Drosophila Larval Chemotaxis Assays

Vosshall Laboratory 8/15/2002

Text by Daniel Baez, Summer Outreach Program (High School: Institute for Collaborative Education)

Adapted from Monte, P. et al. Behav. Genet. **19**, 267-283 (1989).

Larva harvesting. Fifty larvae are collected for each test. Four tests are performed for each odorant. Larvae are collected using a sucrose solution of 15% sucrose in H_2O (15 grams in 100 mL of H_2O). To collect the maximum amount of larvae the sucrose solution may have to be increased to 60 grams of sucrose in 400 mL of H_2O. The sucrose solution is then poured directly into the vials containing larvae. To guarantee the maximum amount of larvae from one vial a #6 brush is used to gently mix the food on the bottom up. Once the larvae float to the top using a transfer pipet, that has been cut to make the hole wider, the larvae are sucked out of the vials and put into an empty petri dish (nothing in it at all, no agarose). Tap water is then added into the petri dish, which acts to rinse the larvae and also to reduce the osmotic strain of the sucrose solution. After enough larvae have been collected into the empty dish, fifty larvae are then picked out using tweezers to be tested and are transferred to an empty 35mm Petri dish until they are tested.

Olfactory tests on control larvae. Olfactory tests were done on petri dishes (diameter 100 mm) covered with a layer of 1% agarose. To make the agarose plates, first 200 mL of filtered H_2O (Barnstead Nanopure) was poured into a beaker (200 mL beaker is the easiest to use). Next 2 grams of agarose (Promega V3125) was added into the beaker that contains the water. The agarose solution in the beaker was then heated in a microwave for four minutes (or until solution is clear). The agarose solution was then poured into plates using a 10 mL serological pipet pouring exactly 10 Ml into the 100 mm petri dishes. This solution (2 grams of agarose in 200 mL of H_2O) makes ~20 dishes. Petri dishes are then left to cool. Before testing however, the petri dishes are left out to be air dried in order to simulate a fairer test. Next two clear lids (from 1.5-mL micro test tubes) are placed on both sides of the petri dish. On top of each clear lid, one small filter disk (Schleicher & Schuell, #740-E) is placed. On one of these filter disks 1 to 2 µl of a pure odorant (sometimes it needs to be increased to 3 µl when an odor did not result in a response after the odor was increased to 2 µl.) is pipetted. The pad that has the odor is recorded as not to confuse which filter disk has the odor and which does not, to keep things organized the pad is put down on the same side throughout the experiment. For example if using the right filter pad to put an odor down on make sure all experiments have the odor on the right filter pad. The control filter receives no solution, unless the odorant came in solid form and had to be dissolved in paraffin oil. In that case, pipet an equivalent amount of pure paraffin oil on the control side. Using a fine brush (size 10/0) fifty larvae were put into the middle of the petri dish. The petri dish was then immediately covered and the response was recorded for five minutes taking one shot

every six seconds. The recordings are made using a Fluorchem High Resolution Chemiluminescene and Fluorescence Imaging System (Alpha Innotech Corporation) and using the computer program AlphaImager 2200 v. 5.5 (Alpha Innotech Corporation). This program has the capability to capture 50 frames. By varying the interval between frames, one can record either 2 minutes 30 seconds (1 frame/3 seconds) or 5 minutes of data (1 frame/6 seconds)

Measuring response. Once the video is done recording images taken at 2 minutes and thirty seconds (023) and five minutes (050) (the middle and the end shots) are printed [printed on Digital graphic printer (Alpha Innotech Corporation) on thermal paper (Mitsubishi K650- CE)]. On the printed pictures the side with an odor is labeled with an O and the side without (the control) is labeled with a C. A ruler was used to measure the print out of the petri dish (which measures out to be six millimeters) and a compass was used to make a semicircle from the half point of the picture (3 millimeters on either side). A semicircle is made on both sides where the lids of the larvae are. Then the larvae on both sides are counted; this is to find the Response Index (RI) of the larvae. A formula is used to find the Response Index it is; O subtracted by C divided by the sum of O and C (O-C/O+C). This then gives the response index, which is used to figure out which odors produce the best response.