Drew University
College of Liberal Arts

The Search for Reliable Behavioral Assays to Model Parkinson's Disease in the Nematode *Caenorhabditis elegans*

A Thesis in Neuroscience

by

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Abstract

Parkinson's disease is a nervous system disorder associated with abnormal damage to dopamine neurons that play a role in reward and movement. This disease is localized to the substantia nigra region of the basal ganglia and the ventral tegmental area, with projections to the striatum (caudate and putamen). This disease is the second most common neurodegenerative disorder, after Alzheimer's disease, with an increased predisposition combining both environment and genetics. Apart from long-term pesticide and toxin exposure, mutations in leucine-rich-repeat kinase 2 (LRRK2) and α-synuclein proteins are shown to greatly influence the onset of Parkinson's. Animal models have been intensively used for the study of Parkinson's disease including rodents, non-human primates and the nematode worms, *Caenorhabditis elegans.* This research involves studying Parkinson's behaviors in *Caenorhabditis elegans* mutants because they are good models with a well-defined dopamine system, which is relevant to the mechanism of Parkinson's. In this study, we experimented with and modified multiple behavioral assays, including the swim-to-crawl assay, the movement-over-alternate-terrain (MOAT) assay, the ethanol avoidance assay, and the swimming-induced-paralysis (SWIP) assay to track and model behaviors of *C. elegans* mutants. We hypothesized that *C. elegans* worms expressing a mutant form of human leucine-rich repeat kinase 2 (LRRK2), would display a loss of dopamine neurons over time, compared to the wild-type worms and this hypothesis was tested using the ethanol avoidance assay, as previously established in literature. Based on the results, we concluded that the SWIP and MOAT assays were not well suited as an assay to model Parkinson's disease. The results we obtained from the

MOAT assay were conclusive, yet not useful since we learned that *C. elegans* do not spontaneously transition from swimming to crawling. The SWIP assay was found to be unsuitable to examine the age-related loss of dopamine neurons because older wildtype worms lose the SWIP phenotype. In the future, we plan to focus on testing older age LRRK2 worms in the ethanol avoidance assay and reproducing the literature results for the basal-slowing response assay, as these are good models for studying Parkinson's. Potential treatments for Parkinson's have been focused on developing LRRK2 kinase inhibitors, that directly inhibit LRRK2 kinase and lead to a decreased loss of dopamine neurons, increasing neuroprotection. We plan to use our worm model of Parkinson's to identify new LRRK2 inhibitor compounds.

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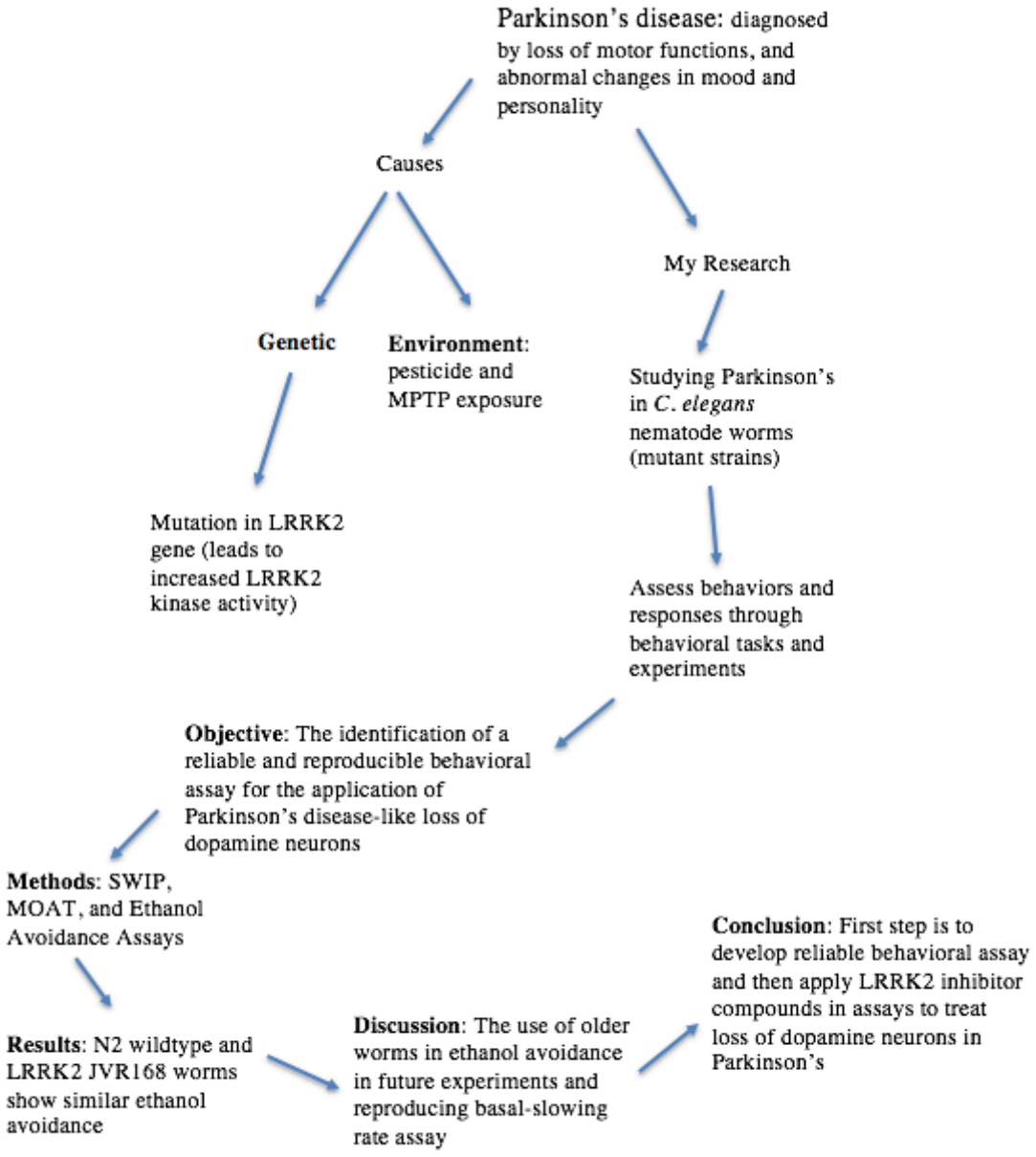


Figure 1: Overview of the Structure of My Paper

Introduction

Parkinson's Disease

Understanding Parkinson's disease (PD) is one of the most relevant objectives to healthcare workers and the field of neuroscience. Parkinson's was named after Dr. James Parkinson who first identified it as a neurological syndrome in 1817 (Goetz 2011). Jean-Martin Charcot and other contributors helped differentiate Parkinson's from other neurological conditions and came up with a rationale based on the physiology and observable symptoms of the disease. Some of the main symptoms exhibited by patients are deficits in motor control, with uncontrollable hand motions called tremors, muscle stiffness, and difficulty in starting and stopping motions (DeMaagd and Philip, 2015). Parkinson's disease is one of several nervous system disorders known to cause damage to specific regions of the brain, leading to diminished motor function in patients. The regions of the brain greatly affected include the substantia nigra in the basal ganglia and parts of the ventral tegmental area and the striatum. The underlying causes of Parkinson's are still under study because Parkinson's can be caused by a wide range of known genetic and chemical factors (Nandipati and Litvan 2016).

While most affected individuals are above the age of 60, Parkinson's has developed in some in their 30s as well (DeMaagd and Philip 2015). Individuals living with the illness for more than 5 years can also advance to a more progressed stage of PD and experience gaps or loss in memory. Neurons in the substantia nigra produce dopamine, which communicates with muscle cells to produce muscle movements (DeMaagd and Philip 2015). Parkinson's disease is characterized by the damage or death

of neurons in the substantia nigra region of the brain greatly affecting the nervous system, but also impacting the gastrointestinal tract and other associated organ-systems.

Diagnosis

Neurologists typically diagnose Parkinson's based on not only motor symptoms, but also from psychological and other health-related problems. The hallmarks of Parkinson's can suggest potential treatment options based on the severity of the motor or psychological symptoms. Along with the dopamine neuron degeneration that can be detected through imaging, neurologists and researchers also look for Lewy bodies, created from insoluble proteins that often form tangles with α-synuclein protein filaments (DeMaagd and Philip 2015). Along with Lewy bodies, dopamine neuron degeneration continues to serve as the major cellular hallmark in the diagnosis of Parkinson's. Patients who show hallmarks of Parkinson's may also experience depression, abnormal sleep, and the loss of cognitive abilities because the pathogenesis of Parkinson's is not only limited to the dopaminergic system, but also encompasses serotonin, noradrenergic, and cholinergic neurons (Nandipati and Litvan 2016).

The prevalence of PD increases greatly in individuals over 60 and twice as much in individuals over the age of 80 (Antony et al., 2013). Parkinson's takes many decades to develop, even in individuals that have inherited forms of PD. Older individuals are generally less susceptible to the physiological influences of the mutations causing PD early on in their life, compared to younger individuals. Several processes that take place during normal aging have also been shown to occur in the pathogenesis of PD, including the aggregation of protein filaments, an increase in reactive oxygen species, decline in

mitochondrial function and impairments in the normal autophagy processes of the body (Antony et al., 2013).

Dopamine Signaling in Humans

Tyrosine hydroxylase is the enzyme that catalyzes the synthesis of dopamine from tyrosine molecules. The DAT-1 dopamine transporter is responsible for enabling the release of dopamine and regulating extracellular dopamine levels (Felton and Johnson 2014). As seen in Figure 2, once dopamine is in the synapse, it can bind to two types of dopamine receptors, the excitatory D1 and inhibitory D2 dopamine receptors (DeMaagd and Philip 2015). Binding of dopamine to DOP-1 results in an activation or excitatory response in the muscle cell, while binding of DOP-2 or DOP-3 receptors results in an inactivation or inhibitory response in the muscle cell.

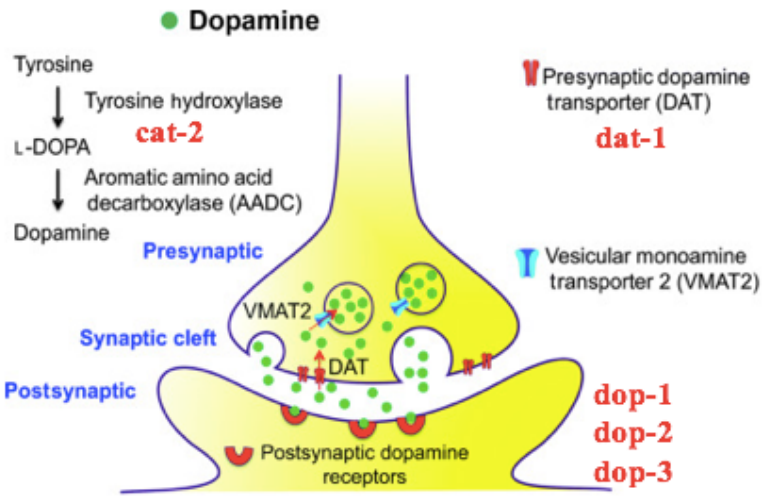


Figure 2: Schematic of Dopamine Signaling Mechanism in Humans and *C. elegans* (Relevant *C. elegans* mutants are highlighted in red)

Genetic Influences of PD

Years of research and analysis have led researchers to the hypothesis that the role of genetic factors in PD is significant to its onset and progressive nature. According to the National Human Genome Research Institute, there are seven genes that have been identified to cause Parkinson's disease (NIH 2014). Mutations in glucocerebrosidase (GBA) and ubiquitin carboxy-terminal hydrolase L1 (UCHL1) are implicated as the cause of Parkinson's and associated with increased risk of developing Parkinson's if previous family members were already diagnosed with the disorder (NIH Genetics Home Reference). Individuals confirmed to have a family history of Parkinson's may express mutations in leucine-rich repeat kinase 2 (LRRK2), parkinsonism associated deglycase (PARK7), PTEN-induced kinase 1 (PINK1), parkin RBR E3 ubiquitin protein ligase (PRKN), and synuclein alpha (SNCA). The inheritance patterns in PD can be classified into autosomal dominant and autosomal recessive depending on which of the genes are expressed by the individual. Mutations in these genes are the most common genetic risk factors for Parkinson's disease, which all lead to alterations in either protein structure and/or expression of proteins that are necessary for the regulation of normal physical processes.

The LRRK2 mutation is most relevant to the investigation of Parkinson's disease because mutations in the LRRK2 gene are found to be the greatest factor in causing Parkinson's (Yao et al., 2010). The effect of LRRK2 mutations on dopamine neurons is a topic widely studied by researchers in order to investigate the mechanism of Parkinson's. The most prevalent LRRK2 variant is the G2019S mutation, which appears in people of

all nationalities, excluding the populations in Asia (Rui et al., 2018), while, other LRRK2 variants are more common in Asian populations from Singapore, Taiwan, and China. Understanding the cellular biology of Parkinson's disease is relevant to understanding these genetic risk factors that contribute to Parkinson's, and the biological effects of these genetic risk factors may also help us understand the cellular biology of Parkinson's. Mutations in LRRK2 have been linked to high LRRK2 kinase activity, and this in turn results in the loss of dopamine neurons (Rui et al., 2018).

LRRK2

Higher levels of LRRK2 expression are seen in dopamine-innervated regions of the brain such as the cerebellum and hippocampus, while lower levels are found in dopaminergic neurons of the substantia nigra and ventral tegmental area (Rui et al., 2018). Proper functioning of LRRK2 is important for the completion of several cellular processes in the human body. Regulation of protein translation, phosphorylation of proteins in the cytoskeleton, and responding to cellular stressors are some of the potential cellular mechanisms LRRK2 is involved in (Drolet et al., 2011). The LRRK2 mutation in Parkinson's has been associated with increased kinase activity which has led to the development of LRRK2 inhibitors for the treatment of Parkinson's (Taymans and Greggio 2016). However, the exact role of LRRK2 activity is still under investigation, and is being determined via the pathogenesis of Parkinson's disease with the help of animal models studies.

LRRK2 protein has two enzymatic domains capable of catalytic activity. Its kinase domain is composed of serine and threonine subunits, involved in the auto-

phosphorylation and heterologous substrates (Berwick et al., 2019). LRRK2 also has a Roc domain, mediated by the Rab GTPase proteins. There are two predominant reasons shown to increase kinase activity: first, a group of Rab GTPases are involved in the hyperactivity of LRRK2 kinase and second, variants of the enzymatic LRRK2 kinase domain have also been shown to increase auto phosphorylation of serine subunits (in particular, Ser1292) and Rab GTPases (Berwick et al., 2019). This also explains why researchers are invested in the development of LRRK2 kinase inhibitors as treatments for Parkinson's.

α-synuclein

Patients confirmed to have Parkinson's may express mutations in the SNCA gene, which codes for the α-synuclein protein (Rui et al., 2018). High levels of α-synuclein protein are found in Lewy bodies, which are found in the neurons of individuals with Parkinson's. α-synuclein protein is necessary for the docking of synaptic vesicles and neurotransmitter release for proper neurotransmission and synaptic function (Rui et al., 2018). Therefore, α-synuclein dysfunction can impact neurotransmitter release and neurotransmission and overall communication to and from the central nervous system.

LRRK2 mutations have been shown to exacerbate the aggregation of α-synuclein in Parkinson's. Attempts to identify the presence of LRRK2 protein in Lewy bodies were made after LRRK2 was identified as a cause of PD. The interplay of LRRK2 and α-synuclein also led researchers to isolate samples of dead brain tissue, in which both LRRK2 and α-synuclein were found from protein extracts (Liu et al., 2012). Some in-vitro studies indicate that LRRK2 may be involved in the formation of α-synuclein

aggregates due to its kinase activity (Liu et al., 2012). The interplay of these two proteins also affects several biological markers including actin and microtubule assembly, ER and Golgi transport, the role of mitochondria, the ubiquitin-proteasome system, and cellular autophagy pathways (Antony et al., 2013).

Environmental Influences in Parkinson's

Even though most individuals are affected by the genetic causes of Parkinson's, there are some who are affected from an interplay of both genetic and environmental factors, such as toxins in the natural environment. Frequent and long-term exposure to pesticides and heavy metals can also lead to the onset of Parkinson's disease. Long-term pesticide uses and exposure in the farming occupation has led to a higher incidence of PD suggesting links between environmental toxins and PD (Nandipati and Litvan 2016). Risk factors for Parkinson's are not limited to the genetic causes mentioned above or these environmental toxins (carbon disulfide, cyanide, pesticide and herbicides, etc.) but also can also include elevated cholesterol levels and bodily stress from other diseases.

An interesting link to the study of neurotoxins in Parkinson's is the agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which was widely under study in animal models. In the early 1990s MPTP was identified as a contamination in heroin commonly abused by young adults who were soon found to present with severe Parkinson's symptoms (Nandipati and Litvan 2016). After this discovery, MPTP was used in animal models ranging from worms to primates for the better study of Parkinson's disease. Rodent animal models showed that MPTP mimicked the action of Parkinson's in that it destroyed the dopamine neurons in the substantia nigra region of the

brain (Nandipati and Litvan 2016). After MPTP, researchers examined the use of several environmental toxins including pesticides, rotenone, organophosphates and other heavy metals for the study of Parkinson's with the help of animal models.

Caenorhabditis elegans as Animal Models

The study of Parkinson's and any disorder would be incomplete without animal research subjects. Going back to the most primitive organisms allows neuroscientists and researchers to compare human nervous systems with the nervous systems of model organism. When scientists are in the search for suitable compounds that can treat nervous system related disorders, they first test the efficacy and effectiveness of these potential therapies in in vitro and animal models. One of the main goals of conducting research on animal subjects is to answer questions relevant to the field of neuroscience and medicine. The findings from pre-clinical and clinical trials is a necessary step in preventative medicine and healthcare today, and animal subjects prove to be excellent models, for genetic mapping and other behavioral analyses.

An important animal model for the study of Parkinson's disease is *Caenorhabditis elegans*. *C. elegans* belong to the class of nematode worms, commonly used for studying diseases of the central nervous system. One of the primary advantages of studying *C. elegans* models is to allow us to compare our CNS of 86 billion neurons to the *C. elegans* nervous system of only 302 neurons. Several years of research has led to the mapping of the entire *C. elegans* nervous system (Wormatlas 2021), and with the help of mutants provided by the *C. elegans* genetic center, neuroscientists can study the effects of these altered genes on basic functions of the organisms. In addition, researchers can generate

mutant *C. elegans* that express mutant human genes in specific neurons to create worm models of human diseases.

Dopamine Signaling in *C. elegans*

The *C. elegans* nervous system contains 8 dopaminergic neurons (6 anterior dopamine and 2 posterior dopamine), compared to an estimated 400,000 dopaminergic neurons in the human nervous system. Mutants (shown in Table 1) available from the *C. elegans* genetic center enable researchers to compare the loss of dopamine neurons between mutant worms expressing different genes. The mechanisms of dopamine signaling in *C. elegans* include some of the same molecules seen in humans and other mammals (Figure 2). In *C. elegans*, the same tyrosine hydroxylase enzyme is responsible for synthesizing dopamine from tyrosine. The DAT-1 dopamine transporter in *C. elegans* is responsible for regulating extracellular dopamine levels (Felton and Johnson 2014). Once dopamine is in the synapse, it can bind to two types of dopamine receptors, the excitatory D1 and inhibitory D2 dopamine receptors (DeMaagd and Philip 2015). In *C. elegans*, dopamine binds to either of these receptors, including D1 subtype DOP-1 and D2 subtypes DOP-2 and DOP-3 (Felton and Johnson 2014). Binding of dopamine to DOP-1 results in an activation or excitatory response in the muscle cell, while binding of DOP-2 or DOP-3 receptors results in an inactivation or inhibitory response in the muscle cell.

Table 1: *C. elegans* Mutants Used for Study of PD

Strain	Genotype
N2	wildtype (normal dopamine signaling)
MT15620	cat-2 (mutation in tyrosine hydroxylase gene – responsible for biosynthesis of dopamine)
LX703	dop-3 (mutation in dopamine receptor 3)
RM2702	dat-1 (loss of activity mutation in the dopamine transporter)
JVR168	pdat-1::GFP, pdat-1::LRRK2 (G2019S); age-dependent loss of dopamine neurons
JVR203	pdat-1::GFP, pdat-1::α-synuclein (A53T); age-dependent loss of dopamine neurons
VM6365	pdat-1::GFP, pdat::ICE (loss of GFP in dopamine neurons in late L1 stage) and no GFP staining in adults

Dopamine's Role in *C. elegans* Behaviors

While the mechanism of dopamine signaling has been implicated in the regulation of several human behaviors, its role across various organismal species, including the *C. elegans* nematode species has also been elucidated. Studies have shown how dopamine's role in learning and memory is observed when worms slow down in the presence of food, which they may detect through its odor and texture (Vidal-Gadea and Pierce-Shimomura 2012). Across most animals, dopamine plays an important role in mediating the transition from slow to fast and fast to slow motor movements, and in *C. elegans* we observe these dopamine-regulated behaviors as the nematodes transition between swimming to crawling when on land vs. in water. *C. elegans* display what are known as distinct "locomotory gaits", isolating their crawling behavior from their swimming behaviors,

which resulted from the earlier finding of how *C. elegans* display discontinuous cycles of crawl and swim-like motions (Vidal-Gadea and Pierce-Shimomura 2012). In *C. elegans*, dopamine-modulated behaviors are often times inhibited by serotonin-modulated behaviors and vice versa (Vidal-Gadea and Pierce-Shimomura 2012). For example, serotonin promotes egg-laying, feeding, and is involved in mediating swimming in *C. elegans.*

C. elegans **Neurotoxin models of Parkinson's (6-OHDA and MPTP)**

Both 6-OHDA and MPTP toxins are commonly used in research to mimic Parkinson's in rodents, primates, and *C. elegans* models. The degeneration of the dopaminergic neurons can be studied using Green Fluorescent Protein (GFP) analysis in *C. elegans* quite distinctly due to their small dopaminergic system. Expression of GFP can be restricted to the worm's 8 dopaminergic neurons using the dopamine neuron specific promoter from the dat-1 gene (Figure 3).

Figure 3: GFP staining of dopamine neurons in *C. elegans* (image is from Bayne Lab)

Application of MPTP in *C. elegans* models resulted in observable defects including decreased mobility, death, and degeneration of the dopaminergic neurons (Braungart et al, 2004). Treated wild-type N2 animals displayed uncoordinated

movements, mimicking the akinesia and rigidity Parkinson's like symptoms (Braungart et al, 2004). Dopaminergic neurons degradation and observable behavioral defects were rescued with the application of Parkinson's treatments (co-administration of neurotoxin 6-hydroxydopamine (6-OHDA) with a DAT antagonist (DAT-1 inhibitor) by lowering DAT expression, presenting *C. elegans* as a novel animal model for MPTP studies.

In Parkinson's studies, neurotoxin (6-OHDA) is injected into animals to destroy dopamine or noradrenergic neurons of interest in the brain. The injection of 6-OHDA also led to mitochondrial dysfunction and axonal degeneration in dopamine neurons, leading to a subsequent increase in reactive oxygen species (ROS) (Lu et al., 2014). Unlike MPTP, 6-OHDA does not cross the blood brain barrier, and instead has to be injected directly into the brain. 6-OHDA is taken up by the dopamine transporter into dopaminergic neurons, and eventually blocks Complex I of the mitochondrial electron transport chain, resulting in oxidative stress (Offenburger et al., 2018). In response to 6-OHDA, cell death and apoptosis pathways were commonly investigated, where the cell bodies of dopaminergic neurons showed condensed chromatin, and the influence of glial cells nearby, consistent with phagocytosis from 6-OHDA treatment (Offenburger et al., 2018). In *C. elegans*, two hours 6-OHDA treatment resulted in blebs around anterior deirides (ADE neurons) and rounded somas, and 72 hours 6-OHDA treatment showed complete loss of green fluorescent protein expression in dopamine neurons (Nass et al., 2002). Researchers then tested whether 6-OHDA-induced toxicity could be prevented, in which case they co-incubated worms with 6-OHDA and with a 6-OHDA or DAT-1 inhibitor (a DAT antagonist), which resulted in a highly decreased loss of GFP

expression in the dopaminergic neurons, compared to worms that were incubated with 6-OHDA alone (Nass et al., 2002).

C. elegans Genetic models of Parkinson's: JVR168 and JVR203

Genetic models of Parkinson's are important to understanding the disease progression and familial history of Parkinson's. The LRRK2 models remain applicable to both the behavioral and genetic analysis of Parkinson's. As a reminder, among several pathogenesis studies of Parkinson's, there are some that suggest that mutations in the LRRK2 gene are linked to abnormal LRRK2 kinase activity, which later results in the degeneration of dopamine neurons. Among the thirty LRRK2 sequence variations, the G2019S mutation found within the kinase domain is found to be the most significant to Parkinson's (Yao et al., 2010). JVR168 worms express human LRRK2 with the G2019S kinase domain, which has been linked to increased kinase activity. The G2019S mutation as well as a GFP marker is specifically expressed in the worm's 8 dopamine neurons (Cooper et al., 2015). These worms display an age-dependent degeneration of dopamine neurons as monitored by the loss of expression of GFP in these neurons. JVR168 contains the G2019S mutation driven by the dopamine neuron specific promoter from the dat-1 gene. JVR168 also expresses GFP from the dat-1 promoter. An example of a worm expressing GFP in its dopamine neurons is shown in Figure 3. JVR168 worms have been used to study the effects of LRRK2 inhibitors in *C. elegans* (Yao et al., 2013).

SNCA was identified as the first linked gene to PD, which encodes for the α-synuclein protein (Lakso et al., 2004). A53T is a point mutation (substitution of alanine to threonine) in the α-synuclein gene. In humans, this mutation leads to an increase in α-

synuclein protein and formation of Lewy bodies. Substantial dopaminergic loss and reduction in dendrites was observed when *C. elegans* models overexpressing human α-synuclein were used (Lakso et al., 2004). JVR203 worms express the A53T mutant of SNCA and the GFP marker specifically in the worm's 8 dopamine neurons and display age-dependent degradation of dopamine neurons (Cooper et al., 2015).

Possible Behavioral Assays to Assess Damage to Dopamine Signaling in *C. elegans*

Based on the roles of dopamine signaling in *C. elegans*, several behavioral assays have been proposed to monitor the defects in the signaling pathways.

Swim-to-Crawl Assay

Swim-to-crawl behavioral assays are the foundation of assessing Parkinson's-like motor deficits and motor symptoms. Swim-to-crawl assays were conducted on young adult worms at the L4 stage: N2 wildtype, VM6365 worms (exhibit a loss of green-fluorescent protein staining in dopamine neurons after late L1 stage), and cat-2 worms (Table 1). The worms were collected in M9 buffer and then placed on a plate with bacteria, with a puddle. The worms were allowed to crawl on agar to get to the puddle and then swim into the puddle. Worm movements, including rate of body bends, were observed and recorded over 2-minute intervals (Vidal-Gadea et al., 2011). In this assay when the puddle dries up, the wildtypes resume crawling, while the VM6365 worms become paralyzed and only begin crawling after being prodded with a platinum wire. Similar results were obtained with cat-2 worms (Vidal-Gadea et al., 2011). Studying

mutants that display deficits in the dopamine signaling pathway will enable us to obtain similar results in swim-to-crawl behavioral assays.

Swimming-induced Paralysis (SWIP) assay

C. elegans exhibit a swimming-induced paralysis (SWIP) phenotype when they are exposed to a norepinephrine transporter inhibitor (NET inhibitor), a compound that blocks the action of the norepinephrine transporter. In addition to the dopamine transporter, the norepinephrine transporter also helps by increasing uptake of extracellular dopamine and NET compounds are prescribed to Parkinson's patients experiencing pain and psychological issues (Nishijima and Tomiyama 2016). NET inhibitors can help us understand the roles of the norepinephrine transporter when the activity of the dopamine transporter in Parkinson's patients is decreased. SWIP is initiated when the dat-1 transporter carries dopamine to synaptic sites. This activates the dop-3 receptor on cholinergic muscle cells, which leads to the paralysis of *C. elegans* dat-1 mutants, inducing the SWIP phenotype. The dat-1 transporter and dop-3 receptors are responsible for inducing SWIP. The SWIP mechanism observed in *C. elegans* can be used to mimic the loss of motor functions observed in Parkinson's.

Ethanol Avoidance Assay

The neurodepressive effects of ethanol consumption are prevalent not only in mammals, but also reliably noteworthy in *C. elegans*; worms develop ethanol tolerance with continuous ethanol exposure (Lee, Jee, and McIntire 2009). Scientists discovered that normal dopamine and serotonin levels are necessary in order for *C. elegans* to show ethanol avoidance behaviors. This finding led to the testing of dopamine and serotonin-deficient mutants, including the cat-2 and tph-1 mutants (Lee, Jee, and McIntire 2009).

Several research studies have shown ethanol avoidance assays to be the most reliably reproduced of most behavioral assays modeled for Parkinson's disease.

Basal-Slowing Rate Assay

C. elegans slow their rate when they are placed on a plate with bacteria after being food-deprived for thirty minutes. This locomotory behavior is called basal-slowing response and is dependent on dopamine-sensing pathways in the animal's neural circuit in response to food (Sarwin, et al. 2000). The disruption of dopamine-sensing pathways is the basis of Parkinson's disease. Studying these basal slowing responses in *C. elegans* serves as an important component in correlating the loss of dopamine neurons, and behavioral deficits.

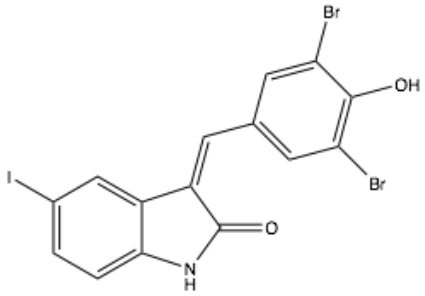
Using the genetic models, Cooper et al., show that JVR203 and JVR168 mutants show an attenuated response of the basal-slowing rate behavior, in the presence of food. Based on the methods by Yao et al., age dependent loss of dopamine neurons can be modeled in *C. elegans* using LRRK2 (JVR168) because at adult day 0 the worms display normal basal-slowing response behavior. However, due to a progressive loss of dopaminergic neurons, the worms lose the basal-slowing response at adult day 2 and adult day 4 (mimicking the condition with no food) (Yao et al., 2010). This provides a correlation in dopaminergic neuronal loss and loss of basal-slowing response behavior in the presence of food. Based on the results from literature studies, this assay is a remarkable and robust assessment for the LRRK2 mutants (Cooper et al., 2018).

LRRK2 Inhibitors Reverse Some Behavioral Deficits in LRRK2 Transgenic worms

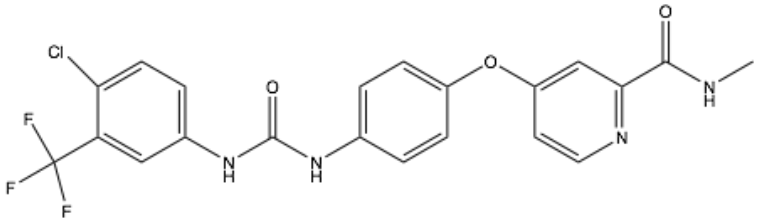
Research studies show evidence for the reversal of Parkinson's-associated behavioral deficits in *C. elegans* mutants expressing LRRK2 using LRRK2 inhibitors (Yao et al., 2013). These inhibitors were known to attenuate neurodegeneration and rescue neurotoxicity in *C. elegans* expressing human R1441C and G2019S mutations (Yao et al., 2013). In addition to neurodegeneration and cell toxicity, the LRRK2 inhibitors TTT-3002 and LRRK2-IN1 also rescued behavioral deficits caused by dopaminergic loss (Yao et al., 2013). These inhibitors were evaluated relative to the onset and progression of Parkinson's-behavioral symptoms in *C. elegans* expressing R1441C and G2019S mutants (Yao et al., 2013). They were administered before or after symptoms appeared to determine if the compounds could protect against behavioral deficits, and also worked to reverse dopamine degeneration. Behavioral responses were not altered by inhibitors during L1 to L4 stages (confirmed with basal-slowing rate assay) because they were defective in basal slowing response (Yao et al., 2013). They assessed the reversal of behavioral deficits via the progressive loss of the basal-slowing response which begins on adult day 2, with a complete loss of basal-slowing response on adult day 4. Based on the results from the basal-slowing response behavioral assays, treatment with both inhibitors during adult day 2 through adult day 4 showed a rescue of the basal-slowing response in *C. elegans* mutants, displaying the effectiveness of these inhibitors in preventing loss of dopamine behaviors caused by R1441C and G2019S mutations (Yao et al., 2013).

After evaluating behavioral rescue in *C. elegans*, they also monitored the survival of dopamine neurons on adult day 9. There were significant improvements in neuronal

survival in R1441C and G2019S worms treated with both inhibitors at lower concentrations (this was true with L4 to adult day 2 transition and adult day 2 to adult day 3 transition) (Yao et al., 2013). Whereas adult day 5 to adult day 7 worms had significant neuronal protection, but at higher concentrations of inhibitors (Yao et al., 2013).



GW5074 (Liu, et al.)



Sorafenib (Liu, et al.)

Figure 4: LRRK2 Kinase Inhibitor Compounds (chemical structure of GW5074 shown above and chemical structure of Sorafenib shown on the bottom)

Eight inhibitors were revealed to inhibit LRRK2 kinase activity, but GW5074 and Sorafenib were seen to strongly inhibit LRRK2. The loss of GFP expression was used as an indicator of dopamine neuron degeneration in these experiments. In 7-day old LRRK2 G2019S mutants, different concentrations of GW5074 and 25uM of Sorafenib significantly rescued over 90% of dopamine neurons (Liu et al., 2011).

The objective of the research described in this work is to identify a reliable and reproducible behavioral assay to assess deficits in dopamine signaling that result from damage to dopamine neurons in *C. elegans*.

Materials and Methods

C. elegans strains were obtained from the *Caenorhabditis* Genetics Center (University of Minnesota), Dr. Vans Raamsdonk (McGill University, Canada) and Dr. Maricq (University of Utah) (Table 1). Several mutant strains (Table 1) were kept and analyzed during the duration of this research project, including the *C. elegans* wildtype worms (N2), which have a properly functioning dopamine system.

The upkeep of these individual *C. elegans* strains included growing them on Nematode Growth Media (NGM) agar plates, seeded with *E. coli* OP50 as a primary food source. *C. elegans* were kept at room temperature at (20°C - 25°C range). For behavioral assays, batch bleaching was performed to obtain eggs and synchronize the worm population, with the treatment of Alkaline Hypochlorite Solution in order to synchronize worms at L1 stage, which has to be generated weekly. After a day of rocking at room temperature, L1 worms were plated on NGM seeded plates so they can develop into larval stage L4 worms, as they transition into the adult stage.

For imaging purposes, worms are mounted onto a 2% agar pad on a glass slide, and immobilized using levamisole, then enclosed using a coverslip. The imaging of immobilized worms was carried out with the help of a Zeiss Axiovert 200M fluorescence microscope with a Hal100 camera.

MOAT Assay

To assess the swim-to-crawl behaviors displayed by *C. elegans*, the MOAT (Movement-Over-Alternate-Terrain) assay was invented as modification of the swim to crawl assay. The objective was for worms to crawl, swim through a moat, and then crawl to the other side of the plate towards the chemoattractant diacetyl.

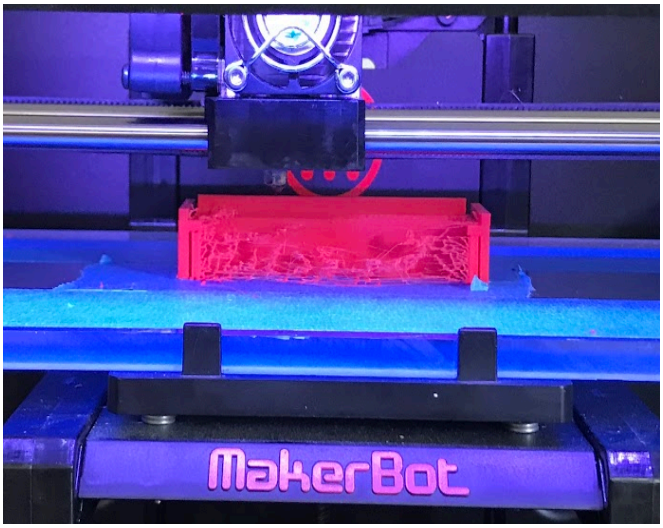


Figure 5: 3D printing of comb (combs were printed using the MakerBot platform seen here)

3D printing was used to create combs to make a ditch in nematode growth media plates to test swim to crawl behaviors on different *C. elegans* strains. With the help of AutoDesk Fusion 360 and MakerBot platforms, a design of the comb was created and then printed using the MakerBot platform (Figure 5). Wildtypes, dopamine deficient cat-2 mutants and age-dependent dopamine mutants were all used with the help of diacetyl and other attractants to perform the MOAT assays (Figure 6). The number of worms used to conduct the assay varied from each assay, but they all were at the L4 stage. The assay

was conducted for at least an hour. We hypothesized that N2 wildtype worms would easily cross over to the diacetyl side, while cat-2 worms would have difficulty crossing over.

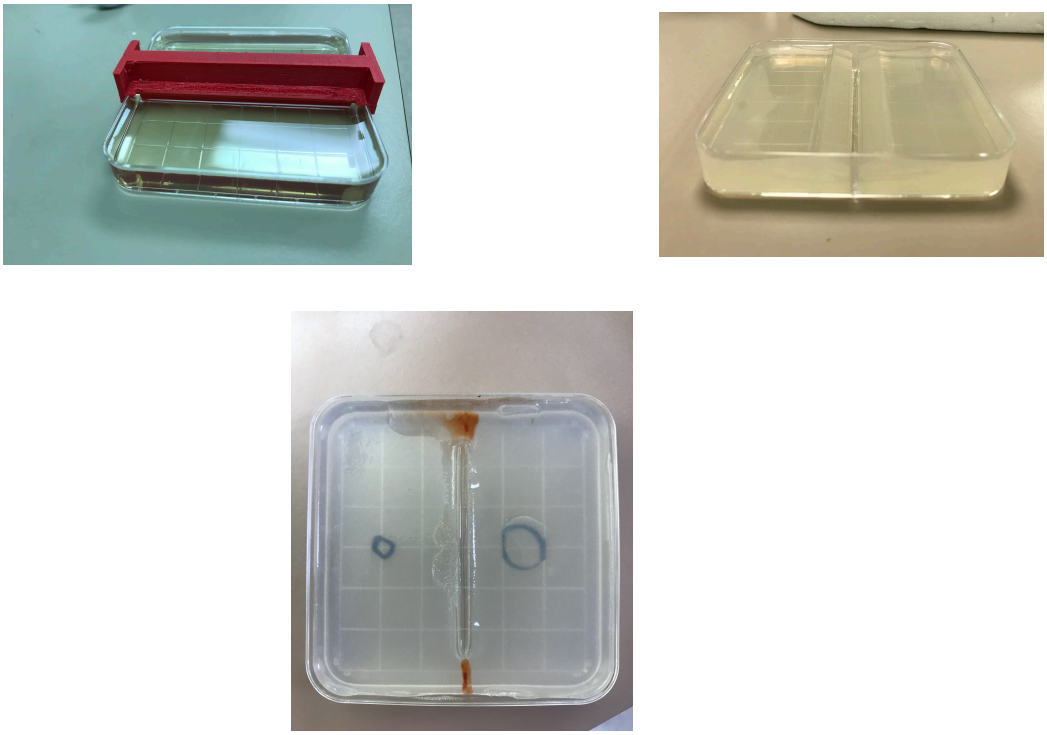


Figure 6: Preparation of Plates for the MOAT Assay. (Hot agarose gel was poured in plate with comb (on top left), image of nematode growth media plate after comb was taken out (top right) and an assay preparation plate with worms plated on the right and attractant on the left and the ditch in the middle of the plate (bottom center)).

SWIP Assay

The SWIP assay was also performed to induce paralysis in N2 wildtypes and

dat-1 *C. elegans* mutants using nisoxetine hydrochloride (NIS), a norepinephrine transporter (NET) inhibitor. For the SWIP assay, 5-10 worms at the L4 stage were placed in Pyrex spot plates in water (no NIS) or NIS solution and observed for paralysis. The worms were observed and recorded for 10 minutes. As shown in Table 2, the scale below was used to rank and characterize the SWIP phenotype on individual worms.

Table 2: Scale Used for Analysis of SWIP Phenotype in *C. elegans*

Scale	Characterization
0	**No visible movement for at least 1 minute**
1	**Light and infrequent movement over 10 min.**
2	**Light and frequent movement over 10 min.**
3	**Infrequent thrashing behavior over 10 min.**
4	**Frequent thrashing behavior over 10 min.**

Ethanol Avoidance

To perform the Ethanol Avoidance Assay, Nematode Growth Media (NGM) agar plates were prepared. Batch bleaching was performed to obtain eggs, and after a day of rocking at room temperature, L1 worms were plated on NGM plates with *E. coli* OP50 so they can develop into larval stage L4 worms. As displayed in Figure 7, clean NGM Plates were divided into four quadrants, two control quadrants and two ethanol quadrants.

Figure 7: Assay plate for ethanol avoidance assay (NGM plates were divided into 4 quadrants and ethanol was plated on ethanol quadrants)

50uL of 100% ethanol was added to each ethanol quadrant, spreading it carefully to ensure it does not seep into the control quadrants. Plates were then sealed with parafilm and were allowed 15-20 minutes to dry. This was repeated one more time, plates were sealed with parafilm and plates sat at room temperature to dry for at least an hour. After sitting at room temperature for more than an hour, 100uL of M9 buffer was added to a worm plate with L4 worms and then collected in a microcentrifuge tube. Being careful not to touch the bottom, about 70uL of M9 buffer was removed. About 10uL of worms remaining at the bottom of the tube were plated on the center of the ethanol-seeded plates. After the liquid in the center of the plates dried up, the worms were given 20-30 minutes to move from the center of the plate into quadrants. Plates were then placed at 4 degrees Celsius for five minutes to immobilize worms, so the worms could stay in place until they were counted. Ethanol avoidance is calculated as ((number of worms in control quadrants) − (number of worms in ethanol quadrants))/total number of

worms. The protocol was followed from the authors of a recent paper (Cooper et al., 2015).

Ethanol Avoidance was calculated as follows:

$$\left\{ \frac{\text{\# of worms on clean quadrants} - \text{\# of worms on ethanol quadrants}}{\text{total number of worms on plate}} \right\} * 100$$

Sample Calculation: [(91- 21)/112] *100 = 62.50%

Results

MOAT Assay

Swim-to-crawl behavioral assays are the foundation of assessing Parkinson's-like motor deficits and motor symptoms. This assay was modified to the movement-over-alternate-terrain assay (MOAT), to analyze worm swim-to-crawl behaviors. Our initial goal was to have more than 20% of N2 wildtype worms get to the attractant side, crossing the ditch, in order to make sure the conditions we designated were appropriate. We also wanted to standardize the moat assay, in terms of time and other parameters, in order to publish the methods we used, so that they could be replicable in future behavioral assays with *C. elegans*.

The first comb we made, in Figure 8, helped us assess whether the comb held firmly in the assay plate, which it did.

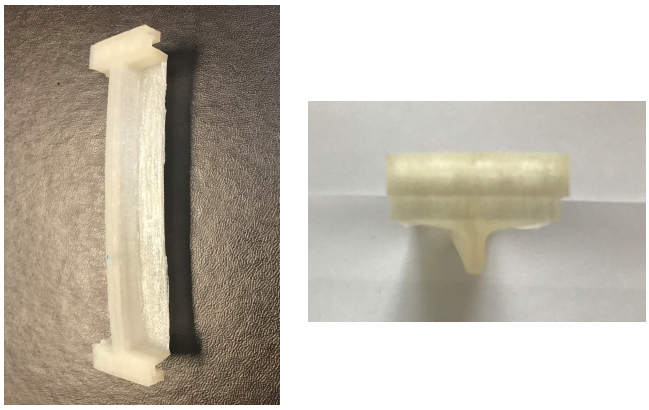


Figure 8: 1st 3D constructed comb for MOAT assay (a. This is an image of the comb; b. This is the cross-section showing the slope and depth of the comb)

However, when we ran the assay we observed that the worms were still having difficulty swimming across the ditch. We changed the parameters of the comb so that the worms could easily swim across, and then re-printed a new version (Figure 9).

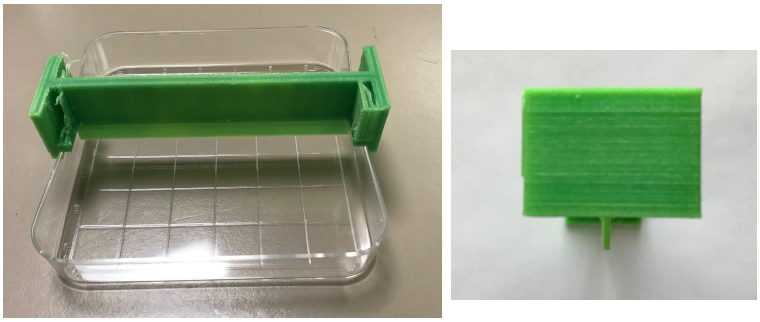


Figure 9: 2nd 3D constructed comb for MOAT assay (a. This is an image of the comb; b. This is the cross-section showing the slope and depth of the comb)

The worms were still not crossing the ditch so we decided to modify the measurements of the comb once again, and re-printed the comb seen in Figure 10.

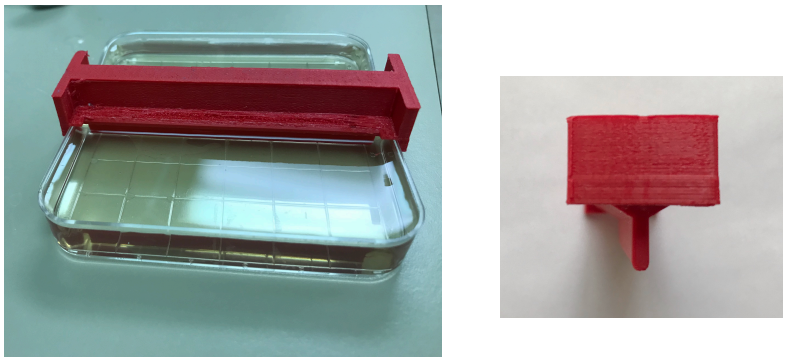


Figure 10: 3rd 3D constructed comb for MOAT assay (a. This is an image of the comb; b. This is the cross-section showing the slope and depth of the comb)

Our last attempt involved making the ditch as shallow as possible, so we tried making a longer walkway for the worms with less space in the middle so the swimming portion would be reduced (Figure 11).

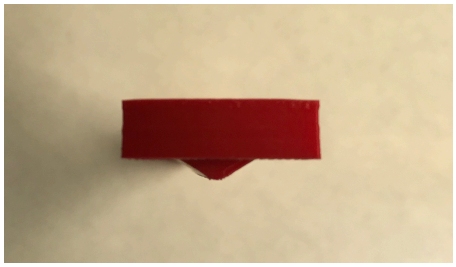


Figure 11: Cross-Section of 4th 3D constructed comb for MOAT assay (this shows the cross-section of the last comb we printed)

Despite the modifications that were made to the comb (in Figures 9, 10, and 11), we observed that *C. elegans* apparently do not spontaneously transition from swimming to crawling despite the presence of the volatile diacetyl as an attractant, the worms continue to swim for an extended period of time. After several modifications to the comb,

we found that 0 N2 worms made it to the diacetyl or attractant side, and we did not run any assays with cat-2 worms.

SWIP Assay

Data was collected for N2 wildtypes and JVR203 worms at different developmental stages and then organized in the graph below. The average percent paralysis of the strains was used as the measure for analyzing the SWIP phenotype in worms.

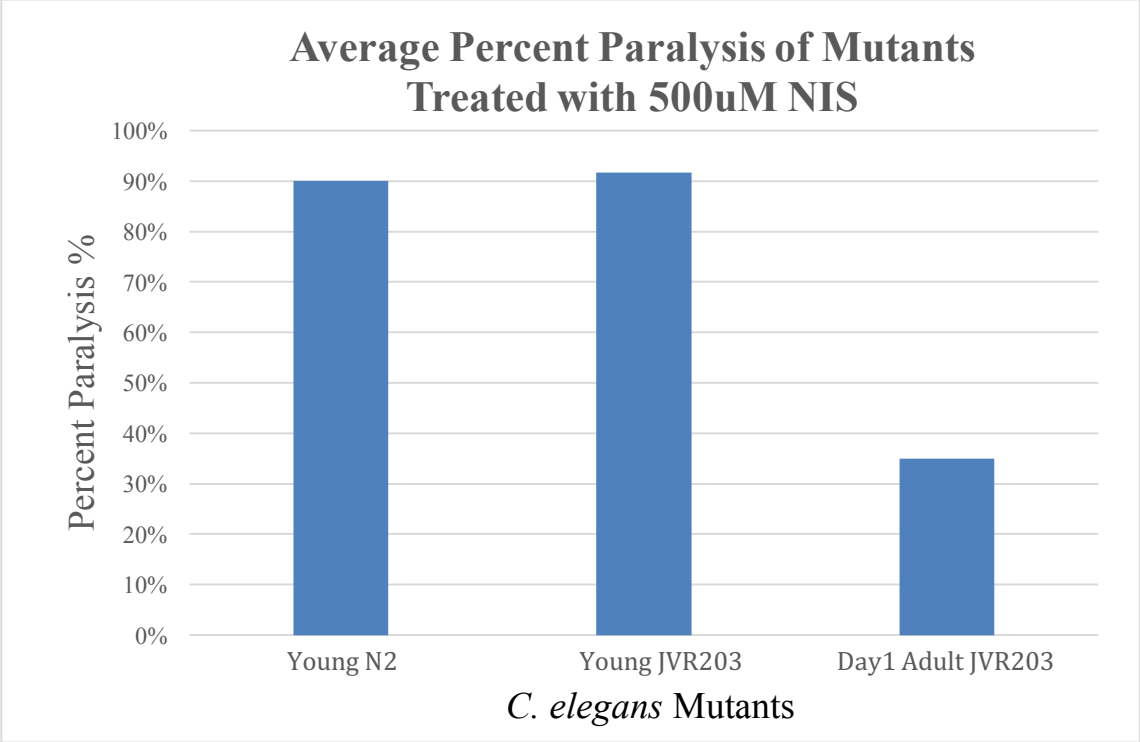


Figure 12: Average Percent Paralysis of Worms under 500uM NIS conditions (N2 wild-type worms and JVR203 worms were at the L4 stage. JVR203 worms at the day 1 adult stage were also used to observe and test paralysis.)

According to Figure 12, L4 N2 worms displayed a strong SWIP phenotype when treated under with 500uM NIS, paralyzing above 80% of worms. Under these conditions, L4 JVR203 worms also had a high paralysis of above 90% (Figure 12). However, older JVR203 worms showed an estimated 35% percent paralysis when treated with 500uM NIS. Along with analyzing the SWIP phenotype using the SWIP scale, we also wanted to analyze the movement of N2 and cat-2 worms with the help of Matlab software (Figures 13 and 14). The Matlab image acquisition tool helped us to analyze the mean variance per frames at time intervals.

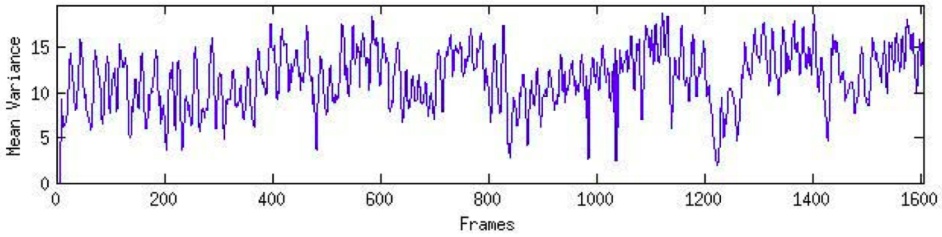


Figure 13: Movement of cat-2 worms under 500uM NIS conditions

AVI films of the cat-2 worms at the L4 stage were recorded after they were treated with 500uM NIS. According to Figure 13, the cat-2 worms displayed continuous movement during the recording when exposed to 500Um of NIS.

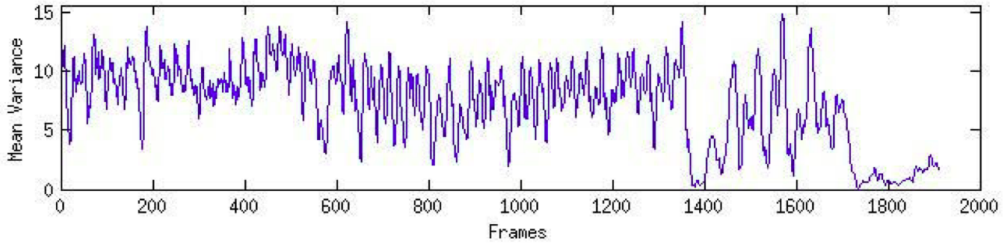


Figure 14: Movement of N2 wildtype worms under 500uM NIS conditions

AVI films of the N2 worms at the L4 stage were recorded after they were treated with 500uM NIS. According to Figure 14, the N2 worms began to slow down towards the end of the 10-minute period and as you can see, the mean variance begins to bottom out at the end of the recording.

Ethanol Avoidance Assay

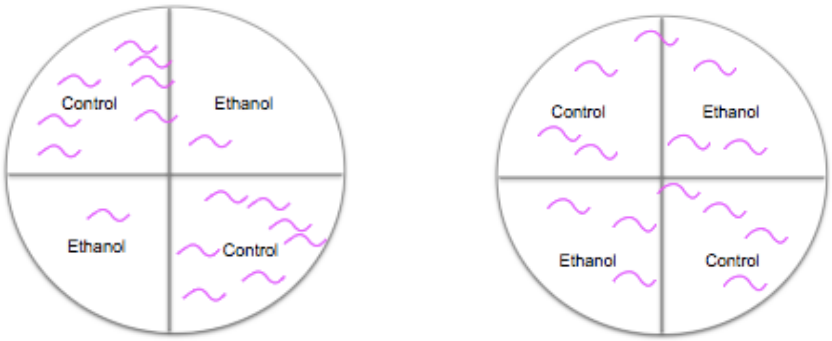


Figure 15: Ethanol Avoidance of N2 wildtypes (on left) vs. mutants (on right)

According to Figure 15, my goal for these experiments was to show that N2 wildtypes would prefer to avoid ethanol quadrants while the LRRK2 JVR168 mutants will show no preference to ethanol or no ethanol quadrants. In Figure 15, the image on the left shows the expected ethanol avoidance results for N2 wildtype worms, while the image on the right shows the expected lack of ethanol avoidance results for JVR168 LRRK2 *C. elegans.*

Table 3: Ethanol Avoidance Results for N2 Worms at the L4 stage Across Trials

	Worms in Clean Quadrants	Worms in Ethanol Quadrants	Total Worms on Plate	% of Ethanol Avoidance
Trial 1	166	127	293	13.31
Trial 2	315	65	380	65.79
Trial 3	91	21	112	62.50
Trial 4	159	19	178	78.70
Trial 5	87	131	218	-20.18
Trial 6	87	22	109	59.60
Trial 7	65	41	106	22.60
Trial 8	133	82	215	23.70
Trial 9	138	98	236	16.90
Trial 10	195	83	278	40.00
Trial 11	67	20	87	54.00
Trial 12	153	38	191	60.00

According to Table 3, we obtained expected ethanol avoidance results for the majority of the trials done using N2 worms at the L4 stage. An outlier that displayed negative ethanol avoidance is seen in Trial 5 and included in Table 3 and Figure 16. As seen in Table 3 and Figure 16, Trial 1, 7, 8, and 9 had very low ethanol avoidance percentages, which we believe could be associated with the technical difficulties in setting up and conducting the assay.

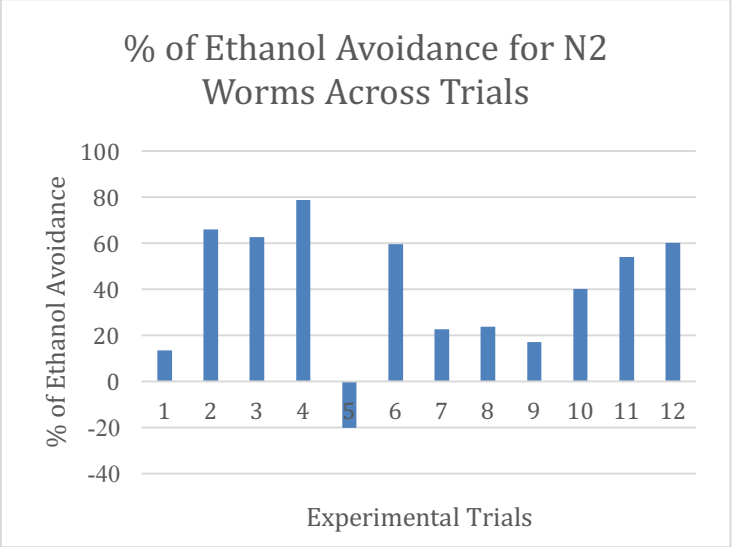


Figure 16: Percent of Ethanol Avoidance of N2 Worms Across Trials

Table 4: Ethanol Avoidance Results of JVR168 Worms Across Trials

	Worms in Clean Quadrants	Worms in Ethanol Quadrants	Total Worms on Plate	% of Ethanol Avoidance
Trial 1	110	16	126	74.60
Trial 2	114	1	115	99.10
Trial 3	67	25	92	45.70
Trial 4	59	37	96	22.92
Trial 5	69	58	127	8.70
Trial 6	58	34	92	26.09
Trial 7	60	20	80	50.00

According to Table 4, we obtained unexpected results for JVR168 worms at the L4 stage, because we expected these mutants to display a lack of ethanol avoidance, where they will not prefer ethanol over clean or clean over ethanol quadrants. However, for the JVR168 worms we obtained four high values (74.60%, 99.10%, 45.70%, and 50.00%) for ethanol avoidance, two medium values (22.92% and 26.09%), and one low ethanol avoidance value (8.70%). On average, N2 worms preferred to move to the control quadrants and displayed a strong avoidance to ethanol. LRRK2 mutants also displayed ethanol avoidance and the majority counted were on the control quadrants.

Table 5: Ethanol Avoidance Results of N2 and JVR168 Worms Across Trials

Trial 1	Worms in Clean Quadrants	Worms in Ethanol Quadrants	Total Worms on Plate	% of Ethanol Avoidance
N2	91	21	112	62.50
JVR	110	16	126	74.60
Trial 2				
N2	159	19	178	78.70
JVR	114	1	115	99.10
Trial 3				
N2	138	98	236	16.90
JVR	69	58	127	8.70
Trial 4				
N2	153	38	191	60.00
JVR	60	20	80	50.00

On some days, both N2 and JVR168 worms were run simultaneously, and we combined the findings from these experiments in Table 5. A scale of 0 to 1 or 0% to 100% was used to rank ethanol avoidance in worms, while 0 to -1 or 0% to -100% was associated with negative ethanol avoidance. Overall, we could detect no difference between the N2 worms and JVR168 worms at the L4 stage.

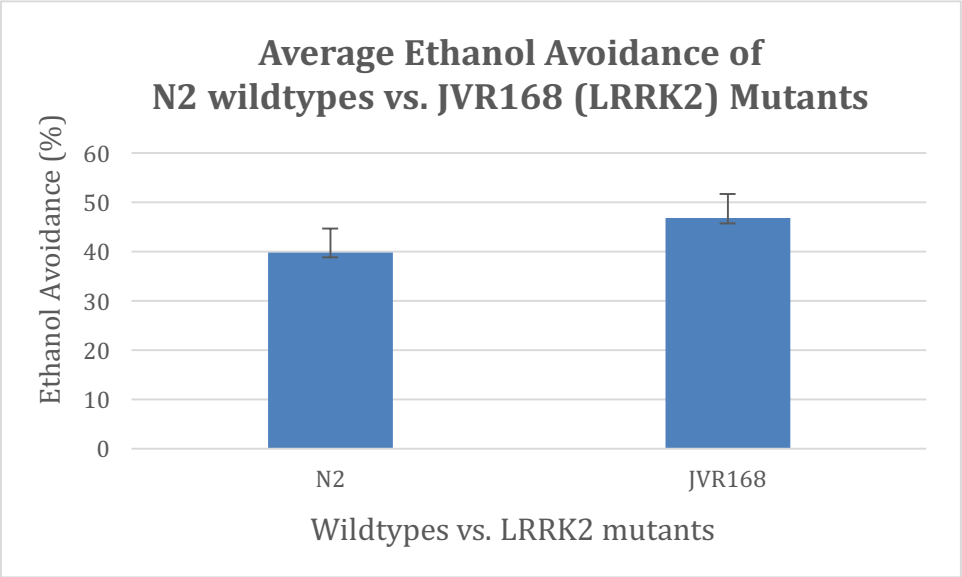


Figure 17: Statistical Analysis of Ethanol Avoidance Assay

Based on the ethanol avoidance results and the similar number of trials run across both N2 and JVR168, there is some overlap between the error bars, which indicates that there is not a significant difference between the ethanol avoidance of N2 vs. JVR168 worms. The average calculated ethanol avoidance is: N2 worms (~40.0%) and the LRRK2 JVR168 worms (~45.0%), which indicates strong ethanol avoidance. The results for the behavioral assays carried out in my research have been compiled into the table below (Table 6).

Table 6: Overall Mutants Used Results for Behavioral Assays

Behavioral Assay	Description/Results
Ethanol Avoidance	N2 wildtypes (control) and LRRK2 with (G2019S) mutation worms tested. Results: Both N2 and LRRK2 worms display similar ethanol avoidance.
Swimming-Induced Paralysis (SWIP)	N2 wildtypes (control) and LRRK2 (G2019S) worms at L4 stage and adult day 1 worms. Results: In wildtype worms, the SWIP phenotype decreases with age; this assay is unsuitable for age-dependent degradation of dopamine neurons.
Movement-over-alternate-terrain (MOAT)	Prototypes for comb were modified and 4 versions were 3D printed. Results: Worms do not spontaneously transition from swimming to crawling; therefore this is not a suitable assay to model Parkinson's disease.

Discussion

MOAT Assay

In swim-to-crawl assays when the puddle dried up, the worms were able to spontaneously transition from swimming to crawling (Vidal-Gadea and Pierce-Shimomura 2012). For the MOAT assay, we discovered that the worms do not spontaneously transition from swimming to crawling after observing that most of the worms could not get to the other side of the ditch but stayed swimming in the ditch. Even with the help of attractants like diacetyl, we observed that the worms will continue to swim in the ditch filled with buffer and will not spontaneously transition to crawling. It is unlikely that diacetyl would leach into the moat because standard chemotaxis assay conditions show that the attractant stays concentrated in the circle where it is added, which is marked on the plate.

SWIP Assay

Understanding the effects of NIS inhibitor on different *C. elegans* mutants is an important aspect of the SWIP assay because a long-term goal was to screen compounds to find an optimal antagonist like mazindol to reverse the effects of SWIP that could be applied to Parkinson's research.

We hypothesized that the JVR203 worms show an age-dependent loss of dopamine neurons, so a greater loss of dopamine neurons would result in lower levels of observed paralysis and a reduced SWIP phenotype. Whereas, the N2 wildtype worms will display a strong SWIP phenotype because they exhibit normal dopamine signaling. The results we obtained indicated that young N2 wildtypes and young JVR203 worms at the L4 stage strongly exhibited the SWIP phenotype with treatment of 500uM NIS. However, day 1 adult JVR203 worms showed low levels of paralysis and display a loss of the SWIP phenotype. A review paper on SWIP assay discussed that while SWIP is prominent in L4 animals, it becomes less observable with age in the N2 wildtype animals (Cooper et al., 2018), so we decided that it was not the best assay to assess the age-dependent loss of dopamine neurons in LRRK2 mutants.

Ethanol Avoidance Assay

Overall, N2 wildtype worms (L4 stage) and JVR168 LRRK2 worms (L4 stage) both exhibited strong ethanol avoidance and there was no statistically significant difference detected, as seen in Figure 17. These results did not support our hypothesis that the LRRK2 mutants should show an age-dependent loss of dopamine neurons, because dopamine facilitates sensory behaviors and ethanol sensing behaviors. However,

we did not test older LRRK2 JVR168 worms, which we believe was important to our hypothesis to obtain the expected lack of ethanol avoidance and age-dependent loss of dopamine neurons. We ran a couple trials using Day 1 adults but we still did not observe a lower ethanol avoidance. As the dopaminergic degradation is greater with age, we expect to observe the lack of ethanol avoidance in adult day 2 or adult day 3 LRRK2 mutants, compared to the day 1 adult LRRK2 worms. In the future, we would like to separately experiment adult day 2, adult day 4, and adult day 6 LRRK2 JVR168 mutants, because we believe this will lead us to observing the loss of ethanol avoidance in worms.

Our data is inconsistent with previously reported data (Cooper et al., 2015) which reported that LRRK2 and α-synuclein mutants display a lack of ethanol avoidance. Figure 18 shows the results taken from Figure 1 from that manuscript. Day 1 adult worms were used in this experiment.

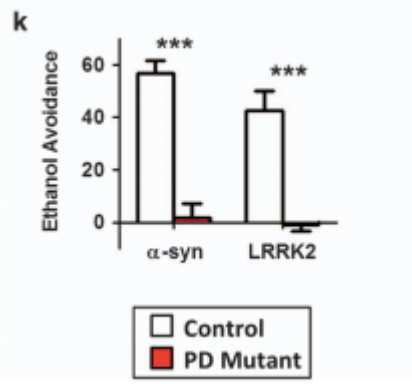


Figure 18: Ethanol avoidance based on the ethanol avoidance assay of control vs. JVR168 (LRRK2) and JVR203 (α-synuclein) worms.

We believe these inconsistent findings and outliers may be in part due to the technical difficulties associated with setting up the assay plates and running the assay. One factor may be the large numbers of worms we ran in the assay, and controlling the

sample of worms for each trial was very difficult. We modified the protocol we used from the literature to give some time to the worms to settle to the bottom of the tube. We would then remove the supernatant and resuspend the worms in M9 buffer and repeat this process a couple times as we believed this was causing the worms to clump together when they were placed in the center of the assay plate. This assay also took more time than estimated to run and sometimes we would wait over an hour for the worms to move to the quadrants from the center.

In addition to LRRK2 mutants, we also wanted to analyze the ethanol avoidance behaviors of α-synuclein JVR203 mutants, cat-2, and other strains. With more time, it would have been interesting to compare the ethanol avoidance behaviors of these other strains, especially since the cat-2 worms have an inactive biosynthetic dopamine system. Studies correlating the loss of dopamine neurons with GFP indicated that the LRRK2 worms lose GFP staining as they age so we need to test older age LRRK2 mutants.

Basal-Slowing Rate Assay

We aim to perform this assay in future work based on the results published by Yao et al (Yao et al., 2012). The researchers used compounds to attenuate neurodegeneration and reverse the behavioral deficits caused by the G2019S and R1441C LRRK2 mutations. Our future goal is to treat these mutants with effective LRRK2 inhibitor compounds to reverse behavioral deficits and neurodegeneration observed by GFP staining. In addition, we are looking forward to using the dat-1 mutants, alongside the cat-2's as additional controls for the experiment.

Lessons Learned:

We realized that we need to have a negative control when we conduct these experiments in the future, because in the ethanol avoidance assay, our positive control N2's did display the expected avoidance, but the JVR168 mutants did as well and we did not have a negative control to validate the assay. We will continue to use N2's as our positive control and in the future, we will designate cat-2 mutants, dop-3, or VM6365 worms as our negative controls. As discussed previously, these three mutants have deficits in the dopamine signaling pathway, so they will display a lack of ethanol avoidance at any developmental stage, so they can serve as a good negative control when looking at adult LRRK2 worms. When conducting behavioral assays, there may have been several external factors which made it difficult for us to obtain the expected results, including the room temperature at which the assays were conducted, and this is why having positive and negative controls, along with our experimental variables is very important so that we can obtain results that are both reliable and valid.

Reliable Behavioral Assays

Based on the behavioral assays that were performed, we think that the ethanol avoidance and basal-slowing rate behavioral assays are the most ideal in terms of reliability. If some experimental parameters are modified, the ethanol avoidance assay may help lead us to observing the lack of ethanol avoidance in adult LRRK2 worms, as seen in literature. Currently, there is no literature support for the use of LRRK2 kinase inhibitors to rescue the ethanol avoidance lost in LRRK2 mutants, but this is something

that we may try in the lab when we receive the library of LRRK2 kinase inhibitor compounds.

In addition to the ethanol avoidance assay, the basal-slowing rate assay has showed consistency in restoring the lost basal-slowing response behavior in *C. elegans* worms with G2019S and R1441C mutations in LRRK2. Since the basal-slowing response behavior was restored with the treatment of LRRK2 kinase inhibitor compounds by Yao and other researchers, this assay is very reliable. Once we are able to perform this assay, and we receive the LRRK2 compounds for testing, we can screen several compounds, and establish this assay for high-throughput screening of LRRK2 kinase inhibitor compounds for the study of Parkinson's disease.

Conclusion

Performing behavioral assays can enable us to screen LRRK2 inhibitor compounds to correlate LRRK2 toxicity and the loss of GFP observed in dopamine neurons to prevent or treat the loss of dopamine neurons in Parkinson's disease. The results obtained from conducting these behavioral assays shows promise towards ethanol avoidance and basal-slowing response assays for future research. Mutations in LRRK2 cause increased LRRK2 kinase activity and the degeneration of dopamine neurons (Liu, et. al). The most potent LRRK2 inhibitors identified in literature for the treatment of Parkinson's disease are GW5074 and Sorafenib, and both have been shown to restore the levels of dopamine neurons. Proposed future goals would be to screen a LRRK2 inhibitor compound library and to connect this with the behavioral work done by researchers in the past few decades to display the overall progress in PD research. We want to be able to

correlate the loss of dopamine neurons with behavioral assays and once we have observed this, to test the most potent LRRK2 kinase inhibitor compounds.

Future Directions

A thorough and sensitive assay can be useful for screening for LRRK2 kinase inhibitors, and the resulting hits can generate a tool kit of compounds that can be applied in animal models for Parkinson's research. Whether in behavioral assays or in cellular and molecular research, the treatment of the most up-to-date inhibitors can enable us to assess toxicity and efficacy. As researchers, we not only want to see if the compound does more harm, but we also want to see if the compound can increase the survival of dopamine neurons and overall provide neuroprotection, both in LRRK2 models and following 6-OHDA treatment.

Acknowledgements

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