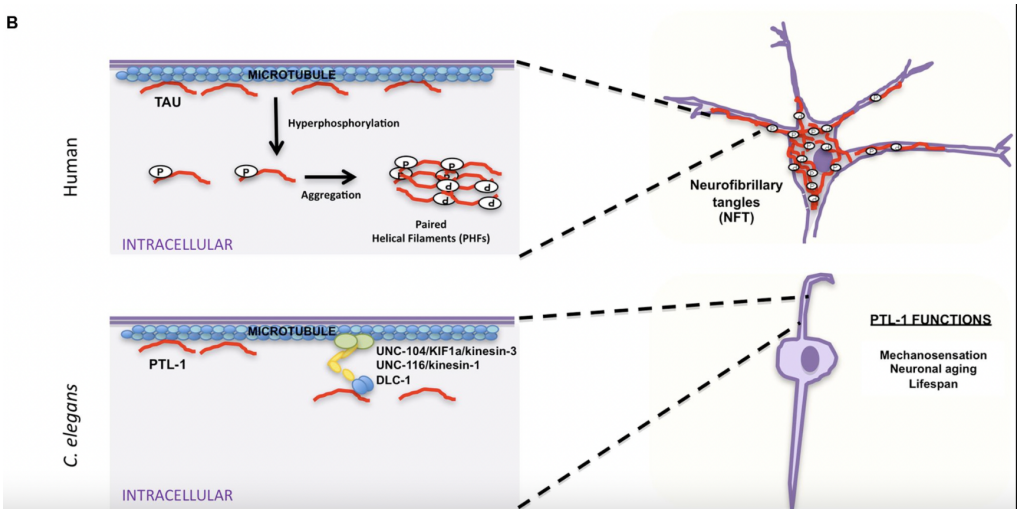

*The left figure shows the 1mm nematode C. elegans as pictured by the Chuang Lab. The right figure shows the life cycle of a C. elegans* (Sharma, Yashaswi)

Prior experimental research proves that *C. elegans* are good to study as model organisms for neurological disorders. Many neurodegenerative issues and disorders are partially caused by the cell bodies' accumulation of neurofibrillary tangles. These tangles have proteins called tau's present, which are part of the microtubule-associated protein (MAP) family. The domains of these MAPs have regions of tandem amino acids and a proline-rich domain. These MAPs are responsible for the assembly and stability of the microtubules, as they are the proteins that bind the microtubules. In the axons, tau is the predominant MAP that gets expressed and controls microtubule stability. *C. elegans* are commonly used as model organisms in research looking to study human neurological patterns due to the similarities in human tau isoforms and *C. elegans* PTL isoforms (30). This is shown in the figure below:




*This figure shows the similarities in proline rich regions and tandem repeats for human taus and C. elegans PTL isoforms (30).*

Human tau proteins and *C. elegans* PTL isoforms both attach and bind to the microtubules. In humans, the taus get phosphorylated and then aggregate to form paired helical filaments. In *C. elegans*, the PTL works with other proteins such as kinesins to move along the microtubules (30). These two processes are shown below:




*This figure shows the pathways of the MAPs (30).*

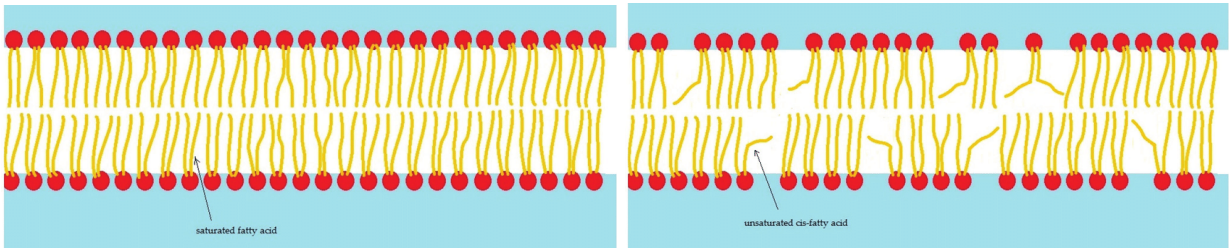
With both of these mechanisms, the loss of tau or PTL causes either penetrant lethality of a shortened lifespan with decreased touch response (30).

These organisms can be subjected to genetic manipulation and other experimental manipulation that would not be ethical to perform on human subjects. They are simplistic in their

genomic and biological nature. They are good for studying diseases because they have many of the same functional counterparts as humans (9). This is due in part to the fact that *C. elegans* and humans use homologous receptors in chemical signaling and have monoamine receptors that function similarly. Common neurotransmitters found in both organisms include octopamine, dopamine, a norepinephrine homolog, and serotonin (10).

## Lipids

Lipids are a hydrophobic macromolecule that are insoluble and made up of long fatty acids. They are made of hydrocarbon chains that have a methyl and carboxylic acid group on either end. There are both saturated and unsaturated fatty acids. For saturated fatty acids, the carbons are completely saturated with hydrogen atoms and have no double bonds to create kinks in the chain, allowing them to tightly pack together. For unsaturated fatty acids, there is at least one carbon double bond, which inhibits the chain from tightly packing and bends and kinds are formed. The differences in the membrane between saturated and unsaturated fatty acids is shown in the images below:




*The left graphic shows a membrane composed of saturated fatty acids. This creates a viscous environment. The right graphic shows a membrane composed of unsaturated fatty acids. This created a fluid environment,* (Nagy, Katalin).

The function of lipids ranges widely, spanning from serving as membranes' structural components to being energy sources to being a critical bioactive signaling molecule (47). The two major types of lipids observed are triacylglycerols and phospholipids. The triacylglycerols are key in maintaining fat homeostasis and in nematode growth. They are neutral lipids that are uncharged and due to their ability to be easily metabolized into energy glucose molecules they

serve as fat stores (11). The phospholipids are important in the membrane's structure and fluidity. They make up cellular membranes and allow them to pack tightly, which allows them to have good membrane fluidity and structure. The fluidity of the membrane is an important factor for cellular functioning, which depends on the composition of saturated and unsaturated fatty acids (12). Lipid molecules are found to have a monolayer of phospholipids that surround the neutral triacylglycerols (11).

## Lipid Metabolism

In most organisms, the lipid metabolism system is highly conserved, thus studies on C. elegans lipid metabolism can be related to that of other species, such as humans. The lipid composition is affected by which proteins are in the lipid membrane. Changing proteins such as those listed in the lipids section leads to cell signaling changes, and thus affects neurotransmission. These two membrane lipids are critical in this research as they regulate the membrane proteins' function, which includes the signaling and transportation of neurotransmitters. This is vital to the research that will be performed in these experiments because neurotransmission is implicated in depression. C. elegans primarily store their lipids in epidermal cells and in the intestine (48). Changes in the lipid composition will alter the fluidity of the membrane, so understanding these changes will help us better understand the way that monoamine oxidase inhibitors impact neurotransmission (11).

An increase in the presence of unsaturated fatty acids causes a change in the membrane fluidity which allows the proteins in the membrane to release additional neurotransmitters. This makes polyunsaturated fats a precursor for signaling molecules. Low levels of saturated fatty acids in the worms is thought to allow better growth. While C. elegans do not require cholesterol (cholesterol auxotrophs) to maintain their integrity in the membrane, they are precursors for various signaling molecules Elongase I and elongase II are enzymes that are involved in the biosynthetic process of regulating unsaturated and saturated fatty acids (48).

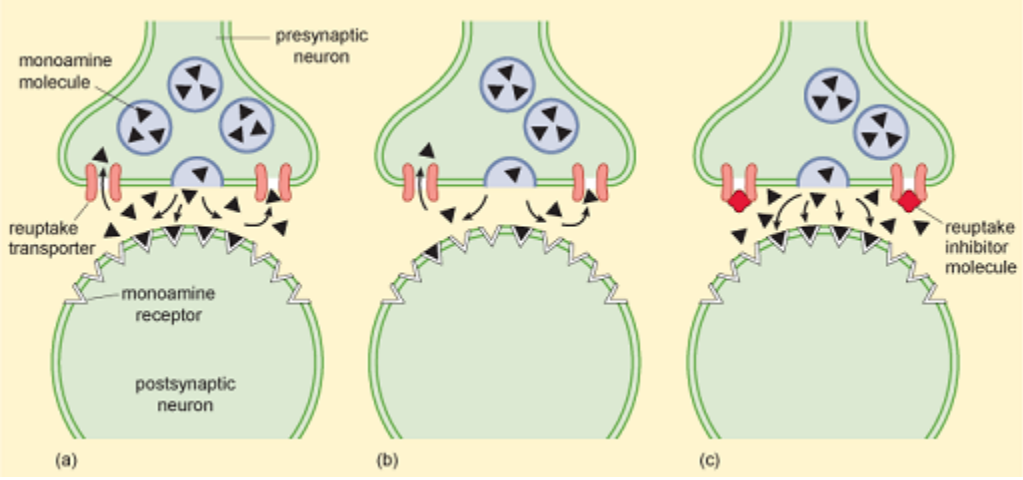
## Neurotransmitters

Neurotransmitters, which in humans include monoamines, peptides, amino acids, and catecholamines, are cell-to-cell signaling molecules that work through chemical reactions to communicate across synapses by transmitting signals to the junctions between the neurons (2).

Our central nervous system uses them to deliver signals between nerve cells throughout the brain and the body (33). Neurotransmitters are released from the brain to allow the cells throughout the body to communicate. The neurons communicate with each other via synapses, which are gaps at the end of the nerve cells that pass the signal along. These neurotransmitters are able to travel from the presynaptic to postsynaptic neuron and induce an activity, where it will then be degraded or return via reuptake back to the presynaptic neuron. Lack of regulation of these neurotransmitters leads to neurological disorders, including depression (13). They are the primary target in antidepressant therapies. The monoamine neurotransmitters are also present in *C. elegans* and are what will be studied for these experiments.

## *Monoamines*

Monoamines are one class of neurotransmitters that include epinephrine, norepinephrine, dopamine, and serotonin; all of which are connected with depression (3). These transmitters get released between the neurons into the synapse and bind to the corresponding receptors, aiding in the cell signaling process. It is hypothesized that a depleted level of these monoamines causes depressive symptoms, hence why it is believed that antidepressants work to target neurotransmitters and increase monoamine availability (5). While this theory has been hypothesized, there is no current research that can identify how a specific monoamine pathway dysfunctions for patients with depression. This hypothesis is shown in the image below.

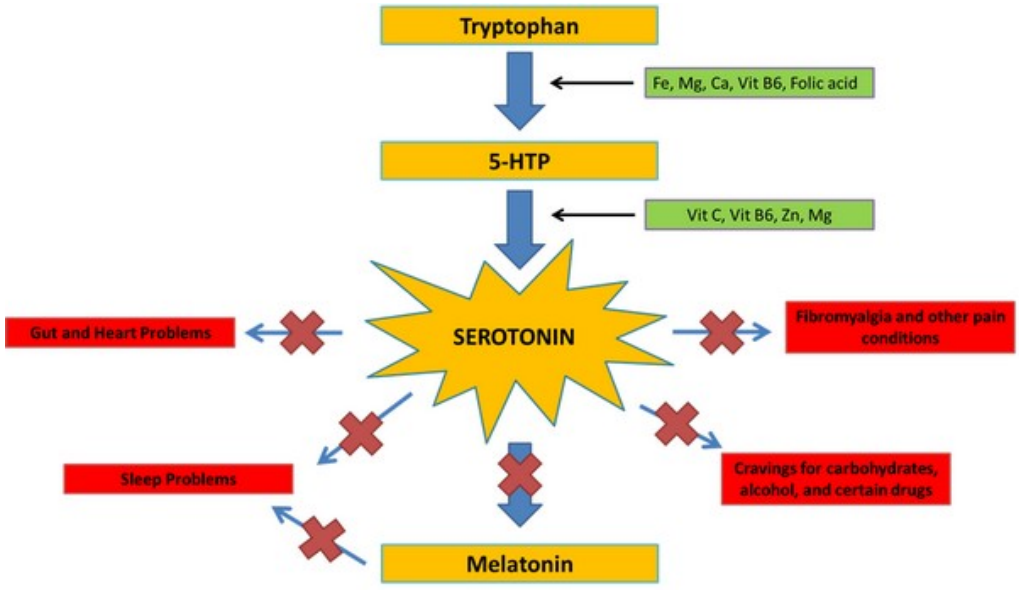



*This figure shows three different receptors. (a) shows a normal brain that allows for the release of the monoamines, which allow them to be brought to nearby neurons. (b) shows the brain of someone with depression. With these neurons, fewer of the molecules of monoamine can*

*begin to reach the receptors, which leads to mood disorders. (c) shows a possibility of how monoamine oxidase inhibitors work. They work to increase the number of monoamines that are present in the synaptic gap, which allows more of these molecules to bring to the neighboring neuron's receptors and thus fix the mood disorder* (Open University).

This lack of research to prove this functionality suggests the possibility that the physiology of depression has more complexity than just a simple neurotransmitter pathway failure (1). The levels of monoamines play a role in depression, as well as other molecule pathways such as that of dopamine and serotonin. While there are multiple studies that show the interactions between serotonin, dopamine, and norepinephrine, studying their combined effects on a patient's mood is difficult, and there are no current studies showing the total system's impact on depression's pathophysiology (14).

One monoamine particularly important is serotonin. This molecule is synthesized in the presynaptic neurons of the central nervous system, where it is also stored (15). Serotonin is released when a neuron is depolarized, where it gets released into the synaptic cleft and binds the receptors. There is a transporter that is located in the vesicle of the presynaptic neuron and this allows for reuptake of the neurotransmitter so that it can either be degraded or converted to another molecule (15). This is an important monoamine for this study, as it has many roles in modulating activity that includes mood, reproduction, eating, locomotion, and cognition (16).



*This figure shows the amino acids and molecules that are important in the serotonin pathway, along with how lack of this monoamine can lead to various health issues (Ablan, Deane).*

In terms of monoamine pathways, the norepinephrine and dopamine pathways are also relevant when looking at depression. For norepinephrine, the molecule gets synthesized from dopamine and then binds to the adrenergic receptors on the sympathetic nerve (17). This monoamine works to help regulate the nervous system along with responding to the body's stress. This is the "fight or flight" branch of the nervous system and helps regulate vasoconstriction, increases in heart rate, and decreases in digestion. When the norepinephrine system is dysregulated, there is proof that this can lead to depressive disorders. This is especially true when the interactions dysregulated are associated with serotonin pathways (17). The dopamine pathway is another important pathway associated with depressive disorders. This pathway helps modulate reproductive behavior and pathways, cognitive functioning, and motor control. When dopamine is produced in either the central or peripheral nervous system, the receptors are widely expressed (17). Studies have shown correlation between dysfunction in this pathway with neurological disorders that include Huntington's Disease, Hyperactivity Disorder, Parkinson's Disease, and Attention Deficit (17). Due to the wide range of implications that dopamine levels have on the body, including its role in depression, it is important that researchers work to fully understand its mechanism of action.

## *Monoamine Oxidase (MAO) and Monoamine Oxidase Inhibitors (MAOIs)*

Monoamine oxidases (MAOs) are intracellular enzymes that are associated with the mitochondria's outer membrane, where they insert themselves via a single C-terminal hydrophobic helix (35). There are two classes, MAO-A and MAO-B, that are reversible enzymes that have around a 70% sequence identity to each other, and which work to metabolize monoamine neurotransmitters such as serotonin and dopamine. A and B differ from each other in terms of both tissue distribution and substrate specificity, and it is theorized that between the two active sites there is no cross-talk (35). In humans, type A is mostly found in intestinal MAOs and type B is predominantly found in the brain (29). Monoamine oxidase inhibitors (MAOIs) work to target one or both of these classes. Currently for selective inhibitors, only MAO-B inhibitors are FDA approved (MAO-A inhibitors are not). These inhibitors are more specific, as they only

target one of the two MAO types. The other class of MAOs are irreversible, and these drugs form covalent adducts within the active site of the MAO-B to the flavin adenine dinucleotide cofactor (35).

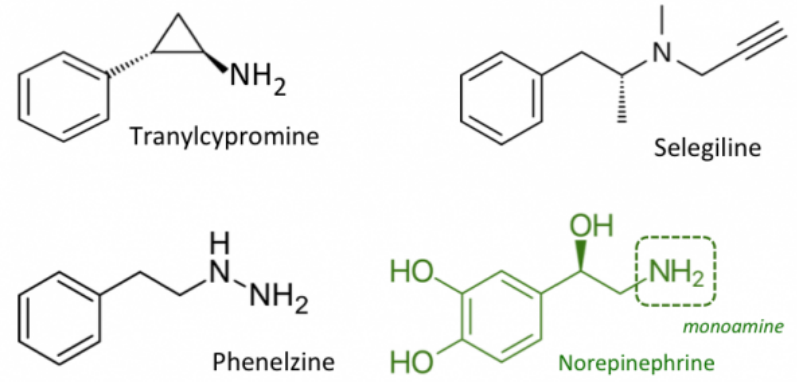
Monoamine oxidase inhibitors are a class of antidepressants that look to target monoamine receptors and affect the degradation (20). They work by targeting the monoamine oxidase, which is an enzyme that is responsible for degrading monoamines. The oxidase catalyzes the monoamine neurotransmitter's degradation process and directly targets the monoamine receptors (21). This enzyme family metabolizes monoamines and indoleamine neurotransmitters which inactivates them. They are present in the nervous system as well as the GI tract, mitochondrial membranes, liver, and platelets (25). The irreversible inhibitors work as "suicide substrates" for the MAO enzyme, as the MAO converts the drug to an active state where it can combine with an essential FAD cofactor and with the active site (29). For weeks after the patients stop taking the drug, they affect the neurology of the patients, so the drug can not unbind from the enzyme's active site; thus continues to cause monoamine levels to rise until the enzyme itself has been degraded (44).

MAOIs can be irreversible ("old" MAOIs) or target one of the two pathways ("new" MAOIs) - either MAO-A or MAO-B. Drugs that are irreversible inhibitors of MAO include phenelzine (Nardil), isocarboxazid (Marplan), and tranylcypromine (Parnate). Seeing as the enzyme forms covalent bonds, they inhibit irreversibly, so the body is forced to regenerate MAOs in order for the body to get back to the previous enzymatic activity. Due to these covalent bonds in which the molecules share electrons and become more stable, this bond is especially hard to break, as breaking the bond will make the molecule more unstable. Seeing as this bond can not be broken, irreversible MAOI remains attached to the MAO enzyme until the complete breakdown of the enzyme, which takes a few weeks (44). Because the regeneration process can take weeks, which means that the MAOI can affect the body even after the drug leaves the bloodstream. This can lead to issues in drug administration drugs, as patients are often prescribed with an inadequate dose (25).

For the reversible MAOs, MAO-A inhibiting drugs include moclobemide (Amira, Aurorix, Clobemix, Depnil and Manerix), a drug not FDA approved but used in other countries. MAOI-B drugs include selegiline (Eldepryl and Zelapar), rasagiline (Azilect), and safinamide (Xadago) (25).

All of these MAOI drugs are important because the deficiency in catecholamines such as dopamine, norepinephrine, and serotonin may be the result of depression; and they help increase levels of catecholamines. This theory is stemmed from studies that show depletion of neurotransmitter precursors, such as tryptophan. These drugs work by specifically inhibiting the catabolism of norepinephrine, serotonin, and tyramine. It is theorized that they are directly linked with MAOIs antidepressant activity (25). While it was previously believed that the MAOI antidepressants caused an increase in the amount of neurotransmitter amines at the nerve terminals, new hypotheses focus on receptor-mediated presynaptic and postsynaptic events (25).

For selegiline, it is theorized that it can either increase the secretion by their metabolites or increase the production of neurotrophin and that combining selegiline with other monoamine inhibitor drugs will further increase these results (22).




*These figures show the chemical composition of four monoamine oxidase inhibitors, two of which are used in these studies* (Pharmwiki).

## *Antidepressant Drugs and their Neurological Impact*

There are 5 major types of antidepressant drugs that are clinically prescribed to help treat depression: monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors, tricyclics, serotonin norepinephrine reuptake inhibitors, and atypical antidepressants. This study focuses on MAOIs. Monoamine oxidase inhibitor drugs not only impact the level of monoamine present, but also also break down tyramine. This is not a neurotransmitter and does not impact the brain, but can have an impact on the nervous system. It leads to cerebral vasoconstriction and then results in vasodilation as a response. This is caused because tyramine triggers the nerve cells

to release norepinephrine, which leads to rising heart rates and blood pressure (45). This is the reason that for people taking MAOI antidepressant drugs they are told to avoid things such as cheese and wine, as they have high levels of tyramine. For MAOI-B drugs such as selegiline, this is not as much of an issue because the A type MAO is not impacted, and this is the main enzyme responsible for tyramine breakdown (because it's located in the stomach) (44).

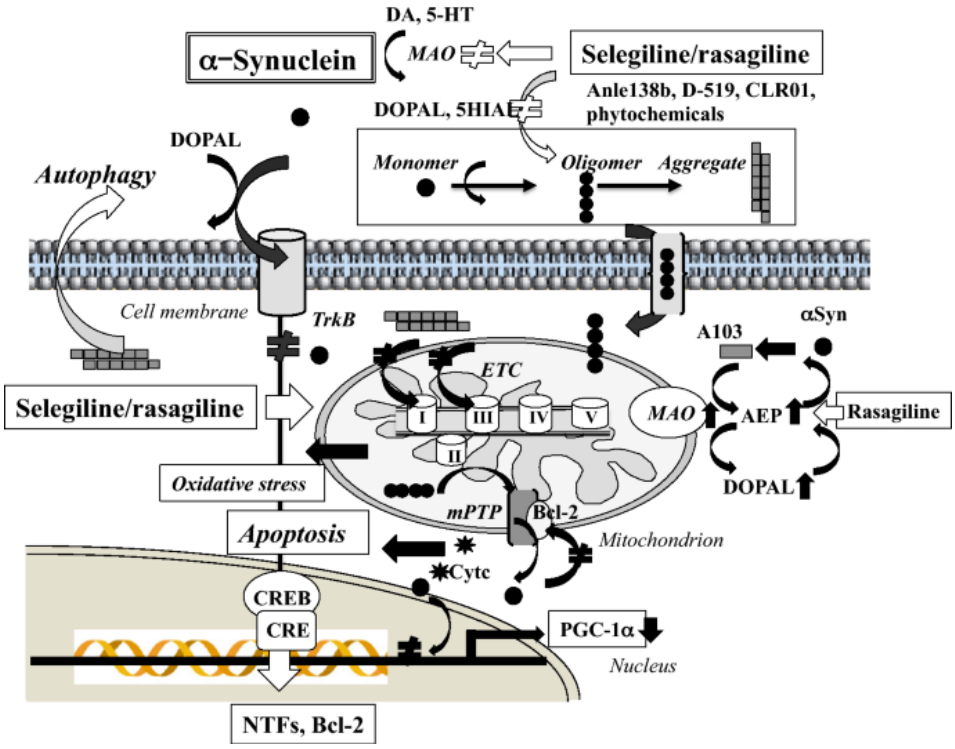
## *Pharmacology of Selegiline*

Selegiline is the R-optical enantiomer of deprenyl (phenyl-isopropyl-methyl-propargylamine). Its molecular formula is $C_{13}H_{17}N$, and the common brand names it is sold as include Eldepryl, Emsam, and Zelapar (29). Selegiline is stored at 20-25 ℃ and must be protected from light. It is a MAO-B inhibitor that is used to treat both depression and Parkinson's disease. It helps manage Parkinson's by prolonging the need to administer levodopa, a dopamine precursor. When administered, selegiline is metabolized rapidly by microsomal enzymes that convert the drug to methamphetamine, amphetamine, and desmethyl-deprenyl (26). It is the only MAOI prescribed in the US that does not require dietary restrictions. These metabolites interfere with neuronal uptake which results in an increased release of neurotransmitters such as dopamine as well as norepinephrine and serotonin (28). Inhibiting the B form of MAO also causes blockage of dopamine reuptake from the synaptic cleft and increases dopamine levels in the substantia nigra (a portion of the midbrain that when neuron function decreases is associated with Parkinson's). Both these actions are important as they lead to an increase in the overall dopamine concentration in the brain (27).

Selegiline's neuroprotective effect has a biphasic character, which leads to issues in clinical administration of the drug. At low concentrations ($10^{-9}$ to $10^{-13}$ M), there is no effect on MAO-B, however anti-apoptotic activity is induced. However, MAO-B selectivity gets lost at high concentrations, and above $10^{-7}$ M, pro-apoptotic activity increases. Additionally, clinical usage is often hindered due to selegiline's metabolites. These anti-apoptotic and pro-apoptotic effects can lead to physicians considering other MAO-B inhibitors such as rasagiline, which has no amphetamine-like metabolites (26).

These changing conditions make the dosage of selegiline hard to determine and it is done very specifically on a patient-to-patient basis. The pharmacokinetics are largely variable on this

dosage (27). The drug can be ingested orally or administered with a transdermal patch. When given through the skin, the drug achieves higher blood levels with less metabolite exposure (than if orally ingested) (28). No more than 10 mg/day is recommended, as there are associated risks with non-selective MAO inhibition (29).




*This figure shows the pathway of selegiline or rasagiline, two monoamine oxidase inhibitors that work by selectively inhibiting MAO-B* (24).

## *Pharmacology of Phenelzine*

For the experiments performed in these studies, phenelzine sulfate was used. This is the same as what is in Nardil, the phenelzine drug prescribed to patients in the US who suffer from depression. The drug has a molecular weight of 234.27 g and a chemical composition of $C_8H_{12}N_2 * H_2SO_4$. In patients, the effective dose used is 1mg/kg of body weight. This is administered daily, with the greatest improvements seen after a 6-week time frame. Phenelzine is metabolized via MAO by oxidation. It is a hydrazine, so can be used as an antidepressant and anxiolytic. It is a nonselective and irreversible MAOI that is FDA approved. Its primary use is for people with major depressive disorder, as it is proven to have a good response rate in people who are

categorized as “neurotic”, “nonendogenous”, and/or “atypical”. Phenelzine causes an increase in the extracellular concentration of the neurochemicals, thus it alters the neurochemistry and neurotransmission of the patient. It also inhibits two other enzymes, alanine transaminase and γ-aminobutyric acid transaminase. This causes a rise in the levels of alanine and GABA in the brain, which are contributors to depressive symptoms. The drug also metabolizes phenethylamine, a releasing agent of dopamine and norepinephrine. Concerns when administering this drug include the risk of headache and acute hypertension, as well as the “cheese reaction” that one can incur if they consume a lot of dietary tyramine (46).

## *Combined Drug Therapy*

A current health concern in the field of depression is treatment-resistant depression. For patients treated for major depressive disorder, over 40% of them do not adequately respond when administered the normal dose of a single antidepressant drug. In order to combat this issue, there have been a few records where adults are treated with multiple psychotropic agents. This includes using either an antidepressant or stimulant medication. In these studies, it was found that some of the individuals saw increased success when they used inhibitors that targeted the two different types of monoamine oxidases: monoamine type A and monoamine type B. 21% of the patients in a 29 person study shows significant improvement with no complications when combined therapy was used. Other patients lost the benefits that just selegiline alone provided them, while others developed a lot of negative side effects and did not see noticeable improvements. It is currently not recommended that monoamine oxidase inhibitors be used with other antidepressants due to the lack of research on its impact on the body and inconsistent results. However, for those patients who continue to struggle with single-drug treatments, this method is an option to try. Current literature reviews and clinical studies do support the use of combined-drug treatment for patients who have failed multiple single-treatment options (40). Risk of these treatments is not known and more studies are needed (23). The research proposed here could help strengthen the argument that combined therapy should be considered in treating patients with major depressive disorder who do not respond well to monotherapy.

One thing that can cause issues with this therapy is when patients develop “serotonin syndrome”. This is when the drug causes a bad reaction because of a very high level of

serotonin. This over-stimulation often occurs when multiple drugs are combined that contain serotonin or stimulate its uptake (44).

## *Hermaphroditic Specific Neurons (HSN) and Ventral C (VC) Neurons*

Both serotonin and NLP-s neuropeptides are released by HSNs, which both induce and regulate *C.elegans*' egg-laying behavior. When HSNs are removed, there is a significant decrease in the egg-laying rate (39). Without HSNs, animals are shown to have a reduced level of serotonin, which provides evidence that HSNs release serotonin as a neuromodulator in order to promote egg-laying. *C. elegans* that are serotonin deficient are shown to have a less severe change in their egg-laying rate than those that lack HSNs. This indicated that there are likely other neurotransmitters that the HSNs release on top of serotonin (40).

The muscle contraction and opening of the valval during *C. elegans* egg-laying events require VC neurons. There are cholinergic neurons that can express many neuropeptides, and may possibly also release serotonin. Additionally, researchers believed that they may also work to release monoamines such as serotonin, as they express a vesicular monoamine transporter (41). While lack of HSN neurons had an effect on egg-laying, lack of VC neurons show little on this behavior. This may be explained by the VC neurons' inhibitory role on egg-laying - while the success rate of egg-laying is higher, there are fewer unlaid eggs and thus fewer overall eggs (42).

It is not currently understood how these two groups of neurons (HSN and VC) work together on a neurochemical level. One key difference between the two is that while muscarinic receptors inhibit feedback of the HSNs, the VC neurons stimulate can valval muscular contraction by releasing acetylcholine though the nicotinic receptors. These signaling abilities or lack thereof may play a role in egg-laying behavior, but it can not be certain (40).

## *Behavioral Assays*

Behavioral assays are a way to observe changes in an organism's behavior. When doing these experiments, it is important that the conditions of the animal are such that they do not change their natural lifestyle. Things that can affect this way of life include inducing excessive stress of the organisms, forcing food competitions between the individuals (36). In the study of

*C. elegans* behavior, changes to their behavior can not be directly correlated to a specific neuron activation/deactivation, however they may indicate the pathways that the certain treatment or change in condition affected. Egg-laying assays allow for the observation of the organism's reproductive behavior, and can be used to predict which neuronal pathways are affected by a certain treatment (the antidepressant drug(s)).

In the lifespan of the *C. elegans*, they lay around 250 eggs. These eggs get fertilized in groups of around 10-15, where they are self-fertilized in the uterus and then expulsion occurs via the vulva (37). There are multiple environmental factors that regulate egg-laying behavior, including both mechanical and vibrational stimulations of the culture, both of which inhibit egg laying. On the other hand, egg laying increases with an overabundance of food. Overall, the observation of egg laying can indicate changes in the behavior of the organism (38).

## Egg Laying Patterns

*C.elegans* have a circuit of alternating states - active and inactive. In the active state, the organisms exhibit rhythmic activity. The command, HSN neurons, and the VC motor neurons are initiated. The other way for the HSN to be promoted is independently of this cycle, with the accumulation of unlaid eggs. The VC neurons drive egg-laying muscle contraction and the release of the eggs, slowing locomotion. In the NSH neurons there is a tyramine gated Cl- channel that contains uv1 neuroendocrine cells. These are able to sense eggs that mechanically pass through the vulva and release tyramine to stop egg laying. Worms are also seen to increase egg laying in the abundance of food (43).

There are two mechanisms that can be manipulated to change the activity of egg laying in *C. elegans*. The first is the encoding of the ion channels, which regulate electrical excitability of the cell and the synapse. The second way is to encode parts of the G-protein signaling pathway. When the HSNs release serotonin, the G-protein coupled receptors are the ones to signal an increase in excitability on the valval muscles. Many behavioral studies have been performed, and it can be concluded through these studies that the neuromodulators use G proteins to signal and regulate the cells excitability and thus can control both the circuit activity and egg laying patterns (43).

# Methodology

### Worm maintenance

For all the egg-laying behavioral and lipid composition assay experiments, *C. elegans* of the N2 wild type was used as the model organism. The *C. elegans* were transferred to new plates at least twice a week by using a sterile titanium pick. They were maintained on 10 cm plates with nematode growth medium. They were seeded with a total of 50 µL of OP50 E. coli and drug. In order to spread the OP50 and drug around, sterile glass beads were used. These plates were left to dry in a 20 ℃ refrigerator for 24 hours.

In order to prepare the OP50 E. coli, a vial of OP50 from the -20 ℃ freezer was thawed. A pipette tip was dipped in the OP50 vial, and was then streaked around a 10 cm plate. This plate was put in the incubator for 24 hours. This plate of bacteria was stored in a 20 ℃ refrigerator and sealed with parafilm. It is important to not add parafilm when the plate is placed in the incubator because the bacteria is aerobic and needs oxygen. All of these steps should be done over a flame to help avoid contamination.

## Objective 1: Behavioral Assays - Egg Laying Behavior

### Varying the concentration of selegiline

The plates were top seeded with OP50 and selegiline. Once the plate was seeded, worms were picked and transferred to each of the plates. The worms were given 4-5 days to grow on *e. Coli* fed plate along with selegiline with a total feeding of 50 µL. One plate had 50 µL *e. Coli*, one had 40 µL *e. Coli* and 10 µL selegiline, one had 30 µL *e. Coli* and 20 µL selegiline, one had 20 µL *e. Coli* and 30 µL selegiline, and one had 10 µL *e. Coli* and 40 µL selegiline. After the worms grew to full adults, M9 1X buffer was put on each plate and swirled around in order to detach the worms from the agar. This solution of worms and buffer was then collected in a 15 mL conical tube. From here, the tube was aspirated leaving only around 1 mL of worm solution, and then the tube was filled with the buffer again. The tube was centrifuged for 1 minute at 2000 rpm and again aspirated to 1 mL. Next, 12.5 mL of a bleach solution was added to the tube. The bleach has 2.5 mL of KOH, 5 mL of bleach, and 17.5 mL of di$H_2O$. Next, the tube was vortexed and left for 6 minutes, allowing the solution to detach the carcass of the worm and expose the eggs inside of them. After this, the tube was centrifuged for 1 minute at 2000 rpm and then

aspirated and filled with a buffer. The tube was then centrifuged and the aspiration and buffer process was repeated twice more. From here, the tube was placed in a rotator for 12-24 hour in a 20 ℃ refrigerator. Next, 10 µL of the worms and 1 mL of M9 buffer was placed in a tube and then tube was vortexed. 10 µL of this solution was then pietted onto a plate and the number of worms was counted. In order to determine the amount of worms per µL, the average number of worms was multiplied by 10.

**Selegiline and Phenelzine**

The same general procedure will be used as listed in objective 1 will be used to perform the behavioral assays and analyze the egg laying behavior. The difference for these experiments are the concentrations of various drugs used. For one plate it had 30 µL *e. Coli* and 30 µL selegiline, one had 20 µL *e. Coli* and 20 µL selegiline and 20 µL phenelzine, and one had 30 µL *e. Coli* and 30 µL phenelzine.

## Objective 2: Observe how combining selegiline with another monoamine oxidase inhibitor antidepressant drug impacts behavioral and lipid composition assays

**Lipid metabolism assays**

Worms grown on a 10 cm plate with OP50 and selegiline and/or phenelzine were grown for 4-5 days. For one plate it had 30 µL *e. Coli* and 30 µL selegiline, one had 20 µL *e. Coli* and 20 µL selegiline and 20 µL phenelzine, and one had 30 µL *e. Coli* and 30 µL phenelzine. From there, M9 buffer was used to collect the worms, they were centrifuged down to pellet the worms at the bottom, and they were transferred to a conical tube and frozen in a -80 ℃ freezer. The worms were then removed from the freezer and thawed. 975 µL of methanol and 25 µL of sulfuric acid were added to a glass tube in the fume hood using glass syringes. Next, a glass pipette was used to remove as much M9 buffer from the tube of thawed worms as possible. The worms were added to the glass tube, and 40 µL of standard (kept in a -20 ℃ freezer) was added to the same glass tube using a glass syringe. The solution was then baked at 80 ℃ for one hour, vortexing the tube every 15 minutes. If the solution started to evaporate during this time, methanol was added to maintain ~1 mL total solution. Next, a micropipette was used to add 1.6

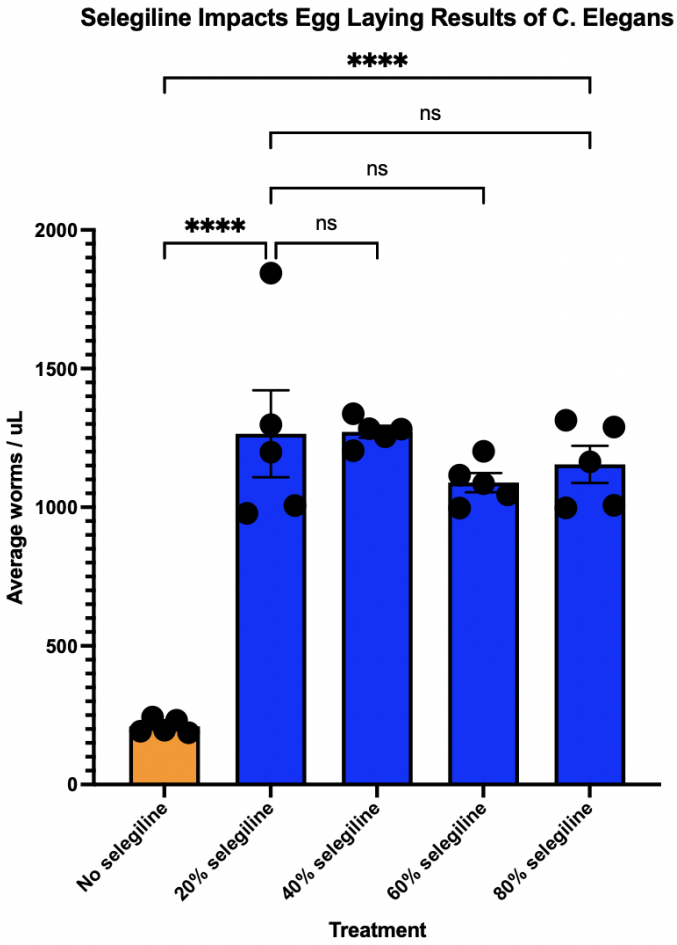
mL HLP graded $H_2O$ (to draw all the non-fatty acids) and a glass syringe was used to add 200 µL hexane (nonpolar so draws fatty acid) to the solution. The tube was vortexed to allow the fatty acid to mix with the hexane. The tube was centrifuged @ 2000 prm for 2 minutes and then the tube was placed in dry ice for 10-15 minutes to allow everything except the hexane to freeze. Small glass tubes were prepared - one for each sample, one for hexane, and one with standard. The liquid solution was poured into the sample glass tube and hexane and standard were added to the other. From here MS was run and the results were analyzed.

# Results

## *Objective 1:* Behavioral Assays - Egg Laying Behavior

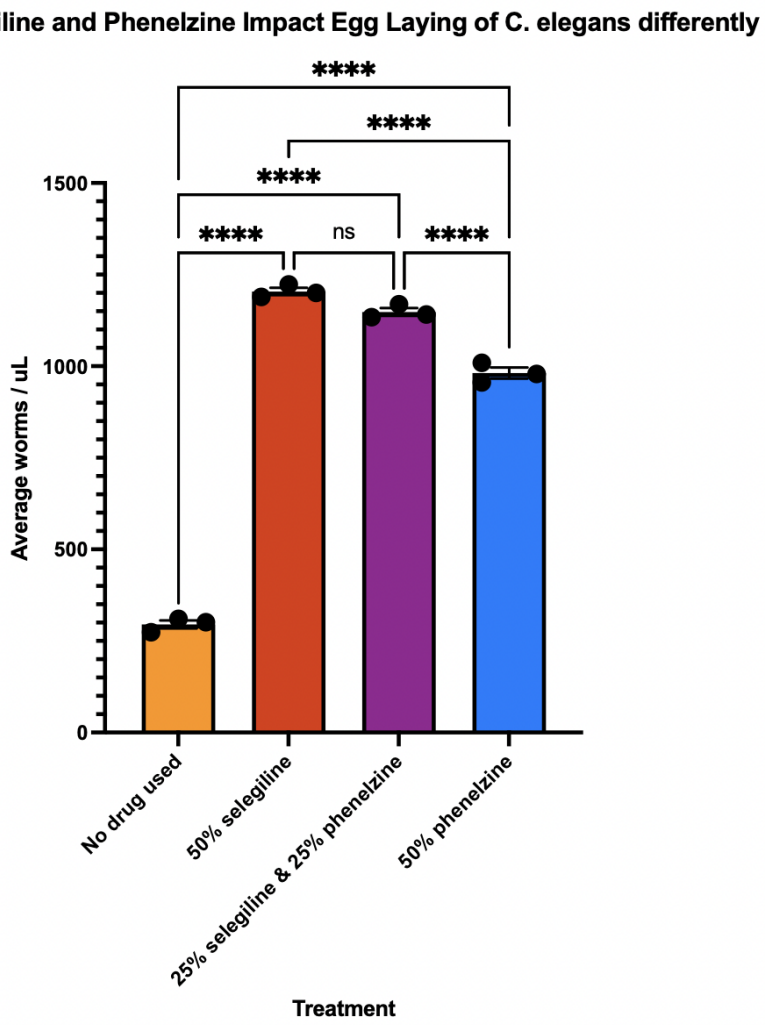
**Varying the concentration of selegiline**

For each trial run, three *C. elegans* were plated on each dish. The results were run 5 times, and each time three different samples were taken and an egg-count was performed. The data shows a significant increase in the number of worms laid per uL when selegiline was introduced to the culture. For the cultures with selegiline present, it was administered in four different amounts. One had 20% selegiline, one had 40% selegiline, one had 60% selegiline, and one had 80% selegiline. All of the data sets passed the Sharpiro-Wilk normality test, so an ordinary one-way ANOVA was performed. This analysis shows that there is a statistical difference between the no selegiline data and any of the trails that included any percentage of selegiline.



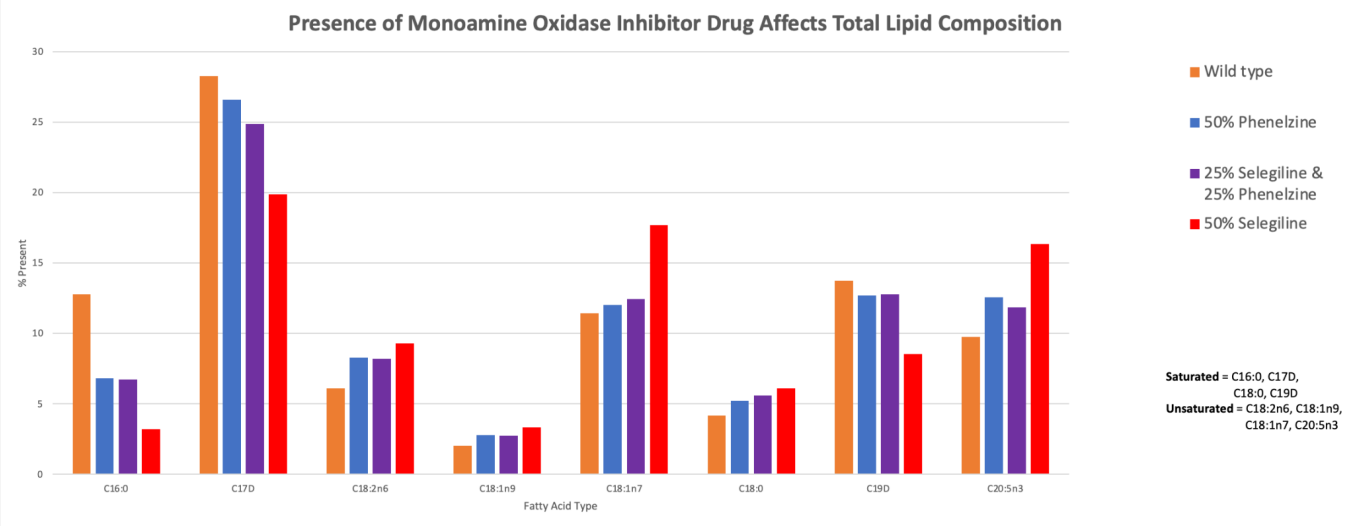

**Selegiline and Phenelzine**

For each trial run, three *C. elegans* were plated on each dish. The results were run 3 times, and each time three different samples were taken and an egg-count was performed. All of the data sets passed the Sharpiro-Wilk normality test, so an ordinary one-way ANOVA was performed. This analysis shows that there is a statistical difference between the no selegiline data and any of the trails that included any percentage of selegiline. It also shows that there is also a statistical significance between the trail with just selegiline as compared to the trail with just phenelzine.




*Objective 2:* Observe how combining selegiline with another monoamine oxidase inhibitor antidepressant drug impacts behavioral and lipid composition assays

**Lipid metabolism assays**




For each trial run, three *C. elegans* were initially plated on the *E.coli* fed dish. This analysis shows that in the presence of a monoamine oxidase inhibitor(s), there was an increase in polyunsaturated fatty acids and a decrease in the presence of saturated fatty acids. All the delta fatty acids also saw a decrease. All the results show that when just selegiline was used there was the largest change from the wild type worms. This was followed by the combined drug trials and then the just phenelzine (least amount of change induced as compared to the wild type).

# Discussion

## *Objective 1:* Behavioral Assays - Egg Laying Behavior

### Varying the concentration of selegiline

It can be seen through egg laying experiments that look at the neuronal pathway of *C. elegans* that selegiline alters neurotransmitter's availability. Egg laying assays were performed to demonstrate how varying selegiline concentration affects the behavior of the C. elegans. The egg-laying behavior is largely driven by the presence of hermaphroditic specific neurons (HSN), and when the amount of these neurons decreases, there is a direct correlation to the amount of egg laid by *C. elegans*. The presence of HSN's is directly correlated to the amount of neurotransmitters available (with serotonin being one of but not the only neurotransmitter impacted) (40). This is important because these conditions are key in neurotransmitter signaling. Selegiline is a drug that inhibits the enzyme that is responsible for the breakdown of serotonin

and other monoamine oxidases. This data shows that selegiline likely works to increase serotonin's availability. When selegiline was introduced to the culture, there was a significant increase in the worm's egg laying. Studies show that in animals without hermaphroditic specific neurons, there is a significant decline in the rate of egg laying. It is likely that the selegiline stimulates these neurons and thus creates an increase in egg-laying.

It is currently unknown how varying amounts of selegiline will impact the neurological pathways. For patients, the amount of selegiline administered depends on the severity of the depressive symptoms that the patient is experiencing (8). The results show that there was no significant difference in the worm's egg-laying behavior when the concentration of selegiline changed. Seeing as the egg laying amount did not increase when more drug was present, it is possible that the selegiline is more selective in inhibiting the MAO-B at lower concentrations, and thus as the concentration of the drug increases, the binding selectivity does not increase and thus does not result in a high-amount of eggs laid. This is important because it presents the possibility that increasing the dosage of selegiline for a patient may not cause an increase in the inhibition of the MAO, thus the amount of monoamines may not increase with an increased dosage of the drug. Seeing as a higher dose of selegiline medication is often the option physicians turn to when the original dose is not effective, there are likely other things than just the HSN and VC neurons impacted when this drug is administered.

**Selegiline and Phenelzine**

Prior studies have proven that selegiline impacts the behavior and lipid composition of *C. elegans*. However the effect of multiple monoamine oxidase inhibitors on the neurological system is yet to be determined. Studies have been performed that show that the use of multiple monoamine oxidase inhibitors can increase the effectiveness in treating depression for some patients (7). Some physicians choose to use combined drug therapy in treating depression, especially for those patients who appear to have developed a resistance to treatment. Treatment-resistant depression is a major medical issue, and studies have shown that over 40% of patients who have major depressive disorder are resistant to their initial medication. Studies are currently being performed to test the use of multiple monoamine oxidase inhibitors and preliminary results show that in 21% of the patients there was a significant increase in effectiveness with no complications (9).

When there is a low concentration of serotonin, the egg laying process of *C. elegans* is shown to decrease. Seeing as the presence of monoamine oxidase inhibitors will cause an increase in the concentration of serotonin, it is expected that egg laying would be increased with a high concentration of the drug. However, a previous study showed for animals that are deficient in serotonin, a common monoamine, the change in the egg laying rate was slight. This study implied that there are multiple neuromodulators (not just serotonin) that are associated with the egg laying process. This is important because there is evidence that indicates that multiple monoamines may be involved in the induction of egg laying. These experiments show that when the same amount of overall drug is administered, it does make a difference as to what percentages of the various MAO-Is are used. The data shows that there is a higher rate of egg-laying when all selegiline is used as compared to when it is combined with phenelzine, or when phenelzine was the only drug used. It is likely with the presence of phenelzine, serotonin syndrome occurs, in which a bad drug reaction occurs. This has been observed in patients on combined drug therapy treatment plans.

## *Objective 2:* Observe how combining selegiline with another monoamine oxidase inhibitor antidepressant drug impacts behavioral and lipid composition assays

**Lipid metabolism assays**

It was observed through lipid composition assays that antidepressants such as selegiline and phenelzine treat depression by changing the fluidity of the membrane to create a more favorable environment for neurotransmitters to be produced and released through membrane proteins. Lipid assays were performed to determine how the lipid composition of the membrane is altered based on the concentration of drug used. In order for the monoamines to be released and uptaken, lipids are key as they serve as transporters. Thus this assay can help determine the impact of the selegiline mechanism and the possible neurotransmitter transporter(s) involved, as it shows an increase in unsaturated fatty acids and a decrease in saturated fatty acids when any monoamine oxidase drug was present. The results show that there was a transition from C:16:0 to C:18:0 to the unsaturated fatty acids, indicating a notable shift in the lipid composition. These results propose the possibility that the enzymes involved are responsible for not only fatty acid synthesis and elongation but also in regulating mood and behavior. These enzymes include elongase I and elgonase II, which get upregulated when a monoamine oxidase inhibitor is

introduced. This enzyme is critical in lipid metabolism regulation. There was also a decrease in the level of all the delta fatty acids when any MAOI drug was introduced. These are bacterial fatty acids, so a decreased level present in the worms likely indicates that there was alleviated food stress on the environment when the drug was introduced.

Not only do these drugs cause a change in the metabolic pathway of the fatty acids, it is also true that a decrease in the saturated fatty acids and an increase in unsaturated fatty acids impacts the fluidity of the membrane. This causes a crease in the melting temperature of the membranes. The most abundant lipid type in the cell membranes are the phospholipids, so the desaturation of C. elegans in the membrane decreases with the decrease in saturated phospholipids. This increases the fluidity of the membrane, a function shown to impact neurotransmission and effectively the severity of depressive symptoms.

## Conclusions and Further Directions

The results from these experiments hope to show important data to further our understanding of how monoamine oxidase inhibitor drugs work to treat depression. These results aim to provide a better understanding of how the concentration of selegiline impacts the drug's effectiveness and the effectiveness of using combined drug therapy to treat depression. They show that the use of a monoamine oxidase inhibitor drug (selegiline, phenelzine, or a combination of both) causes increased egg-laying behavior and membrane fluidity. They also show a shift in which signaling molecules are being expressed, as the polyunsaturated fatty acids serve as precursors and are more prominent when the drug is present.

This study also provides additional evidence that individualized care for depressed patients is critical. How different drugs or combinations of both of them affected the worms varied from case to case. This along with the different changes in lipid metabolism behavior demonstrates that multiple neurological pathways are at work and that these get altered differently from one test subject to the next.

Future work from here could involve studying if *C. elegans* suffer complications or negative side effects when being treated with a varied concentration of selegiline or when being treated with multiple monoamine oxidase inhibitor antidepressant drugs. Other behavioral assays (thrashing, etc) could be performed to see what other neurons are impacted by the drug as the

concentrations change. Additionally, it would be interesting to perform the same experiments on worms that are serotonin deficient, so that the impact of the various different monoamines can be further understood. It would also be interesting to determine if and what the correlation is between the motor neuron activity (egg laying) and fatty acid composition. Performing these experiments would allow the team of researchers to get closer to determining how neurological pathways are affected when a monoamine oxidase inhibitor drug is introduced.

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