A Lab Using *C. elegans* to Investigate the Nervous System and Behavior

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Biography

Jessica Goldstein received a BA in Biology from Macalester College and a PhD in Molecular Cell Biology from Washington University in St. Louis, MO. She is currently a Lecturer in the Biology Department at Barnard College in New York City where she is responsible for coordinating Introductory Biology Laboratory courses for science majors and non-majors.

Introduction

The animal, *Caenorhabditis elegans (C. elegans),* a member of the Phylum Nematoda, is a small (~1 mm) non-parasitic worm that lives in the soil and eats bacteria. Scientists study *C. elegans* because much is known about its anatomy and behavior, and it is small and easy to work with in the lab. In fact, it is so small that it has only ~1000 cells, about a third of which are part of the nervous system (Riddle et. al., 1997). For these reasons, it is an excellent organism in which to study how the nervous system develops and functions.

This lab focuses on the sensory nervous system which is responsible for detecting external, environmental signals. These environmental signals can come in many forms: light, sound, heat, etc. Chemosensation, the ability to detect different chemicals in the environment, is one very important sense that provides many organisms with information about their environment. To detect chemicals in the environment, humans use specialized sensory neurons called chemoreceptors in their mouth (taste receptors) and our nose (olfactory receptors). Similar to humans, *C. elegans* use specialized chemoreceptors located in their head to sense chemicals in their environment. These chemoreceptors are open to the external environment and relay information to the nematode about the type of environment it is in (whether there is food, toxins, other worms, etc).

C. elegans uses the information that it receives from its chemosensory neurons to modify many aspects of its behavior. For instance, certain bacterial metabolites (its food source) stimulate the feeding response, certain pheromones affect mating behavior, and certain toxins induce the worms to migrate away from them. Different chemosensory neurons can detect different types of chemicals; certain sets of neurons mediate the detection of volatile chemicals (such as alcohols), whereas different sets of neurons mediate the detection of water-soluble chemicals (such as ions or amino acids) (Riddle et al., 1997). This is similar to how humans have different chemoreceptors for different types of tastes (sweet, sour, salty, etc).

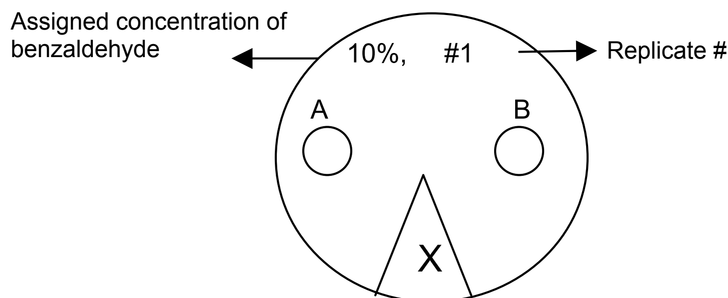
In this lab, we will examine one behavioral response mediated by the nervous system in *C. elegans*: chemotaxis, which is the movement towards or away from specific chemicals. To do this, we will use a previously-established assay to determine if a chemical can be sensed by a worm. In this assay, the test chemical is added to one end of a Petri dish filled with agar, and a control chemical (ethanol) is added to the other end. A group of worms is placed an equal distance from each chemical (test and control) and allowed to move freely for a certain period of time (in our case, 30 minutes). At the end of the specified time period, the number of individuals that have migrated to each chemical is determined. To compare the response of these animals to different chemicals, a quantitative value to represent amount of "attractiveness" has been developed: the chemotaxis index. The chemotaxis index for a test chemical is calculated by using the following formula: Chemotaxis Index (CI) = (# at test chemical - # at control chemical) / total # on dish. If the chemotaxis index is positive, then we can conclude that the animal was attracted to the chemical. If it is negative, then the animal was repulsed by the chemical. If it is close to 0, then the animal is neither repulsed nor attracted to the substance (Bargmann et al., 1993).

In this lab, we will investigate the chemotactic response of *C. elegans* to different concentrations of the chemical benzaldehyde. These organisms are attracted to benzaldehyde because it is a bacterial

metabolite, and they eat bacteria. Today we will investigate if *C. elegans* can detect only one concentration of benzaldehyde or many different concentrations. We will examine if they respond to high concentrations in the same way as they do to low concentrations, and we will determine if there are concentrations that the animals avoid.

Student Outline

Preparing the Petri Plate:
Label the bottom of an empty (no worms) agar test plate as shown in the following diagram. Measure 1 µL of 1 M sodium azide (caution: POISON) and gently dispense the liquid on the agar in the Petri dish above the spot labeled "A". Repeat, dispensing another 1 µL of 1 M sodium azide above the spot labeled "B".



Preparing *C. elegans*:
Use a transfer pipet to add approximately 2 mL of salt solution directly to the agar of the Petri dish containing the worms. Swirl to mix the worms with the salt solution, and transfer 1.5 mL of the salt solution (containing worms) into a 1.5 mL microcentrifuge tube. Place the microcentrifuge tube upright in a rack, and wait 3-5 minutes to let the worms settle. Remove the top portion of the liquid from the microcentrifuge tube without disturbing the worms at the bottom. Discard this liquid in the waste beaker. Repeat this procedure once with salt solution, and once with water. After the water wash, remove most of the water, leaving ~250 µL of worms-in-water.

Measuring Chemotaxis Index:
To distribute worms to your test plate, pipet 10 µL of worms directly above the label "X", making sure to mix up the worm/water solution so that the worms are evenly distributed before pipetting. Immediately after dispensing the worms, distribute 1 µL of ethanol onto the agar test plate above "B", and 1 µL of assigned benzaldehyde concentration above "A". After 30 minutes, examine the worms on the Petri dishes. Count the worms that are paralyzed in quadrant "A" and those in quadrant "B". Then, count the total number of worms on the Petri dish (both paralyzed and not paralyzed).

Data Analysis:
Calculate the Chemotaxis Index (# worms at "A" - # worms at "B" / total # worms) for your concentration of benzaldehyde (in triplicate). Calculate means and standard deviations for each concentration of benzaldehyde (class data). Construct a bar graph of the class data, plotting mean +/- SEM for each concentration of benzaldehyde tested.

Notes for Instructor

Solutions for this lab:
Information about *C. elegans* handling and recipes for solutions can be found in Riddle et al., 1997.

Nematode Growth Medium (NGM)—Preparation Protocol for 250 mL
For each flask, weigh out:
0.75 g NaCl
0.63 g peptone
4.25 granulated agar

Place these dry ingredients into one 1-Liter flask. Add 250 mL Milli Q water, and 0.25 mL of cholesterol (5 mg/mL in 200 proof ethanol). Cover flask opening with double layer of aluminum foil secured with autoclave tape.

In a separate container, mix up one bottle (10mL) of potassium phosphate mix (must be made fresh each time):
1.3 mL 1M K_2HPO_4
8.7 mL 1M KH_2PO_4

Autoclave both solutions on liquid cycle for 30 minutes. Check for clear solution upon removal from the autoclave. (If solution is not clear, i.e. granules are present, then autoclave for another 30 minutes). While the solutions are autoclaving, set a water bath to 60^oC. Place NGM agar solution in water bath and cool to 60^oC. Cooling takes approximately 30 minutes.

When the NGM is approximately 60^oC, aseptically add three sterile solutions:
0.25 mL of 1M $CaCl_2$
0.25 mL of 1M $MgSO_4$
6.25 mL of Potassium Phosphate Mix

Shake each flask to evenly mix contents before dispensing agar to mix (very important). Fill plates (approximately 7mL/plate) and then flame the plates to remove bubbles. Stack plates and allow them to solidify overnight. Store at 4^oC for up to one month.

Seeding Plates
To "seed" plates with bacteria, obtain *E. coli* strain OP50 from the CGC and aseptically grow a culture overnight at 30-37°C in Luria Broth (no antibiotics). Using a sterile pipet, distribute ~1mL

of culture to the top of each plate, and let the drop sit on top of the plate. Make sure to leave space around the edges free of bacteria (or the worms are likely to crawl up the side of the Petri dish and desiccate). Let the *E. coli* grow on the nematode growth media agar plates overnight at room temperature. The next day, move the plates to 4°C storage (can be stored seeded or unseeded for approximately one month).

Salt solution (M9 Salts; 1 L)

Na_2HPO_4	5.8 g
KH_2PO_4	3.0 g
NaCl	0.5 g
NH_4Cl	1.0 g

Add dH_2O to 1 Liter and autoclave.

Benzaldehyde:
0.01 - 10% in ethanol; store in glass containers because it dissolves plastic

Sodium Azide:
1M solution in dH_2O (caution: POISON)

Agar test plates:
(1.5% agar in dH_2O; autoclave and distribute ~7mL per Petri dish)

Materials needed (per pair of students)
Micropipetter (1-10µl range) and tips
3 agar test plates
6 µL 1M sodium azide
3 µL benzaldehyde (various concentrations)
3 µL ethanol
1 NGM plate with many adult N2 worms
6 mL M9 salt solution
1 microcentrifuge tube
5 transfer pipets (1-3 mL size)
1 dissecting microscope (without a scope, just have students count the # of white specks – these are the worms)

Tips for Class:

As a class, four different benzaldehyde concentrations will be examined: 10%, 1%, 0.1%, and 0.01%. Each pair will be assigned one concentration of benzaldehyde to test in triplicate.

When distributing any solutions to the agar Petri dish, do not poke into the agar surface– if you poke holes in the agar, the worms will borrow in them and skew the results.

Students should use caution and wear gloves when using sodium azide: it is a dangerous toxin that inhibits mitochondrial respiration – this will paralyze the worms, but will also affect your own cells. Using small quantities such as outlined in this lab protocol greatly reduce the risk of serious injury.

As students "wash" the worms for this assay, they should work quickly; if they let the animals sit in liquid too long, they get sluggish and do not perform well.

After initially dispensing the worms on the "X", wait approximately 5 minutes and then use the dissecting microscope to watch the worms as they "crawl" on the agar. If the worms have not moved and are either clumped at the "X" or still "swimming" in a pool of water, open the lid and gently disperse them using a pipet tip. Be careful not to poke the agar as you disperse the worms. Continue to check and disperse at 5 minute intervals until the worms are no longer clumped nor stuck in a pool of water.

Literature Cited

Bargmann, C. I., Hartwieg, E., and H. R. Horvitz. 1993. Odorant-Selective Genes and Neurons Mediate Olfaction in C. elegans. Cell, 74:515-527

Riddle, D. L., Blumenthal, T., Meyer, B. J., and J. R. Priess. 1997. C. elegans II. First edition. Cold Spring Harbor Laboratory Press, Plainview, New York, 1222 pages.

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Caenorhabditis elegans

Caenorhabditis elegans (C. elegans) are small (~1 mm) non-parasitic nematodes that live in the soil in nature but can be grown on nutrient media in the lab. These nematodes are comprised of approximately 1000 cells (a third of which are part of the nervous system), making them excellent organisms in which to study how the nervous system functions.

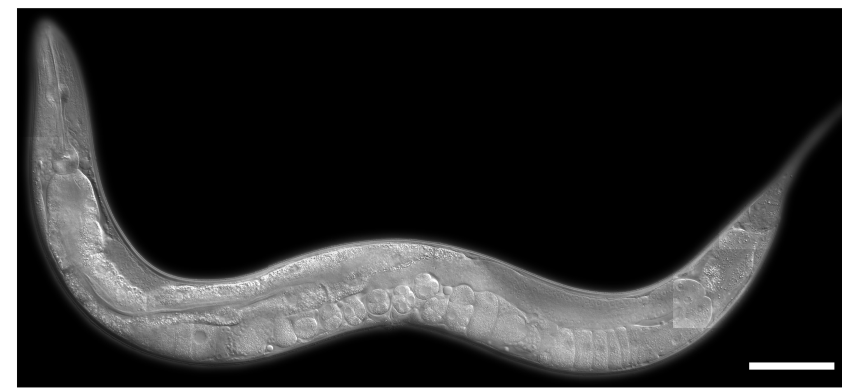


Figure 1. An adult *C. elegans* hermaphrodite. Adapted from: http://www.nematode.net/IMAGES/c_elegans.jpg

Chemosensation

The sensory nervous system is responsible for detecting environmental signals, such as light, sound, heat, etc. Chemosensation, the ability to detect different chemicals in the environment, is an important sense that provides organisms with information about their environment. *C. elegans* use specialized chemoreceptors in their heads to detect chemicals in their environment (food, toxins, other worms, etc). Because these nematodes do not have a visual system, they must rely on chemosensation to detect this type of information. *C. elegans* can use the information that it receives from its chemosensory neurons to modify its behavior.

Chemotaxis

Chemotaxis is the movement towards or away from specific chemicals. To examine this behavior, we will add a test chemical to one end of an agar dish and a control chemical to the other end. A group of nematodes will be placed an equal distance from each chemical (test and control) and allowed to move freely for 30 min. To determine if these animals can sense the test chemical, a quantitative value, the **chemotaxis index (CI)**, has been developed. The CI is calculated using the following formula:

Chemotaxis Index (CI):
(# at test chem - # at control chem) / total #

Positive CI → attraction towards chemical
Negative CI → repulsion away from chemical

Lab Questions

This lab investigates the chemotactic response of *C. elegans* to different concentrations of benzaldehyde, a bacterial metabolite (food source of *C. elegans*).

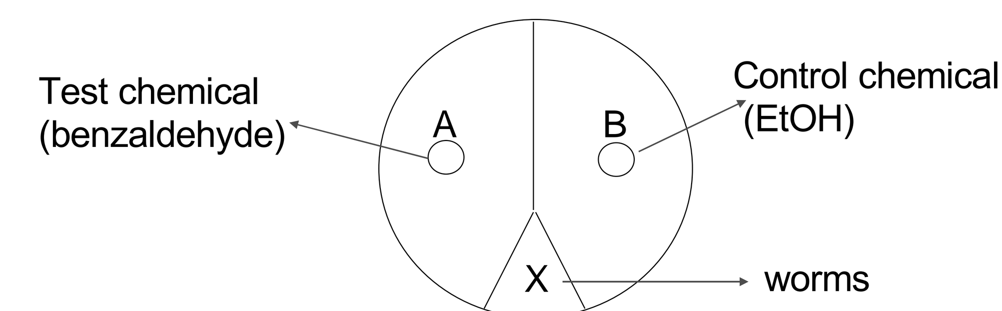
Do *C. elegans* use chemosensation to locate their food source?

Can *C. elegans* detect different concentrations of benzaldehyde?

If so, will they respond to high concentrations in the same way as they do to low concentrations?

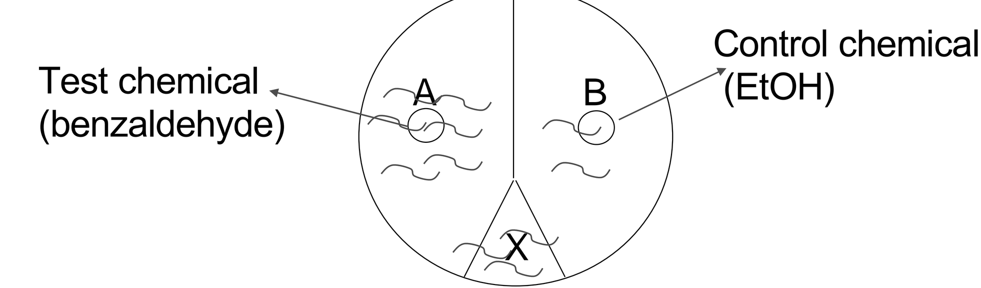
Methods

- Label the bottom of a Petri dish (with agar) as shown in the following diagram.



- Dispense sodium azide (caution: POISON) above spots “A” and “B”.
- Next, prepare worm samples by adding salt solution directly to a dish containing worms. Swirl to mix.
- Transfer the salt solution (with worms) into a 1.5 ml tube. Place the tube upright in a rack, and wait ~5 min for the worms to settle to the bottom.
- Wash the worms 2X with salt solution.
- Wash the worms 1X with water. Remove most of the water, leaving a concentrated worm solution in your tube.
- Dispense worms on to your test plate above spot “X”.
- Dispense control chemical (ethanol) above spot “B” and test chemical (benzaldehyde) above spot “A”.
- After 30 min, count the worms in quadrants “A” and “B” (should be paralyzed). Count the total worms on the Petri dish (both paralyzed and not). Calculate CI.

Example Results:



CI: (6-2)/(6+2+3) = 0.36

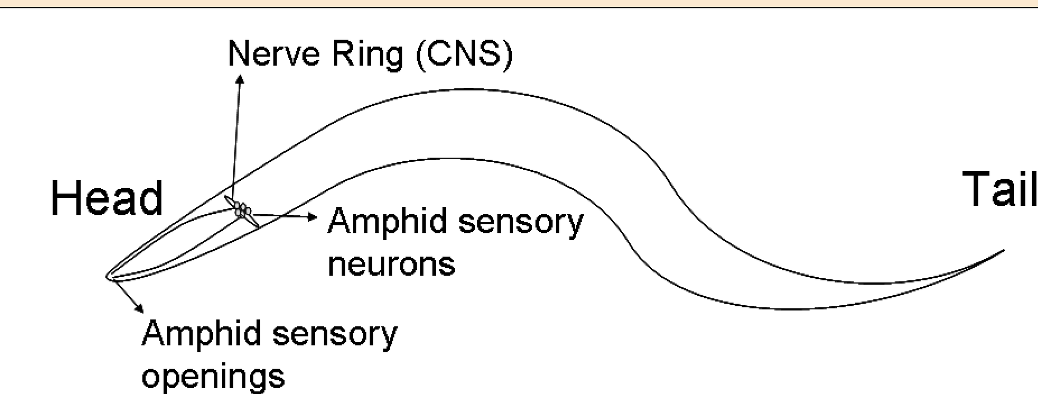


Figure 2. Simplified diagram of a *C. elegans* hermaphrodite. The head, tail, and amphid chemosensory neurons are indicated.

Results

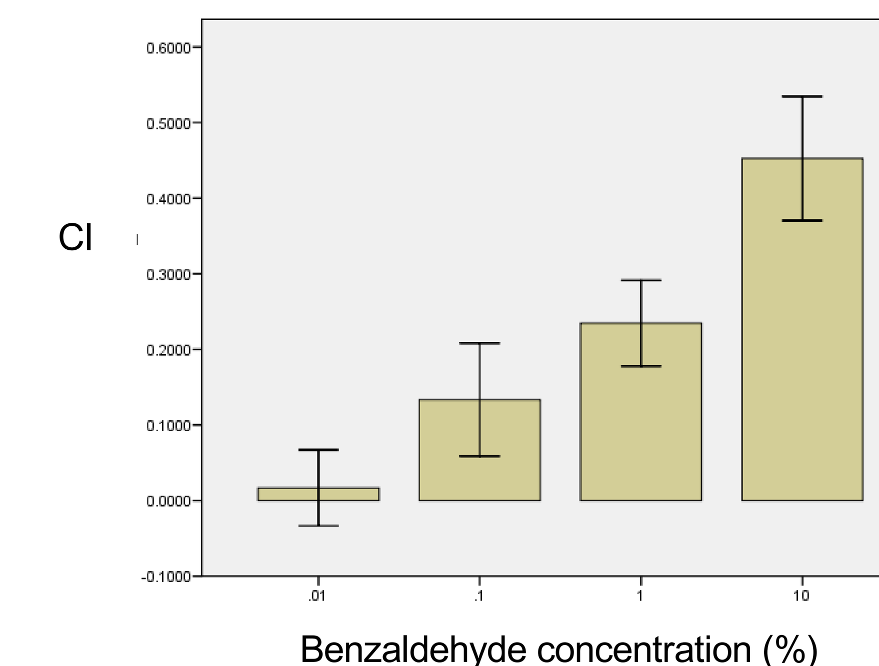


Figure 3. *C. elegans* display a dose-dependent attraction to benzaldehyde. Mean CI +/- SEM was graphed for 4 different benzaldehyde concentrations. This data was collected by Introductory Biology Laboratory students at Barnard College in 2006 and 2007.

Discussion and Reflection

- Describe the relationship that appears to exist between different concentrations of benzaldehyde and *C. elegans* chemotaxis behavior.
- What type of statistical test could you perform to determine if there is a statistically significant relationship between the concentration of benzaldehyde and the chemotaxis index? Explain your answer.
- Based on the amount of variation that occurred in your individual data set, why do you think you were asked to repeat the assay three times? Do you think it would be better to repeat the assay more or fewer times? Explain.
- *C. elegans* are neither attracted to nor repulsed by ethanol. Explain why this is important for this experiment.

Obtaining and Culturing *C. elegans*

The standard laboratory strain of *C. elegans* is referred to as “N2” and can be obtained from the Caenorhabditis Genomics Center (CGC): http://www.cbs.umn.edu/CGC/

C. elegans can be maintained between 15 - 25°C. They have a ~4 day life cycle at 20°C and ~2.5 day lifecycle at 25°C.

C. elegans can be maintained on Nematode Growth Medium (NGM) agar . This NGM agar is generally poured into Petri dishes that are “seeded” with OP50 *E. coli* bacteria. For detailed information and instructions for preparing NGM agar and OP50 *E. coli,* see Riddle, *et al*., 1997.

C. elegans Websites

- http://www.cbs.umn.edu/CGC/
- http://elegans.swmed.edu/
- http://www.wormbase.org/

Literature cited

Bargmann, C. I., Hartwieg, E., and Horvitz, H. R. 1993. “Odorant-Selective Genes and Neurons Mediate Olfaction in *C. elegans*” Cell v74 (3) p515-527

Brenner, Sydney. 1974. “The Genetics of *Caenorhabditis elegans*” Genetics 77: 71-94

Riddle, Donald L., Blumenthal, Thomas, Meyer, Barbara J., Priess, James R. 1997. “*C. elegans* II” Cold Spring Harbor Laboratory Press, New York.

Acknowledgments

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