Effect of isoamyl alcohol on aggregation of wild-type and mutant *Caenorhabditis elegans*

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Abstract

Caenorhabditis elegans respond to chemical stimuli through olfaction involving transfer of chemical signals via synapses to produce an elicited response which results in moving towards, away, or producing a neutral response to the chemical stimuli (Bargmann 2006). Using isoamyl alcohol as an attractant, both mutant *C. elegans* and wild type were exposed for a set amount of time and aggregation was observed after 30 minutes. Isoamyl alcohol was found to affect the aggregation of *C. elegans* when compared to control conditions (p=0.0005), the aggregation of the mutant phenotype was different than wild type (p=0.0051), and both the wild type and mutant responded differently to the attractant (p=0.0051). It is suggested that *unc-2* regulating odorant receptor gene *str-2* explains the mutant's minimal response to attractant.

Introduction

Caenorhabditis elegans are free-living organisms with a complex nervous system consisting of 302 neurons, of which 282 belong to the somatic nervous system (Bozorgmehr *et al.* 2013). Chemosensory and mechanosensory neurons dictate the worm's ability to move and respond to various external stimuli (Bargmann 2006). These neurons send electrical signals to interneurons and motor neurons to initiate movement (Hart and Chao 2010). Locomotion occurs by the head bending in the desired direction, followed by contraction of the anterior and posterior muscles lining the body wall (Riddle *et al.* 1997). Their transparent, 1-mm long body can then be propelled forward or backwards resulting in wave-like bending of the body (Bono and Maricq 2005). In the presence of a chemical stimulus, the olfactory neuron, AWC, prompts the *C. elegans'* body to turn as a response to search for food as illustrated in Figure 1 (Chalasani *et al.* 2007).

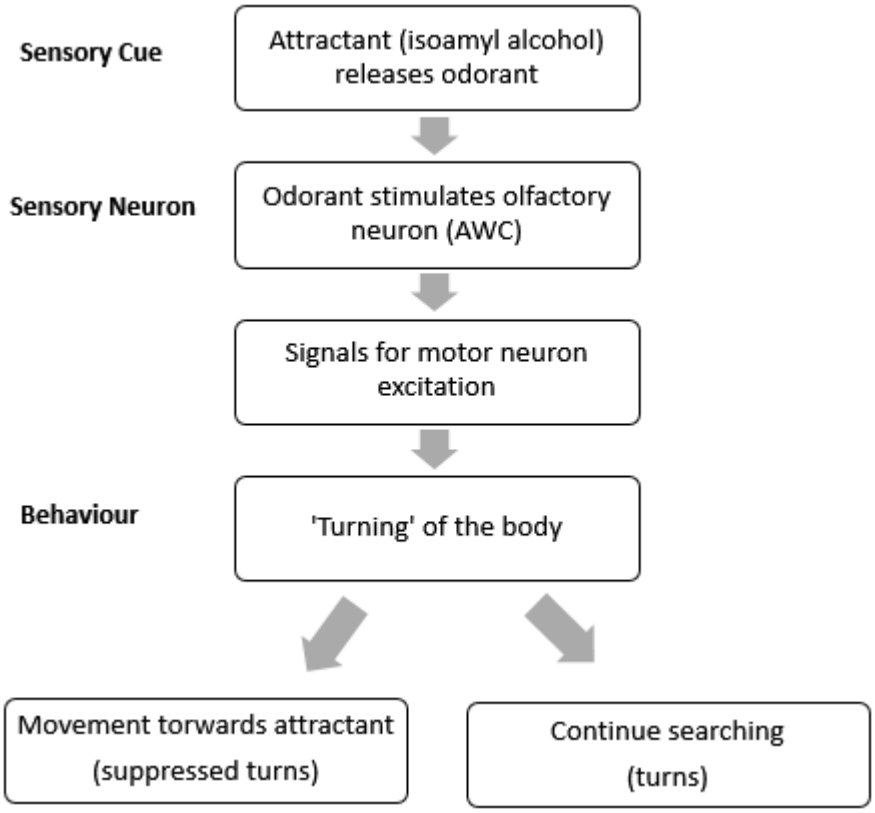

Figure 1. Biological model pathway of *C. elegans* when exposed to an attractant. The motor neuron initiates turning of the body and when moving towards an attractant, the turns are suppressed.

The objective of this experiment was to determine how the *unc-2* mutant (VC854) responds to the attractant isoamyl alcohol in comparison with the wild-type response. The mutant used in this experiment appears physically identical to the wild type (N2) and only differs in a deletion in the *unc-2* gene. Unc stands for uncoordinated and is found primarily in the motor neurons of *C. elegans* where it regulates the release of neurotransmitters (Bargmann 2006). This gene mutation leads to a decrease in motility and desensitization to dopamine, which regulates movement and emotional response (Mathews *et al*. 2003).

For the experiment, we had three sets of hypotheses. The first null hypothesis is presence of attractant has no effect on the aggregation of *C. elegans*. The first alternative hypothesis is presence of attractant has an effect on the aggregation of *C. elegans*. The second null hypothesis is presence of mutation has no effect on the aggregation of *C. elegans*. The second alternative hypothesis is that the presence of mutation has an effect on the

aggregation of *C. elegans*. The third null hypothesis is the effect of the presence of the attractant on the aggregation of *C. elegans* is the same in wild type and mutant. The third alternative hypothesis is the effect of the presence of attractant on the aggregation of *C. elegans* is not the same in wild type and mutant.

We predicted that both the wild type and mutant *C. elegans'* aggregation would be affected when exposed to the attractant and therefore they would respond to the attractant equally despite the *unc-2* gene mutation. This prediction is derived from a study by Bargmann (2006) which tested the response of wild- type *C. elegans* when exposed to various types and concentrations of attractants and repellants. Because the *unc-2* gene mutation mainly affects the mobility of *C. elegans* (Mathews *et al.*, 2003), we predicted the mutants would exhibit a response similar to the wild type.

Methods

To test our hypotheses, we prepared 1-mL solution of 10% isoamyl alcohol diluted from a 100% stock solution. Additionally, 1 mL of sterile distilled water was used as the control treatment. To create an area for counting nematodes, the field of view was calculated by setting the total magnification of the dissecting microscope to 25x. This resulted in a field of view with a diameter of 1.5 cm. A 1.5 cm diameter circle cut-out was made to ensure consistency when counting on different agar plates by tracing it on the bottom of each plate. A point was then marked in the middle of each outlined field of view to maintain consistency of the location of the water or isoamyl alcohol droplet. Each group member was assigned one of the four following treatments: mutant control, mutant attractant, wild-type control, and wild-type attractant. Four replicates were used for each treatment. Each member had an area set up as seen in Figure 2. Treatments began by preparing an agar plate with marked field of view and removing *C. elegans* (either mutant or wild type) from prepared agar plates which included a growth medium. *C. elegans* were transferred to treatment agar plates using sterile

technique until a total of 10 were present. Transferred individuals were placed randomly around the treatment plate.

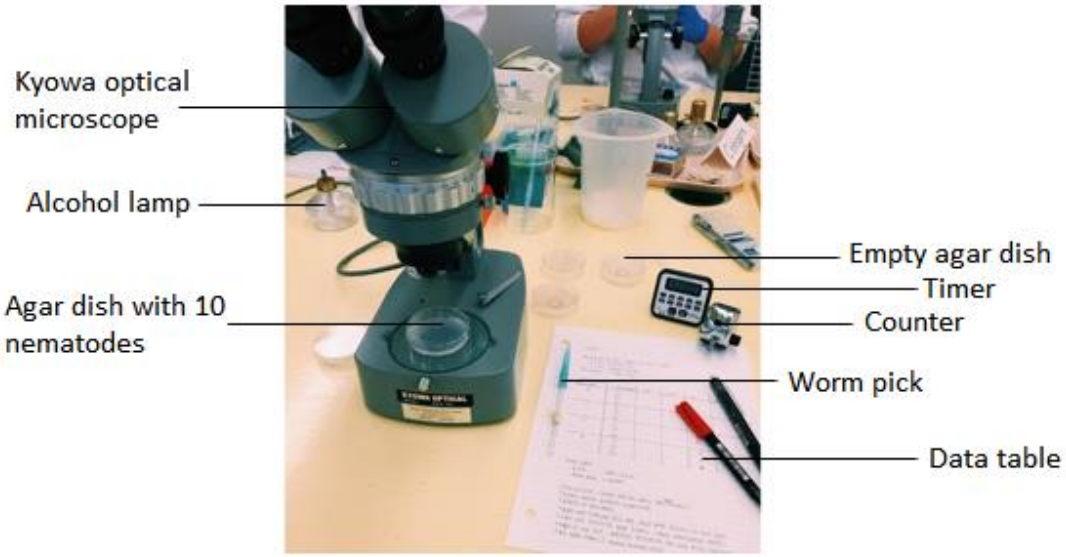

Figure 2. Set-up of the experiment. The agar dish on the microscope stage contains freshly transferred nematodes.

After transferring the *C. elegans*, 1 µL of diluted isoamyl alcohol or sterile distilled water was applied to the centre of the plate using a micropipette and the plate lid was immediately put over top. Observation began immediately following lid placement and the total number of *C. elegans* was counted inside the marked field of view to produce the time = 0 observation data. Additionally, qualitative data were obtained following the counting of the number of individuals in the field of view to measure the differences in location and movement including those not within the field of view. Observations continued at 15 and 30-minute time points after which the plate was removed from the dissecting microscope and observations stopped. A 30-minute time period was chosen because wild-type *C. elegans* have been shown to ignore the attractant after 30 minutes (He *et al.* 2012). Treatment plates undergoing experimentation were gently placed to the side of the dissecting microscope between observation times to reduce the effects of vibration disturbing the nematodes and

heating of the agar plate. After successful preparation of the agar plate with treatment conditions met and initial observations noted, a new plate was prepared and the process repeated.

Following observation and data collection for all four treatments, proportions were determined by dividing the total number of nematodes observed at 30 min by 10. Proportions of nematodes in replicates for all four treatments at 30 min were analyzed using a two-way ANOVA to determine the effects of the mutation and attractant on aggregation.

Results

Analysis of presence of attractant on the proportion of *C. elegans* after 30 minutes using two-way ANOVA ($p = 0.0005$) where $p<0.05$ resulted in a statistically significant difference between the proportion of *C. elegans* and presence of attractant. Analysis of phenotypes on the proportion of *C. elegans* after 30 minutes using two-way ANOVA ($p = 0.0051$) where $p<0.05$ resulted in a statistically significant difference between mutant and wild type. Analysis of change in proportion between phenotypes in the presence of attractant using two-way ANOVA ($p = 0.0051$) where $p<0.05$ resulted in statistically significant difference between phenotypes as both do not respond at the same rate.

Mean proportion of *C. elegans* after 30-minute exposure for all treatments are as follows: wild-type control (n=4) is 0.1 ± 0.1, wild-type attractant (n=4) is 0.7 ± 0.3, mutant control (n=4) is 0.1 ± 0.1, and mutant attractant (n=4) is 0.2 ± 0.1 (Figure 3). As indicated above and shown in Fig. 3, both wild-type control and mutant control have the same mean proportion and the same 95% confidence interval.

Trends indicate both wild type and mutant do not have high response at control treatments (Figure 3). When exposed to the attractant, the wild type displays a much greater response than the mutant with attractant and the control. The mutant attractant treatment is

different than control as the response is much less than wild type, although more variable as shown by larger error bars (Figure. 3).

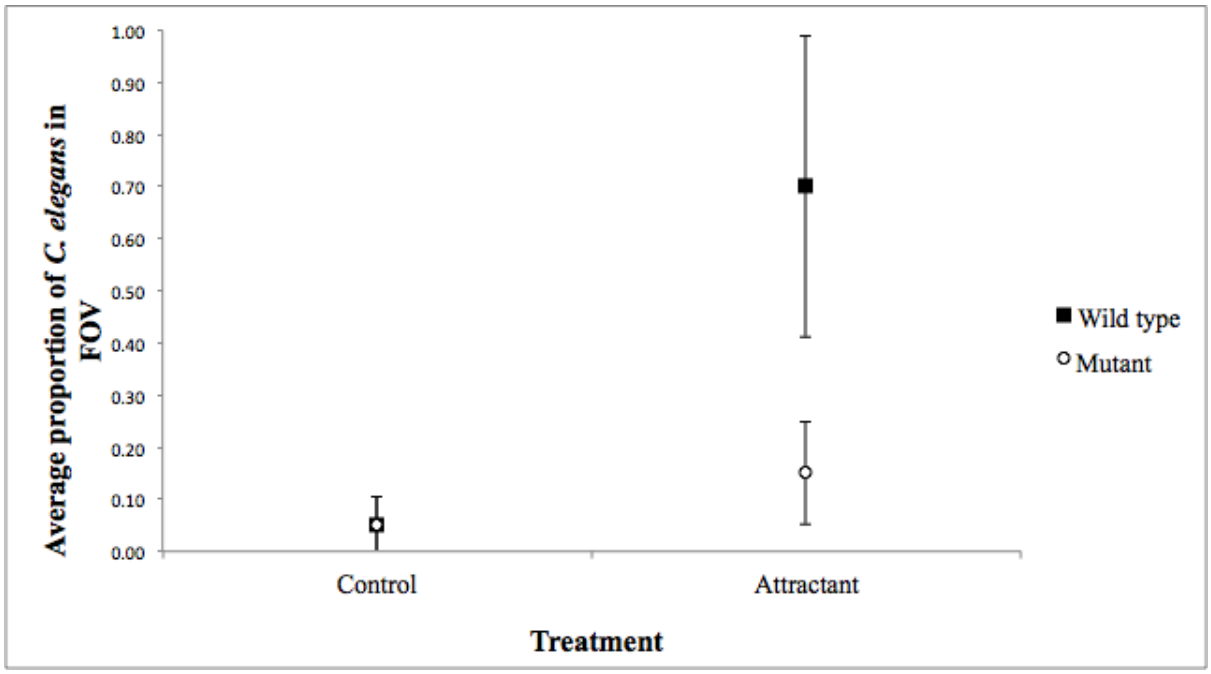

Figure 3. Proportion of wild type (N2) and mutant (VC854) *C. elegans* in field of view (FOV: 1.5 cm) after 30-minute exposure to control (water) or attractant (10% isoamyl alcohol) treatment. Error bars represent 95% confidence interval of mean for each treatment group (n=4).

Discussion

We reject our first null hypothesis that the presence of isoamyl alcohol has no effect on the aggregation of *C. elegans* (p<0.05), and provide support for our alternative hypothesis. Additionally, we reject our second null hypothesis that the presence of mutation has no effect on the aggregation of *C. elegans* (p<0.05) and provide support for our alternative hypothesis. Lastly, we reject our third null hypothesis that wild-type and *unc-2* mutant have the same response to isoamyl alcohol (p<0.05), and provide support for our alternative hypothesis. These results show that overall *C. elegans* is attracted to isoamyl alcohol, but that presence of *unc-2* mutation affects the response, and that isoamyl alcohol has a different effect on the wild type than the mutant. Our wild-type results are consistent with other research which found isoamyl alcohol to be an attractant for *C. elegans* (Bargmann *et al.* 1993, Pereira and

Kooy 2012). The results for the *unc-2* mutant do not have a comparable experiment in the literature.

These results are mostly consistent with our predictions; however we did not expect the *unc-2* mutants to show a different response to isoamyl alcohol. Upon further research, it was found that *unc-2* is necessary for a Ca-dependent pathway which controls expression of the *str-2* odorant receptor gene (Mathews *et al*. 2003), providing a possible explanation for why the mutants showed a decreased response to isoamyl alcohol. The odorant receptor protein STR-2 is not the main receptor for *C. elegans*, as it is only expressed in one of the two AWC neurons. STR-2 has been suggested to be a receptor for attractive odorants (Troemel et al. 1999), providing further insight as to why *unc-2* mutants respond differently to isoamyl alcohol than wild type.

The olfactory response of *C. elegans* is dependent on the AWC odorant receptors binding an odorant and stimulating a motor neuron response as seen in Figure 1 (Chalasani *et al.* 2007). This motor neuron stimulates 'turning' to determine if the odorant is an attractant and causes either movement towards the attractant, which involves UNC-2 protein channels, or continued searching (Chalasani *et al*. 2007). A reliance on 'turning' to respond effectively to attractants may also help explain why the *unc-2* mutant shows less response to isoamyl alcohol than the wild type, as it has uncoordinated movement and is missing UNC-2 channels important for locomotion.

Aside from a biological explanation for why *unc-2* mutants responded differently than wild type to isoamyl alcohol, there may be some experimental design flaws that contributed to this. For example, our experiment gave the same time frame of 30 minutes to mutants and wild type, when it is possible this is insufficient time for the mutants to aggregate to the attractant. Also, our experiment didn't quantify displacement of nematodes, so any

nematodes that moved closer to attractant without entering the circle did not contribute to our data.

There were multiple sources of variation in this experiment that could have affected our results. Firstly, transfer of nematodes was done by hand and there could have been slightly more or fewer than 10 nematodes transferred by mistake. Also, during transfer some nematodes may have died or been shocked enough to not respond normally to the attractant, as nematodes are motion sensitive (Syntichaki and Tavernarakis 2004). The placement of nematodes is also a possible source of variation, as placement was pseudorandom with the intention of having nematodes spread evenly over the petri dish. If certain replicates had placement closer to the center of the dish it is possible a greater response would be observed. Regarding the treatment, the drop of isoamyl alcohol was approximately, but not exactly, centered in the dish. The circles used to count nematodes were made using a template that was not perfectly circular and could have had a small impact on the data.

Future studies may wish to look at the aggregation of *C. elegans* at increasing concentrations of isoamyl alcohol to determine if olfactory organs and sensory neurons are affected by increased amount of attractant. Studies may also wish to alter experimental design to permit one *C. elegans* individual per replicate in order to remove the potential of group effects.

Conclusion

All three null hypotheses were rejected leading to the conclusion that presence of attractant and the mutation have an effect on the aggregation of *C. elegans*. Furthermore, the aggregation of *C. elegans* was not the same for the wild type and the mutant after 30 minutes. We initially predicted that the aggregation of *C. elegans* will vary after 30 minutes for both wild type and mutant when exposed to an attractant because the *unc-2* gene does not affect

olfactory senses. However, *unc-2* mutants were shown not to respond significantly to an attractant, suggesting olfaction may not be the sole determinant in response to attractants.

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