Science Education Collection

C. elegans Chemotaxis Assay

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Abstract

Chemotaxis is a process in which cells or organisms move in response to a chemical stimulus. In nature, chemotaxis is important for organisms to sense and move toward food sources and move away from stimuli that may be toxic or harmful. Chemotaxis is also important at the cellular level. For example, chemotaxis is required for the movement of sperm toward an egg prior to fertilization. In the lab, chemotaxis is frequently examined in the nematode, *C. elegans*, which is known to migrate towards food sources in soil, but away from toxins such as heavy metals, substances with a low pH, and detergents. This video demonstrates how to perform a chemotaxis assay, which includes preparing the chemotaxis plates and the worms, running the assay, and analyzing the data. Then, we discuss examples of how chemotaxis assays can be used in *C. elegans* as a tool to understand learning and memory, olfactory adaptation, and neurological disease such as Alzheimer"s disease. Chemotaxis experiments in *C. elegans* have near-limitless possibilities for learning more about the cellular and genetic mechanisms of many biological processes, and may lead to a greater understanding of human biology, development, and disease.

Transcript

The movement of a cell or organism in response to a chemical stimulus is a behavior called chemotaxis. In this video, we will learn how to perform a chemotaxis assay using the nematode, *C. elegans*. We will also discuss how chemotaxis assays in *C. elegans* are applied to study learning and memory, olfactory adaptation, and Alzheimer's disease.

Let's first discuss two different types of chemotaxis. Movement toward a chemical stimulus is called positive chemotaxis. In contrast, movement away from a chemical stimulus is called negative chemotaxis, allowing organisms to move away from harmful chemicals.

Chemotaxis can occur at the organismal level, as organisms move toward a food source. Chemotaxis also takes place at the cellular level, within organisms. For example, immune cells migrate toward pathogens or sites of inflammation. In another example, sperm cells move toward the egg in response to a chemo-attractant released by the egg. Chemotaxis is also an important process during development, in which cells migrate in response to a chemical stimulus, forming tissues and organs in the developing organism.

For wild, soil-dwelling C. elegans, chemotaxis is important for detection and movement toward bacteria, their main food source. In contrast, *C. elegans* are repelled by heavy metals, substances with a low pH, and detergents, which are toxic to the organism.

Chemotaxis assays typically begin by preparing chemotaxis plates. Using a ruler and a marker, divide a 5 cm plate with nematode growth medium into four equal quadrants. Then, draw a circle with a 0.5 cm radius around the center of the quadrant. This will be the starting point for the worms. Mark and label a point in each quadrant, such that each point is equidistant from the center, and from each other.

When preparing worms for the assay, it's critical to use age synchronized young adult worms so that differences in chemotaxis are not an artifact of the developmental stage. Once worms are synchronized, collect them by first pipetting 2 ml of S-basal buffer onto a plate containing young adults. Swirl and tilt the dish to wash the worms from the plate.

Next, pipette the worm/S-basal solution into a microcentrifuge tube. Wash the worms by briefly centrifuging the worm/S-basal solution, removing the supernatant, and adding another milliliter of S-basal solution to the worm pellet. Invert the tube and repeat the wash two more times. After washing, remove all but approximately 100 µl of the S-basal solution. Next, add 2 µl of the worm/S-basal mixture to an NGM plate. Using a microscope, count the number of worms present. Ideally, there will be between 50-250 worms per 2 µl of S-basal.

Now that the chemotaxis plates and the worms are ready, we can get started on the chemotaxis assay. First, mix equal volumes of your test solution with 0.5 M sodium azide, an anesthetic that will paralyze worms once they reach their destination. Do the same with your control solution. Next, pipette 2 µl of worm/S-basal mixture onto the center of your chemotaxis plate. Then, pipette 2 µl of the test or control solution and place on appropriately labeled points on the chemotaxis plate. Once the test and control solutions have been absorbed, place the lid back on, invert the plate, and set a timer for 1 hour.

After the worms have been given one hour to respond to the chemical stimuli on the plate, the data can be analyzed. Manually count the number of worms within each quadrant. If the worms are attracted to the test chemical, there will be more worms present in those quadrants. If they are neutral towards that chemical, worms will be present in each quadrant equally.

Use these data to calculate the chemotactic index, which is the number of worms in the test quadrants minus the number of worms in the control quadrant, divided by the total number of worms. A chemotactic index close to +1 suggests attraction, while a chemotactic index close to -1 indicates repulsion.

Now that we've learned how to set up a chemotaxis assay, let's have a look at how these experiments are applied to answer scientific questions.

One of the ways chemotaxis assays in *C. elegans* have been applied is for studying learning and memory. For example, worms can be conditioned to associate a chemical stimulus with a food source. Well-fed worms are starved for one hour, and then they are conditioned with food, as well as a chemical such as butanone.

Next, the worms are held on a plate with food, but without butanone. Running a chemotaxis assay will then determine whether the worms have learned to associate butanone with food. Many variations of this experiment can be performed to determine other information such as which genes or neurons are important for learning and memory.

Olfactory adaptation is a phenomenon that occurs when sensory neurons decrease their response to a stimulus over time, allowing the animal to respond to other, possibly more important, stimuli. For example, wild-type *C. elegans* exposed to an odor for a period of time, will ignore that odor during a chemotaxis assay due to olfactory adaptation, rather than be attracted to it. Therefore, high throughput genetic screens can be performed to reveal the genetic regulators of olfactory adaptation, such as egl-4. Additionally, transgenic worms expressing fluorescently tagged proteins can be observed for changes in localization during olfactory adaptation.

Finally, chemotaxis assays can be used in *C. elegans* to study Alzheimer's disease. Scientists can express fluorescently tagged human amyloid beta peptide - a hallmark of Alzheimer's disease - in the neurons of C. elegans. Interestingly, chemotaxis assays revealed that worms expressing amyloid beta in a population of neurons show reduced chemotaxis towards a chemo-attractant compared to the control. Many variations of this experiment could be performed, including expressing amyloid beta in other neuron populations or tissues, or determining whether any compounds can alleviate the effects of amyloid beta expression, ultimately leading to a potential therapy.

You've just watched JoVE's introduction to chemotaxis in C. elegans. First, we defined what chemotaxis is and why it is important in nature for organisms and cells. Then we demonstrated how to perform a chemotaxis assay with C. elegans. Finally, we discussed how chemotaxis can be applied to understand learning and memory, olfactory adaptation, and Alzheimer's disease. Thanks for watching!