Effect of potassium chloride concentration on the sinusoidal movements of the wild-type (N2) and mutant (*unc-2* VC854) strains of *Caenorhabditis elegans*

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Abstract

The objective of our study was to measure the chemotaxis response of the wild type (N2) and mutant (*unc-2* VC854) strains of *C. elegans* at three different potassium chloride (KCl) buffer concentrations. The experiment was performed by transferring *C. elegans*, to an agar plate with 20μL of KCl treatment buffer and recording their sinusoidal head movements after a 10 second acclimatization period. We counted the number of sinusoidal head movements of the *C. elegans* in the videos. We then performed a two-way ANOVA analysis and found that the interaction between the type of strain and the response to the KCl concentration level had a *p* value of 0.08. This indicated that the difference in chemotaxis response to increasing KCl concentration levels between the wild-type (WT) and the mutant strains of *C. elegans* was not significant. We observed no trend in the chemotaxis response of *C. elegans* at increasing KCl concentrations. We observed a trend of higher chemotaxis response rate in WT as compared to mutant. As for the effect of mutation on response to increasing KCl concentration, we observed no trend for the WT; however, there was a trend of increasing chemotaxis response with increasing KCl for the mutant.

Introduction

Caenorhabditis elegans is a transparent, terrestrial roundworm that exists either as a self-fertilizing hermaphrodite or, much less commonly, a male (Wixon et al. 2000). *C. elegans* has an intricate stress response system which enables it to tolerate fluctuations in environmental factors such as osmotic stress, temperature and salt concentration, despite it being only about 1mm in length (Félix and Braendle 2010). Its nervous system consists of a primitive brain made of a ring of nerves, as well as about 1000 G protein-coupled receptors that are involved in the organism's gustation (taste) and olfaction (smell) systems (Félix and Braendle 2010). Much of *C. elegans'* behavior and function is regulated through recognition and discrimination of environmental chemosensory cues (Sengupta 2007). Among such cues are chemoattractants (food sources) and chemorepellents (toxins). In order to survive, the organism depends on its ability to detect,

locate, and navigate toward or away from such cues. *C. elegans'* response to chemosensory stimuli (referred to as chemotaxis response), involves locomotory behavior which is regulated by the organism's motor neurons. Our mutant strain of *C. elegans* (VC854 *unc-2*) has a deletion in the *unc-2* gene, a gene that is required for regulation of dopamine and serotonin levels (WormBase n.d.). This gene is expressed primarily in motor neurons, most sensory neurons in the head region, as well as neurons that control egg laying (Mathews et al. 2003). In our experiment, we investigated the effects of KCl concentration on the chemotaxis response of WT adult *C. elegans* as well as adult *unc-2* mutant *C. elegans.* The chemotaxis response was measured by the number of sinusoidal contractions (Bargmann 1993) in a given period of time. Our hypotheses are as follows:

H_{01}: Increasing KCl concentration has no effect on the chemotaxis response of *C. elegans* as measured by their sinusoidal head movements for both WT and mutant.

H_{a1}: Increasing KCl concentration does have an effect on the chemotaxis response of *C. elegans* as measured by their sinusoidal head movements for both WT and mutant.

H_{02}: Presence of the mutation (*unc-2*) has no effect on the number of sinusoidal head movements of *C. elegans* as measured by their sinusoidal head movements.

H_{a2}: Presence of the mutation (*unc-2*) has an effect on the sinusoidal head movements of *C. elegans* as measured by their sinusoidal head movements.

H_{03}: The effect of KCl concentration on the chemotaxis response of *C. elegans* is the same in WT (N2) and mutant (*unc-2*)) as measured by their sinusoidal head movements.

H_{a3}: The effect of KCl concentration on the chemotaxis response of *C. elegans* is not the same in WT (N2) and mutant ((*unc-2*)) as measured by their sinusoidal head movements.

For H_{01}, we predicted that increasing KCl concentration would result in an increased chemotaxis response of *C. elegans*. We anticipated this because *C. elegans* have demonstrated a locomotory response to chemorepellents (Sengupta 2007) and this would be observed as the concentration approaches 11.51 g/L, which is the highest level tolerated by *C. elegans* (Khanna et al. 1996, see Figure 1).

For H_{02}, we predicted that the presence of the mutation would result in fewer sinusoidal head movements of *C. elegans*. We predicted this due to the diminished function of motor neurons caused by the deletion of *unc-2* gene sequence (Mathews et al. 2003). This is further demonstrated below in our model (Figure 2).

For H_{03}, we predicted that the effect of KCl concentration would produce a lower chemotaxis response in mutant *C. elegans*, as compared to WT. Considering Mathews et al. (2003), demonstrated that the deletion in the *unc-2* sequence hinders the propagation of sensory neuron synapses, hence the *C. elegans*' ability to detect chemical stimuli may be impeded, as further demonstrated in Figure 1 and 2 below.

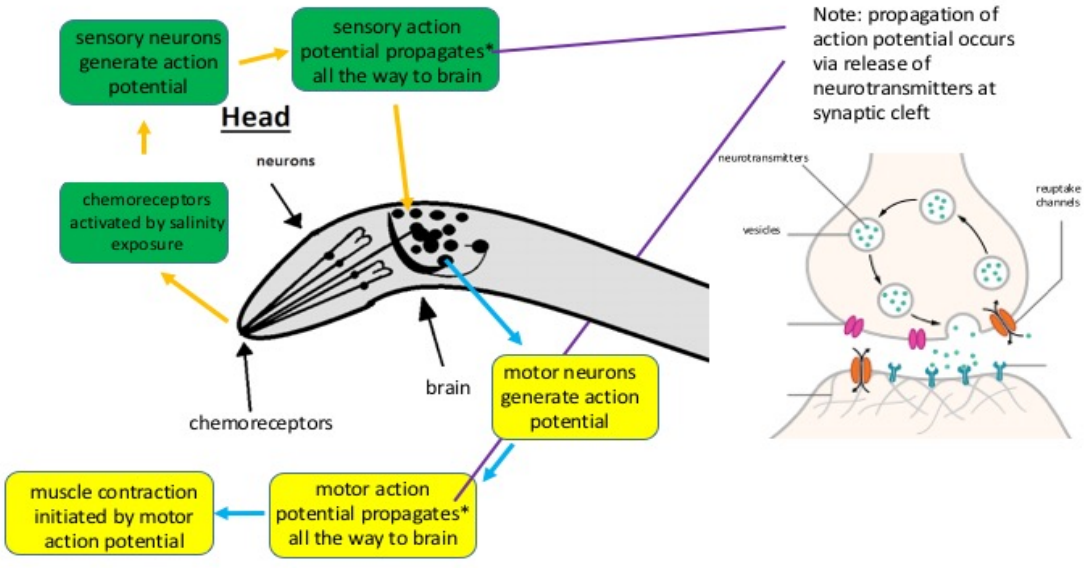

Figure 1. Model of chemotaxis response pathway in WT (N2) *C. elegans* (Figure 1 prepared by Gulaab Sara).

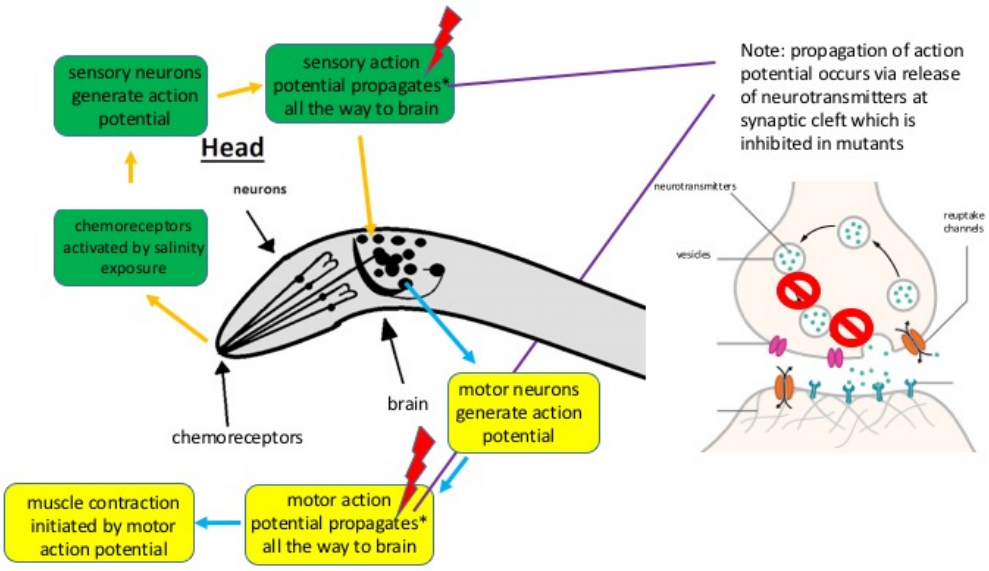

Figure 2. Model of chemotaxis response pathway in mutant (*unc-2*) *C. elegans* (Figure 2 prepared by Gulaab Sara).

Methods

We conducted our experiment using treatments of 0 g/L (control), 5 g/L and 10 g/L KCl buffer solutions. Given that the *C. elegans* are not able to survive in pure water (Nelson and Riddle 1984), we decided to use storage buffer, rather than pure water, as our control and also as the solvent used in our dilutions for the two treatments. 500 mL of storage buffer contained 21.25 mL 0.15M K_2HPO_4, 21.775 mL of 1 M KH_2PO_4, and 2.93g of NaCl. As the storage buffer is used as the solvent in all treatments, we decided to consider the amounts of K and Cl ions already present in the stock buffer solution as the baseline. We conducted our experiment in two separate three-hour- lab periods. During the first lab period we obtained four stock petri dishes which contained agar (medium), adult WT *C. elegans*, and *E.coli* bacteria (food). We used a sterile worm pick to transfer five *C. elegans* (pseudoreplicates) from each stock dish to a petri dish containing only agar, for three seconds. We did this to prevent contaminating the assay dish with *E. coli*. Then, we transferred each *C.elegans* to the assay dish where we had added 20µL of treatment solution. We placed the assay dish under a dissecting microscope, which we had

previously set up with DinoXcope camera. We allowed each *C. elegans* to acclimatize to the new environment for 10 seconds before recording its sinusoidal movements for 20 seconds using the DinoLite software.

To facilitate this process, we marked the microscope's stage plate (Figure 3) as well as the assay dish (Figure 4). This was an efficient way to find the *C. elegans* and monitor their movements under the microscope after we transferred them.

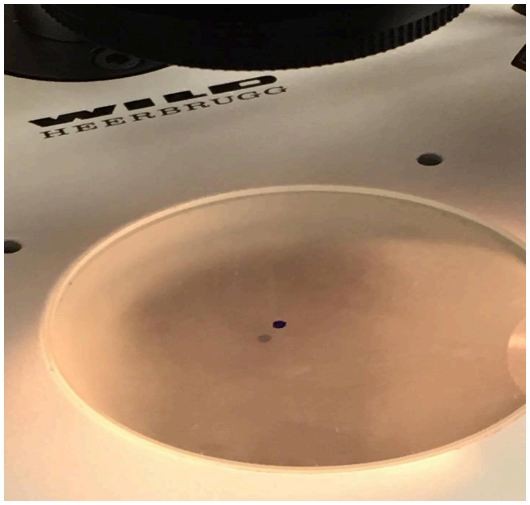

Figure 3. Dissecting microscope stage plate with the marking that was used to quickly find the correct field of view.

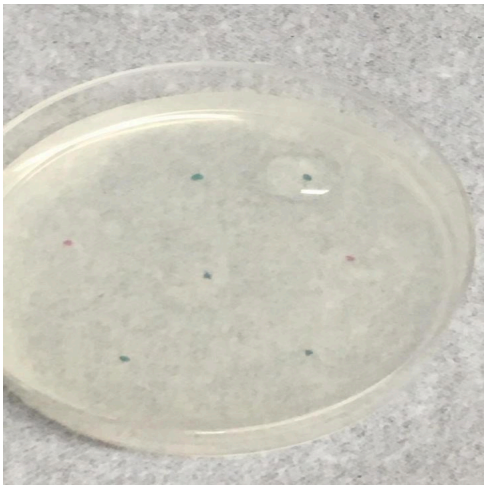

Figure 4. Treatment plate with markings indicating the spacing and amount of KCl used on each plate prior to worm transfer.

We repeated all steps for all three treatments. During the second lab period, we repeated the same procedure using the mutant stock dishes. The entire procedure (outlined in Figure 5) was done in the same room, at the same bench, at the same time of the day to keep the environmental factors consistent. The light intensity of the microscopes was to set to the same setting to keep the amount of heat and light the *C. elegans* were exposed to (from being viewed under the microscope) consistent. After the experiment was complete, we measured the chemotaxis response of each pseudoreplicate by counting the number of sinusoidal contractions the worm experienced in the 20-second window of each video recording (screenshot of video

recording shown in Figure 6). We considered each lateral head movement (90° to the left or right) as a single response, and two group members at a time measured the movements using tally counters. Lastly, because we are investigating two factors, we performed a two-way analysis of variance (ANOVA) on the measurements to test the significance of each factor and also the effect of the factors when interacting together.

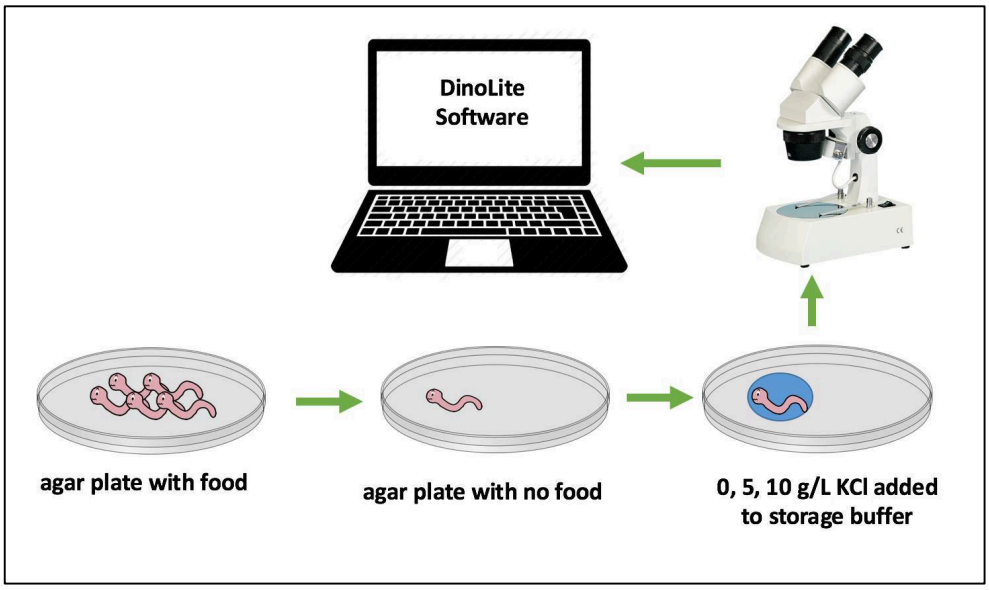

Figure 5. Procedure showing the technique used for each of the four replicates for both mutant and WT *C. elegans*. One *C. elegans* was transferred from the stock plate to a plate with no food and then to the plate where the treatment was applied. Its movements were then recorded using the DinoLite software (Figure 5. prepared by Gulaab Sara)

Figure 6. Mutant (*unc-2*) *C. elegans* as viewed in the dissecting microscope through the DinoXcope at 43.3 X magnification photographed using DinoLite software.

Results

In Figure 7, the mean number of head movements for the WT is significantly higher than the mutant strain. From the two-way ANOVA test, we found that the significant difference between the mean number of head movements of WT and mutant corresponds to a *p* value of 2 x 10^{-16}. The WT response has no obvious trend, since the head movements increased from 0 to 5g/L, but decreased from 5 to 10 g/L. The 95% confidence intervals overlap for the WT mean head movements at the three treatment levels. In contrast, the mutant response increased as KCl concentration increased from 0 to 5g/L and from 5 to 10 g/L. For the mutant, as seen in Figure 7, the 95% confidence intervals do overlap from 0 to 5 g/L and from 5 to 10 g/L, but they do not overlap between 0 and 10 g/L. Figure 7 also shows that the error bars are large for the WT, while they are noticeably smaller for the mutant. Using the two-way ANOVA analysis, the effect of the treatment KCl concentration on the mean head movements produced a *p* value of 0.29. For the interaction between the type of strain and the response to KCl concentration had a *p* value of 0.08.

We observed that WT *C. elegans* were generally larger in size and had thicker bodies compared to the mutants. While in the stock dish, WT *C. elegans* were quite active, whereas the mutants showed generally limited motion. Once we transferred the *C. elegans* to the solution on the assay dish, the WT worms began swimming right away while the mutant *C. elegans* remained motionless for a while and showed limited movement.

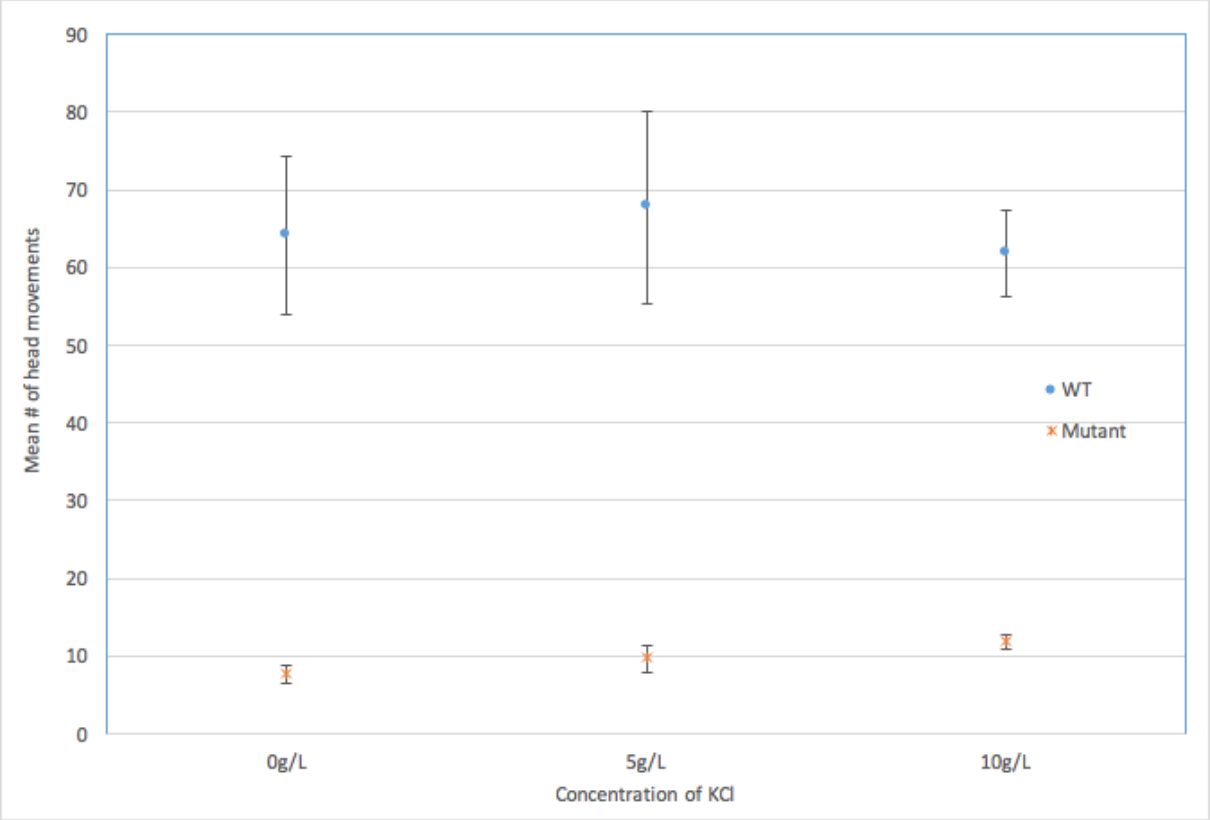

Figure 7. Mean number of sinusoidal head movements, measured for 20 seconds after five seconds of exposure to 0g/L, 5g/L, and 10g/L KCl buffer solution in the WT (N2) and mutant (*unc-2*) strains of *C. elegans*. Error bars show 95% Confidence Intervals. n=4 for WT and n=4 for mutant. Two-way ANOVA *p* values for treatment, type and interaction respectively are: 0.29, $2x10^{-16}$ and 0.08.

Discussion

The results from two-way ANOVA gave *p* values of 0.29 for treatment, $2x10^{-16}$ for the type (WT or mutant) and 0.08 for interaction. These *p* values correspond to hypotheses 1, 2 and 3 respectively.

Hypothesis 1

We failed to reject H_{01} because the *p* value of 0.29 is greater than 0.05. This indicates that increasing KCl concentrations had no significant effect on the chemotaxis response of both mutant and WT *C. elegans* as measured by their sinusoidal head movements. Thus, the results from our experiment show that for both mutant and WT strains, there is no significant increase in the sinusoidal head movements in response to increasing KCl concentrations in their direct

environment. For H_{01}, we predicted that increasing KCl concentration would result in an increased chemotaxis response of *C. elegans*. We anticipated this because *C. elegans* have demonstrated a locomotory response to chemorepellents (Sengupta 2007), we took this as evidence that as KCl concentration increased to a toxic level (Khanna et al. 1996), the chemotaxis response would become pronounced as *C. elegans* would attempt to move away from KCl solution. In particular, Khanna et al. (1996) demonstrated that *C. elegans* could tolerate up to 11.51 g/L KCl without significant lethality. We expected to observe this increased chemotaxis response displayed as an increase in the sinusoidal head movements of *C. elegans* (Bargmann 1993).

However, the results from the experiment are not consistent with our prediction where we expected there to be a significant increase in sinusoidal head movements as KCl concentrations increased. This can be explained by the fact that *C. elegans* have a distinct difference in swimming locomotion in comparison to their regular 'crawling' locomotion (Pierce-Shimomura et al. 2008). It is possible that the *C. elegans* could have already been swimming as fast as they could to try escaping the more concentrated KCl environment. This explanation is further supported by our observations that the *C. elegans* were clearly swimming to the edges of the fluid droplets.

Although our prediction was not supported by the results of the experiment, it could be due to the method with which we measured the chemotaxis response of the *C. elegans*. Perhaps there could be a better way to measure the movement of *C. elegans* that does not involve the counting of sinusoidal head movements, as this may not translate very well to a chemotaxis response. A better way to track movement would have been using the distance travelled by the *C. elegans* as a numerical representation of how far they moved in response to the increasing KCl

concentrations. This method could be done using a camera and computer tracking program, then calculating the distance moved by each worm; this is similar to what Melstrom & Williams, (2007), did in their experiment.

Hypothesis 2

We rejected H_{02} and provided support for H_{a2} because the *p* value of $2x10^{-16}$ is much smaller than the significance level of 0.05. This indicates that the presence of the mutation has a significant effect on *C. elegans* as measured by their sinusoidal head movements. At all treatment levels, the WT *C. elegans* had a significantly higher sinusoidal head movement count than the mutant *C. elegans*. For H_{02}, we predicted that the presence of the mutation would have an effect on the number of sinusoidal head movements of *C. elegans*. We made this prediction considering the effect of the mutation (deletion of *unc-2* gene sequence) on sensory and motor neurons (Mathews et al. 2003). According to Mathews et al. (2003), the *unc*-2 gene encodes the UNC-2 protein, which plays a significant role in the presynaptic release of neurotransmitters such as acetylcholine (ACh) and gamma-aminobutyric acid (GABA). The *unc-2* deletion inhibits the proper functioning of motor and sensory neurons in *C. elegans*. Therefore, we predicted that our mutant *C. elegans* would exhibit a lower rate of locomotion as measured by the sinusoidal head movements. The results from our experiment are consistent with our prediction.

Hypothesis 3

We failed to reject H_{03} because the *p* value of 0.08 was larger than the significance level of 0.05. This result shows that there is no significant difference between WT and mutant *C. elegans* in sinusoidal head movement count in response to increasing KCl concentrations. This implies that the mutant strains do not respond more or less to increasing KCl concentrations as measured by sinusoidal head movements than the WT strains do, and that their sensory

capabilities may not be affected by the presence of the mutation. For H_{03}, according to Mathews et al. (2003), this means that the propagation of nervous impulses could be hindered by deletion of the *unc-2* sequence; hence the *C. elegans*' ability to detect chemical stimuli may be impeded. Due to these reasons, we predicted that the deletion of the *unc-2* gene in the mutant strain (VC854) should negatively affect the organism's chemotaxis response when exposed to different concentrations of KCl, which is a chemical stimulus.

The results from the experiment are not consistent with our prediction that the WT *C. elegans* would have a significantly higher chemotaxis response as measured by sinusoidal head movements, to increasing KCl concentrations as compared to the mutant *C. elegans*. A possible explanation for this is similar to the explanation for hypothesis 1 where the distinct difference between *C. elegans* swimming locomotion and regular locomotion, coupled with the observation that the *C. elegans* were trying to swim away from the solution indicated that the *C. elegans* may have already reached their swim speed limit and could not swim faster and thus, the movement as measured by sinusoidal head count did translate accurately as a chemotaxis response.

Similar to the case in hypothesis 1, the method with which we measured the chemotaxis response may not have been sufficiently accurate and the method mentioned above might be a better way to measure the response. Additionally, an alternative method would be to measure the acclimatization time for the mutant strain in comparison to the WT strain as this would better indicate the ability to detect the difference in KCl concentrations for the respective strains and respond to it. This would also help to overcome the problem of measuring sinusoidal head movements, which is limited to the *C. elegans* maximum swim speed.

Sources of Uncertainty and Variation:

A source of uncertainty or variation is the criteria by which we judged a movement or behavior to be considered a response. Since one person would be judging each replicate at a certain treatment, this could result in an over or under estimation of responses. To minimize the variation, we had two people analyze the DinoLite recording at a time and report the quantitative data after coming to a consensus of what was considered a response. Another source of variation and uncertainty in the experiment is that there could be food contamination, which could affect the response of the *C. elegans*. To minimize this, a blank plate was used in between transfers from the source petri dish to the observational petri dish. However, there could still be food contamination despite the precautions taken, especially in the case of the mutants where it was difficult to transfer them without also picking up some agar along with them. Another variation could be the age of the *C. elegans* used. We minimized this by using only adult *C. elegans*; *however,* although this reduces possible variation it does not eliminate it.

Conclusion

In summary, we failed to reject H_{01} and H_{03}; however, we rejected H_{02} and provided support for H_{a2}. By failing to reject H_{01}, it is shown that increasing KCl concentrations does not cause a significant difference in the chemotaxis response of either the WT or mutant *C. elegans* as measured by sinusoidal head movements. This is inconsistent with our prediction. In the case of hypothesis two, we provided support for our prediction that the presence of the mutation would cause diminished locomotive abilities as measured by sinusoidal head movements. With regards to H_{03}, the results indicate that the mutant strains do not have a difference in response to increasing KCl concentrations as measured by sinusoidal head movements, from the WT strains. This may suggest that their sensory capabilities are not affected by the presence of the mutation; this is inconsistent with our prediction.

Acknowledgements

We would like to thank Dr. Carol Pollock for the unwavering support, dedication and direction she has provided us throughout our experiment. We would also like to thank our lab technician, Mindy Chow, for assisting us in determining the materials we would need as well as preparing them for us in a timely manner. We would also like to acknowledge our teaching assistant Jason Wong for always being there for us when we had a question and helping to oversee that our experiment was going as planned. Lastly, we want to thank the University of British Columbia for providing us with the opportunity to take Biology 342.

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