Video Article

C. elegans Chemotaxis Assay

Olivia Margie[1], Chris Palmer[2], Ian Chin-Sang[3]

[1]Life Sciences, Queen's University

[2]Department of Chemical Engineering, Queen's University

[3]Department of Biology, Queen's University

Correspondence to: Ian Chin-Sang at chinsang@queensu.ca

URL: http://www.jove.com/video/50069
DOI: doi:10.3791/50069

Keywords: Behavior, Issue 74, Biochemistry, Cellular Biology, Molecular Biology, Developmental Biology, Physiology, Anatomy, Chemical Engineering, chemotaxis, Caenorhabditis elegans, C. elegans, chemotaxis assay, nematode, chemotactic index, worm movement, animal model

Date Published: 4/27/2013

Citation: Margie, O., Palmer, C., Chin-Sang, I. *C. elegans* Chemotaxis Assay. *J. Vis. Exp.* (74), e50069, doi:10.3791/50069 (2013).

Abstract

Many organisms use chemotaxis to seek out food sources, avoid noxious substances, and find mates. *Caenorhabditis elegans* has impressive chemotaxis behavior.

The premise behind testing the response of the worms to an odorant is to place them in an area and observe the movement evoked in response to an odorant. Even with the many available assays, optimizing worm starting location relative to both the control and test areas, while minimizing the interaction of worms with each other, while maintaining a significant sample size remains a work in progress [1-10]. The method described here aims to address these issues by modifying the assay developed by Bargmann *et al.*[1]. A Petri dish is divided into four quadrants, two opposite quadrants marked "Test" and two are designated "Control". Anesthetic is placed in all test and control sites. The worms are placed in the center of the plate with a circle marked around the origin to ensure that non-motile worms will be ignored. Utilizing a four-quadrant system rather than one 2 or two 1 eliminates bias in the movement of the worms, as they are equidistant from test and control samples, regardless of which side of the origin they began. This circumvents the problem of worms being forced to travel through a cluster of other worms to respond to an odorant, which can delay worms or force them to take a more circuitous route, yielding an incorrect interpretation of their intended path. This method also shows practical advantages by having a larger sample size and allowing the researcher to run the assay unattended and score the worms once the allotted time has expired.

Video Link

The video component of this article can be found at http://www.jove.com/video/50069/

Introduction

Ward first developed the chemotaxis assay in 1973 [5], and since then it has had far reaching applications. Neurobiology is one field that has benefitted from using a variety of chemotaxis assays. Olfactory adaptation, a simple form of learning and memory, has been demonstrated in *C. elegans* using chemotaxis assays [6]. They have also been used to show that *C. elegans* can develop ethanol tolerance-a result that not only demonstrates the behavioral plasticity of the worms, but that also shows that the worms can be very useful in the study of alcohol dependence in humans [3]. Assays have even been developed to demonstrate the ability of *C. elegans* to store short and long term memory by showing that associations are made by the worms between chemoattractants and food (OP50) [7]. Additionally, given the extensive information currently available regarding the *C. elegans* genome, the chemotaxis behavior of *C. elegans* has been altered numerous times by inducing mutations [1,8]. This allows for many exciting engineering possibilities, such as the development of *C. elegans* as a bioremediation tool. Thus, since the initial development of the chemotaxis assay in 1973, it has been frequently altered and used to elucidate mysteries in a variety of disciplines.

Certain assays aimed to discover the specific route taken by the worms toward a target. The prototypical assay of this kind was developed by Ward [5]. Three worms were placed on melted agar for 15 min. Their movements were traced by the imprint they left as they travelled from the periphery of the plate up a gradient to an attractant at the center of the plate. All worms on the plate were arrested using chloroform at the end of each trial. One descendant of this method placed a single worm in the middle of the plate with the attractant and the control at equal and opposite distances from the origin [2].

Pierce-Shimomura *et al*. developed an assay to observe the exact nature of the movement involved in chemotaxis[9]. Individual worms were placed in 9 cm Petri dishes either containing a uniform concentration of the attractant or a radial shaped gradient, culminating in the source of the attractant. A computer software program that recognized the worm was used to record the observed behavior. A video camera attached to a microscope worked in conjunction with the stage to adjust the Petri dish automatically as the assay ran to ensure the worm remained in the field of view. From this, more detailed information was discovered regarding the cause of pirouettes displayed by *C. elegans*.

Other assays, more similar to the one described here, tested the response of a large population of worms to test compounds. Two-quadrant chemotaxis assays have been used to explore the roles that various neurons, receptors, and signal transduction molecules played when *C. elegans* was exposed to various compounds [1]. Between 20-50 washed worms were placed near the center of the plate with an attractant and a control at polar ends along with the anesthetic, sodium azide (NaN_3). After 60 min, a chemotactic index with values from -1.0 to +1.0 was generated based on the difference between how many worms were affixed to the attractant or the control. A similar chemotactic index was used in the assay reported in this article, although the earlier assay failed to strictly evaluate non-motile worms. This assay was then further applied to testing the effects of neuronal ablation on chemotaxis.

Another variation of the aforementioned assay was performed where 100-200 worms were placed at the center of a plate containing four quadrants [3]. Adjacent quadrants either contained the test or control substance. As in previous assays, the worms were immobilized by the action of sodium azide before being scored. A similar method is described here as a way of evaluating the response of *C. elegans* to various compounds. However, the method below has the added benefit of only evaluating worms that have passed a threshold distance separating mobile from immobile worms.

Other assays have incorporated similar guidelines for ignoring immobile worms. Frøkjær-Jensen *et al*. developed a versatile assay which can be used to test both volatile and water soluble compounds [10]. A Petri dish was divided into four quadrants. The top and bottom quadrants did not contain solvents. The left quadrant contained water, and the right contained the attractant. When testing volatile odorants, the analyte was placed on the lid of the dish, over the proper quadrant, whereas water soluble compounds were placed directly on the agar.

The methods currently in existence for evaluating the chemotactic response of *C. elegans* are constantly being refined to optimize their ease of use, efficiency, and accuracy. So, while the assay described here has the capability of assessing the greatest number of worms (maximum throughput: 250 worms/hour per plate, slightly greater than the throughput demonstrated by Lee *et al*. [3]); the real strength of this method is the succinct culmination of many of the attributes of earlier assays (**Table 1**).

Protocol

1. Preparing/washing the Worms

1. Synchronize worms to young adult[11].
2. Pipette 2 ml of the S Basal onto a 5 cm Chemotaxis plate of staged worms that have just cleared the lawn of OP50 *E. coli*. Tilt the plate as needed to ensure the worms are washed from the plate surface into the buffer.
3. Pipette 1 ml of the worm-S Basal solution into a microcentrifuge tube.
4. Centrifuge for 10 sec using a PicoFuge at 6,600 rpm.
5. Aspirate the S Basal, leaving the pellet of worms undisturbed.
6. Add 1 ml S Basal solution to the microcentrifuge tube, invert a few times to wash the worms.
7. Repeat steps 1.4 to 1.6 another three times.
8. Centrifuge for 10 sec a fifth time, this time aspirating the supernatant to a final volume of ca. 100 µl.
9. Pipette 2 µl of the worms onto an NGM plate to ensure there are between 50 and 250 worms in each 2 µl sample. Adjust the concentration of worms in the S Basal as needed by resuspending the worms in a smaller or larger volume of S Basal.
10. Use the worms in the assay immediately after washing the worms for up to 1 hr.

2. Preparing the Test Plates

1. Marking the underside of a 5 cm plate, divide the dish into 4 equal quadrants. Chemotaxis agar or NGM may be used. A minimum of 3 trials are required per genetic strain being tested.
2. Mark a circle of radius 0.5 cm around the origin (**Figure 1**).
3. Mark a point in each quadrant with either a "T" for "Test" or a "C" for "Control," ensuring that the sites are equidistant from the origin and each other. The points must be at least 2 cm away from the origin. Mark the top left and bottom right quadrants as test quadrants and the top right and bottom left quadrants as controls (**Figure 1**).

3. Running the Assay

1. Mix the test solution by combining equal volumes of the test compound and 0.5 M azide (an anesthetic used to arrest the worms upon reaching a quadrant). Mix the control solution by combining the solvent used to dilute the test compound with 0.5 M azide.
2. Pipette 2 µl of the worm solution (prepared in 1.8) from the pellet onto the origin (where the two lines intersect).
3. Immediately after, pipette 2 µl of the test solution onto the two "T" sites. Likewise, pipette the same amount of the control solution onto the two "C" sites.
4. Once the worm and odorant drops have been absorbed in the agar, replace the lids and invert the plates.
5. After 60 min, place the worms in a 4 °C incubator. Only remove a plate from the incubator when it can be counted. Leaving worms at room temperature may allow them to mobilize again.
6. Record the number of worms in each quadrant that completely crossed the inner circle.
7. Repeat steps 3.2 to 3.6 using the control solution in both the test and control quadrants. This will serve as the control plate. Three such plates should be made and run to serve as an appropriate control.

4. Interpreting the Scores/Determining the Chemotaxis Index

1. Calculate the chemotaxis index using Equation 1. This will yield a chemotactic index between -1.0 and +1.0.

(1)

Chemotaxis Index = (# Worms in Both Test Quadrants - # Worms in Both Control Quadrants) / (Total # of Scored Worms)

A +1.0 score indicates maximal attraction towards the target and represents 100% of the worms arriving in the quadrants containing the chemical target. An index of -1.0 is evidence of maximal repulsion. Similar assay methods have already been employed [3] (**Table 1**).

2. Report the mean Chemotaxis Index (CI) and standard error of mean (SEM). Perform a Student's T-test comparing the data from the test and control plates.

Representative Results

Comparing wild-type (N2) *C. elegans* to the *odr-10(ky10)* mutant.

We used diacetyl, a known *C. elegans* chemoattractant, to compare wildtype worms to that of a mutant that lacks the receptor for diacetyl [1,12]. For *wildtype (N2)* worms the chemotactic index was 0.100±0.066 to ethanol, and 0.839±0.031 to 0.5% diacetyl. As expected, diacetyl elicits a significant chemoattractive response from wild-type worms (P<0.003). In contrast the *odr-10(ky10)* mutants which lacks the diacetyl receptors had a diacetyl CI that was not significantly different from the controls (**Figure 3**).

Author	# worms/plate	Duration (min)	1 hr throughput (max # worms)	Pros	Cons
Ward[5]	3	15	12	• track movement • account for immobile worms by clear observation	dependent on researcher involvement @ short time intervals (15 min)
Bargmann & Horvitz[2]	1	60	1		• low throughput • attractant diffuses for 12-24 hr
Bargmann, Hartwieg, Horvitz[1]	20-50	60	50	• minimal researcher involvement (NaN_3 used) • moderate # worms	• no discreet immobile scoring method • doesn't track movement
Lee, Jee, McIntire[3]	100-200	60	200	• minimal researcher involvement (NaN_3 used) • high # worms	
Pierce-Shimomura,Morse, Lockery[9]	1	20 (or until worm hits edge of plate)	3	• track movement • account for immobile worms by clear observation • detailed, permanent record of worm movement	requires advanced computer tracking system
Margie, Palmer, Chin-Sang (this method)	50-250	60	250	• minimal researcher involvement (NaN_3 used) • high # worms • accounts for immobile worms	• doesn't track movement

Table 1. Comparison of assay methods described.

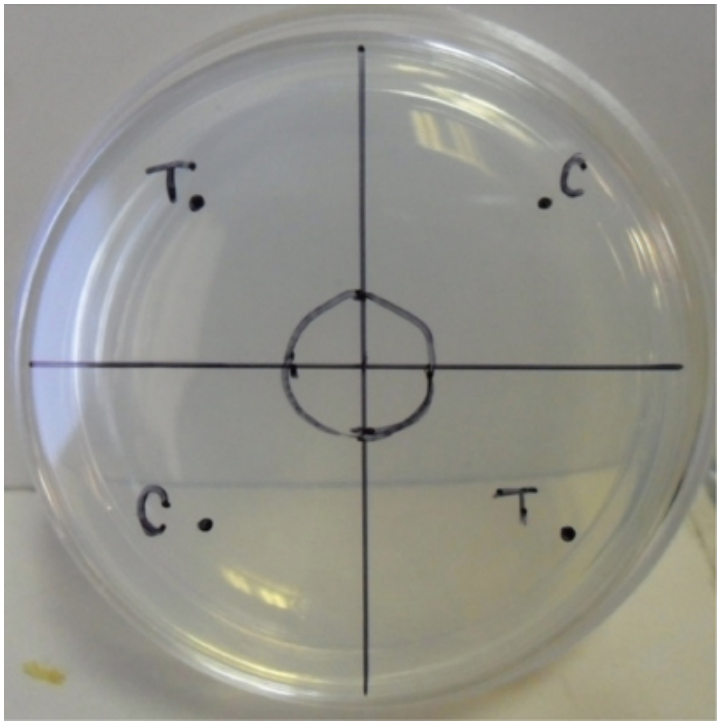

Figure 1. The 5 cm Petri dish is divided into four quadrants, each designated either as Test ("T") or Control ("C") area. The inner circle delineates an area where movement is not scored. This prevents immobile worms from skewing the data. The quadrants create an alternating pattern of Test and Control to prevent any bias that may occur from the initial placement of the worms.

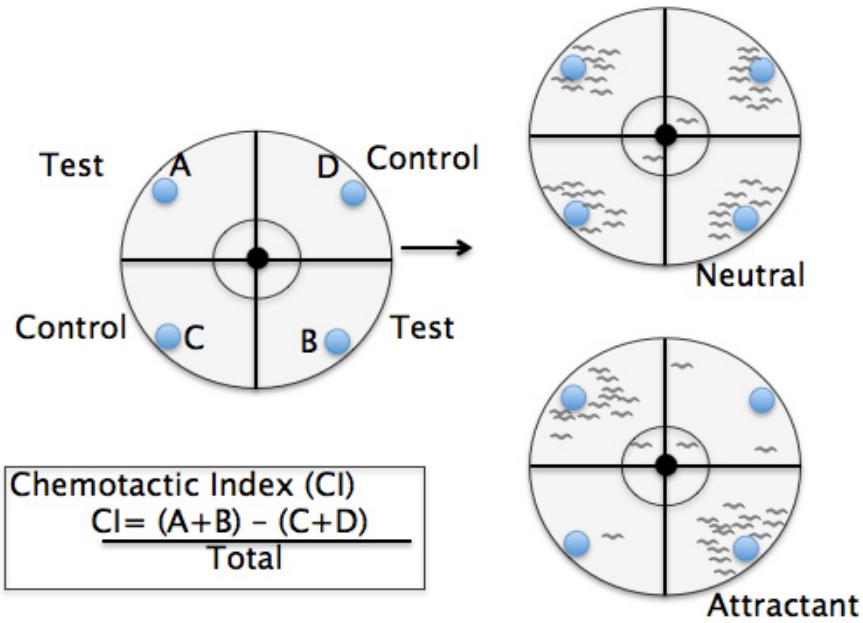

Figure 2. A schematic of the assay adapted from Lee *et al.* (2009). Plates are divided into quadrants two test (A&B) and two controls (C&D). Sodium azide is also included with the Test and Control spots to paralyze worms. Worms are placed at the center of the plate and after 60 minutes worms are counted on each quadrant. Individuals that did not cross the inner circle are not scored. Schematic examples of neutral and attractant are indicated. The Chemotactic Index (CI) calculation is shown below.

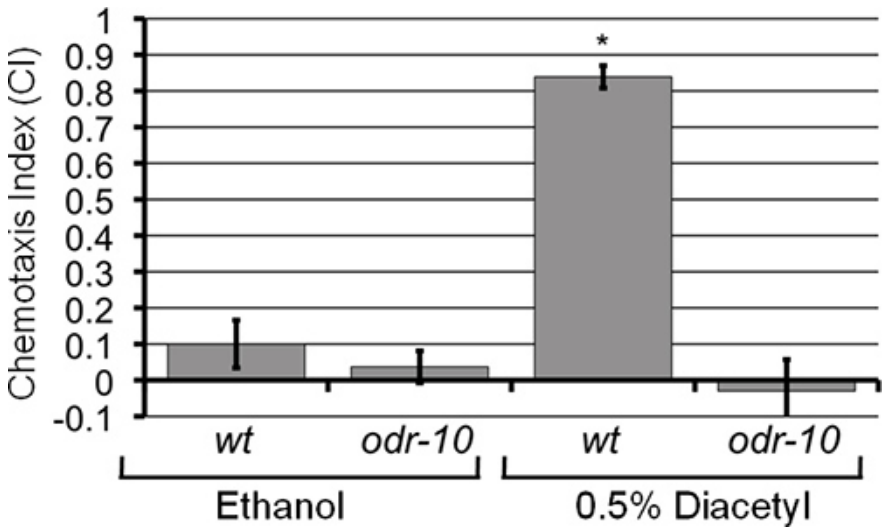

Figure 3. Chemotactic indices generated from assays performed with *wild-type (wt)* and *odr-10(ky10)* worms using either diacetyl as a test attractant or ethanol (control plates). Values are averages from at least 3 independent experiments (n>200 worms) for each condition. Error bars reflect the standard error of the mean (SEM) *=P<0.003 Student's T-test.

Discussion

Chemotaxis, although controlled by a complex set of neuronal and cellular mechanisms, can be easily and objectively quantified using chemotaxis assays. To obtain the best results from the assays, certain critical steps must be taken. Firstly, staging the worms is essential in yielding consistent experimental results. Worms at different life stages behave differently [13]; so mixed stage worms may skew experimental results. Secondly, ensuring all *E. coli* is washed off the worms is crucial, as residual bacteria may interfere with the assay. It is also important to note that the anesthetic, 0.5-1 M-sodium azide, will arrest worms within a 1 cm radius of where it was placed. Given this, it is paramount that the azide be placed at least 2 cm from the origin. If the azide is spotted 1.5 cm or less from the origin the worms will be paralyzed within the inner circle and none will be scored. The further the azide is placed, the further into the quadrants the worms can move. Plates should also be kept level to ensure consistency across trials and to ensure results are not confounded by gravitational biases. In addition, no more than 250 worms should be used in the experiment. Crowding may impede worm movement for a number of reasons. We found that clustered worms tend to remain together, in favor of traveling to a quadrant. Finally, placing the assay plates at 4 °C after the 60 min keeps the worms from moving, allowing the researcher to preserve the state of the plate until it is able to be counted. Plates can stored at 4 °C and scored several days later without affecting the results.

A few procedural modifications can further enhance the utility of this protocol. As is, this protocol can serve as a method of testing a variety of phenotypes in separate trials. Ideally, the results of the mutant trials should be compared under the same conditions. Using strains that express fluorescent proteins such as GFP or RFP would accomplish this. This modification would give the researcher greater experimental consistency by placing the various strains on the same assay plate and the benefit of scoring one plate instead of scoring test versus control plates.

Chemotaxis assays may also be used to characterize the chemotactic behavior of genetically engineered nematode worms. This allows the binding affinity of receptors taken from other species to be tested. If these receptors are expressed in the chemoattractant or chemorepulsive neurons of the worm in a compatible manner, expected chemotactic results should be observed [8].

This protocol may be further adapted to suit other needs as well. Currently, this method does not allow the investigator to track the movement of worms. Because 5 cm plates are acceptable for these assays, a video camera attached to a stereomicroscope can effectively film the movement of the worms toward one quadrant of the assay without the use of a motorized stage and worm tracking software. Finally, although chemoattractants are primarily referred to as the test compounds in the protocol, chemorepellents may also be used. This protocol, as is or modified, is simple and efficient and can serve as an effective tool for quantifying the chemotactic behavior of *C. elegans*, or for demonstrating the behavior to students at the high school or undergraduate level.

Disclosures

We have nothing to disclose.

Acknowledgements

We thank Life Sciences and the Faculty of Arts and Science at Queen's University for funding this work. As well, we thank the Chin-Sang laboratory for providing the necessary reagents, equipment and technical support. We also thank QGEM 2011, especially Tony He for his contribution to the discussion.

References

1. Bargmann, C.I., Hartwieg, E., & Horvitz, H.R. Odorant-selective genes and neurons mediate olfaction in *C. elegans. Cell.* **74**, 515-527 (1993).
2. Bargmann, C.I. & Horvitz, H.R. Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in *C. elegans. Neuron.* **7**, 729-742 (1991).
3. Lee, J., Jee, C., & McIntire, S.L. Ethanol preference in *C. elegans. Genes Brain Behav.* **8**, 578-585, doi:10.1111/j.1601-183X.2009.00513.x (2009).
4. Swierczek, N.A., Giles, A.C., Rankin, C.H., & Kerr, R.A. High-throughput behavioral analysis in *C. elegans. Nat. Methods.* **8**, 592-598, doi:10.1038/nmeth.1625 (2011).
5. Ward, S. Chemotaxis by the nematode *Caenorhabditis elegans*: identification of attractants and analysis of the response by use of mutants. *Proc. Natl. Acad. Sci. U.S.A.* **70**, 817-821 (1973).
6. Colbert, H.A. & Bargmann, C.I. Odorant-specific adaptation pathways generate olfactory plasticity in *C. elegans. Neuron.* **14**, 803-812 (1995).
7. Kauffman, A., Parsons, L., Stein, G., Wills, A., Kaletsky, R., & Murphy, C.C. elegans Positive Butanone Learning, Short-term, and Long-term Associative Memory Assays. *J. Vis. Exp.* (49), e2490, doi:10.3791/2490 (2011).
8. Troemel, E.R., Kimmel, B.E., & Bargmann, C.I. Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in *C. elegans. Cell.* 91, 161-169 (1997).
9. Pierce-Shimomura, J.T., Morse, T.M., & Lockery, S.R. The fundamental role of pirouettes in *Caenorhabditis elegans* chemotaxis. *J Neurosci.* **19**, 9557-9569 (1999).
10. Frokjaer-Jensen, C., Ailion, M., & Lockery, S.R. Ammonium-acetate is sensed by gustatory and olfactory neurons in *Caenorhabditis elegans. PLoS One.* **3**, e2467, doi:10.1371/journal.pone.0002467 (2008).
11. Porta-de-la-Riva, M., Fontrodona, L., Villanueva, A., & Cerón, J. Basic Caenorhabditis elegans Methods: Synchronization and Observation. *J. Vis. Exp.* (64), e4019, doi:10.3791/4019 (2012).
12. Sengupta, P. & Bargmann, C.I. Cell fate specification and differentiation in the nervous system of *Caenorhabditis elegans. Dev. Genet.* **18**, 73-80, doi:10.1002/(SICI)1520-6408(1996)18:1<73::AID-DVG8>3.0.CO;2-Z (1996).
13. Hart, A.C. Behavior WormBook, doi:10.1895/wormbook.1.87.1 (2006).

