

Behavioral Characterization of a *daf-18* mutation in *Caenorhabditis elegans*

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INTRODUCTION

Autism Spectrum Disorder is a range of neurodevelopment disorders that are marked by impaired social interaction. The most severe disorder in this spectrum is Autistic Disorder. Children with Autism often have difficulty interacting with other children because they do not understand social cues. Centers for Disease control and prevention estimate that 1 out of 88 children age 8 will have an Autism Spectrum Disorder (Autism Fact Sheet). Children with Autism Spectrum Disorder may have co-occurring conditions such as Fragile X syndrome, tuberous sclerosis, epileptic seizures, Tourette syndrome, learning disabilities, and attention deficit disorder (Autism Fact Sheet). The cause of Autism Spectrum Disorder is still mostly unknown. Research has suggested that the disorder is a result of disruption in fetal brain development caused by defects in genes that control brain growth and brain communication (Autism Fact Sheet).

One gene that has been identified as a potential cause of Autism Spectrum Disorder is *PTEN*. *PTEN* is a tumor suppressor gene that codes for a dual specificity phosphatase affecting G1 cell cycle arrest and apoptosis (Butler *et al.*, 2005). A study involving 18 Autism Disorder Spectrum subjects revealed that 3 out of the 18 subjects had a germline *PTEN* mutation (Butler *et al.*, 2005). Germline cells are cells with genetic material that can be inherited by offspring. This study suggests that *PTEN* mutations may be associated with Autism. The low percentage of subjects with *PTEN* mutation suggests that the *PTEN* mutation alone does not result in Autism. More likely there is a combination of factors that lead to the disorder.

Another study used yeast samples to test the effects of a *PTEN* mutation. They found that the yeast with a *PTEN* mutation suffered a modest loss of growth compared to the yeast without that mutation (Rodriguez-Escudero *et al.*, 2011). However they found that the yeast with that mutation was able to recover their growth. The researchers of this study found that the mutation in *PTEN* that they were studying was not necessarily linked to Autism Spectrum Disorder *PTEN* mutations This is a reason that the researchers also described the need for more models to study the effect of *PTEN* mutations (Rodriguez-Escudero *et al.*, 2011). This suggests that different types of *PTEN* mutations may lead to different results. More models of the effect of *PTEN* gene mutations will help researchers to determine the specific implications of a *PTEN* mutation. Model

organisms such as *C. elegans* are useful in observing the effects of a mutation on a gene such as *PTEN*.

In this study I used *daf-18* mutations in *C. elegans* to model the effect of a *PTEN* mutation in humans. *Daf-18* is the homologue of *PTEN* so it should exhibit similar functions in *C. elegans* as *PTEN* exhibits in humans. *C. elegans* are microscopic worms that have a short life cycle. Their short life cycle will allow me to fully observe the effect of a *daf-18* mutation on *C. elegans*. *Daf-18* mutation in *C. elegans* can be caused by a deletion to the section coding the catalytic domain of the protein (Mihaylova *et al.*, 1999). Mutations on their *daf-18* gene have already been shown to cause problems in the development of *C. elegans* (Fukuyama *et al.*, 2006). More specifically I will observe whether or not a *daf-18* mutation will affect the worms' ability to eat. I believe that the mutation should prevent the mutant worms from positively associating NaCl with food. In order to determine the effect of the mutation I completed five experiments on mutant and wild type *C. elegans*. The first experiment simply tested if the mutation would prevent the worms from associating NaCl with food. The second experiment tested if more time would allow the worms to associate NaCl with food. In the third experiment I increased the time intervals in order to better gauge the amount of time would affect the worm's ability to associate NaCl with food. In the fourth experiment I observed the effect of temperature on the behavior of the *C. elegans*. For the fifth experiment I tested for the effect of NaCl concentration on the behavior of *C. elegans*. These experiments not only provide information on the effect of a *daf-18* mutation on *C. elegans* but they also provide possible insight onto how a *PTEN* mutation could affect a human.

Principal Approaches

The Bio Rad *C. elegans* behavior kit was used to design experiments to study the learning behavior of *C. elegans*. The kit provided a population of wild type *C. elegans* and a population of *daf-18* mutant *C. elegans*. The kit also provided the basic materials and instructions on how to prepare *C. elegans* for experimentation. Before experiments were performed on the *C. elegans*, agar plates were made. NGM lite agar plates were prepared based on instructions from the *C. elegans* behavior Kit and standard NGM lite agar preparation procedures.

NGM lite agar plates were prepared to create a habitat for the *C. elegans* to grow and populate. The two populations of *C. elegans* were grown on separate plates. Along with the *C. elegans*, *Escherichia coli* were placed on the plates to provide the *C. elegans* with food. The NGM lite agar plates contained sodium chloride. The sodium chloride present in the agar plates was intended to allow *C. elegans* to positively associate sodium chloride with food. The *C. elegans* grew on the NGM lite agar plates for a week. While the *C. elegans* were growing and populating additional NGM lite agar plates and sodium chloride free assay plates were prepared. The sodium chloride free assay plates were used to perform all of the experiments. The omission of sodium chloride in the assay plates allowed for the isolation of sodium chloride onto one part of the plate. In order to maintain a healthy population of *C. elegans* while completing experiments the worms were sub cultured every week. Sub-culturing the *C. elegans* involved moving a sample of *C. elegans* from an NGM lite agar plate to a new NGM lite agar plate with a fresh lawn of *E. coli* for food.

Once the *C. elegans* had populated the plate for a week the first experiment was completed. Using the assay plates a line was drawn down the center of the bottom of the assay plate. A 2.5 M NaCl solution was placed on one side of the plate and an equal amount of *C. elegans* buffer on the other side of the plate to be a control. Assay plates were prepared the same way for each experiment except for experiment 5. A sample of mutant *C. elegans* was transferred onto the center of the agar of the assay plate. Using another assay plate a sample of wild type *C. elegans* was placed onto an assay plate. After thirty minutes part of the assay plates the *C. elegans* moved to was observed. In order to represent the data statistically a chemotaxis index was calculated. The chemotaxis index essentially is a statistical representation of the amount of *C. elegans* that moved toward the

chemical sodium chloride. A positive chemotaxis index indicated that more *C. elegans* moved toward the chemical and a negative chemotaxis index indicated that more *C. elegans* moved toward the control. A chemotaxis index near zero indicated that there was similar movement toward both sides. In the second experiment the behavior of the *C. elegans* was monitored over the course of an hour. The procedure for the second experiment was the same as the first experiment except the *C. elegans* were counted after thirty minutes, forty five minutes, and sixty minutes.

In the third experiment the time period before counting *C. elegans* positions on the assay plate was increased in order to learn more about how time affects the behavior of *C. elegans*. The *C. elegans* were counted every hour over a three hour period. In experiment 4 the *C. elegans* were placed in an Enviro-Genie incubator to measure the effect of temperature on *C. elegans*' behavior. Mutant and Wild type *C. elegans* were placed onto the center of two separate assay plates. Both assay plates were then placed in the incubator at 13.5 degrees Celsius. After thirty minutes the amount of *C. elegans* on each side of the assay plates were counted. Then mutant and wild type *C. elegans* were placed on two more separate assay plates. These assay plates were then placed in the incubator at 33.5 degrees Celsius. The amount of *C. elegans* on each of the plates was counted after thirty minutes in order to calculate the chemotaxis index. In experiment 5, three different concentrations of NaCl solution was placed on the NaCl side of the assay plates. The three concentrations were 1.5 M, 2.5 M, and 3.5 M. A sample of Mutant and Wild type *C. elegans* were placed on separate assay plates with each different concentration of NaCl solution. The amount of worms on each side of the assay plates were counted after thirty minutes.

Present Knowledge

Experiment 1

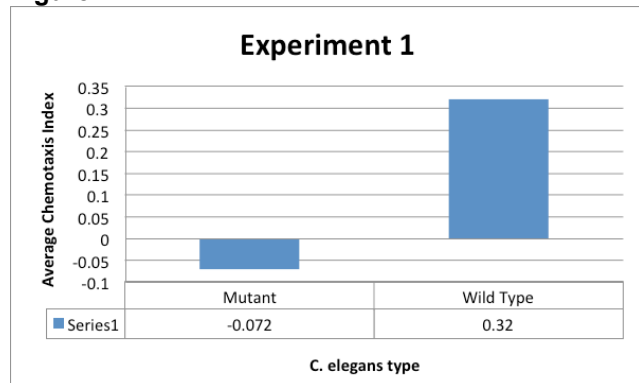
As shown in Table 1. The results of experiment 1 show that the mutant *C. elegans* had negative chemotaxis indices near zero. The wild type *C. elegans* showed positive chemotaxis indices that are still closer to zero than one but reveal a stronger association.

The average mutant chemotaxis index was -0.072. The average wild type chemotaxis index was

0.32. The average mutant chemotaxis index suggests that the mutant *C. elegans* in this experiment were mostly spread evenly across the assay plates. The average wild type chemotaxis index suggests that most of the wild type *C. elegans* moved toward NaCl. These average chemotaxis indices are shown in Figure 1.

Table 1. Expt. 1 Effect of NaCl on *C.elegans* behavior

Trial	<i>C. elegans</i> type	#NaCl side	#Control	Total	Chemotaxis Index
1	Mutant	16	17	33	-0.03
	Wild Type	32	13	45	0.42
2	Mutant	49	63	112	-0.125
	Wild Type	40	22	62	0.29
3	Mutant	102	130	232	-0.12
	Wild Type	88	54	142	0.24

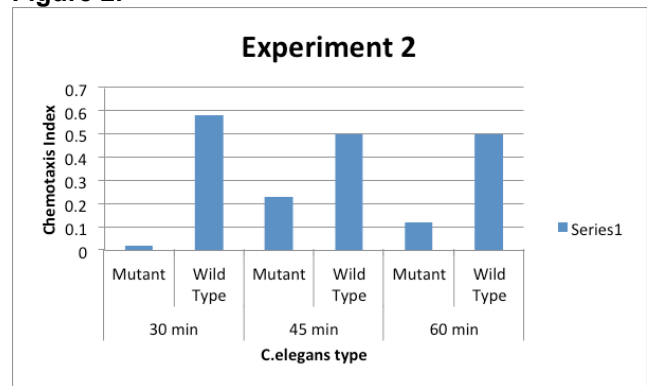
Figure 1.**Experiment 2**

The mutant *C. elegans* chemotaxis indices were all positive and close to zero. The wild type chemotaxis indices were all positive and midway between zero and one. These results are shown below in Table 2.

The mutant chemotaxis index after thirty minutes was 0.018. The wild type chemotaxis index was 0.58. This suggests a moderately strong positive association with NaCl. The mutant chemotaxis index after forty- five minutes was 0.23. This suggests a moderately weak positive association with NaCl. The wild type chemotaxis index after forty- five minutes was 0.5. This suggests a moderate positive association with NaCl. The mutant chemotaxis index after sixty minutes was 0.12. This suggests a weak association with NaCl. The wild type chemotaxis index after sixty minutes was 0.5. These results are also shown in Figure 2.

Table 2. Expt. 2 *C. elegans*' behavior over an hour

Time	<i>C. elegans</i> type	#NaCl side	#Control	Total	Chemotaxis Index
30 min	Mutant	111	107	218	0.018
	Wild Type	19	5	24	0.58
45 min	Mutant	134	84	218	0.23
	Wild Type	18	6	24	0.5
60 min	Mutant	122	96	218	0.12
	Wild Type	18	6	24	0.5

Figure 2.**Experiment 3**

In trial 1 all of the chemotaxis indices were negative except for the wild type *C. elegans* chemotaxis index after 60 minutes. This chemotaxis index was close to zero. All of the mutant chemotaxis indices were negatively close to zero. The two negative wild type chemotaxis indices were negatively midway between zero and one. These results are shown below in Table 3.

In trial 2 the mutant chemotaxis indices were all close to zero but the mutant chemotaxis index after 60 minutes was positive whereas the chemotaxis indices after 120 minutes and 180 minutes were negative. The wild type chemotaxis indices were also close to zero but the wild type chemotaxis index after 60 min was negative and the wild type chemotaxis index after 180 minutes was positive. The wild type chemotaxis index after 120 minutes was zero. These results are shown below in Table 4.

In experiment 3 trial 3 all of the chemotaxis indices were negative. They all exhibited chemotaxis indices between 0.5 below zero and 1 below zero. These results are shown in Table 5.

In experiment 3 trial 4 all of the chemotaxis indices were negative except for the wild type *C. elegans* after 180 minutes. In this trial all of the mutant chemotaxis indices were closer to negative one than zero. The wild type chemotaxis index after 120 minutes was slightly closer to negative one than zero but the other two chemotaxis indices were closer to zero. These results are shown in Table 6.

The average mutant chemotaxis index after sixty minutes was -0.334. The average wild type chemotaxis index after sixty minutes was -0.1. The average mutant chemotaxis index after one hundred and twenty minutes was -0.46. The average wild type chemotaxis index after one hundred and twenty minutes was -0.38. The average mutant chemotaxis index after one hundred and eighty minutes was -

0.378. The average wild type chemotaxis index after one hundred and eighty minutes was -0.27. These results are shown in Figure 3.

Table 3. Expt. 3 trial 1: *C. elegans*' behavior over a three hour period

Time	<i>C. elegans</i> type	#NaCl side	#Control	Total	Chemotaxis Index
60 min	Mutant	41	44	85	-0.035
	Wild Type	10	7	17	0.18
120 min	Mutant	39	46	85	-0.082
	Wild Type	5	12	17	-0.42
180 min	Mutant	42	43	85	-0.012
	Wild Type	5	12	17	-0.42

Table 4. Expt. 3 trial 2

Time	<i>C. elegans</i> type	#NaCl side	#Control	Total	Chemotaxis Index
60 min	Mutant	102	76	178	0.15
	Wild Type	6	4	10	0.2
120 min	Mutant	67	111	178	-0.25
	Wild Type	5	5	10	0
180 min	Mutant	80	98	178	-0.101
	Wild Type	4	6	10	-0.2

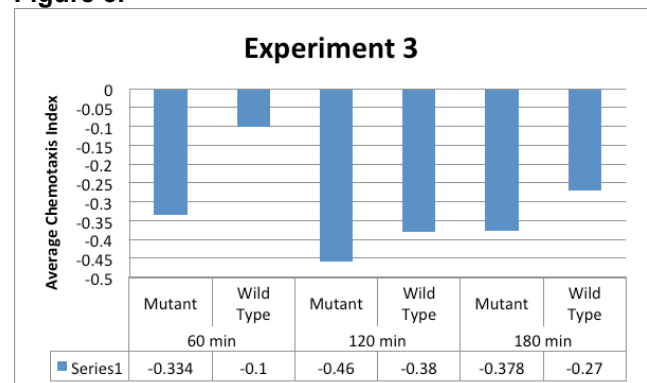
Table 5. Expt. 3 trial 3

Time	<i>C. elegans</i> type	#NaCl side	#Control	Total	Chemotaxis Index
60 min	Mutant	14	103	117	-0.761
	Wild Type	3	9	12	-0.5
120 min	Mutant	16	101	117	-0.73
	Wild Type	3	9	12	-0.5
180 min	Mutant	18	99	117	-0.69
	Wild Type	3	9	12	-0.5

Table 6. Expt. 3 trial 4

Time	<i>C. elegans</i> type	# NaCl side	# Control	Total	Chemotaxis Index
60 min	Mutant	22	122	144	-0.69
	Wild Type	36	66	102	-0.29
120 min	Mutant	16	128	144	-0.78
	Wild Type	48	54	102	-0.59
180 min	Mutant	20	124	144	-0.72
	Wild Type	53	49	102	0.04

Figure 3.



Experiment 4

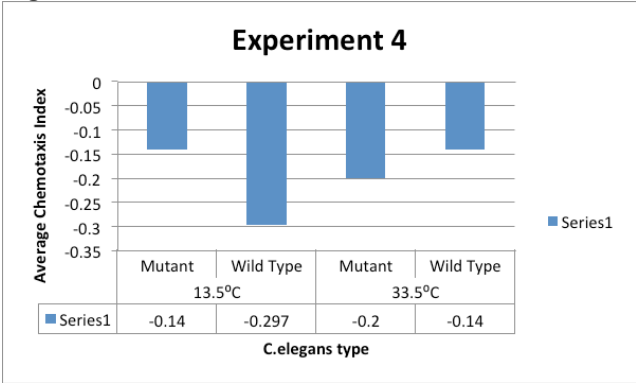
The chemotaxis indices were all negative except for the wild type *C. elegans* in the third trial at 33.5 °C. All of the mutant *C. elegans* chemotaxis indices were closer to zero than negative one. Most of the wild type chemotaxis indices were also closer to zero than one. The wild type *C. elegans* in trial two of both temperature conditions were the only groups to have a chemotaxis index completely in between zero and negative one. These results are shown in Table 7.

The average chemotaxis index of mutant worms at 13.5 degrees Celsius was -0.14. The average wild type chemotaxis index at 13.5 degrees Celsius was -0.297. The average mutant chemotaxis index at 33.5 degrees Celsius was -0.2. The average wild type chemotaxis index at 33.5 degrees Celsius was -0.14. These averages are represented below (Figure 4)

Table 7. Expt. 4. Effect of Temperature on behavior

trial	Temperature(°C)	<i>C. elegans</i> Type	#NaCl side	#Control side	total	Chemotaxis Index
1	13.5	Mutant	141	196	337	-0.16
		Wild Type	5	10	15	-0.33
2		Mutant	78	127	205	-0.24
		Wild Type	5	15	20	-0.5
3		Mutant	75	77	152	-0.013
		Wild Type	35	40	75	-0.06
1	33.5	Mutant	44	87	131	-0.33
		Wild Type	2	6	8	-0.5
2		Mutant	122	154	276	-0.12
		Wild Type	31	28	59	0.051
3		Mutant	105	141	246	-0.15
		Wild Type	31	30	61	0.016

Figure 4.



Experiment 5

In trial 1 the mutant *C. elegans* chemotaxis indices were negative at 1.5 and 2.5 M NaCl concentrations and positive at 3.5 M NaCl concentration. The wild type chemotaxis indices were negative at 1.5 M NaCl concentration and positive at 2.5 M and 3.5 M NaCl concentrations. These results are shown below in Table 8.

In experiment 5 trial 2 all of the chemotaxis indices were negative. They were all also closer to negative one than zero. The results are shown in Table 9.

The average mutant chemotaxis index with 1.5 M NaCl was -0.53. This suggests a moderate negative association with NaCl. The average wild type chemotaxis index with 1.5 M NaCl was -0.54. This suggests a moderately negative association with NaCl. The average mutant chemotaxis index with 2.5 M NaCl was -0.7. The average wild type chemotaxis index with 2.5 M NaCl was -0.125. This suggests a weak negative association with NaCl. The average mutant chemotaxis index with 3.5 M NaCl was -0.415. This suggests a moderately negative association with NaCl. The average wild type chemotaxis index with 3.5 M NaCl was -0.185. This suggests a weakly negative association with NaCl. These averages are shown in Figure 5.

The average chemotaxis indices for each experiment except experiment 2, which did not have averages, are found in Appendix 1. The sample average length of the *C. elegans* and the average amount of *C. elegans* per experiment are found in Appendix 2.

Table 8. Expt. 5 trial 1 NaCl concentration and *C. elegans* behavior

NaCl concentration (M)	<i>C. elegans</i> type	# NaCl side	# control	total	Chemotaxis Index
1.5	Mutant	47	223	270	-0.65
	Wild Type	10	68	78	-0.74
2.5	Mutant	28	165	193	-0.71
	Wild Type	12	53	65	-0.63
3.5	Mutant	20	215	235	-0.83
	Wild Type	12	96	120	-0.6

Table 9. Expt. 5 trial 2

NaCl concentration (M)	<i>C. elegans</i> type	# NaCl side	# control	total	Chemotaxis Index
1.5	Mutant	10	24	34	-0.41
	Wild Type	5	10	15	-0.33
2.5	Mutant	2	11	13	-0.69
	Wild Type	9	4	13	0.38
3.5	Mutant	5	5	10	0
	Wild Type	16	10	26	0.23

Figure 5.

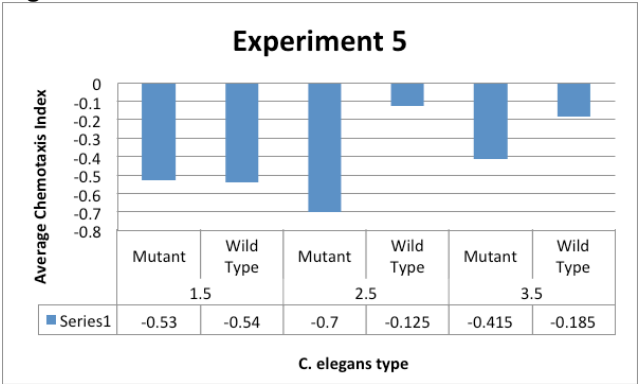


Figure 6.

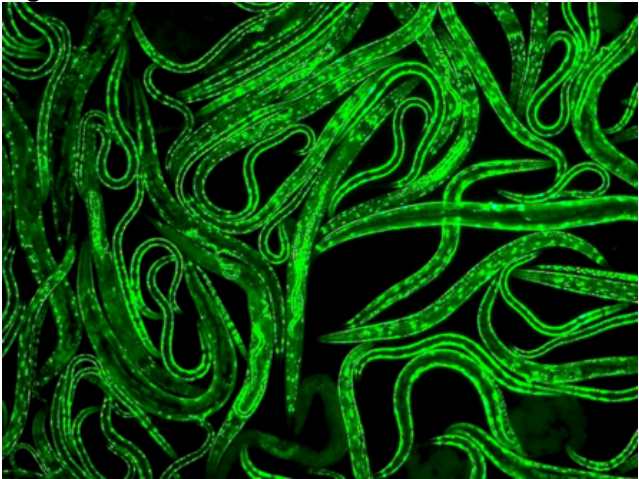


Figure 6. Image taken of GFP *C. elegans* using a compound fluorescent microscope

Conclusion

The results of experiment 1 were mostly consistent with my original expectation that a *daf-18* mutation would prevent *C. elegans* from positively associating NaCl with food. The mutant *C. elegans* had an average chemotaxis index of 0.072. Since the chemotaxis index is close to zero the mutant *C. elegans* most likely were not able to associate NaCl with food. However, the average chemotaxis index of 0.32 for the wild type *C. elegans* is low which would suggest that a large portion of wild type *C. elegans* are not associating NaCl with food. Some of the wild type *C. elegans* may be having trouble associating NaCl with food due to their individual exposure to the chemical on the NGM lite agar plates.

The results of experiment 2 were consistent with experiment 1. Even after an hour the wild type *C. elegans* were attracted to the NaCl side of the assay plates. In experiment 2 the wild type chemotaxis index was even higher than experiment 1. The mutant chemotaxis index was still close to zero which suggests that the mutant worms were not able to associate NaCl with food even after an hour. The results of experiment 3 are not consistent with the original hypothesis. The average mutant chemotaxis indices suggested a negative response to NaCl. The mutant worms should not have a strong negative or positive reaction to NaCl. The average wild type chemotaxis index were negatively close to zero which suggested that the wild type *C. elegans* were not strongly associating NaCl with food.

One possible explanation for these results is that the control side of the assay plates may have contained trace amounts of a contaminant that the *C. elegans* may have used as food. The *C. elegans* need to be in a completely starved environment in order to observe if they associate NaCl with food. The results of experiment 4 are also not consistent with original expectations. Both wild type and mutant *C. elegans* had negative chemotaxis indices near zero for both temperature experimental conditions. These results also suggest that the *C. elegans* may have had contaminants on their assay plates. The fifth experiment produced more negative chemotaxis indices. Food present on assay plates may also be a reason for these results. However, the concentration of NaCl did seem to have some effect on the behavior of the wild type *C. elegans*. At 2.5 M and 3.5 M the wild type *C. elegans* chemotaxis index was closer to zero. This suggests that some of the *C. elegans* may have been associating NaCl with food. Although NaCl was present on these assay plates a lot of the *C. elegans* would most likely still travel to wherever the food was located.

Another possible explanation for the results of the last three experiments could be that some of the wild type *C. elegans* were associating the buffer with food. They also could have been associating NaCl with starvation from crowded plates (Biotechnology explorer team). Another possible factor that could have affected the results of all my experiments is sub culturing. In order to subculture the majority of the worms I used a chunking method. The razor blade I used to cut chunks of agar with *C. elegans* was not sterilized after each use. The lack of sterilization may have been the cause of contamination found in some of the agar plates. Also I may not have gotten enough worms to populate through using the chunking method. *C. elegans* should be transferred properly in order to maintain a growing population (Steirnagle, 2006). The frequency of *C. elegans* sub culturing could also have affected my results. *C. elegans* need to be transferred before food becomes scarce (Steirnagle, 2006). If the *C. elegans* had been living in their optimal experimental environment for survival then the effect of NaCl on their behavior could have been better observed. Overall these experiments suggest that the behavior of *C. elegans* toward NaCl could be impacted by many different environmental and physiological factors

In order to better study *C. elegans* more focus would be put on preventing any source of contamination. All of the experimental material would be made to be of the best quality for the experiment. I would like to complete more experiments on the effect of NaCl concentration on *C. elegans*. Another aspect of the experiment I would like to improve is the sample size for mutant *C. elegans* and wild type *C. elegans*. As shown in Appendix 2, the average experimental amount of mutant *C. elegans* was a lot higher than the average amount of wild type *C. elegans*. Equal sample sizes would make comparing the results more valid. Also as shown in Appendix 2 the sample average size of the wild type *C. elegans* was much greater than the sample average size of the mutant *C. elegans*. I would like to try to determine if *C. elegans* size could affect their learning behavior. I would also like to observe the behavior between wild type *C. elegans* and mutant *C. elegans* on the same agar plate. In order to identify the different types I would use Green Fluorescent Protein, abbreviated as GFP. I have taken an image of GFP worms which shows that GFP can be used as a tagging mechanism for distinguishing between different types of worms. In addition to identification GFP could be used to better study how the physiology of *C. elegans* might affect behavior. GFP could even allow me to visualize *C. elegans* gene expression patterns (Boulin

et al., 2006). Using GFP to study *C. elegans* works well due to the animals thin transparent bodies. The anatomy of *C. elegans* infused with GFP can be viewed without dissection or high powered microscopes (Boulin *et al.*, 2006). With GFP I could observe the effects of a *daf-18* mutation on the physiology of a *C. elegans*. Using GFP would not only allow me to better understand the effects of a *daf-18* mutation on the physiology of the animal but also how their physiology could affect their behavior.

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Appendix 1

Expt. 1 Average Chemotaxis Index

Mutant	-0.072
Wild Type	0.32

Expt. 3 Average Chemotaxis Index by time

Time	<i>C. elegans</i> type	Average Chemotaxis Index
60 min	Mutant	-0.334
	Wild Type	-0.1
120 min	Mutant	-0.46
	Wild Type	-0.38
180 min	Mutant	-0.378
	Wild Type	-0.27

Expt. 4 Average chemotaxis by temperature

Temperature(°C)	<i>C. elegans</i> type	Average chemotaxis
13.5	Mutant	-0.14
	Wild Type	-0.297
33.5	Mutant	-0.2
	Wild Type	-0.14

Expt. 5 Average Chemotaxis Index by NaCl concentration

NaCl Concentration (M)	<i>C. elegans</i> type	Chemotaxis Index
1.5	Mutant	-0.53
	Wild Type	-0.54
2.5	Mutant	-0.7
	Wild Type	-0.125
3.5	Mutant	-0.415
	Wild Type	-0.185

Appendix 2

Sample *C. elegans* length measurements

<i>C. elegans</i>	Wild Type length (mm)	Mutant length (mm)
1	1.2	0.8
2	1.5	0.7
3	1	0.5
4	1	0.4
5	1.2	0.6
6	1	0.7
7	1	0.8
8	1.1	0.6
9	1.2	0.5
10	1.3	0.6
Average	1.15	0.62

Average amount of worms per experiment

<i>C. elegans</i> type	Amount of worms
Mutant	161.05
Wild Type	48.45