Attractant preference between NaCl and KCl in wild-type and mutant *Caenorhabditis elegans*

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Abstract

We investigated preferences between the chemical attractants KCl and NaCl in wild-type (strain N2) and mutant-type (strain VC854) *Caenorhabditis elegans* (*C. elegans)*. As *C. elegans* relies on its chemosensory system for survival, we attempted to gain further insight into its sensory system with our experiment. The mutant has an affected *unc-2* gene, which limits movement and sensory function. 25 wild-type and 25 mutant-type nematodes were subjected to a V-shaped choice maze with a time limit of 1 minute (wild type) and 3 minutes (mutant-type). One arm of the maze contained 0.103 M NaCl solution and the other arm contained 0.103 M KCl solution. For the wild type, our data revealed a significant attractant response (choice or no movement) with a chi-square value (X^2) of 12.56 and a *p*-value of 0.0004. An X^2 value of 0.80 and a *p*-value of 0.3711 indicated no significant preference for either attractant in the wild type; however, there was a slight trend for KCl preference. The mutant showed a significant no movement response with a X^2 value of 7.12 and *p*-value of 0.0076, and only 3 nematodes displayed an attractant response. Therefore, we reject our null hypothesis H_{o1}, but failed to reject null hypothesis H_{o2} for wild type. We failed to reject H_{o3} for the mutant type, and in turn had insufficient data to reject H_{o4} or H_{a4}. Studies in the literature support a no movement response from the mutant strain due to the affected *unc-2* gene. The no-preference result of wild-type *C. elegans* may be due to initial habituation conditions and attractant toxicity levels. Further studies should test a larger range of chemical attractants to determine a statistically significant preference in both wild and mutant-type *C. elegans*.

Introduction

Caenorhabditis elegans (*C. elegans*) is a small, transparent nematode that reaches lengths of 1-2 mm as a fully-grown adult. This organism can be found in most temperate soil environments feeding on dead or decaying organic matter (Felix and Braendle 2010). As *C. elegans* lacks both a circulatory and respiratory system, they rely heavily on their chemosensory system to navigate, locate food, and to avoid predators and toxic substances (Sengupta 2007).

The organism has a total of 302 neurons, 32 of which are chemosensory (Lanjuin and Sengupta 2004). The chemosensory neurons are further categorized into 14 types (Lanjuin and Sengupta 2004). These neurons are bipolar and can extend their dendritic ends within the environment, enabling *C. elegans* to sense environmental stimuli such as attractants or repellents (Lanjuin and Sengupta 2004). The mutant-type *C. elegans* (strain VC854) used in our study has a silenced *unc-2* gene. This mutation causes

uncoordinated movement (Brenner 1974) and impedes the detection of certain environmental stimuli, such as the attractant NaCl, due to affected chemosensory neurons (Worm Atlas 2013).

The objective of this experiment was to elucidate the variations between the wild-type and mutant chemosensory systems. The differences in their chemosensory responses were investigated by placing chemical attractants in opposing arms of a V-shaped choice maze and observing the nematodes' preferences (Figure 1). KCl and NaCl were selected as appropriate chemicals for our study as both are widely recognized, and well-studied ionic attractants for *C. elegans* (Riddle et al. 1997). Specifically testing KCl to NaCl is important because the mutant strain used in our study has difficulty detecting Cl^- and Na^+ ions in their environment (Worm Atlas 2013). We expected the wild-type *C. elegans* to display an attractant choice response between KCl and NaCl, and we expected the mutant nematodes to prefer KCl as explained previously. As such, we composed the following hypotheses:

H_{o1}: *Caenorhabditis elegans* (*C. elegans* strain N2, wild-type) will have a no movement response.

H_{a1}: *Caenorhabditis elegans* (*C. elegans* strain N2, wild-type) will have a chemical attractant response.

H_{o2}: *Caenorhabditis elegans* (*C. elegans* strain N2, wild type) will have no preference between the attractants NaCl and KCl.

H_{a2}: *Caenorhabditis elegans* (*C. elegans* strain N2, wild type) will have a preference between the attractants NaCl and KCl.

H_{o3}: *Caenorhabditis elegans* mutants (*C. elegans* strain VC854, mutant-type) will have a no movement response.

H_{a3}: *Caenorhabditis elegans* mutants (*C. elegans* strain VC854, mutant-type) will have a chemical attractant response.

H_{o4}: *Caenorhabditis elegans* mutants (*C. elegans* strain VC854, mutant-type) will have a greater preference for the attractant NaCl than the attractant KCl or have no preference between attractants NaCl and KCl.

H_{a4}: *Caenorhabditis elegans* mutants (*C. elegans* strain VC854, mutant-type) will have a greater preference for the attractant KCl than the attractant NaCl.

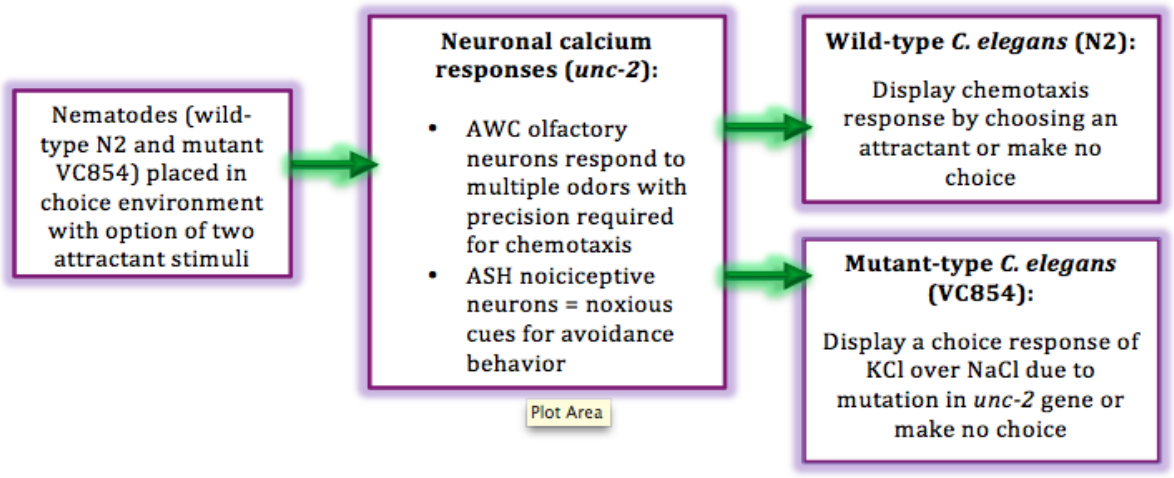

Figure 1: A description of the processes in the chemosensory system of *C. elegans* wild type and mutant type when placed in an environment with chemical stimuli that act as attractants (KCl and NaCl) (Kato et al 2014).

In summary, this study investigates chemical attractant preferences in both wild type and mutant strains of *C. elegans* using a novel experimental method. This is significant as it provides insight into the differences in the chemosensory systems between these two different strains of *C. elegans*.

Methods

A sample size of 25 replicates for mutant type and 25 replicates of wild type was tested. We divided our experiment into four main stages: (1) preparation, (2) data collection, (3) confirmation and (4) data analysis. All replicates were tested and observed at room temperature using the Kyowa dissecting microscope.

(1) Preparation Stage: Set up of mazes

To avoid bias, we randomized the order of the mutant and wild-type nematodes as well as the side of the maze each attractant would be placed. This was done for each replicate by flipping a coin.

Fifty mazes were drawn on the bottom of each agar plate by tracing a V-shaped stencil on the bottom of each Petri dish (Figure 2A). We used a scalpel to make 2.7 cm lengthwise channels in the middle of each arm of the maze approximately halfway into the agar. 10µL of 0.103 M NaCl was pipetted into the top of one channel, and the same was done for 10µL of 0.103 M KCl to the opposing channel (marked X in Figure 2A). The concentrations were selected for both chemicals because they were below the lethal dose for *C. elegans*, yet sufficient enough to be effectively detected (Khanna et al. 1997). Furthermore, NaCl is an optimal attractant in concentrations between 0.1-200 mM (Hart and Chao 2010). Limited research was available regarding the optimal concentration of KCl as an attractant, thus we selected to use a concentration in the mid-range of the NaCl range. After both solutions were injected, we labelled each agar plate according to its corresponding group and replicate number. The agar plates were immediately sealed using Parafilm and the solutions were left to diffuse into the agar for 30 minutes. Thirty minutes was deemed an appropriate amount of time for diffusion based on pre-experimental tests done using food coloring to track the diffusion process (Figure 2B).

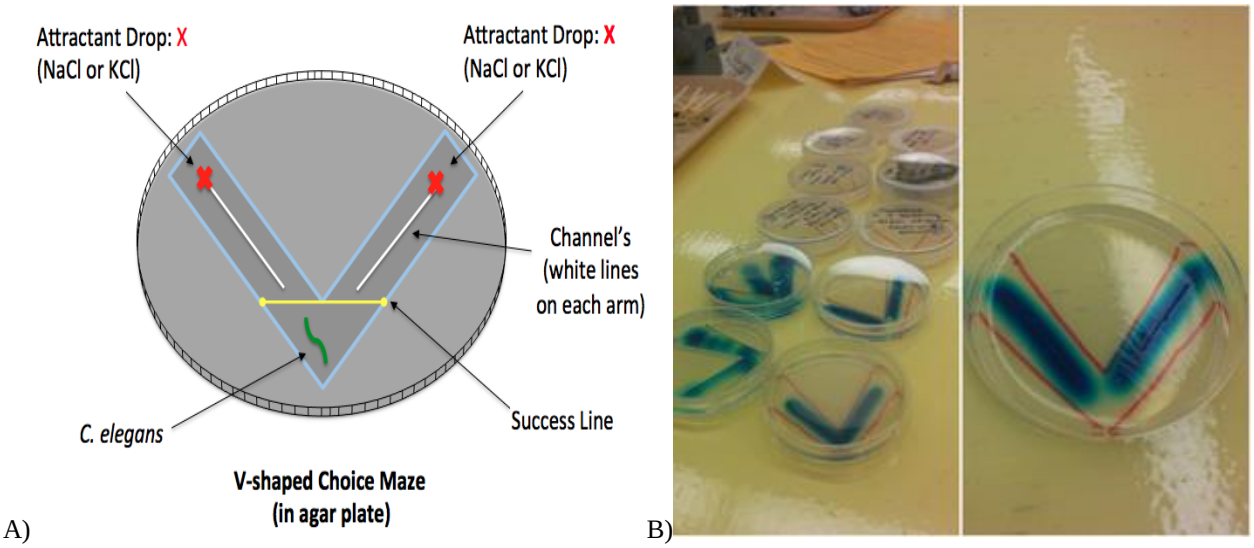

Figure 2: **A) V-shaped stencil for test maze.** This stencil was used to standardize the maze for all replicates. The chemical attractants were pipetted into the top of the channels, marked X, and allowed to diffuse through the agar. The success line was the minimum distance the nematodes had to travel for a chemical attractant response to be determined. **B) Pre-experimental testing for optimal maze construction.** Tested the diffusion rate through the agar medium using food colorants.

(2) Data collection: Monitoring a nematode's choice

For the experiment itself, either a mutant or a wild-type *C. elegans* was transferred from its initial agar plate into its corresponding maze using a worm pick. We attempted to get the nematode to face forward each time when placing them at the starting point to avoid bias towards one side (Figure 2A). For the wild type, each replicate was timed for 1 minute and the mutants were each timed for 3 minutes. We allotted the mutant nematodes with extra time to account for their slow movement as the mutation to the *unc-2* gene impedes *C. elegans* movement. At the end of the timed trial, if the nematode was touching the success line, had travelled past the success line, or had gone into a channel, the trial was considered a success. We documented whether the nematode made a choice and, if applicable, their chemical attractant preference. In addition, any observations on the nematode's behavior were noted throughout the duration of each replicate. These observations included the nematode's initial reaction when placed in the maze, how fast or slow the nematode was moving, and if it had no movement.

(3) Confirmation: Validation of observations

After 30 minutes the replicates were re-examined to ensure their choice was consistent with our initial findings. This technique was used as a method of validation for the initial observations that were recorded.

(4) Data Analysis

The replicate count (i.e. how many nematodes showed a response and made a choice between an attractant) was analyzed with a chi-square test to determine whether the choice of attractant, as well as the preference between attractants, was significant in both wild-type and mutant *C. elegans*. Chemical attractant response (choice or no movement) for the mutant and wild type, as well as attractant preference for the wild-type, were visualized as proportion bar graphs due to the varying sample size between mutant (n=20) and wild-type (n=23) replicates. The replicate count, for both mutant and wild-type choice between right and left sides of the maze, was also analyzed using a chi-square test to check for bias. These results were not depicted graphically.

Results

When exposed to the chemical attractants, wild-type *C. elegans* displayed a significant chemical attractant response with a chi-square value (X^2 (1, N=23)) of 12.56 (critical value=3.84), and a *p*-value of 0.0004, as seen in Figure 3. The chemical attractant response was described in the qualitative data as a movement of the nematode towards a chemical attractant within the trial's times. 20 out of 23 wild-type nematodes (0.87) displayed this response. This was in contrast to a no movement response portrayed by 3 out of 23 (0.13) wild-type nematodes. These 3 nematodes displayed behavior described in the observational data as a "pretzel-like" coiling motion or no movement at all.

When comparing chemical attractant preference of NaCl and KCl, it was determined that there was no significant preference due to a *p*-value of 0.3711 and a chi-square value (X^2 (1, N=20)) of 0.80 (critical value=3.841), as seen in Figure 4. However, there was a slight trend with 12 out of 20 (0.6) wild-type nematodes selecting KCl in contrast to 8 out of 20 (0.4) selecting NaCl.

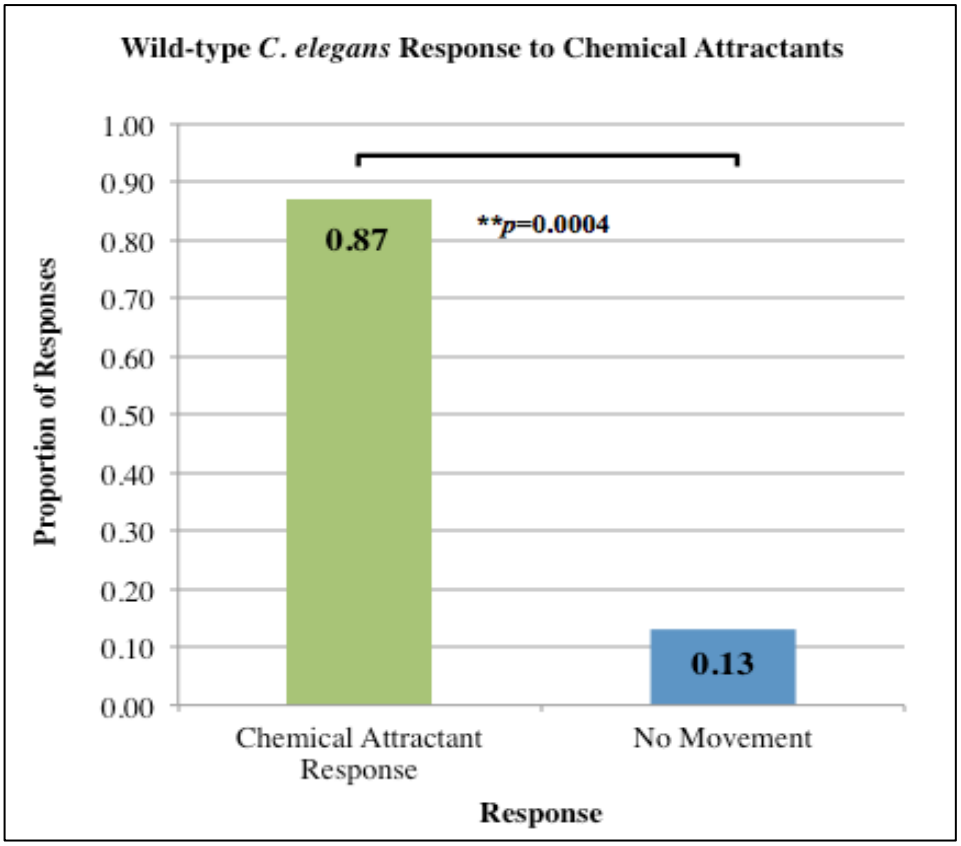

Figure 3: Response of wild-type *C. elegans* exposed to chemical attractants, the wild-type *C. elegans* showed a significantly greater (denoted as **) chemical attractant response than no movement response, X^2 (1, N=23) =12.56 (critical value=3.841), $p< 0.05$ (p=0.0004).

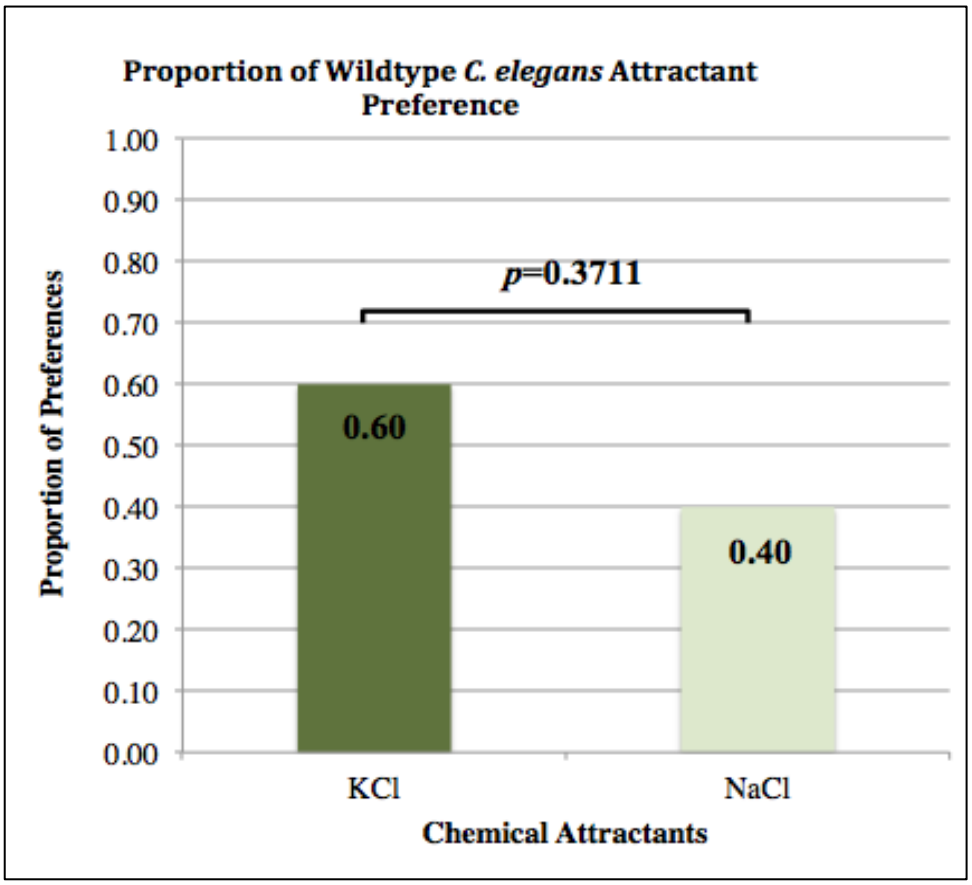

Figure 4: Response of wild-type *C. elegans* exposed to chemical attractants KCl and NaCl, X^2 (1, N=20) =0.80 (critical value=3.841), p>0.05 (p=0.3711); however, there was a slight trend of preference for the chemical attractant KCl.

For mutant *C. elegans*, 3 out of 17 (0.18) nematodes displayed an attractant response and 14 of the 17 (0.82) displayed a no movement response. As seen in Figure 5, a chi-square value (X^2 (1, N=17)) of 7.12 (critical value=3.841) and a *p*-value of 0.0076 indicated that the mutant nematodes significantly performed a no movement response over a chemical attractant response. We were unable to perform a chi-square analysis on the attractant preference response for the mutant-type, as there was insufficient data with fewer than 5 nematodes displaying a response.

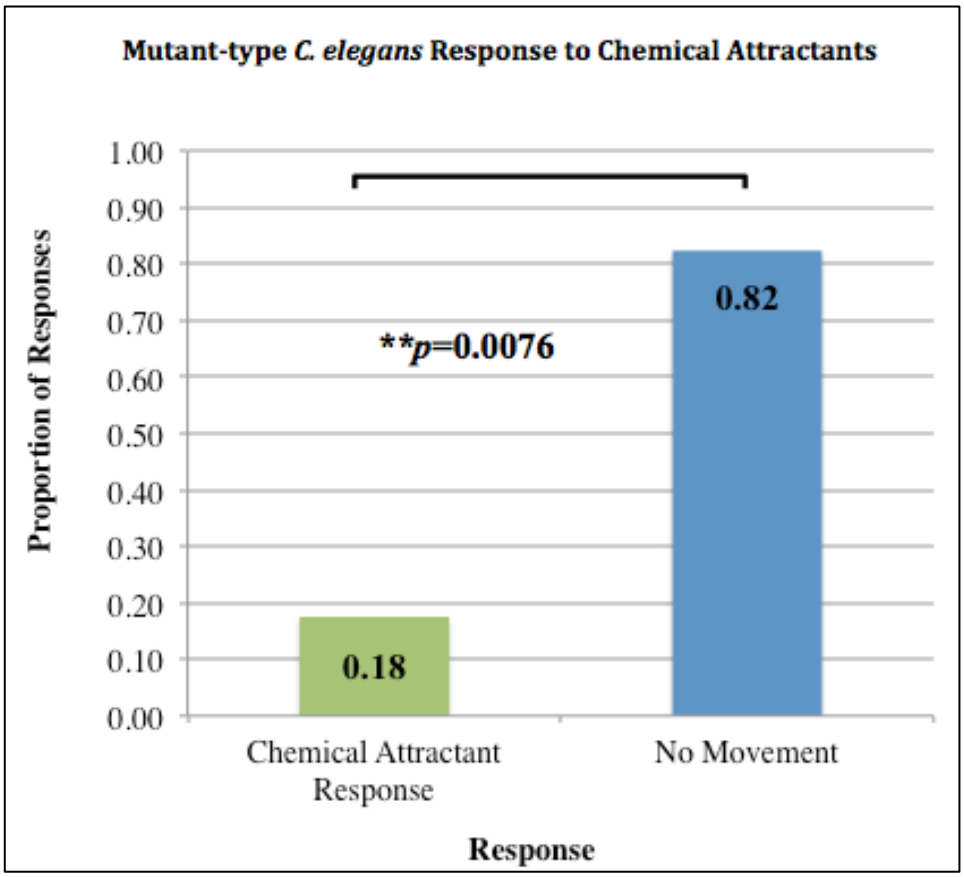

Figure 5: Response of mutant *C. elegans* exposed to chemical attractants, X^2 (1, N=17) =7.12 (critical value=3.841), $p<0.05$ (p=0.0076). As the number of mutant *C. elegans* that did display a chemical attractant preference response was below 5, a chi-square test for attractant preference response was not performed.

To mitigate confounding factors, a chi-square test was performed to ensure that right and left-side preferences were equally distributed in both wild-type and mutant *C. elegans* populations. The results of the analysis indicated that the nematodes did not display a significant preference for either the right or left side of the maze (X^2 (1, N=23) =1.09 (critical value=3.841), p=0.2971).

Discussion

The nematode *C. elegans* relies on its chemosensory system to respond and discriminate between a variety of chemical attractants and repellents (Riddle *et al*. 1997). Many attractants, including Na^+, K^+, and Cl^- are products of bacterial metabolism, suggesting that these ions could be acting as chemical cues in the nematode's environment (Riddle *et al*. 1997). We anticipated that wild-type nematodes would

successfully have a chemical attractant response and, we theorized that their response would be an unbiased chemotactic one (the ability of moving up a chemical gradient in response to a chemical attractant), thereby showing no preference between NaCl and KCl. For the mutant-type *C. elegans,* we expected them to demonstrate a chemical attractant response; however since the mutant's chemosensory system is unable to detect Na^+ and Cl^-, we expected a greater preference for the attractant KCl. The mutant is still able to sense the potassium ion (Chan et al 2012), which should make KCl the more attractive salt.

Our statistical analysis showed that wild-type *C. elegans* did have a significant chemical attractant response over a no movement response (p=0.0004) (Figure 3). Based on this p-value our results were significant and we were able to reject our null hypothesis (H_{o1}) and provide support for our alternate hypothesis (H_{a1}). However, the wild-type *C. elegans* did not display a significant preference between the chemical attractants KCl and NaCl (p=0.3711, Figure 4); therefore, we failed to reject our null hypothesis (H_{o2}).

In regards to the mutant strain of *C. elegans*, exposure to chemical attractants resulted in a significant no movement response in comparison to a chemical attractant response (p=0.0076, Figure 5). Based on this result, we failed to reject our null hypothesis (H_{o3}). As a significant proportion of our mutant nematodes displayed a no movement response, we had insufficient data to determine whether or not there was a preference in the mutants between KCl and NaCl, and thus we could not reject or support either hypotheses (H_{o4} and H_{a4}).

The *C. elegans* wild type demonstrated a choice towards one of the two chemical attractants rather than no movement. This is consistent with previous studies stating that *C. elegans* is capable of moving up a chemical gradient in response to a chemical attractant, a behaviour known as chemotaxis (Appleby 2012). In addition, Troemel (1999) determined that *C. elegans* is attracted to K^+, Na^+, and Cl^-; it follows that the preference between the two attractants should be random. This was indeed the case in our study. However, despite the response not being significant, we did see a slight preference as 12 of the 20 (0.60) nematodes chose KCl over NaCl. Studies in the literature suggest that NaCl is a stronger attractant than KCl for wild-type *C. elegans* (Dusenbery 2005), which is inconsistent within our results.

A possible explanation for the preference of KCl over NaCl could be that the food provided for the wild-type strain had a higher concentration of NaCl. *C. elegans* is capable of acclimating to concentrations of certain chemical attractants (Sanders and Cohen 2012). Acclimation to a high salt concentration reduces *C. elegans'* ability to demonstrate chemotaxis towards that salt (Luo et al. 2014). If the food on the agar plate in which it was grown had a higher concentration of NaCl, the difference between what the wild-type was accustomed to versus the concentration of NaCl that was present in the experimental plate would be much smaller. Thus, the wild-type *C. elegans* would pick KCl rather than NaCl because of its perceived larger chemical gradient. However, if the food had a higher concentration of KCl instead of NaCl, a possible explanation could be that *C. elegans* had learned to associate KCl with food. *C. elegans* is capable of associative learning (Appleby 2012) and it has been shown that if *C. elegans* learns to associate high levels of a salt, such as KCl, with food, they will show a greater chemotactic response towards that salt (Appleby 2012).

Another possible explanation for the slight preference of KCl over NaCl is the varying levels of attraction and toxicity between the two salts. Both KCl and NaCl were present at the same molar concentration of 0.103 M. However, KCl shows significant lethality at 0.15 M whereas NaCl shows significant lethality at 0.27 M (Khanna et al. 1997). The molar concentration we utilized (0.103 M) was closer to the toxicity level of KCl than it was to the toxicity level of NaCl. Studies have shown that NaCl is most attractive to *C. elegans* between 0.0001 M-0.2 M and that this optimal range is directly related to the toxicity level of the salt (Hart and Chao 2010). Assuming KCl follows a similar pattern and that this range is proportional to the toxicity level of the salt, then its optimal range for *C. elegans* attraction should be 0.00006 M-0.1 M. This hypothesized optimal range of attraction for KCl places 0.1 M at the uppermost end of the range. That being said, perhaps KCl was more attractive to *C. elegans* because a molar concentration of 0.103 M was more of an ideal concentration for KCl attraction than it was for NaCl attraction.

The mutant strain of *C. elegans* demonstrated a significant no movement response, thus we failed to reject H_{o3}. As a result we had insufficient data to determine whether or not there was a preference between the two chemical attractants and could not support or reject either hypotheses (H_{o4} and H_{a4}). The

mutation in the strain VC854 of *C. elegans* is a loss-of function allele of the *unc-2* gene (Chan et al. 2012). This causes uncoordinated movement, destabilized serotonin levels and sensitization to dopamine (Chan et al. 2012). Stabilized serotonin levels are needed for proper foraging and movement in the nematodes (Yin et al. 2014). As this mutation alters the movement of the organism, this is most likely the reason why our mutant strain of *C. elegans* failed to move towards either of the two attractants. In addition, mutant-type *C. elegans* also cannot sense or respond to certain environmental stimuli (Chan et al 2012), which may have hindered its ability to sense chemical attractants.

All replicates that took part in the experiment underwent a significant amount of stress from being transferred to and adapting to a new environment. An increase of stress tends to decrease the serotonin levels in *C. elegans* (Yin et al. 2014). The mutant strain is incapable of stabilizing serotonin levels and therefore, if we were to compare a mutant and wild-type nematode under the same amount of stress, we would still see lower serotonin levels in the mutant. Low levels of serotonin, as seen in the mutant, provide another possibility as to why we did not see any movement towards the attractants. In addition, aging decreases the levels of serotonin in *C. elegans* (Yin et al. 2014) and even with the assumption of the mutant and wild-type strains being the same age, we would still see a greater drop in serotonin levels in the mutant (Chan et al 2012).

Sources of uncertainty in our model may have arisen from many factors, such as stress on the nematodes from transferring them from one agar plate to another. Stress in the wild-type could have encouraged a random choice of attractant due to the importance of finding a safer location over moving towards a preferred attractant. An acclimatization period on the new agar plates could have reduced the negative impacts from this transfer. In addition, there may have also been discrepancies between handlers when picking up, transporting and releasing the nematodes. The difference in the level of skill between researchers could have caused varying amount of stress (i.e. transferring the nematode too roughly, creating dents in the agar), which may have been avoided by designating a specific handler to perform the transfers or increased practice with nematode handling, as suggested by laboratory guides (Stiernagle 2005).

Human error within the aspects of our procedure is another potential source of error, such as not placing the organisms in the same initial direction. We also had to consider that because the attractant solutions are transparent, it is impossible to determine whether they were able to diffuse evenly along the channels within the given amount of time. 30 minutes may have not been enough time to allow the solution to diffuse well enough for the nematodes to sense the attractants, which may have attributed to the randomness in attractant preference. The addition of a suitable colorant to the attractant solutions may have aided in determining more exact diffusion times. There were also aspects that were out of our control such as their initial food source and environment, their prior exposure to stress and the age of the nematodes. Although we tried to use worms of approximately equal size, there was no way to ensure they were all identical. Furthermore, we tried to account for possible confounding variables by randomizing our replicates, and analyzing our data for right and left side preferences, which revealed no significant effect of side on choice (p=0.2971).

Future studies on this topic should investigate the attractants at varying concentrations and diffusion levels into the agar, as well as considering a different maze design. It may also be interesting to look at a larger range of different chemical attractants for the wild-type to choose from to see if there is significant preference.

Conclusion

When exposed to chemical attractants, the wild-type *C. elegans* displayed a significant chemical attractant response; therefore we were able to reject our first null hypothesis (H_{o1}). However, wild-type *C. elegans* did not display a significant preference between KCl and NaCl, so we failed to reject our second null hypothesis (H_{o2}). This could be due to varying preference for chemical attractant concentrations (Khanna et al. 1997) or habituation to environmental stimuli (Sanders and Cohen 2012). In contrast, the mutant nematodes displayed a significant no movement response when exposed to chemical attractants and as such we failed to reject our third null hypothesis (H_{o3}). This could be due to the fact that the affected *unc-2* gene has an uncoordinated movement mutation (Chan et al. 2012) or due to low serotonin levels which are required for proper foraging and movement (Elkes 1997). As we had a significant no

movement response in mutant *C. elegans* we had insufficient data to determine if there was a preference response between NaCl and KCl (H_{o4} and H_{a4}). This study is significant as it outlines an experimental method for studying chemosensory responses, provides preliminary research regarding chemical attractant preferences in mutant nematodes and further elucidates the broadly studied chemosensory system of *C. elegans*.

Acknowledgements

We would like to thank Dr. Carol Pollock for her guidance in regards to the focus of our research, as well as the methodology for our experiment. We would also like to thank Katelyn Tovey for providing input as to which attractants to use and Mindy Chow for providing us with the lab equipment necessary to conduct the experiment. Finally, we would also like to thank the University of British Columbia (UBC) for the opportunity to take this course and for providing the necessary funding.

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