

Chemotaxis assay

Overview

C. elegans has a highly developed chemosensory system that enables it to find food, avoid noxious conditions, develop appropriately, and mate. It senses chemicals with chemosensory neurons that penetrate the cuticle to expose their sensory cilia to the environment: the amphid, phasmid, and inner labial neurons. Chemosensory behaviors are regulated primarily by the amphid chemosensory organs, which contain eleven pairs of chemosensory neurons. Each amphid sensory neuron expresses a specific set of candidate receptor genes and detects a characteristic set of attractants, repellents, or pheromones. About 500–1000 different G protein-coupled receptors (GPCRs) are expressed in chemosensory neurons, and these may be supplemented by alternative sensory pathways as well. Downstream of the GPCRs, two signal transduction systems are prominent in chemosensation, one that uses cGMP as a second messenger to open cGMP-gated channels, and one that relies upon TRPV channels. These sensory pathways are modulated and fine-tuned by kinases and phosphatases. Chemosensory preferences can be modified by sensory adaptation, developmental history, and associative learning, allowing *C. elegans* to integrate context and experience into its behavior.

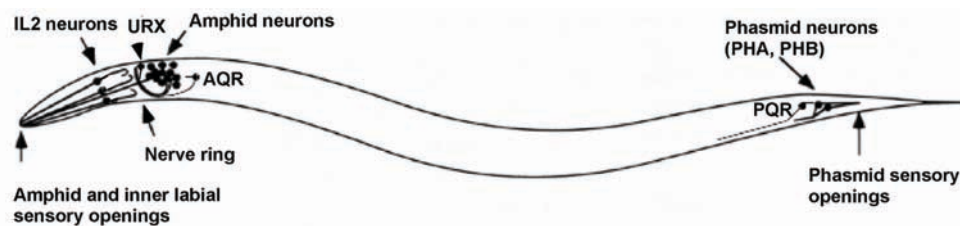


Figure 1. Disposition of chemosensory neurons in *C. elegans*. Each of the two amphids contains 12 associated chemosensory or thermosensory neurons. Each of the two phasmids contains 2 chemosensory neurons, PHA and PHB. There are six inner labial organs, each of which contains one IL2 chemosensory and one IL1 mechanosensory neuron. There are two URX neurons, one AQR neuron, and one PQR neuron; the endings of these neurons are within the animal, and not exposed. http://www.wormbook.org/chapters/www_chemosensation/chemosensation.html

The described chemotaxis assay is used to examine the ability of a worm to sense and move towards or away from a particular chemical in its environment. The sex, age and life cycle stage of the animal tested in an assay is an important factor for behavior. Nervous system development is largely complete by the end of the L1 stage, but some developmental changes are clearly coordinated with the L4/adult molt. Therefore, it is recommended to use young adults for the assay.

Materials

Healthy adult animals from uncrowded plates

Chemotaxis plates

M9 Buffer

Chemotaxis assay buffer

0.5 M Sodium azide

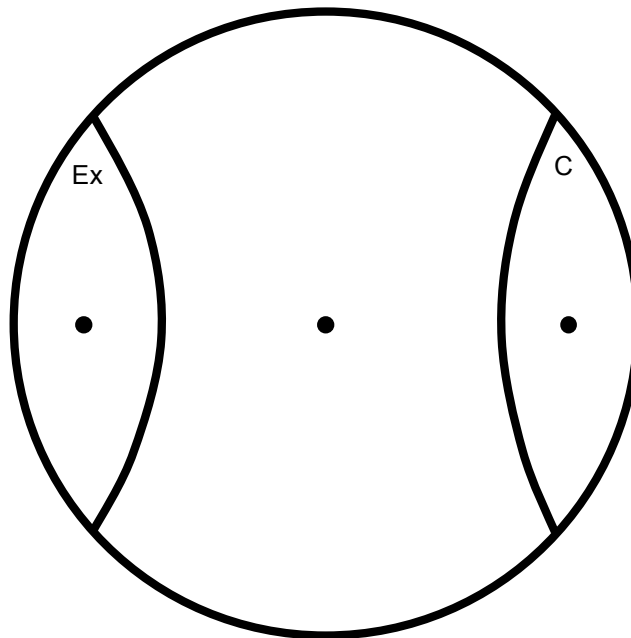
100% Ethanol

Chemicals to test, diluted in Ethanol if needed

Procedure

Note: Use the healthy adult animals from uncrowded plates. Animals should be maintained by transferring 2 to 3 animals to a new NGM plate before starving. Do not use the starved animals or those from a starved agar chunk.

1) *Label chemotaxis plates.* Wipe dry any moisture on the inside of the plate lids with a Kimwipe. Using a Sharpie, trace the design from the template below onto the bottom of each of the chemotaxis plates.



2) *Harvest worms.* Transfer 1 mL of M9 Buffer onto the surface of one of the N2 stock plates. Carefully tilt the plate back-and-forth several times to wash the entire surface of the plate with M9 Buffer, and then tilt the plate to allow the buffer and suspended worms to collect at the lower edge of the plate. Pipet all of the buffer and worms off the plate and transfer to 1.5 mL centrifuge tube.

3) *Wash worms*. Leave the tubes undisturbed for two minutes on the bench to let the worms settle at the bottom of each tube. You should see a “worm pellet” – a mass of worms settled at the bottom of the tubes, visible as a white clump. Carefully remove most of the buffer, trying not to disturb the worm pellet. Add 1 mL M9 Buffer and gently flick the bottom of the tubes to resuspend the worms. Being careful not to disturb the worm pellet, pipette off most of the supernatant.

4) *Wash worms (2nd time)*. Add 1 mL M9 Buffer to each tube and let the worms settle at the bottom of the tubes for one minute. Being careful not to disturb the worm pellet, pipette off most of the supernatant. The washes enrich for larger animals (adults), which is what you want.

5) *Wash worms (3rd time)*. For the final wash, add 1 mL of Chemotaxis Assay Buffer to each tube and allow the worms to settle to the bottom of the tubes for about one minute. Pipette off most of the supernatant from each tube, leaving about 200 μ L of buffer on top of the worms.

6) *Determine worm concentrations*. Resuspend the worms by flicking the bottom of each tube. Using Pasteur pipettes, place one drop of the worm suspension from each tube onto a glass slide. Examine the slide under the microscope to estimate the number of worms in each drop. (You only need a rough estimate – it will be too difficult to get an accurate count as the worms will likely be thrashing rapidly in the drop.) For the chemotaxis assay, you will want to transfer ~100 worms to each chemotaxis plate, so ideally, you want a final worm concentration of ~100 worms per drop. Adjust the volume of buffer in each tube accordingly to obtain the desired worm concentration. If necessary, you can repeat Step 9 to verify that you can transfer about 100 worms per drop with the Pasteur pipette.

7) *Add sodium azide*. Sodium azide is an anesthetic that causes paralysis in *C. elegans*. Add 2 μ L of 0.5 M sodium azide to **both of the outer spots** on all nine plates. Leave the lids off of the plates until all of the sodium azide has soaked into the agar. Note: **Wear gloves and safety goggles when adding the sodium azide**. Once the sodium azide has soaked into the agar, you can remove your goggles, but continue to wear gloves whenever handling the plates as a safety precaution. Do not add the sodium azide too far in advance as it may diffuse through the agar, changing the localized concentration at each spot.

8) *Add worms*. When the worms are ready, use a Pasteur pipette to transfer one drop of worm suspension onto the **center spot** of each of the chemotaxis plates. Check each plate under the microscope to confirm that you transferred about 100 worms (if necessary, you can add more worm suspension to the plate). When adding the worms, be careful not to gouge the agar with the pipette – worms tend to crawl into any break in the agar surface, which will make it difficult to count the worms later.

9) *Add chemicals*. Ethanol is your control chemical since worms are affected by ethanol. Once you've added the worms to each plate, add 2 μ L of ethanol to the “C” (control) outer spot on each of the nine plates. Add 2 μ L of the appropriate experimental chemical to the “Ex” (experimental) outer spot on each of the nine plates.

10) *Remove excess liquid*. Once you've added the chemicals, CAREFULLY use a Kimwipe to touch the edge of the worm drop in the center of each plate. The goal is to wick away the excess liquid from the drop so that the worms will be able to crawl away from the center drop across the surface of the agar. Do not “dab” at the agar with the Kimwipe as you may kill the

worms and/or gouge the agar surface. (You may find it helpful to look at the plate under the microscope while doing this step.) Once the excess liquid is removed or has soaked into the plates, put the lids back on the plates and begin timing a 1-hour incubation.

11) *Incubate plates.* After 10 minutes or so make sure the worms are not all clumped at the origin. If they are, disperse them with a wire pick. Allow the plates to incubate face-up on your bench at room temperature for one hour undisturbed. Keep the plates away from any heat sources (e.g. a microscope light or other equipment) and do not move the plates around during this incubation – worms are sensitive to both temperature gradients and movement, and heating and/or jostling the plates may affect their chemotaxis behavior.

12) *Chill plates.* After the 1-hour incubation, place all nine chemotaxis plates in the 4°C cold room for 15 minutes to chill the worms to stop them from moving.

13) *Count worms.* Take one plate out of the cold room at a time when counting the worms. For each plate, count the worms within the oval area surrounding the “Ex” experimental chemical spot and the worms within the oval area surrounding the “C” control chemical spot. Also count the worms on the remainder of the plate (in the middle section outside either oval areas). (Note: If there is a large immobile clump of worms still in the center spot of your plate, do not include the worms stuck in the clump in your final counts – only count those worms that were actually able to move away from the center spot clump.)

14) *Analyze data.* Using your worm counts, calculate the chemotaxis index (CI) for each of your twelve plates as follows:

$$\text{CI} = \frac{\# \text{ of worms at experimental spot} - \# \text{ of worms at control spot}}{\text{Total \# of worms on entire plate}}$$

For each conditions tested, calculate the mean CI value. A high chemotaxis index (close to 1) indicates that the chemical acts as a strong attractant; a lower chemotaxis index indicates that the chemical is a less effective attractant or does not act as an attractant.

Solutions

Chemotaxis plates

2% agar

5mM KPO₄ [pH6]

1 mM CaCl₂

1 mM MgSO₄

KPO₄, CaCl₂ and MgSO₄ are prepared separately and added after autoclaving. 20 ml of agar is aliquoted into 10 cm Petri plates, and allowed to dry overnight.

Chemotaxis Assay Buffer

5 mM KPO₄ [pH6]

1mM CaCl₂

1 mM MgSO₄

References

Hart, Anne C., ed. Behavior (July 3, 2006), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.87.1, <http://www.wormbook.org>.

MIT OpenCourseWare
<http://ocw.mit.edu>

7.15 Experimental Molecular Genetics
Spring 2015

For information about citing these materials or our Terms of Use, visit: <http://ocw.mit.edu/terms>.