Gender-based Differences in Parkinson's Disease and Amyotrophic Lateral Sclerosis Expression in *C. elegans*

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Abstract

Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) are common neurodegenerative disorders exhibiting male gender biases. The gender-related differences at both symptomatic and therapeutic levels are not understood. We developed *C. elegans* models for studying both disorders, and examined gender differences in symptom presentation using behavioral assays measuring locomotion and chemosensory abilities. Symptoms exhibiting gender bias had limited presentation in our models. Additionally, we investigated the efficacy of two potential treatments, caffeine and vanillin. We found that while treatment with caffeine had mixed results based on disease type, vanillin reduced neuronal deficits in both PD and ALS models.

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Gender-based Differences in Parkinson's Disease and Amyotrophic Lateral Sclerosis Expression in *C. elegans*

Amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD) are two of the most common neurodegenerative diseases (Martin, 2010). The primary symptoms of both are movement difficulties and muscle degeneration, and both diseases are more prevalent in men than women (Harrington et al., 2010; Kiernan et al., 2011). Neither the cause of the gender-related differences nor their full effects on the etiology, pathology, and prognosis of either disease is fully understood. Due to the complexity of these diseases and the human nervous system, researchers have used mice models to gain insight into the mechanisms of the diseases (Gillies, et al., 2014; Suzuki et al., 2007; Hegedus et al., 2009). While mice models have been able to recreate many of the symptoms seen in humans, including gender differences, they are still too complex to fully elucidate the mechanisms behind these effects, partially due to the incomplete understanding of how different areas of the mouse brain function together and separately. In recent years, an even simpler model organism, *C. elegans*, have become a promising tool for basic research into neurodegenerative diseases, particularly ALS and PD (Calahorro & Ruiz-Rubio, 2011). *C. elegans* may be able to provide insights into nuances of the disease that can then be applied to more complex models. Gender differences have yet to be explored within this model system for either ALS or PD. The present study examines the gender differences in symptom presentation and response to two different treatments in *C. elegans* models of ALS and PD in order to determine the potential of the model for future research into the cause and effects of gender differences in these diseases.

C. elegans

Caenorhabditis elegans, a common nematode, was first proposed as a model organism for studying neurodegenerative disorders in the 1960s (Brenner, 1973). This organism shares many characteristics with humans, including neuronal structure, but is simple enough at a cellular and molecular level to be well understood (Hart & Chao, 2010). The nervous system of *C. elegans* contains 302 neurons, a minute number when compared to the 100 billion neurons in the human nervous system or even the 4 million neurons in the murine nervous system (Watts & Strogatz, 1998; Herculano-Houzel, 2010). Because of its simplicity, *C. elegans* are the only organism to date with a completely mapped connectome, meaning the connections of each of its neurons are completely understood (Watts & Strogatz, 1998). Additionally, *C. elegans* have

orthologues to many genes implicated in neurodegenerative disorders (Calahorro & Ruiz-Rubio, 2011). This means that these diseases can be studied using *C. elegans* models, and the results from these studies are fairly straightforward to interpret due to the level of understanding surrounding the organism's nervous system. Furthermore, these findings are translatable into more complex model systems and even have implications for humans because of the orthologous nature of many of the genes of *C. elegans*.

Parkinson's disease and ALS are particularly good candidates for neurodegenerative disorders to be studied using *C. elegans*. The primary manner of data collection from the nematodes comes from studying changes in their movement through locomotion and chemotaxis assays. Locomotion assays involve the study of the speed and type of movement of *C. elegans*. The amount of turns, patterns of tracks, and amount of forward versus reverse motion can all be gained from such assays and used to establish the basic behavioral effects of a variable. Chemosensory assays allow for the study of attraction to and avoidance of stimuli by *C. elegans*. Changes in attraction and avoidance behavior could indicate lack of control over motor movement, preventing the nematodes from avoiding or moving towards stimuli or changes in sensory neuron ability that actually prevent the nematodes from sensing stimuli. Because PD and ALS are both characterized behaviorally by movement difficulties, studies in *C. elegans* are fairly straightforward to analyze and relate to human symptoms in both diseases.

The *C. elegans* model has not yet been used to study gender differences in neurodegenerative disorders. One explanation for this is the androdiecious nature of the species—its two sexes are male and hermaphrodite, not male and female. However, there is reason to believe that the model system could lend insight into both female and male-specific symptoms. The hermaphrodite of *C. elegans* is a "modified female", meaning that it contains only very slight modifications from a true female ancestor (Portman, 2007). Namely, the hermaphrodite lacks male genital structures, meaning while it can produce and store sperm it cannot fertilize other hermaphrodites. In addition, *C. elegan's* closest relatives have true female-male dichotomies, implying that there may not be a large difference between the hermaphrodite and "true female" (Portman, 2007). Furthermore, there is the presence of gender specific neurons between the male and modified female (hermaphrodite) *C. elegans* (Portman, 2007). In fact, recently a team of researchers at Columbia University used the *C. elegans* model to study

how gender-specific neuronal differences emerge. The research supports the continued use of the model as a simple tool to gain insight into the more complex working of the human brain and the ability to use *C. elegans* to study gender differences (Oren-Suissa, Bayer & Hobert, 2016). Despite not exactly following the female-male dichotomy typical for humans, *C. elegans* still exhibit two distinct genders with measurable morphological, neurological, and behavioral differences, and thus should be a valuable model for studying gender differences in ALS and PD.

Parkinson's Disease

Parkinson's disease is a progressive disorder of the central nervous system primarily characterized by the loss of dopaminergic neurons in the brain's substantia nigra (Jankovic, 2008; Piccini et al., 1999). Although the cause of PD is not well understood, the effects of the neuron loss are well-characterized. The cardinal symptoms of PD are shakiness, impaired voluntary movement, rigidity, and postural instability (Jankovic, 2008). As the disease progresses, these symptoms become more severe, and within seven to 14 years, complications from lack of muscle control often prove to be fatal although the disease itself is not (Sveinbjornsdottir, 2016). Symptoms of PD typically present themselves in populations over 60 years of age, and the disease is about twice as prevalent in men compared to women (Sveinbjornsdottir, 2016; Gillies, Pienaar, Vohra & Qamhawi, 2014).

Gender differences extend beyond etiology and into disease pathology. The average age of onset is two years later for women, and they are more likely to present with a milder phenotype of the disease (Haaxama et al., 2007). Men are more likely to present with rigidity of movement and rapid eye movement (Martinez-Martin et al., 2012). The differences present not only behaviorally, but cognitively, as well. Recent research shows that women develop deficits in visuospatial abilities, while men develop deficits in abilities involving emotional recognition and verbal fluency as the disease progresses. The cause of these differences remains unclear (Gillies et al., 2014).

PD research in mice has shown that mouse models can mimic the etiology and chronology of gender differences observed in humans (Gillies, et al., 2014). However, the complexity of the mouse model system makes it difficult to interpret the results. Furthermore, this research focuses on the administration of oxidative toxins to induce stress and cell death in dopaminergic regions, and therefore ignores the genetic factors that may contribute to PD.

Because research indicates that PD is likely caused by both environmental and genetic factors, animal models, like *C. elegans*, that can explore genetic rather than environmentally-induced cases of PD are valuable. A model organism with a genetic defect that mimics the symptoms of PD, like the *cat-2* strain of *C. elegans*, could provide insight into genetic causes of PD and whether gender differences within the disease may stem from genetic factors.

The *C. elegans* gene *cat-2* encodes tyrosine hydroxylase, an enzyme that catalyzes the conversion of tyrosine into L-DOPA, the precursor of dopamine (DA). For this reason, mutant strains of *C. elegans* without functional *cat-2* have greatly reduced levels of DA. One such strain was created by Ringstad Lab at NYU to include a 1010 bp deletion of the *cat-2* locus in order to mimic the effects of Parkinson's disease on the organism (Omura et al., 2012). The Ringstad Lab found that dopamine is required to restrict the ranges of speed at which the *C. elegans* track, and that dopamine-deficient worms typically make large adjustments to their speed. The study also found that the treatment of the *cat-2* mutants with exogenous dopamine restored the worms' ability to make small adjustments to their speed, and to restrict the ranges of speed at which they travelled (Omura et al., 2012). This means that *cat-2* mutants retain the ability to transduce dopamine signals, which indicates that reduced levels of DA in the worms are responsible for the behavioral defects associated with the *cat-2* mutation. The newly isolated strain described by Omura, MT15620 *cat-2* (n4547) and a second strain containing a point mutation, CB1112 *cat-2* (e1112), have been chosen as the model for PD in *C. elegans* for this project due to its success in *cat-2* knockout and mimicry of the disease as a result of DA disruption.

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a motion disorder affecting upper and lower motor neurons (Kiernan et al., 2011). Symptoms begin as muscle weakness and slight difficulties in completing everyday tasks (Zarei et al., 2015). As the disease progresses and more motor neurons die, these difficulties become more severe with inhibited swallowing, difficulties speaking, and other spastic movements (Zarei et al., 2015). Eventually, lung function is lost, and most people with ALS die from respiratory failure or pneumonia within 30 months of the onset of symptoms (Kiernan et al., 2011). The pathology of the disease varies from person to person, with patterns emerging when gender is examined as a variable.

ALS is slightly more common in men than women, with men being 1.3 times more likely to develop the disorder (McCombe & Henderson, 2010). Despite only a slight difference in the prevalence of disease between the genders, ALS presents with a variety of gender differences in terms of pathology. Men begin to show symptoms earlier than women and tend to present more severe forms of the disease initially (McCombe & Henderson, 2010). Men are also more likely to present with symptoms related to limb movement that correspond with limb-onset ALS, while women are more likely to present with bulbar-onset ALS, meaning symptoms beginning in the mouth and head region (McCombe & Henderson, 2010). The cause of these gender differences and the exact extent of their effects remains unknown.

Three proteins have become associated with ALS: superoxide dismutase-1 (SOD1), transactive response DNA binding protein 43 (TDP-43), and the RNA-binding protein, FUS. These proteins are typically found in large aggregates in the motor neurons of those affected by ALS (Deng et al., 2010). SOD1 is associated mainly with familial (inherited) ALS, which occurs in under ten percent of cases (Kiernan et al., 2011). Studies with mouse models of ALS have centered on the SOD1 mutation and have linked this mutation to earlier onset in males but not to gender differences in muscle neuron degeneration (Suzuki et al., 2007; Hegedus et al., 2009). The majority of ALS research involving gender, however, ignores the TDP-43 and FUS mutations believed to be responsible for a large portion of sporadic cases of the disease (Mackenzie et al., 2007).

TDP-43 is responsible for transcription, splicing and translation. The *C. elegans* orthologue of the gene encoding TDP-43 is called *tdp-1*. Researchers are as of yet uncertain whether TDP-43 loss of function or overexpression or both are the source of TDP-43-related issues in patients with ALS (Scotter, Chen & Shaw, 2015). Typically knockouts of the gene encoding TDP-43 in animal models result in severe symptoms and lethality; therefore, the current research will focus on knockouts of the TDP-43 orthologue, *tdp-1*, within *C. elegans* (Saldi et al., 2014).

Treatments

Both Parkinson's disease and amyotrophic lateral sclerosis lack cures and have minimal options for treatment (Harrington et al., 2010; Kiernan et al., 2011). PD symptoms can be treated with the drug Levodopa, but the medication loses effectiveness as the disease progresses and can

cause further spastic movements (Sveinbjornsdottir, 2016). Treatment options for ALS are even less promising. One drug, called Riluzole, is available, but is only effective for only two to three months and is very expensive (Miller, Mitchell & Moore, 2013). Therefore, the discovery of new treatment options for either of these neurodegenerative diseases is both valuable and necessary.

Recently, investigations into herbal extracts and remedies for neurodegenerative disease have gained increasing credibility (Zhang et al., 2014). One method for taking alternative medicine or "natural remedies" and gathering empirical data to assess validity is to examine the monomers or active ingredients within these natural remedies.

For example, drinking coffee and tea is negatively correlated with the incidence of both PD and ALS, meaning coffee and tea drinkers are less often found within ALS and PD populations (Potenza et al., 2013, Prediger et al., 2013). Research into a possible neuroprotective of caffeine has begun, and it has been discovered that caffeine activates dopaminergic, movement-related neurons by inhibiting adenosine receptors in the brain (Potenza et al., 2013, Prediger et al., 2013). Findings indicate that caffeine has positive effects on mouse models of ALS and Parkinson's, with lower amounts of neural cell death in mice consuming caffeine (Potenza et al., 2013; Prediger et al., 2013). Additionally, research into the interactions between gender and the effect of caffeine has been conducted. In mouse models, caffeine has shown a neurprotective effect in male, but not female mouse models of ALS (Seevaratnam, 2009). The same gender investigations have not been done in mouse models of Parkinson's, and investigations of the effects of caffeine in *C. elegans* models of ALS and PD have yet to be carried out.

Another common food, maple syrup has been shown to protect against ALS, bringing the polyphenols comprising it under investigation (Aaron, Beaudry, Parker & Therrien, 2016). Research into two of these polyphenols, catechol and gallic acid show both have a neuroprotective effect in *C. elegans* models (Aaron, Beaudry, Parker & Therrien, 2016). Another polyphenol of maple syrup, vanillin, has shown similar neuroprotective effects on cell lines modeling Parkinson's disease, but has not yet been tested in an animal model (Dhanalakshmi et al., 2015). No research has investigated whether vanillin affects genders differently.

Present Research

Insight into gender differences within ALS and PD could lead to increased knowledge behind the causes of the diseases and even different treatment strategies. Mouse models of ALS and PD remain too complex to elucidate much about any observed gender differences. *C. elegans* present a promising model through which to gain insight about the cause and mechanisms of gender differences due to its simplicity and the knowledge surrounding its genes and neurons; however, this area has yet to be studied within the model for either ALS or PD. The present study will investigate the potential use of *C. elegans* for this purpose. Gender differences in locomotion and in chemotaxis of *C. elegans* in the models for both ALS and PD are expected to be observed. Locomotion assays should reveal differences in speed or amount of turning, while chemotaxis assays should reveal whether motor control involved in either attraction to or avoidance of stimuli is altered based on gender and disease presence. It is predicted that males will display harsher phenotypes of both disease, resulting in faster locomotion, lower avoidance, and lower attraction.

The present research also aims to investigate two potential treatments, caffeine and vanillin, in *C. elegans* models of ALS and PD. The influence of *C. elegans* gender on the effectiveness of the treatment will also be examined. It is predicted that vanillin will have similar, restorative effects in both Parkinson's and ALS due to the neuroprotective effects seen in past research at the cell level (Dhanalakshmi et al., 2015). Caffeine is predicted to have positive effects on male and hermaphrodite models of ALS and Parkinson's due to its role in the adenosine/dopamine interactions in the brain (Prediger et al., 2013).

If *C. elegans* are an applicable model for studying differences in ALS and PD between genders, it could lead to critical breakthroughs in understanding the diseases and developing treatments. The potential also exists for *C. elegans* to become a tool for studying gender differences in other neurological disorders--like attention-deficit hyperactivity disorder or Autism spectrum disorders, both of which are more prevalent in males.

Materials & Methods

C. elegans Selection and Maintenance

All *C. elegans* strains (N2; *him-5*; *cat-2* knockout, MT15620; *cat-2* deletion, CB1112; *tdp-1* knockout; *tdp-1* knockout, VC549; *tdp-1* complex substitution, RB929) were obtained from the Caenorhabditis Genetics Center (CGC). The N2 strain is the original strain derived by Brenner (1964) and was used as a control strain for behavior comparison as nearly all genetic and behavioral baseline research has been performed on this strain (Riddle, Blumenthal & Meyer, 1988).

Strains Chosen. Strains of study were chosen based on previous studies on Parkinson's disease (PD) and ALS performed using only hermaphrodites. The *cat-2* gene encodes for an enzyme responsible for production of dopamine precursors (Omura et al., 2012). Strains with mutated *cat-2* genes have reduced dopamine and can be used to study the loss of dopaminergic neurons seen in PD. The MT15620 strain contains a complete knockout of the *cat-2* gene, while the CB1112 contains only a partial deletion. Both strains were studied in order to examine the effects of different mutations that can cause PD. The *tdp-1* strain VC549 contains a complete knockout of the *tdp-1* gene, the worm orthologue of the human *tdp-43*, a gene implicated in many sporadic (non-inherited) cases of ALS (Zhang et al., 2012). The *tdp-1* strain RB929 contains a complex substitution that disrupts the *tdp-1* gene.

Male Creation. The prevalence of males in N2 and other wild type strains is 0.1 to 0.2% (Hodgkin, Horvitz & Brenner, 1979). However, a strain with a higher incidence of males (*him*-5) and no other detected behavioral differences was developed by Hodgkin, Horvitz, and Brenner (1979). In order to generate a higher incidence of males in each strain of study, the *cat-2* (MT15620) deletion strain were crossed with the *him-5* strain, as detailed in Figure 1, below, which demonstrates the cross with the *cat-2* strain. The presence of both sets of genes in the new strains was confirmed via PCR and gel electrophoresis.

The parent lineage (P_0) consisted of five plates each containing two hermaphrodites from the strain of interest and between seven and nine males from the *him-5* strain. After three to five days, the first progeny (F_1) plates were created, consisting of seven new plates each containing one hermaphrodite from the parent plates containing a high number of males. On the right of

each step in the cross, the possible genetic combinations within the cross are listed. This process repeated as detailed in the diagram until the fourth progeny generation, at which time PCR and gel electrophoresis analysis looking for the desired genotype (circled in red) was performed.

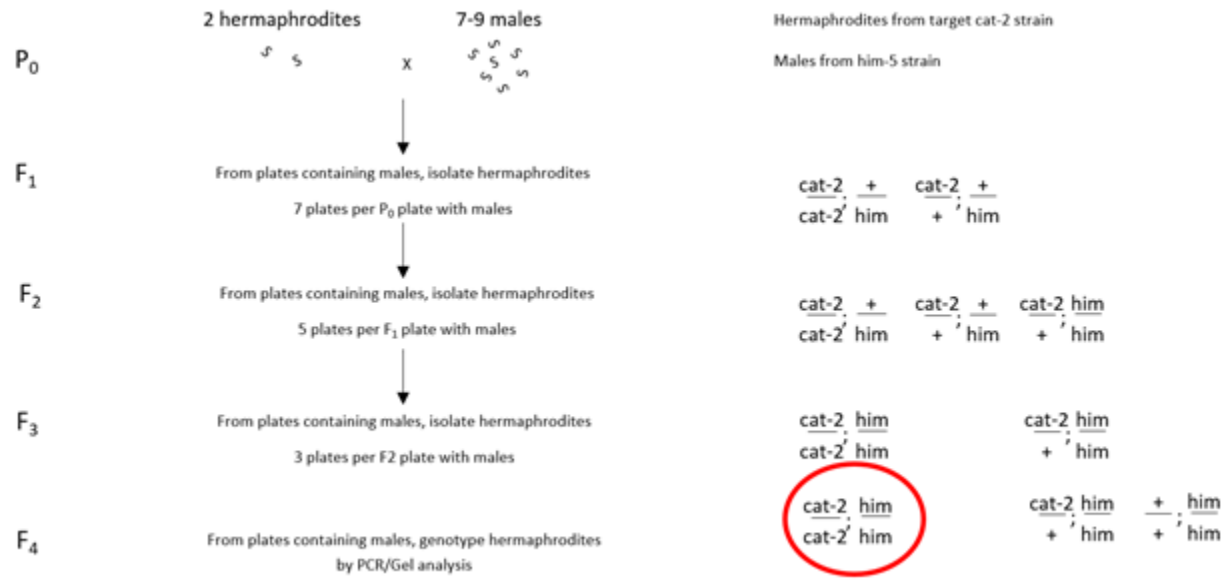

Figure 1: Crossing the *him-5* and *cat-2* (MT15620) strains for male generation

Males of the N2 and VC549 strain were generated via a heat shock procedure, as described by Sulston & Hodgkin (1988). The method involves forcing nondisjunction of the X chromosome by inducing stress in the worms through exposure to heat. Nondisjunction creates the X0 genotype which defines male *C. elegans*. Due to the complexity and time involved in generating male strains, males were not able to be generated for the *cat-2* point mutation (CB1112) nor the *tdp-1* complex substitution.

Strain Maintenance. Strains were maintained on agar plates seeded with OP50 *E. coli.* Three to four hermaphrodites were passed from one plate to another every three to five days. In order to maintain a high number of males, the cross strains and N2 heat shock line were passed at a ratio of seven males to two hermaphrodites every three to five days. All strains were stored at 20 degrees Celsius.

Locomotion Assay

Locomotion assays evaluating the speed and turning patterns of *C. elegans* were performed in order to determine the effect of mutations and treatments on the worms as well as to establish behavioral differences between males and hermaphrodites. Locomotion assays were carried out on 35 mm plates with five worms (either five males or five hermaphrodites) per plate. These plates were seeded with OP50 *E. coli* the day before the assay. Thus, the assay was carried out "on food" to prevent variations in starvation state from affecting results. Worms (10 to 15) were picked to a separate, larger plate five hours prior to performing the assay. To carry out the assay, five worms were placed on a plate, allowed to sit for five minutes and then filmed using the WormLab program from MBF Bioscience. Plates were filmed for 20 minutes at a time. Video analysis was then carried out using the WormLab software. The speed (um/s) and track length (um) were extracted for each of the tracked worms. Tracks under 5,000 um were removed in order to eliminate software errors in tracking. The average speed was then determined for each plate to use as a measure of comparison.

Chemosensory Assays

Chemotaxis Assay. An assay designed to measure chemotaxis (attraction) in *C. elegans* was selected and used for this study to determine the effect of mutations and treatments on the ability of the worms' to sense and respond to attractive stimuli and determine if there were gender differences in this ability. It was decided that isoamyl alcohol (IAA) at a 10^{-2} dilution would be the chemical used for this protocol based on a previous project using the same assay to study Alzheimer's disease (Coyle, Nikolaki & Ong, 2016).

Assay plates were made from 2% agar, 5 mM KPO_4 pH 6.0, 1 mM $CaCl_2$, and 1 mM $MgSO_4$, and were poured into a 3.5 cm petri dish. Two marks, labeled A (attractant) and C (control), were made 180 degrees apart on the bottom of the plate. One μL of 1 M sodium azide (NaN_3) was placed on each mark in order to paralyze worms once they came in contact with the location of the IAA or the DI water.

Approximately 30-50 worms of one gender were taken from a seeded plate, and were washed three times using S. Basal in a 1.5 mL microcentrifuge tube to remove traces of food or eggs that may interfere with chemotaxis from the worms. After three washes, the worms were washed once with distilled water to remove any residual S. Basal. The excess water was then

pipetted off, leaving a pellet of worms at the bottom of the microcentrifuge tube. Two μL of the washed worms were then transferred to the origin point (located in the center of the plate). Excess liquid was then removed using a KimWipe. One μL of IAA and DI water was placed on the A and C marks, respectively. The lid of the plate was closed following the placement of the chemicals in order to contain the odor of IAA. If clumping was observed initially, the worms were dispersed with a pick. The assay was then run for one hour. At the end of the hour, the number of paralyzed worms at each location was counted. Each plate is considered to be an N of 1, and at least 10 plates were tested for each strain. The chemotaxis index was calculated using the following equation:

$$\text{Chemotaxis Index} = \frac{\text{(Worms at Attractant - Worms at Control)}}{\text{(Total Number of Worms on Plate)}}$$

Avoidance Assay. The ability of *C. elegans* with varying mutations to avoid negative stimuli and the effect both gender and different treatments have on this ability was evaluated through an avoidance assay. Avoidance assays were carried out on plates containing approximately 30 worms at a time. Twenty to 30 young adult males or hermaphrodites were picked onto a foodless plate. SDS (0.1%) was used as the solvent of avoidance, as it is well-documented as a *C. elegans* repellant (Hilliard, Bargmann & Bazzicalupo, 2002). A low dilution was chosen in order to be sensitive to small differences in male and hermaphrodite chemosensory behavior (i.e. disease-affected males may be less sensitive to avoidant stimuli due to gender bias), thus even slight gender differences would be detected. M9 buffer was used as the solvent control, as it does not typically produce an avoidance response. Under magnification, a drop of SDS or M9 was placed at the tail of a forward-moving worm using a mouth pipette. Capillary action carried the liquid up the body of the worm, to the head region where the cilia of the worm are located. An avoidance response was considered to be a full sinusoidal reverse motion by the worm or a +/- 90 degree angle turn of the worms head within four seconds. Around ten worms per plate were tested for both the solvent and the solvent control, with a two minute waiting period before switching between the liquids. Each plate of approximately 20 nematodes was considered to be an N of 1. At least three separate plates were tested over at least three separate days for each strain to minimize the possible effects of atmospheric conditions.

The avoidance index was calculated using the following equation:

$$\text{Avoidance Index} = \frac{(\text{Number of Worms Avoided SDS} - \text{Number of Worms Avoided M9})}{(10 \text{ Worms per condition})}$$

Creation of Treatment Media

Both vanillin and caffeine have been shown to have promising neuroprotective effects that could treat ALS and PD (Potenza et al., 2013, Predinger et al., 2013). In order to determine the effects of caffeine and vanillin on the symptoms of ALS and Parkinson's-like mutations in *C. elegans* and determine if there was a differential effect based on gender, worms were grown on LB agar medium seeded with OP50 *E. coli* and a 10 millimolar concentration of either vanillin or caffeine. This concentration was determined from examining past literature. A 10 millimolar concentration of caffeine was found to improve longevity in *C. elegans* (Sutphin et al., 2012). While no direct past research had been similarly done on vanillin, related compounds in a 10 millimolar concentration were found to have neuroprotective effects (Ma et al., 2016).

Caffeine Media. An amount of caffeine was dissolved into a volume of sterilized LB medium such that the final concentration was 10 millimolar caffeine. The solution was sterilized by autoclaving and inoculated with OP50 *E. coli* which was allowed to grow overnight at 35 degrees Celsius and then stored at 4 degrees Celsius.

Vanillin Media. An amount of vanillin was dissolved into an amount of distilled water. The resulting solution was sterilized by autoclaving and added to a volume of sterile LB media previously inoculated with OP50 *E. coli* such that the final concentration of vanillin was 10 millimolar. The final solution was stored at 4 degrees Celsius.

Results & Discussion

Establishing the Model

The locomotion and chemosensory abilities of wild type strains (N2 and *him-5*) and mutant strains modeling PD and ALS (MT15620, CB1112; VC549, RB929) of *C. elegans* were examined to gain an understanding of the traits of the disease models and investigate the presence of gender bias in symptom presentation. Due to time constraints only hermaphrodites of the CB1112 and RB929 strains could be tested. All statistical analysis was performed at significance level, alpha, equal to 0.05.

Parkinson's Disease. Both mutations modeling the dopaminergic neuronal loss associated with PD affect the *cat-2* gene of *C. elegans*. The deletion strain (MT15620) and the point mutation strain (CB1112) were both compared to *him-5* and N2 wild type strains.

Locomotion Assay. The speed of wild type *C. elegans* and PD models was measured and visualized graphically in Figure 2, below.

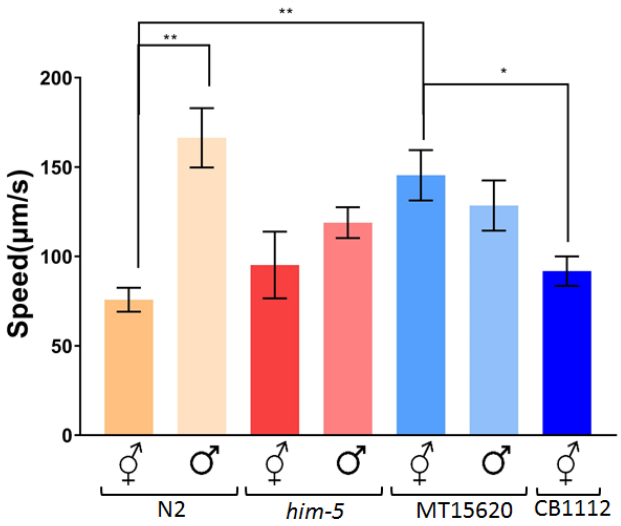

Figure 2: Locomotion of PD models

Figure 2 displays the average locomotion speed in micrometers per second for each strain assayed. Both males and hermaphrodites were assayed, when possible. Exact means and standard deviations for each strain and gender can be found in Table 1, below.

Table 1: *Means and Standard Deviations of Locomotion Speeds (µm/s) for PD models*

Strain	Gender	Mean	Standard Deviation
N2	Hermaphrodite	72	20
	Male	166	28
him-5	Hermaphrodite	95	49
	Male	118	17
MT15620	Hermaphrodite	145	31
	Male	128	37
CB1112	Hermaphrodite	91	18

A Shapiro-Wilk normality test was completed on the locomotion data seen in Figure 1, with the N2 hermaphrodites (*W=0.7619, p=0.0012*), N2 males (*W=0.75, p<0.0001)* and MT15620 males (*W=0.8055, p=0.0465)* all found to be nonparametric. A Kruskal-Wallis test was then performed on the data set to test for significance in the strain speed differences. The test indicated that significance was present in the strain data, *H(7,46)=22.69, p<0.001*. Post-hoc analyses were then performed to identify which strains had significant differences in speeds. Mann-Whitney U tests were used for nonparametric data sets and Welch's t-tests were used for parametric data.

Examining gender differences in speed first, wild type N2 hermaphrodites (*M=72μm/s, SD=20*) moved significantly slower than N2 males (*M=166 μm/s, SD=28*), Mann-Whitney U test, *U=1, p=0.005*. This disparity was expected, as it is well supported in the literature (Mowrey, Bennett & Portman, 2014). This difference was not seen to such a degree in the *him-5* hermaphrodites (*M=95μm/s, SD=49*) and males (*M=118, SD=17*) due to lower male speed in this strain, Welch's T test, *t(1.51)=8.11, p=0.282*. This could be a result of the mutations that created the *him-5* strain. There was no significant gender difference seen between the MT15620 hermaphrodites (*M=145μm/s, SD=31)* and the MT15620 males (*M=128μm/s, SD=37)* Mann-Whitney U test, *U=13, p=0.530.*

Locomotion differences between wild type nematodes and the disease models were also analyzed. Hermaphrodites of the *cat-2* deletion MT15620 (*M=145μm/s, SD=31*) moved significantly faster than N2 hermaphrodites (*M=72μm/s, SD=20*), showing a decreased control over their locomotion, Mann-Whitney U test, *U=4, p=0.002.* There was no significant difference in the male worm speed between N2 males (*M=166μm/s, SD=28)* and MT15620 males (*M=128μm/s, SD=37),* Mann-Whitney U test, *U=4, p=0.175,* showing the damage to locomotive ability had a gender bias. However, the bias operated the opposite direction of what was expected. Given the male bias present in PD, it was expected that the speed of the male disease model would be higher than that of the hermaphrodite. However, not only was the hermaphrodite speed higher than the male speed for the MT15620 disease strain, but the male's speed was not even affected in the disease model. No difference was seen when comparing the both the MT15620 and CB1112 diseased strains to the *him-5* strain (*p values>0.106).* However, the CB1112 worms (*M=91μm/s, SD=18*) moved much significantly slower than the other *cat-2*

knockout, a result that can be linked to the nature of the mutation, Welch's T test, *t(3.28)=6.453, p=0.015*. A point mutation in the *cat-2* gene is found in the CB1112 strain, which could affect the movement to a lesser degree than a full deletion of the gene.

Overall this locomotion analysis showed an increase in the movement speed of only the MT15620 hermaphrodite, indicating that only this strain's ability to properly control its movement was negatively affected by the disease genotype. The results indicate that the locomotion of *C. elegans* male models of PD may be unaffected by the disease.

Chemosensory Assays. The ability of the PD model strains to respond to environmental stimuli was also examined. The ability to move towards or away from a stimulus is expressed as an index, with a higher chemotaxis index indicating a greater ability to move toward attractive stimulus and a higher avoidance index indicating a greater ability to move away from a repellent stimulus. Thus a higher index is better for both measures.

Chemotaxis Assay. Figure 3, below, shows results from the chemotaxis assay, which measured the ability of the PD models to sense and move toward an attractive stimulus.

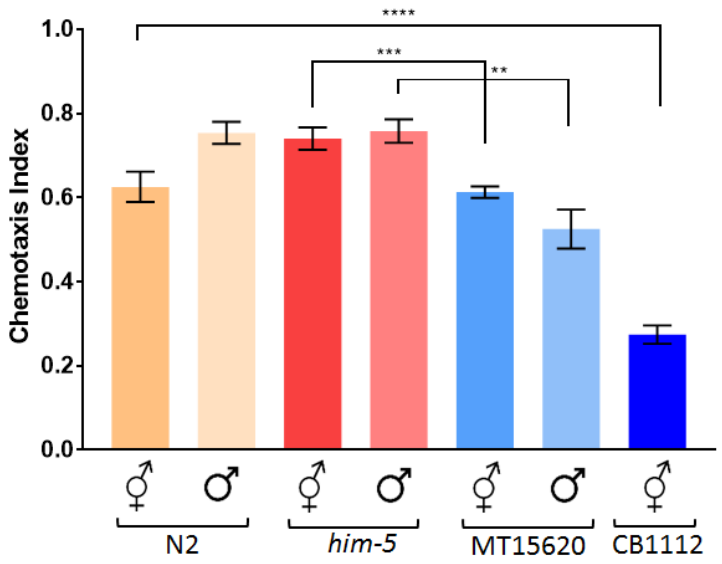

Figure 3: Chemotaxis assay of *C. elegans* models of PD using IAA (10^{-2} dilution)

Figure 3 shows the average chemotaxis index for each strain and gender of worm tested, including the wild type *C. elegans* (N2 and *him-5*) and the PD models (MT1620 *cat-2* deletion

and CB1112 *cat-2* point mutation). Table 2, below, describes the means and standard deviations of each strain's chemotaxis index.

Table 2: *Means and Standard Deviations of Chemotaxis Indices for PD Models*

Strain	Gender	Mean	Standard Deviation
N2	Hermaphrodite	0.63	0.11
	Male	0.75	0.59
him-5	Hermaphrodite	0.74	0.08
	Male	0.76	0.06
MT15620	Hermaphrodite	0.62	0.04
	Male	0.53	0.10
CB1112	Hermaphrodite	0.27	0.07

A Shapiro-Wilk normality test revealed that the male *him-5* data was nonparametric (*W(10)=0.76, p=0.04*). Therefore, a Kruskal-Wallis test was used to examine the significance of the differences in the chemotaxis indices. The test showed significant differences present in the data, *H(7, 55)=40.20, p<0.001*. Post-hoc analyses were then performed in order to determine which nematode gender and strains differed significantly. The Mann-Whitney U test was used in any circumstance when one or both of the data sets was nonparametric, while Welch's t-test was used when both data sets were parametric.

Looking at the strains based on gender, the N2 wild type hermaphrodites (*M=0.63, SD=0.11)* had a lower chemotaxis index than males (*M=0.75, SD=0.59*) nematodes, indicating a lesser ability to sense and respond to an attractive stimulus (Welch's t-test, *t(12.89)=2.97, p=0.01*). However, no gender bias was present in the *him-5* wild type strain. Hermaphrodites (*M=0.74, SD=0.08)* had chemotaxis indices similar to males (*M=0.76, SD=0.06)*, Mann-Whitney U test, *U=20, p = 0.57*. In addition, there were no differences in chemotaxis indices for the MT1560 strain (hermaphrodites: *M* =0.62, *SD=0.04*, males: *M=0.53, SD=0.10*, Welch's t-test, *p=0.12*).

Differences between comparable strains were also examined. The N2 hermaphrodite chemotaxis index is significantly lower than that of the *him-5* hermaphrodite, Welch's t-test, *t(16.48)=2.56, p=0.02*. However, the N2 hermaphrodite chemotaxis index was significantly higher than that of the CB1112 *cat-2* point mutation (*M=0.27, SD=0.07*), Welch's t-test,

t(14.74)=8.34, p<0.001. Hermaphrodites and males of the MT15620 strain showed similar reductions in their ability to sense and respond to an attractive stimulus in comparison to the wild type. The chemotaxis index of the MT15620 hermaphrodite was significantly lower than that of the *him-5* hermaphrodite, Welch's t-test, $t(13.53) = 4.27$, $p<0.001$. Similarly, the chemotaxis index of the MT15620 male was significantly lower than that of the *him-5* male, Mann-Whitney U test, $U=0$, $p = 0.008$.

Overall, analysis of the chemotaxis assay show that there was a significant deficit in all of the PD models' ability to sense and respond to an attractive stimulus; however, no significant gender bias was observed within the disease models.

Avoidance Assay. Figure 4 shows the results of the avoidance assay for PD models, which measures the ability to sense and avoid a repellent stimulus.

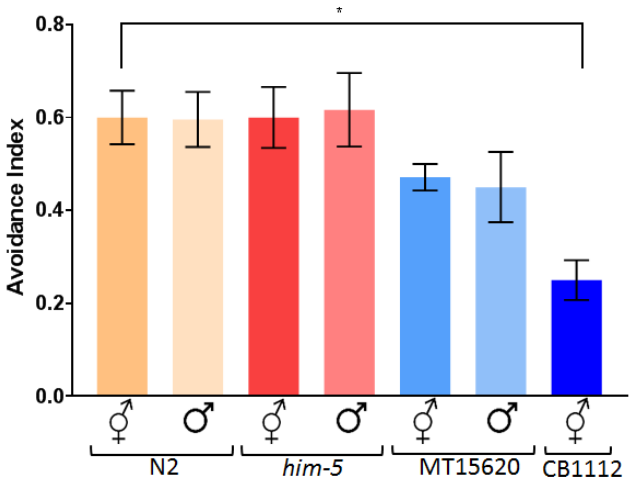

Figure 4: C. elegans Parkinson's disease models' avoidance of 0.1% SDS

Figure 4 shows the average avoidance indices of the wild type (N2 and *him-5*) and PD (MT15620 and CB1112) model worms tested. Table 3, below, describes the means and standard deviations of each strain's avoidance index.

Table 3: *Means and Standard Deviations of Avoidance Indices for PD models*

Strain	Gender	Mean	Standard Deviation
N2	Hermaphrodite	0.60	0.14
	Male	0.60	0.15
him-5	Hermaphrodite	0.60	0.19
	Male	0.62	0.19
MT15620	Hermaphrodite	0.47	0.08
	Male	0.45	0.21
CB1112	Hermaphrodite	0.25	0.10

A Shapiro-Wilk normality test revealed that the N2 male data was nonparametric in nature ($W=0.73$, $p=0.01$), as was the MT15620 male data set ($W=0.80$, $p=0.03$). Therefore, a Kruskal-Wallis test was used to examine the potential significance of differences in the avoidance indices. The test showed significant difference between the avoidance index of each worm-type $H(7, 46) = 16.84$, $p = 0.01$. Post-hoc analyses were then performed in order to determine which nematode gender and strains differed significantly. The Mann-Whitney U test was used in any circumstance when one or both of the data sets was nonparametric, while Welch's t-test was used when both data sets were parametric.

Analysis of gender differences revealed the wild type strain, N2, had similar avoidance indices between hermaphrodites ($M= 0.60$, $SD=0.14$) and males ($M=0.60$, $SD=0.15$) nematodes, Mann-Whitney U test, $U=16.5$, $p=0.88$. There was also no significant difference between avoidance indices of *him-5* hermaphrodites ($M=0.60$, $SD=0.17$) and males ($M=0.62$, $SD=0.19$), Welch's t-test, $t(10.2)=0.16$, $p=0.87$. Like the wild types, the MT15620 *cat-2* point mutation strain also showed no significant gender bias in the ability to sense and avoid a negative stimulus between hermaphrodites ($M=0.47$, $SD=0.08$) and males ($M=0.45$, $SD=0.21$), Mann-Whitney U test, $U=25$, $p=0.71$.

Differences between comparable strains were also examined. Only the CB1112 hermaphrodites ($M=0.25$, $SD=0.10$) displayed a significantly reduced ability to sense and respond to the negative stimulus when compared to their N2 wild type counterparts, Welch's t-test, $t(8.20)=4.87$, $p<0.001$. There was no significant difference in the ability of the wild type hermaphrodites (N2 and *him-5*), Welch's t-test, $t(10.99)=0$, $p>0.99$), nor the wild type males

(Mann-Whitney U, *U=15.5, p=0.71*) to respond to the negative stimulus. Although the MT15620 strain showed reduced ability to respond to a stimulus, this reduction in ability was not significantly when compared to the *him-5* model for hermaphrodites (Welch's t-test, *t(8.21)=1.8, p=0.11*) or males (Mann-Whitney U, *U=12.5, p=0.15*).

Overall, analysis of the avoidance assay shows that although there was reduction in all the PD model's ability to sense and respond to a negative stimulus, the defect was only significant in the CB1112 *cat-2* point mutation. Additionally, no gender bias was present in the avoidance capabilities of the strains tested.

PD Discussion. Both PD models generated interesting results. The deletion, MT15620, and point mutation, CB1112, showed greater effects of the disease, dependent upon the assay tested. While the locomotion of the CB1112 strain wasn't affected significantly, the effects of both chemosensory assays were seen most significantly within this strain. It's likely, therefore that the point mutation of the *cat-2* gene is located in an area of the gene implicated in sensation. As expected, based on the literature, the locomotion of the hermaphrodite *cat-2* deletion, MT15620, was greatly affected, due to the importance of dopamine in locomotor control (Harrington et al., 2010). However, the lack of significant effect in the males of the deletion strain was unexpected, especially given the male gender bias of Parkinson's disease. The present research marks the first time the males of this strain have been tested, and the reasoning for the lack of effect remains unclear. Male *C. elegans* have a number of extra neurons; it's possible that some of these are responsible for rescuing the effect of the *cat-2* deletion. Aside from this lack of significant locomotion change in the male MT15620 strain was no significant gender bias seen in the untreated Parkinson's models.

Amyotrophic Lateral Sclerosis. Two strains modeling the loss of function of human TDP-43 served as ALS models. The VC549 strain is a deletion of the *tdp-1* gene, while the RB929 strain contains a complex substitution within the gene. The locomotion and chemosensory behaviors of these ALS models were tested and compared to the wild type strains.

Locomotion Assay. Figure 5, below, shows the effects of the disease on the locomotion of the ALS models.

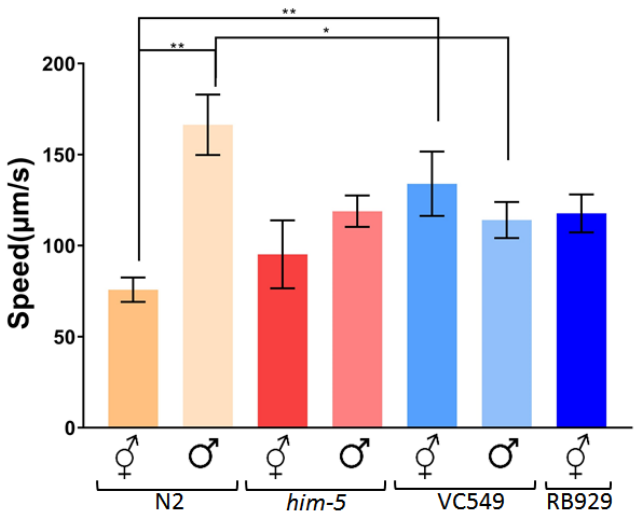

Figure 5: Locomotion of ALS strains

Figure 5 displays the average locomotion speed in micrometers per second for each strain assayed. Both males and hermaphrodites were assayed, when possible. Exact means and standard deviations for each strain and gender can be found in Table 4, below.

Table 4: *Means and Standard Deviations of Locomotion (µm/s) of ALS Strains*

Strain	Gender	Mean	Standard Deviation
N2	Hermaphrodite	72	20
	Male	166	28
him-5	Hermaphrodite	95	49
	Male	118	17
VC549	Hermaphrodite	134	43
	Male	114	24
RB929	Hermaphrodite	117	18

After locomotion speed was collected, a Shapiro-Wilk normality test was performed on the data. This revealed that the N2 hermaphrodites (*W=0.7619, p=0.001*), N2 males (*W=0.75, p<0.001)* and the RB929 hermaphrodites (*W=0.76, p=0.020)* were all nonparametric. A Kruskal-Wallis test was then performed to test for significance. This test showed significance in the ALS locomotion speed data, *H(7,44)=18.79, p=0.005).* Post-hoc analyses were then completed to test for significant differences between individual strain speeds. The Mann-

Whitney U test was used in any circumstance when one or both of the data sets was nonparametric, while Welch's t-test was used when both data sets were parametric.

The two ALS disease strains locomotion speed was compared to the wild type N2 and *him-5* strains. Similar results were seen to the Parkinson's disease strains, with the diseased VC549 knockout hermaphrodites (*M*=134μm/s, *SD*=43) showing a significantly higher speed, Mann-Whitney U test, *U= 10, p=0.005)* than the wild type N2 hermaphrodites (*M=72*μm/s, *SD*=20). The males of the VC549 strain (*M*=114μm/s, *SD*=24) showed a significantly slower speed, Mann-Whitney U test, *U=1, p=0.047,* than the N2 males (*M*=166μm/s, *SD*= 28). This displays an opposite reaction for the hermaphrodite and male worms in the disease state. The hermaphrodites increased significantly, while the male worms slowed significantly. The RB929 hermaphrodites (*M*=117μm/s, *SD*=18) were also seen to move significantly quicker, than the N2, but to a lesser degree than the VC549 deletion, Welch's t-test, *t(3.39)=3.89, p=0.029*. This could be due to a lessened effect on the gene activity due to the genetic editing of a complex substitution as opposed to a full deletion of the gene. Neither the VC549 strain nor the RB929 strain was seen to be significantly different from the *him-5* strain (*p-values>0.181).*

Chemosensory Assays. The ability of the ALS model strains to respond to environmental stimuli was also examined.

Chemotaxis Assay. Figure 6 shows the results of the chemotaxis assay of the *tdp-1* ALS models, which measures the nematode's ability to sense and move toward an attractive stimulus.

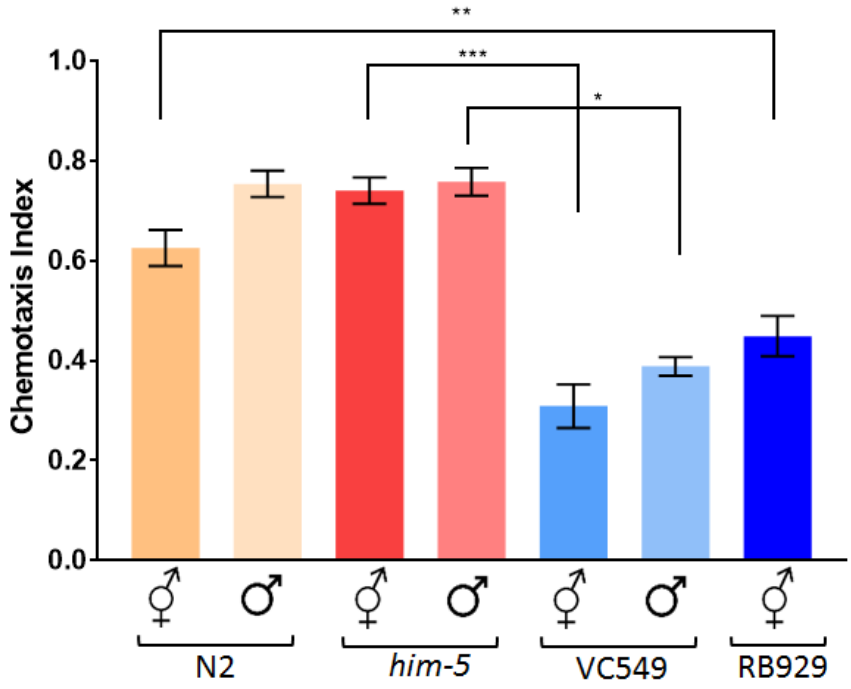

Figure 6: Chemotaxis of *tdp-1* strains using IAA (10^{-2} dilution)

Figure 6 depicts the average chemotaxis index for each wild type and ALS model strain tested, with the hermaphrodite on the left and male on the right for each strain. Table 5, below, contains the mean chemotaxis index and standard deviation for all strains tested.

Table 5: *Means and Standard Deviations of Chemotaxis Indices for ALS Models*

Strain	Gender	Mean	Standard Deviation
N2	Hermaphrodite	0.63	0.11
	Male	0.75	0.08
him-5	Hermaphrodite	0.74	0.08
	Male	0.76	0.06
VC549	Hermaphrodite	0.31	0.13
	Male	0.39	0.03
RB929	Hermaphrodite	0.45	0.13

Analysis with a Kruskal-Wallis test revealed that there were significant differences between the avoidance indices of the worm types, $H(7, 53) = 39.67$, $p < 0.001$. Post-hoc analyses were run in order to determine from where the significance stemmed.

Gender differences within strains were examined first; however none were found, even between the male and hermaphrodite of the VC549 ALS model, (*ps>0.12*). The lack of gender bias is unexpected due to the harsher phenotype and increased gender bias for ALS seen in humans.

Differences in chemotaxis indices were also examined between strains. A Welch's t-test determined that there was a significant difference between the chemotaxis index between the wild-type *him-5* hermaphrodite (*M=0.74, SD=0.08*) and hermaphrodites of the VC549 strain (*M=0.31, SD=0.13*), $t(14.85) = 8.49$, $p < 0.001$. A Mann-Whitney test proved the significance of the difference between the chemotaxis indices of the male *him-5* (*M=0.76, SD=0.06*) and male VC549 strains (*M=0.39, SD=0.03*), *U=0*, $p = 0.036$. The difference between the RB929 chemotaxis index (*M=0.45, SD=0.13*) and its wildtype counterpart N2 (*M=0.63, SD=0.11*) also showed a significant difference, Welch's t-test, *t(17.79)=3.25, p=0.005*.

Overall, both ALS models, VC549 and RB929, showed significant defects in the ability of the mutated worms to respond to or sense an attractive stimulus; however, there is no significant gender bias in the chemotaxis of *tdp-1* worms modeling ALS.

Avoidance Assay. Figure 7, below, shows the results of the avoidance assay of the *tdp-1* ALS models, which measures the nematode's ability to sense and avoid a repellent stimulus.

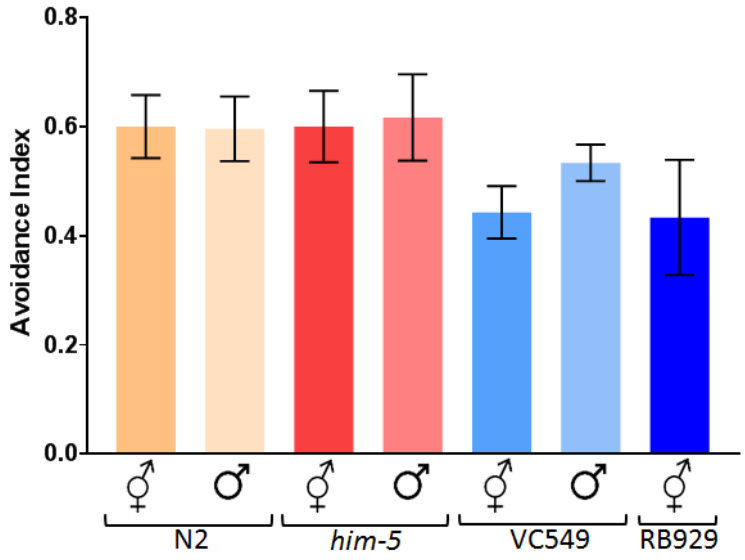

Figure 7: Avoidance of 0.1% SDS by *C. elegans* models of ALS

Figure 7 shows the same average avoidance indices for the N2 and *him-5* strains alongside the average avoidance indices from the two ALS models. Means and standard deviations from chemotaxis indices can be found in Table 6, below.

Table 6: *Means and Standard Deviations of the Avoidance Indices of ALS Models*

Strain	Gender	Mean	Standard Deviation
N2	Hermaphrodite	0.60	0.14
	Male	0.60	0.15
him-5	Hermaphrodite	0.60	0.19
	Male	0.62	0.19
VC549	Hermaphrodite	0.44	0.13
	Male	0.53	0.06
RB929	Hermaphrodite	0.43	0.26

A Shapiro-Wilk normality test revealed that the male N2 data was nonparametric ($W=0.73$, $p=0.01$) as was the VC549 male data ($W=0.75$, $p<0.001$). Therefore, a Kruskal-Wallis test was used to examine the significance of the differences in the avoidance indices. The test showed that there was no significant difference between the avoidance indices of the worms by gender nor by strain, $H(7,41)=7.19$, $p=0.30$. Thus, although the ability of the ALS models to sense and respond to a negative stimulus was lower than the avoidance indices of the wild type worms as seen in Figure 7 and in the values from Table 6, the impact of the disease cannot be determined to be the reason for this deficit, nor does it appear that gender bias is having any effect.

ALS Discussion. The effects of the *tdp-1* mutations varied greatly across the three behavioral assays. The locomotion of the hermaphrodite of the genetic deletion was increased by the mutation significantly; the locomotion of the male VC549 strain actually slowed significantly. This is unexpected, given the male bias of ALS and the expectation that the *tdp-1* knockout would damage locomotive ability in both genders, especially male. Once again, the effect could be due to some sort of rescue effect in the extra neurons of the males. There were no major gender differences observed in the results of any of the behavioral assays, thus the expected gender bias toward more affected male phenotypes was not observed.

Although the RB929 strain showed a slight increase in locomotion, the model doesn't show the same significant effect as the VC549 hermaphrodite. The smaller effect make sense given the nature of the complex substitution over the full deletion of the *tdp-1* gene. In the chemotaxis assay, however, both mutations show significant negative effects on the mutated worms' ability to be attracted to a positive stimulus. However, these same effects are not seen in the worms' ability to respond to a negative stimulus, which was not significantly affected by any mutation. This differential effect on chemosensory behavior is interesting, and implies that the *tdp-1* gene may be linked to attraction but not avoidance responses.

Caffeine Treatment

Caffeine's ability to rescue deficits in locomotion and chemosensory behaviors in *C. elegans* models of PD and ALS was examined, as was the interaction of gender with response to caffeine treatment. All statistical analysis was performed at alpha=0.05.

Parkinson's Disease. The *cat-2* strains modeling PD were treated with caffeine. The same behavioral assays measuring locomotion, attraction, and avoidance were performed to determine the effects of the compound on PD symptoms.

Locomotion Assay. Figure 5, below, shows the effect of caffeine treatment on locomotion speeds of the Parkinson-modeling strains compared to the *him-5* wild type. Due to technical issues with the recording and analysis software and time constraints, the CB1112 and N2 strains previously explored were unable to be tested with caffeine treatment.

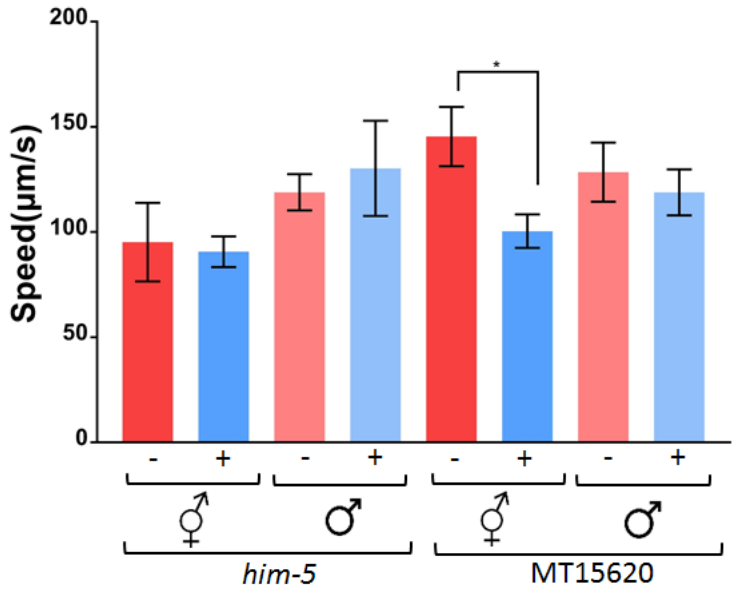

Figure 8: Locomotion of PD strains treated with caffeine

Figure 8 above displays the locomotion speeds of *him-5* wild type and MT15620 *cat-2* knockout strains before and after treatment with caffeine. The untreated worms are represented by red columns, while the treatment is represented by blue columns. The hermaphrodites are shown as darker colors, and the males are the lighter columns. The mean speed and standard deviation for each strain, gender and treatment condition can be seen below in Table 7.

Table 7: *Means and Standard Deviations of Locomotion Speeds (µm/s) for Caffeine-treated PD Models*

Strain	Gender	Treatment	Mean	Standard Deviation
him-5	Hermaphrodite	None	95	49
		Caffeine	90	17
	Male	None	118	17
		Caffeine	130	39
MT15620	Hermaphrodite	None	145	31
		Caffeine	100	17
	Male	None	128	24
		Caffeine	118	26

A Shapiro-Wilk test was completed on the PD caffeine data to test for parametric data. It was found that MT15620 male untreated was the only data nonparametric (*W=0.8055,*

p=0.0465). The *cat-2* knockout strains were then analyzed using t-tests for their locomotion speed after treatment with caffeine.

To start, the *him-5* strain was tested to ensure that caffeine treatment would not have an effect on wild type worms. No significant change in speed was seen between the untreated and treated *him-5* groups for both hermaphrodites and males (*ps> 0.675).* The MT15620 hermaphrodites (*M=145μm/s, SD=31*) were then tested on their movement speed after caffeine treatment (*M=100μm/s, SD=17*). The previously high speed of the diseased hermaphrodites was seen to drop significantly, Welch's t-test, *t(2.78)=6.32, p=0.03,* indicating a partial rescue of healthy behavior. The treated males (*M=118μm/s, SD=26*) saw a small drop from the untreated males (*M=128μm/s, SD=37*), but no significant change was seen, Mann-Whitney U test, *U=19, p=0.836,* partially due to the lessened increase in speed for the males in the diseased state. In addition, no significant difference was seen between the speeds of the treated hermaphrodites and the treated males, indicating that gender did not have an effect on treatment response, Welch's T test, *t(1.36)=8.65, p=0.208)*

Chemosensory Assays. The ability of these caffeine-treated worms to respond to both positive and negative environmental stimuli was examined.

Chemotaxis Assay. Figures 6 shows the effects caffeine treatment had on the worms' ability to be attracted to a positive stimulus.

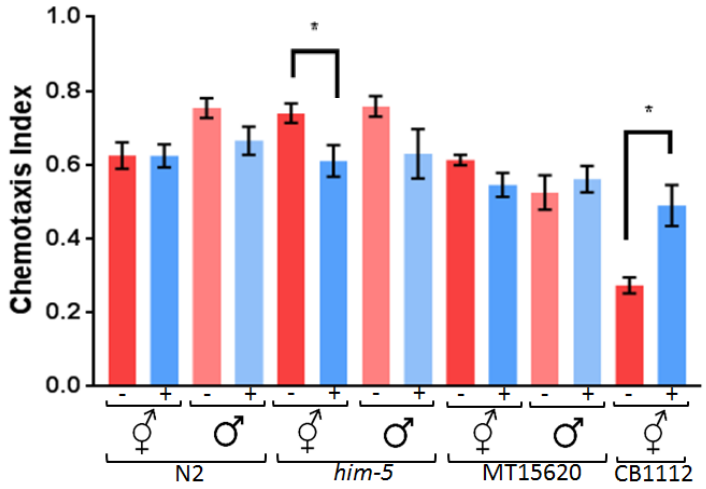

Figure 9: Chemotaxis indices of PD models after caffeine treatment

Figure 9 shows each strain's untreated chemotaxis index on the left in red (dark for hermaphrodite strains and lighter for males), and the chemotaxis index after treatment with caffeine in blue to the right. Table 8, below, shows the exact means and standard deviations of each of the strains and genders before and after caffeine treatment.

Table 8: *Means and Standard Deviations of the Chemotaxis Indices of Caffeine-treated PD Models*

Strain	Gender	Treatment	Mean	Standard Deviation
N2	Hermaphrodite	None	0.63	0.11
		Caffeine	0.62	0.07
	Male	None	0.75	0.06
		Caffeine	0.67	0.07
him-5	Hermaphrodite	None	0.74	0.08
		Caffeine	0.61	0.10
	Male	None	0.76	0.06
		Caffeine	0.63	0.12
MT15620	Hermaphrodite	None	0.61	0.04
		Caffeine	0.55	0.07
	Male	None	0.53	0.10
		Caffeine	0.56	0.06
CB1112	Hermaphrodite	None	0.27	0.07
		Caffeine	0.49	0.12

Multiple t-tests were completed in order to determine whether there was a significant difference in chemotaxis behaviors after each strain was treated with caffeine. All significant data throughout the chemotaxis experiments was parametric, and was therefore analyzed using a Welch's t-test.

The wild-type *C. elegans* typically did not show a significant change in attractive behavior after treatment with caffeine, Welch's t-test, (N2 *hermaphrodites*: *t(0.023) = 12.28, p = 0.982;* N2 *males: t(1.91) = 3.96, p = 0.15; him-5 males: t(1.78) = 2.72, p = 0.183)*. However, there is significant data suggesting that *him-5* hermaphrodites were negatively affected by caffeine treatment, Welch's t-test, *t(2.56) = 7.13, p = 0.037*. Neither males nor hermaphrodites of the *cat-2* deletion strain (MT15620) displayed any significant difference in their ability to be attracted to a positive stimulus after treatment with caffeine, Welch's t-test (*hermaphrodites*: *t(1.93) = 5.51, p = 0.183; males: t(0.63) = 5.96, p = 0.555)*. However, the caffeine-treated *cat-2*

point mutation strain (CB1112) hermaphrodites (*M=0.49, SD=0.12*) showed an increased ability to be attracted to a positive stimulus compared to the untreated CB1112 hermaphrodites (*M=0.27, SD=0.07*), Welch's t-test, *t(3.64) = 5.27, p = 0.014* (*).

Overall, the chemotaxis assays showed that caffeine had no significant positive or negative effect on the *cat-2* deletion strain, however did significantly improve the *cat-2* point mutation strain's chemotaxis index. Gender played no role in response to caffeine treatment.

Avoidance Assay. Figure 10 shows the PD worms' abilities to sense and avoid a negative stimulus after treatment with caffeine.

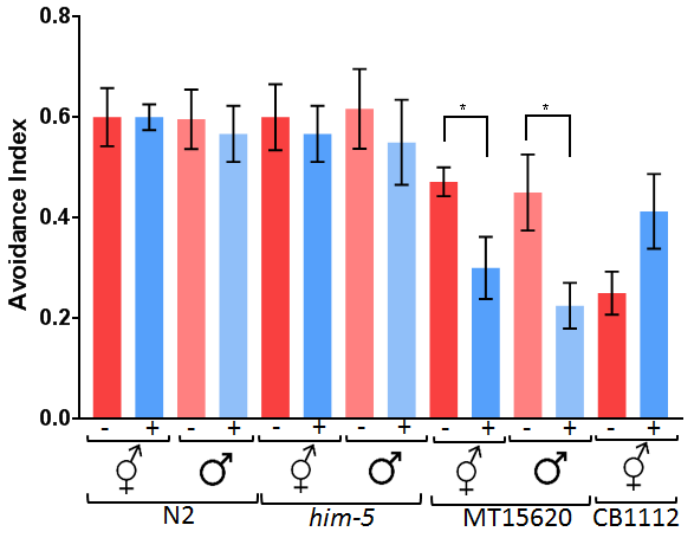

Figure 10: Avoidance of 0.1% SDS by PD models after caffeine treatment

Figure 10 shows each strain's untreated avoidance on the left in red (darker for hermaphrodites, lighter for males) and the avoidance index after treatment with caffeine on the right in blue (darker blue for hermaphrodites, lighter for males). Table 9, below, shows the exact means and standard deviations of each of the strains and genders, before and after caffeine treatment.

Table 9: *Means and Standard Deviations of the Avoidance Indices of Caffeine-treated PD Models*

Strain	Gender	Treatment	Mean	Standard Deviation
N2	Hermaphrodite	None	0.60	0.14
		Caffeine	0.60	0.06
	Male	None	0.60	0.14
		Caffeine	0.57	0.14
him-5	Hermaphrodite	None	0.60	0.17
		Caffeine	0.57	0.14
	Male	None	0.62	0.19
		Caffeine	0.55	0.21
MT15620	Hermaphrodite	None	0.47	0.08
		Caffeine	0.30	0.16
	Male	None	0.45	0.21
		Caffeine	0.23	0.13
CB1112	Hermaphrodite	None	0.25	0.10
		Caffeine	0.41	0.21

Several t-tests were performed in order to determine whether there was a significant difference in avoidance behaviors after caffeine treatment, using Welsh's t-test for parametric data sets and the Mann-Whitney U test for nonparametric data. Only the MT15620 strain showed a significant difference in avoidance index after treatment. The treated hermaphrodite (*M=0.30, SD=0.16*) experienced a significant decrease in its ability to sense and respond to a negative stimulus compared to before untreated data (*M=0.47, SD=0.08*), Welsh's t-test, $t(8.46) = 2.521$, $p = 0.03$. A similar reduction in the males of the MT15620 strain's avoidance behavior was seen when comparing before treatment (*M=0.45, SD=0.21*) and after treatment (*M=-0.23, SD=0.13*), Mann-Whitney U test, *U=12, p=0.03*. No other strains were significantly affected by caffeine treatment nor were there any differences in effect between gender, ($p>0.09$ for all tests).

Overall, the avoidance assays showed that caffeine had a significant negative effect on the ability of one PD model to sense and respond to a negative stimulus and was unable to significantly improve the ability of the second PD model to do so.

Caffeine & PD Summary. Overall, treatment of PD models with caffeine showed mixed results. Although treatment significantly improved the locomotion of the MT15620 hermaphrodites back to wild type level, it significantly reduced the ability of the same strain to respond to a negative stimulus. A reduction in the ability to respond to or sense a positive stimulus was also observed, although this was not significant. Caffeine acts as an antagonist to

adenosine receptors, which act in circuit with dopaminergic neurons in the basal ganglia (Prediger et al., 2013). As caffeine blocks the adenosine receptors, dopaminergic neurons are activated. Given dopamines importance in locomotion, this caffeine-based interaction could be the cause behind the improvements in locomotion. However, it remains unclear why caffeine would have a negative impact on the chemosensory abilities of male and hermaphrodites. The lack of significant response to caffeine in the locomotion of males of the MT15620 strain is likely due to the lack of significant difference from a wild type speed in the model's baseline. Treatment with caffeine also restored the CB1112 strain's ability to sense and respond to an attractive stimulus, but did not improve locomotion or avoidance behavior similarly. Given the mixed results, it appears caffeine may not be the best treatment to address the totality of the symptoms of PD.

Amyotrophic Lateral Sclerosis. The *tdp-1* strains were treated with caffeine as well, and their speed of locomotion and ability to avoid or move towards repellant or attractive stimuli was examined.

Locomotion Assay. Figure 11, below, shows the effects of caffeine treatment on the locomotive speed of the VC549 models of ALS. Once again, due to time and technical constraints, the N2 and RB929 strains were unable to be treated with caffeine.

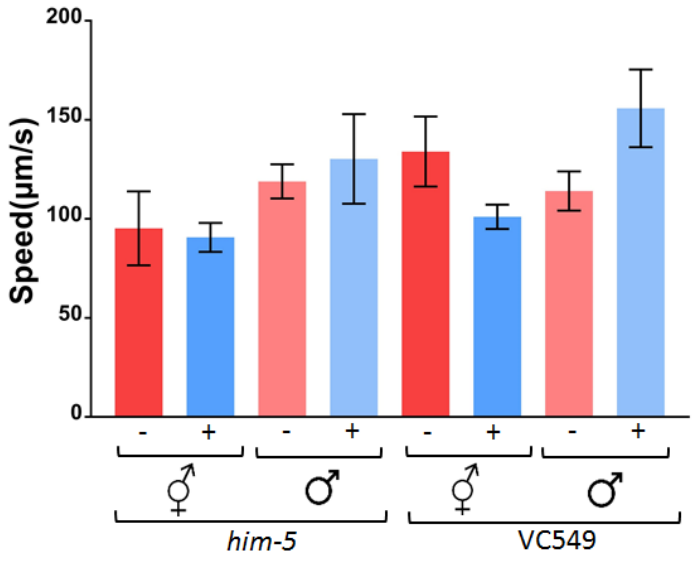

Figure 11: Locomotion of VC549 tdp-1 knockout hermaphrodites after caffeine treatment

Figure 11 above shows the average speed of *him-5* wild type and VC549 ALS disease strain before and after treatment with caffeine. The untreated worms are represented by red columns, while the treatment is represented by blue columns. The hermaphrodites are shown as darker colors, and the males are the lighter columns. The mean speed and standard deviation for each strain, gender and treatment condition can be seen below in Table 10.

Table 10: *Means and Standard Deviations of the Locomotion Speeds of Caffeine-treated ALS Models*

Strain	Gender	Treatment	Mean	Standard Deviation
him-5	Hermaphrodite	None	95	49
		Caffeine	90	17
	Male	None	118	17
		Caffeine	130	39
VC549	Hermaphrodite	None	134	43
		Caffeine	101	15
	Male	None	114	24
		Caffeine	155	33

A Shapiro-Wilk test was completed to test for parametric data sets, and all data groups were found to be parametric. The *tdp-1* knockout strain was tested using Welch's t-tests for its locomotion speed after caffeine treatment. The increased movement speed of disease strain hermaphrodites (*M=134μm/s, SD=43*) was seen to drop after caffeine treatment (*M=101μm/s, SD=15*), although not significantly, Welch's T test, *t(1.76)=6.20, p=0.127*. Similarly, the diseased speed of the male VC549 (*M=114μm/s, SD=24*) was seen to increase (*M=155μm/s, SD=33*), although significantly,Welch's T test, *t(1.90)=3.07, p=0.151*, moving in the opposite direction of the wild type worms. This shows that caffeine was able to partially rescue the increased speed of the hermaphrodites of the diseased *tdp-1* knockout strain VC549, and help rescue the decreased speed of VC549 males in comparison to N2 as seen previously. However, none of the effects of caffeine on the locomotion of ALS models was significant.

Chemosensory Assays. To determine if caffeine treatments impacted the ability of *tdp-1* models of ALS to respond to environmental stimuli, the chemotaxis and avoidance behaviors of the models were examined.

Chemotaxis Assay. Figure 12, below, shows the effects caffeine has on the ability of the *tdp-1* ALS models to respond to an attractive stimulus.

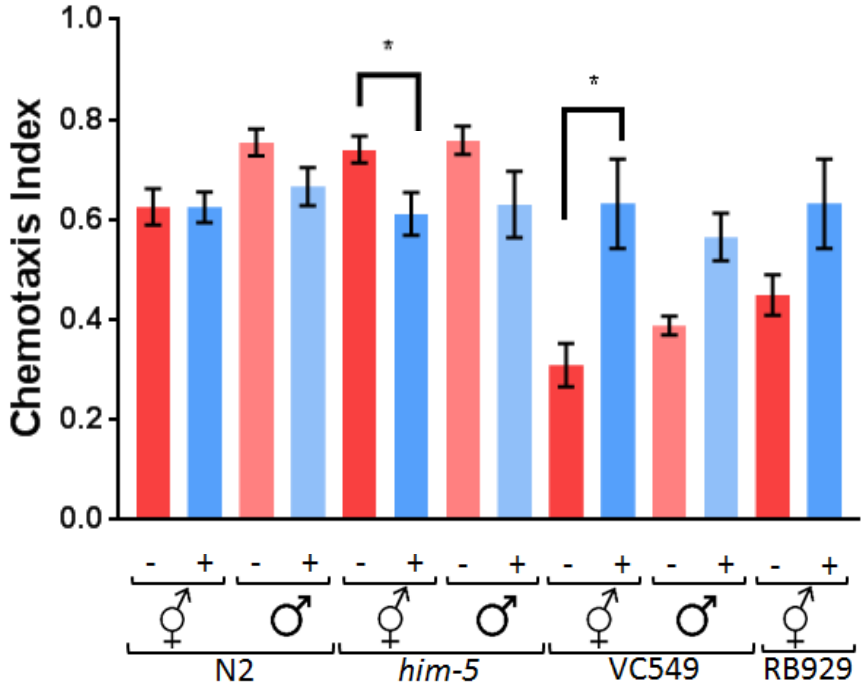

Figure 12: Chemotaxis indices of ALS strains after caffeine treatment

Figure 12 shows each strain's untreated chemotaxis on the left in red (darker for hermaphrodites, lighter for males) and the chemotaxis index after treatment with caffeine on the right in blue (darker blue for hermaphrodites, lighter for males). Table 11, below, shows the exact means and standard deviations of each of the strains and genders, before and after caffeine treatment.

Table 11: *Means and Standard Deviations of the Chemotaxis Indices of Caffeine-treated ALS Models*

Strain	Gender	Treatment	Mean	Standard Deviation
N2	Hermaphrodite	None	0.63	0.11
		Caffeine	0.66	0.05
	Male	None	0.75	0.08
		Caffeine	0.66	0.09
him-5	Hermaphrodite	None	0.74	0.08
		Caffeine	0.64	0.05
	Male	None	0.76	0.06
		Caffeine	0.66	0.14
VC549	Hermaphrodite	None	0.31	0.13
		Caffeine	0.63	0.20
	Male	None	0.39	0.03
		Caffeine	0.57	0.08
RB929	Hermaphrodite	None	0.45	0.13
		Caffeine	0.63	0.20

Multiple t-tests were completed in order to determine whether there was a significant difference in chemotaxis behaviors after each strain was treated with caffeine. All significant data throughout the chemotaxis experiments was parametric, and was therefore analyzed using a Welch's t-test. In the VC549 strain, hermaphrodite treatment with caffeine rescued the ability of the worms to be attracted to a positive stimulus (*M=0.63, SD=0.19*) in comparison to untreated hermaphrodites, Welch's t-test, *t(3.26)=5.974, p=0.017*(*). However, while there was an increase in the chemotaxis index for the male strain, the data was not significant, Welch's t-test, *t(3.44) = 2.61, p = 0.051*. In the RB929 strain, the data is also not significant, however, we do see again that caffeine increased the hermaphrodite's ability to be attracted to a positive stimulus (*M=0.63, SD=0.19*), Welch's t-test, *t(1.87) = 5.70, p = 0.114).*

Overall, the chemotaxis assay revealed that caffeine was only an effective treatment in rescuing the ability of the VC549 hermaphrodite to sense and respond to a positive stimulus. Male deficits in the same strain were not rescued, showing a gender bias in response to treatment.

Avoidance Assay. In contrast to Figure 12, Figure 13 shows the effects caffeine has on the ability of *tdp-1* ALS models to respond to a repellent stimulus.

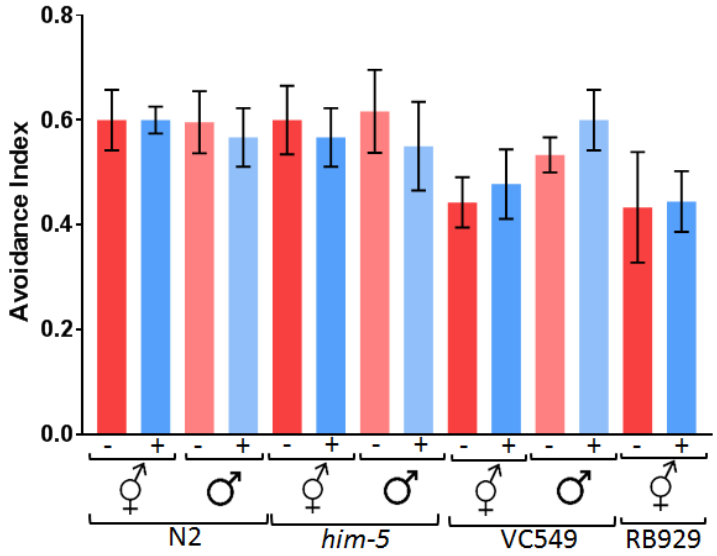

Figure 13: Avoidance indices of ALS models after treatment with caffeine

Figure 13 shows each strain's untreated avoidance on the left in red (darker for hermaphrodites, lighter for males) and the avoidance index after treatment with caffeine on the right in blue (darker blue for hermaphrodites, lighter for males). Table 12, below, shows the exact means and standard deviations of each of the strains and genders, before and after caffeine treatment.

Table 12: *Means and Standard Deviations of Avoidance Indices of Caffeine-treated ALS Models*

Strain	Gender	Treatment	Mean	Standard Deviation
N2	Hermaphrodite	None	0.60	0.14
		Caffeine	0.60	0.06
	Male	None	0.60	0.14
		Caffeine	0.57	0.14
him-5	Hermaphrodite	None	0.60	0.17
		Caffeine	0.57	0.14
	Male	None	0.62	0.19
		Caffeine	0.63	0.21
VC549	Hermaphrodite	None	0.44	0.13
		Caffeine	0.48	0.20
	Male	None	0.53	0.06
		Caffeine	0.60	0.10
RB929	Hermaphrodite	None	0.43	0.26
		Caffeine	0.44	0.17

Several t-test were performed in order to determine if caffeine treatment had any significant effects on *tdp-1* models. The tests revealed that caffeine treatment induced no significant changes in the ALS models ability to avoid a negative stimulus (*p-values>0.70*). Due to caffeine's lack of effect on the avoidance behavior of the *tdp-1* strains in general, there was also no gender bias in response to caffeine treatment.

Caffeine & ALS Summary. The effects of caffeine treatment on *tdp-1* models of ALS were minimal, although there were some interesting results. The treatment did not affect the ability of the worms to respond to a negative stimulus, but possibly because the defects from the mutations weren't far enough from wild type responses to show a change. However, caffeine was seen to rescue the ability of the hermaphrodite of the VC549 strain, but not the male, to respond to and sense a positive stimulus. There were no significant differences seen in the locomotion of the worms after treatment for either gender. Both genders did show a slight positive change in locomotion after caffeine treatment. The hermaphrodites were seen to slow slightly after caffeine treatment, indicating a return to normally slower speed. The males were seen to increase speed slightly after treatment, indicating a return to normally higher speed. This difference in effects as well as the rescue of hermaphrodite, but not male, chemotaxis behavior shows a gender bias in response to treatment. The bias indicates that men may not respond as positively to caffeine treatment for ALS as women. However, the mechanism behind this difference in response is unclear.

Vanillin Treatment

Vanillin's ability to rescue deficits in locomotion and chemosensory behaviors in PD and ALS models was examined as was the interaction of gender with response to vanillin treatment. All statistical analysis was performed at alpha=0.05.

Parkinson's Disease. The *cat-2* strains were treated with vanillin, and the compound's effects on locomotion and chemosensory behavior were examined, as in previous experiments.

Locomotion Assay. Figure 14, below, shows the effects vanillin treatments had on the locomotive speed of the *cat-2* MT15620 strain compared to the *him-5* strain as a wild-type.

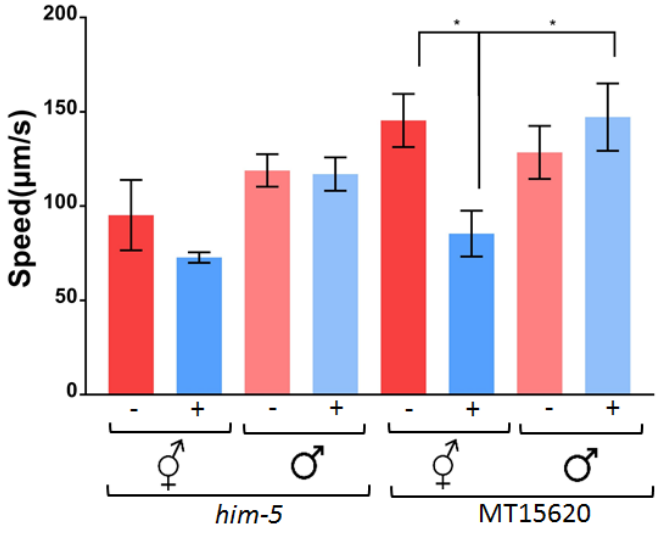

Figure 14: Locomotion speed of PD model with vanillin treatment

Figure 14 above shows the average speed of *him-5* wild type and MT15620 PD disease strain before and after treatment with vanillin. The untreated worms are represented by red columns, while the treatment is represented by blue columns. The hermaphrodites are shown as darker colors, and the males are the lighter columns. The mean speed and standard deviation for each strain, gender and treatment condition can be seen below in Table 13.

Table 13: *Means and Standard Deviations of Locomotion Speeds (um/s) of Vanillin-treated PD Models*

Strain	Gender	Treatment	Mean	Standard Deviation
him-5	Hermaphrodite	None	95	49
		Vanillin	72	4
	Male	None	118	17
		Vanillin	117	21
MT15620	Hermaphrodite	None	145	31
		Vanillin	85	29
	Male	None	128	37
		Vanillin	147	30

A Shapiro-Wilk test was carried out on the PD model vanillin data to test for parametric data. The untreated MT15620 males (*W=0.81, p=0.047)* was again seen to be the only nonparametric data group. To start, the *him-5* wild type hermaphrodites and males were tested

for significant differences in locomotion speed before and after treatment. No significant change in locomotion speed was seen after treatment for both genders of *him-5* worms (*p-values>0.277).* This displays that vanillin treatment has no effect on locomotion of wild type worms. The previously seen increased MT15620 hermaphrodite speed (*M=145µm/s, SD=31*) was seen to drop significantly, Welch's t-test, *t(3.22)=8.43, p=0.011*) after treatment (*M=85µm/s, SD=29*). This shows a partial rescue for normal locomotion behavior in the diseased worms. The untreated male disease worms (*M=128µm/s, SD=37*) were seen to rise slightly after treatment (*M=147µm/s, SD=30*) although not significantly, Mann-Whitney U test, *U=6, p=0.383.* Therefore a significant difference, Welch's t-test, *t(2.86)=3.944, p=0.0468* was seen between the treated hermaphrodites and the treated males. This difference shows that both the disease and the treatment affected the genders differently in the *cat-2* knockout; only hermaphrodites experienced the benefits of vanillin treatment.

Chemosensory Assays. Figures 15 and 16, show the effects of vanillin treatments on the worms' chemosensory behavior, namely their abilities to respond to both a positive and a negative stimulus.

Chemotaxis Assay. Figure 15, below, shows the vanillin-treated *cat-2* strains' ability to move toward an attractive stimulus.

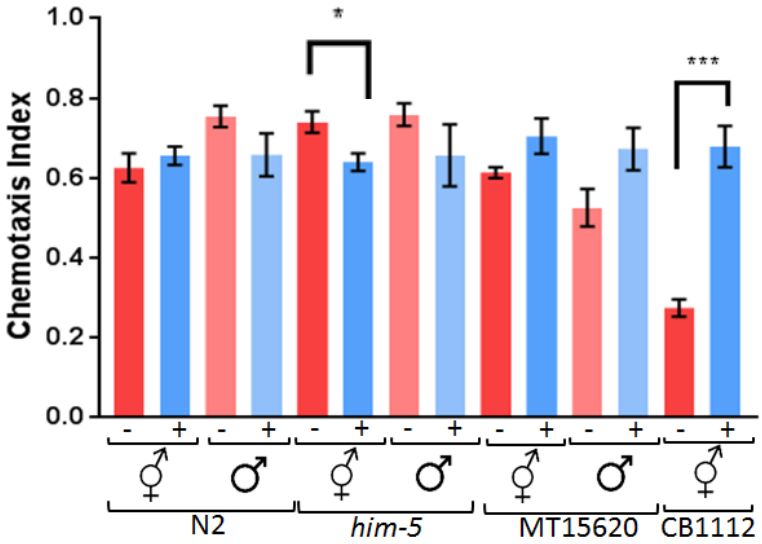

Figure 15: Chemotaxis indices of PD models after vanillin treatment

Figure 15 shows each strain's untreated chemotaxis index on the left in red (darker for hermaphrodites, lighter for males) and the chemotaxis index after treatment with vanillin on the right in blue (darker blue for hermaphrodites, lighter for males). Table 14, below, shows the exact means and standard deviations of each of the strains and genders, before and after vanillin treatment.

Table 14: *Means and Standard Deviations of the Chemotaxis Indices of Vanillin-treated PD Models*

Strain	Gender	Treatment	Mean	Standard Deviation
N2	Hermaphrodite	None	0.63	0.11
		Vanillin	0.66	0.05
	Male	None	0.75	0.08
		Vanillin	0.66	0.09
him-5	Hermaphrodite	None	0.74	0.08
		Vanillin	0.64	0.05
	Male	None	0.76	0.06
		Vanillin	0.66	0.14
MT15620	Hermaphrodite	None	0.61	0.04
		Vanillin	0.70	0.10
	Male	None	0.53	0.10
		Vanillin	0.67	0.09
CB1112	Hermaphrodite	None	0.27	0.07
		Vanillin	0.68	0.12

Multiple t-tests were completed in order to determine whether there was a significant difference in chemotaxis behaviors after each strain was treated with vanillin. All significant data throughout the chemotaxis experiments was parametric, and was therefore analyzed using a Welch's t-test. The figure above displays the chemotaxis index of both *cat-2* strains, MT15620 (the deletion), and CB1112 (the point mutation). The wild type strains, *him-5* and N2, are also displayed, and it can be seen that there is a significant decrease in the *him-5* hermaphrodite worms' ability to be attracted to a positive stimulus, Welch's t-test, $t(2.94) = 12.45$, $p = 0.021$ (*). However, all other wild-type worms were unaffected by vanillin treatment, Welch's t-test, (N2 hermaphrodites: $t(0.705)=12.96$, $p=0.493$; N2 males: $t(1.61)=3.01$, $p=0.205$; *him-5* males: $t(1.24)=2.53$, $p=0.319$). Therefore, MT15620 hermaphrodites post-treatment were compared with only N2 hermaphrodites post-treatment, in order to analyze solely the effects of vanillin and

avoid any confounding effects of the *him-5* mutation. However, while there was an increase in chemotaxis index of the MT15620 strain in both genders after treatment with vanillin, after multiple t-tests it was found that the data was not significant (hermaphrodites: *t(1.98)=4.79, p=0.108; males: t(2.09)=4.86, p=0.092)*. The *cat-2* point mutation, CB1112 was greatly affected. Vanillin rescued the ability of the CB1112 hermaphrodites to be attracted to a positive stimulus (*M=0.68, SD=0.11*), Welch's t-test, *t(7.26)=5.48, p<0.001* (***).

Avoidance Assay. Figure 16, below, shows the ability of *cat-2* worms treated with vanillin to avoid a negative stimulus.

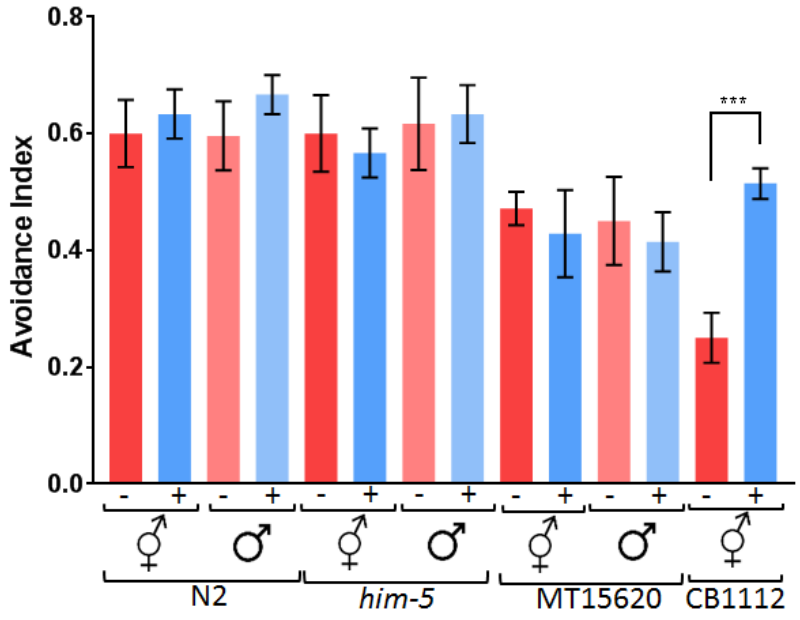

Figure 16: Avoidance indices of 0.1% SDS by PD models after treatment with vanillin

Figure 16 shows each strain's untreated avoidance on the left in red (darker for hermaphrodites, lighter for males) and the avoidance index after treatment with vanillin on the right in blue (darker blue for hermaphrodites, lighter for males). Table 15, below, shows the exact means and standard deviations of each of the strains and genders, before and after caffeine treatment.

Table 15: *Means and Standard Deviations of Avoidance Indices of Vanillin-treated ALS Models*

Strain	Gender	Treatment	Mean	Standard Deviation
N2	Hermaphrodite	None	0.60	0.14
		Vanillin	0.63	0.10
	Male	None	0.60	0.14
		Vanillin	0.67	0.08
him-5	Hermaphrodite	None	0.60	0.17
		Vanillin	0.57	0.10
	Male	None	0.62	0.19
		Vanillin	0.63	0.12
MT15620	Hermaphrodite	None	0.47	0.08
		Vanillin	0.43	0.20
	Male	None	0.45	0.21
		Vanillin	0.41	0.13
CB1112	Hermaphrodite	None	0.25	0.10
		Vanillin	0.51	0.06

A series of t-tests were performed to determine whether treatment with vanillin significantly impacted the ability of the *cat-2* strains to avoid a negative stimulus. The CB1112 hermaphrodite displayed a significant increase it its ability to sense and avoid a negative stimulus between its untreated avoidance index ($M=0.25$, $SD=0.10$) and its avoidance index after vanillin treatment ($M=0.51$, $SD=0.06$), Welch's t-test, $t(8.43)=5.271$, $p<0.001$. The avoidance behavior of no other strains was significantly affected by vanillin treatment ($ps>0.61$).

Overall, the avoidance assay showed that vanillin treatment significantly improved the ability of the CB1112 strain to avoid a negative stimulus, returning its ability to a wild type level. However, vanillin treatment did not affect the ability of the MT15620 PD model strain to respond to the stimulus, regardless of gender.

Vanillin & PD Summary. Vanillin had several positive effects on *cat-2* models of PD. Treatment with the compound returned the speed of hermaphrodite MT15620 locomotion to wild type levels. Although there wasn't a significant effect on the chemosensory abilities of the MT15620 strain, the both chemotaxis and avoidance indices trend toward improvement. However, the CB1112 strain was significantly better at sensing and responding to both negative and positive stimuli after treatment. Interestingly, there was a gender difference in response to treatment present only in how the speed of the MT15620 male was affected. The speed of the

male actually increased, rather than decreasing, although not a significant amount compared to the untreated male. However, this increase in speed did create a significant difference between the treated male and hermaphrodite. It's unclear whether this difference stems from the initial difference in the untreated worms' speed or purely from the effects of the treatment. The multiple positive effects from vanillin treatment make it a candidate for further research as a treatment for PD.

Amyotrophic Lateral Sclerosis. The *tdp-1* strains of *C. elegans* representing ALS models were treated with vanillin and their locomotive speed and ability to respond to a negative and positive stimulus were examined to determine the effects of this treatment.

Locomotion Assay. Figure 17, below, shows the effect vanillin had on the *tdp-1* strains VC549 and RB929's speed.

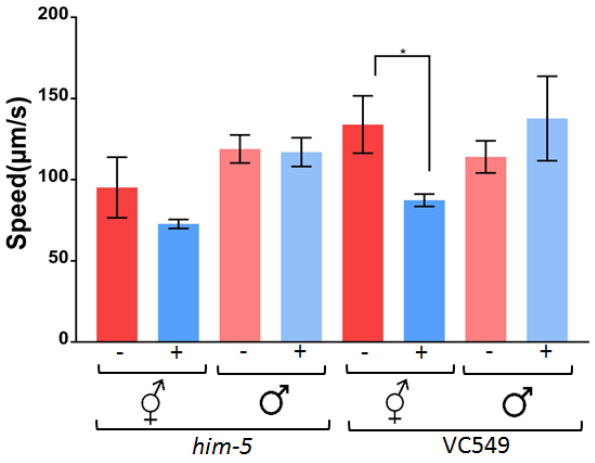

Figure 17: Locomotion speed in *tdp-1* knockout in *C. elegans* after vanillin treatment

Figure 17 above shows the average speed of *him-5* wild type and VC549 ALS disease strain before and after treatment with vanillin. The untreated worms are represented by red columns, while the treatment is represented by blue columns. The hermaphrodites are shown as darker colors, and the males are the lighter columns. The mean speed and standard deviation for each strain, gender and treatment condition can be seen below in Table 16.

Table 16: *Means and Standard Deviations of Locomotion (um/s) of Vanillin-treated ALS Models*

Strain	Gender	Treatment	Mean	Standard Deviation
him-5	Hermaphrodite	None	95	49
		Caffeine	72	4
	Male	None	118	17
		Caffeine	117	21
VC549	Hermaphrodite	None	134	43
		Caffeine	87	9
	Male	None	114	24
		Caffeine	137	45

A Shapiro-Wilk test was initially carried out on the ALS vanillin treatment data to test for parametric data. All data sets were found to be parametric. Therefore, Welch's t-ests were used to detect significant changes in movement speed in ALS model worms after vanillin treatment. The increased speed of the untreated VC549 hermaphrodites (*M=134μm/s, SD=43*) was seen to drop significantly after treatment (*M=87μm/s, SD=9*), Welch's T test, *t(2.58)=5.47, p=0.045*. This significant difference displays a partial rescue of normal movement by vanillin treatment. A negligible change was seen in the male worms before (*M=114μm/s, SD=24*) and after treatment (*M=137μm/s, SD=45*), likely due to lack of a significant disease state and a large variance in the data of treated males, Welch's T test, *t(1.92)=2.09, p=0.190*.

Chemosensory Assays. Chemosensory behavior after treatment with vanillin was also examined.

Chemotaxis Assay. Figure 18 shows the ability of the *tdp-1* ALS models to respond to an attractive stimulus.

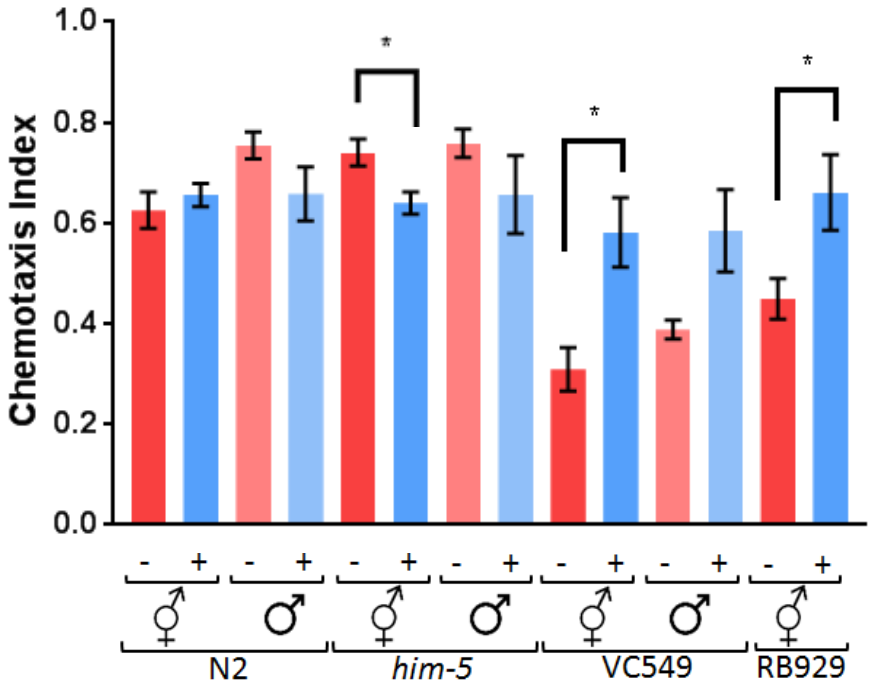

Figure 18: Chemotaxis Indices of ALS strains after vanillin treatment

Figure 18 shows each strain's untreated avoidance on the left in red (darker for hermaphrodites, lighter for males) and the avoidance index after treatment with vanillin on the right in blue (darker blue for hermaphrodites, lighter for males). Table 17, below, shows the exact means and standard deviations of each of the strains and genders, before and after vanillin treatment.

Table 17: *Means and Standard Deviations of the Chemotaxis Indices of Vanillin-treated ALS Models*

Strain	Gender	Treatment	Mean	Standard Deviation
N2	Hermaphrodite	None	0.63	0.11
		Vanillin	0.66	0.05
	Male	None	0.75	0.08
		Vanillin	0.66	0.09
him-5	Hermaphrodite	None	0.74	0.08
		Vanillin	0.64	0.05
	Male	None	0.76	0.06
		Vanillin	0.66	0.14
VC549	Hermaphrodite	None	0.31	0.13
		Vanillin	0.58	0.16
	Male	None	0.39	0.03
		Vanillin	0.58	0.14
RB929	Hermaphrodite	None	0.45	0.13
		Vanillin	0.66	0.17

Multiple t-tests were completed in order to determine whether there was a significant difference in chemotaxis behaviors after each strain was treated with caffeine. All significant data throughout the chemotaxis experiments was parametric, and was therefore analyzed using a Welch's t-test. While there is an increase in chemotaxis index of the VC549 strain males after treatment with vanillin, after a Welch's t-test was completed it was found that the data was not significant, *t(2.34)=2.21, p=132*. However, the VC549 (*M=0.58, SD=0.16*) and RB929 (*M=0.66, SD=0.17*) hermaphrodite*s* were greatly affected by treatment. Vanillin rescued the ability of hermaphrodites from both strains to be attracted to a positive stimulus, Welch's t-test, (VC549: *t(3.32)=7.25, p = 0.012* (*)*;* RB929: *t(2.47)=6.39, p=0.046* (*)).

Overall, the chemotaxis assay showed that only hermaphrodites of the diseased strain experienced positive effects from vanillin treatment, showing a gender bias in response to treatment that reflects the negative male bias of PD in humans.

Avoidance Assay. Figure 19, below, shows the ability of *tdp-1* worms to respond to a negative stimulus after treatment with vanillin.

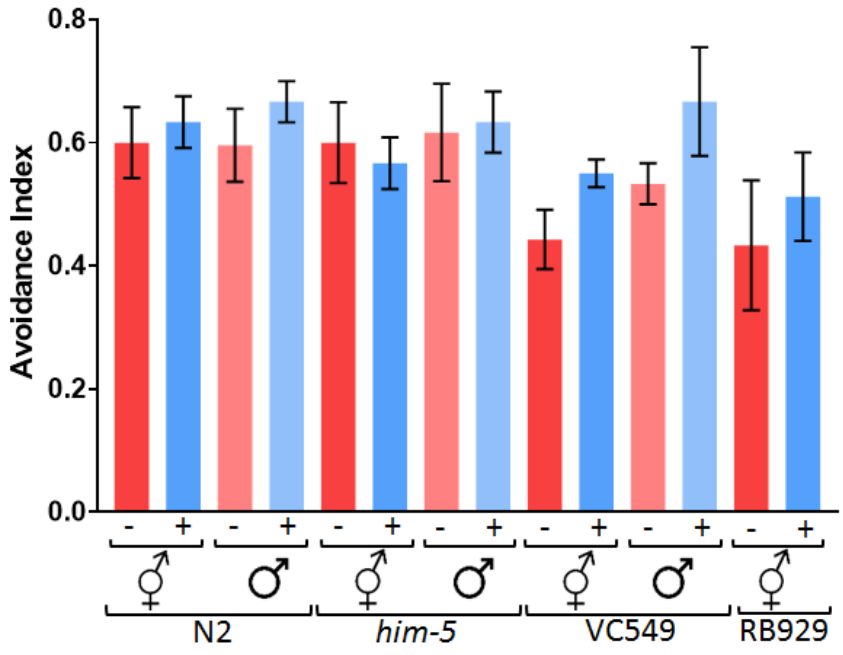

Figure 19: Avoidance of 0.1% SDS by ALS models after treatment with vanillin

Figure 19 shows each strain's untreated avoidance on the left in red (darker for hermaphrodites, lighter for males) and the avoidance index after treatment with vanillin on the right in blue (darker blue for hermaphrodites, lighter for males). Table 15, below, shows the exact means and standard deviations of each of the strains and genders, before and after caffeine treatment.

Table 18: *Means and Standard Deviations of Avoidance Indices of Vanillin-treated ALS Models*

Strain	Gender	Treatment	Mean	Standard Deviation
N2	Hermaphrodite	None	0.60	0.14
		Vanillin	0.60	0.10
	Male	None	0.60	0.14
		Vanillin	0.57	0.08
him-5	Hermaphrodite	None	0.60	0.17
		Vanillin	0.57	0.10
	Male	None	0.62	0.19
		Vanillin	0.63	0.12
VC549	Hermaphrodite	None	0.44	0.13
		Vanillin	0.55	0.05
	Male	None	0.53	0.06
		Vanillin	0.67	0.15
RB929	Hermaphrodite	None	0.43	0.26
		Vanillin	0.51	0.20

A series of t-test were performed to determine if vanillin had significant effects on the avoidance behavior of *tdp-1* ALS models. The tests revealed that vanillin had no significant effects on the ability of the worms to sense and avoid a negative stimulus (*p-values>0.40*). Although there were slight improvements in the ability to avoid a negative stimulus after vanillin treatment in all ALS models tested, these improvements were not significant. It's possible that the effects of the mutations were not significantly different enough from wild type levels for the treatment to improve behavior a significant amount.

Vanillin & ALS Summary. Vanillin treatment of *tdp-1* ALS models displayed promising results. It was able to return the locomotion and chemotaxis behaviors of VC549 hermaphrodites to the wild type levels, and the avoidance behavior of the strain trended in the positive direction. The ability of both the VC549 and the RB929 hermaphrodites to sense and respond to a positive stimulus was also restored to wild type levels by the treatment. No significant effects were seen

in male *C. elegans* after treatment, showing a slight gender bias in response to treatment. Vanillin may be a promising compound for future research as a treatment for ALS, especially for women.

Conclusions & Future Work

C. elegans as a Model for Gender Differences

C. elegans were examined as a potential model system for studying the male gender bias in Parkinson's disease and amyotrophic lateral sclerosis. Results from three different behavioral assays revealed that overall, male *C. elegans* of the PD or ALS strains did not display worse phenotypes than hermaphrodites of PD or ALS strains. Interestingly, while males and hermaphrodites of both diseases experienced similar defects in chemosensory behavior, the locomotion of males was actually slowed in ALS in comparison to the increase in speed seen in hermaphrodites. No significant change in speed was seen for PD males. As a whole, the differences in disease phenotype between genders were not as strong as expected and do not accurately reflect the male bias for both PD and ALS seen in humans.

Despite this lack of main effect from gender, the gender of the worms did appear to have some significant interaction with response to treatment. In several instances only the behavioral deficits observed in hermaphrodites, but not those in males, were rescued by treatment. This bias towards treatment being more effective for hermaphrodites was seen most strongly for vanillin treatment of both PD and ALS. However, the effect was not consistent across strain or assay. Overall, even with the slight gender bias seen in treatment response, *C. elegans* does not seem to be a strong model in which to study gender bias of ALS or PD. However, the inconsistent gender bias observed implies that there could be a difference in the way men and women respond to treatment for both PD and ALS. In future work, gender should be considered as an independent variable when examining potential treatments in order to fully understand the efficacy of a compound.

The results gained from the present study provide further ideas for future research. It's possible that the androdiecious nature of *C. elegans* prevented strong gender bias from being seen. Studying the diseases in close relatives of *C. elegans* with a true female-male dichotomy may reveal a greater gender bias. Alternatively, it's possible that the nematode is too simplistic

of a model system in which to see the gender bias in ALS and PD. The gender biases may stem from something the nematode model lacks, such as certain hormonal interactions unique to mammals. Specific to the lack of gender bias observed in the ALS models, where there are several genes implicated in disease pathology, it's possible that the *tdp-43* gene in humans does not contribute to gender differences. Perhaps another gene thought to be responsible for the disease, such as FUS is implicated in the observed gender differences. Alternatively, the biases could be from environmental factors common sociologically to different genders rather than derived from the genetic basis of gender.

Treatment Conclusions

Caffeine and vanillin were also examined as potential treatments for both PD and ALS. While neither compound rescued every behavioral deficit caused by the disease mutations, both caused some improvements in disease phenotypes.

Caffeine improved the locomotion of PD models, but further damaged their chemosensory abilities. It was also able to improve only the chemotaxis behavior of one ALS model. The mixed results of caffeine treatment for PD and the lack of broad positive effects in the ALS indicate that caffeine may not merit further pursuit as a treatment.

Vanillin treatment, on the other hand, showed many positive results, returning locomotion and chemosensory behaviors to normal levels in both PD and ALS models. Given these benefits, vanillin should be considered in further research. Assays to understand the mechanisms through which vanillin is inducing improved locomotion and chemosensation as well as tests with varying concentrations of the compound could lend more insight into its potential as a treatment. Furthermore, vanillin's effects on other neurodegenerative diseases, such as Alzheimer's disease, could also be examined as could other phenols similar to vanillin, such as syringaldehyde (Aaron et al., 2016).

The different behavioral results and responses to treatment stemming from varying mutations (i.e. differences in speed or chemosensation between the MT15620 and CB1112 mutations) demonstrate the importance of studying several model types for these diseases. The lack of understanding of the cause of both PD and ALS and the multitude of genes that have been implicated in both diseases necessitate that more than one genetic model be examined. This

study's findings indicate that even small differences in the same gene can produce significantly different results in disease expression and response to treatment. For example, the results of assays on the point mutation of the *cat-2* gene often differed greatly from those of the deletion of the *cat-2* gene in unexpected ways--the deletion did not always display the more affected phenotype as was expected. Different mutations in future model systems should therefore be exhaustively exploited in order to capture the intricacies of both PD and ALS.

The complexity of the both of these diseases makes them difficult to study, even in simple models such as *C. elegans*. The mixed results of this research in terms of both gender and treatment reflect this complexity. The research and its results will hopefully add to our understanding of PD and ALS and inspire further research into these diseases, their causes, gender biases and potential treatments. The increasing prevalence of adults over the age of 65 in the United States and around the globe means that the both Parkinson's disease and amyotrophic lateral sclerosis will only become more common in the coming years, making research such as this even more imperative.

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