Proboscis Extension Response (PER) Assay

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Rearing healthy, appropriately aged and starved flies for PER:

1. Rear flies in fresh cornmeal food vials or bottles in the 25°C, 65% humidity incubator. Our lab incubator cycles 12 hr L/D with lights on at 7 AM and off at 7 PM. ***Note:** that flies raised in vials vs bottles show differences in behavior. If possible, rear all behavior flies in either all vials or all bottles.

2. Egg laying adults should be removed from vials 48-72 hours following introduction. This will promote larval health by limiting population size and decreasing competition. Following the first eclosion, wait 2 days to collect flies so that all flies collected are 0-2 days old. Only collect and test flies from the first eclosion. ***Note:** If you add yeast to the food to promote egg laying, do this for every vial.

3. Transfer flies to fresh vials for 72–96 hrs in the 25°C. During the collection for the transfer, do not leave flies on the pad >2 min. ***Note:** Do not use CO_2 following this step. CO_2 can damage chemosensory neurons. As a general practice, make sure ≥24 hours between CO_2 administration and testing the PER.

4. After recovery/aging, starve flies by tapping them into vials with Kimwipes soaked in tap water. To prepare starvation vials, bunch up one or two Kimwipes and add 3 or 6 mL water, respectively. For shorter starvation times, one Kimwipe is sufficient but for starvation ≥18 hrs, use two Kimwipes. Place flies back into the 25°C incubator for the duration of the starvation.

Preparing flies for the assay:

Preparing an aspirator: Attain thin, rubber tubing and insert the base end of a cut P20 pipette tip to openings at the end of each side of the tubing. For the end that will contact the fly add a small ball of cotton in order to stop the fly from coming up towards your mouth. Add a P1000 pipette tip over the end of the tubing with the cotton ball. Cut the tip of the P1000 tip so that a single fly can fit through the hole.

Prepare flies as demonstrated in: Shiraiwa and Carlson, 2007

1. Aspirate fly through the P1000 tip end of the aspirator and immediately cap with a P20 tip.

2. Hold the aspirator vertically so that the fly starts to climb straight up towards the top of the P20 tip. Simultaneously flick the tip downwards and blow so that the fly becomes stuck in the top of the pipette tip. The fly's forelegs should be crossed/folded like a mummy/ X across the thorax.

3. Remove the P20 tip and, using a razor, cut at a 45° angle towards the head from the fly's thorax just below the folding of the forelegs. This should expose the proboscis and head but keep the legs trapped without damaging the fly.

4. Gently insert a small piece of cotton under the fly so that it cannot slip out through the bottom of the tip over time. Insert the cotton so that it is just touching the end of the fly without putting any pressure on the fly which risks damaging or killing it.

5. Prepare ~6-12 flies at a time and place on a tray lined with a moist paper towel to keep the flies from drying out and becoming thirsty.

6. Satiate each fly by allowing them to drink water ad libitum. When the fly is satiated it will no longer extend its proboscis to a water offering. ***Note:** One trick is to leave a small droplet of water on the edge of the tip contacting the proboscis. The fly will drink until satiated and you do not have to satiate each individual fly manually.*

Preparing Kimwipe wicks:

Prepare small pieces of twisted Kimwipes soaked in tastant of interest as demonstrated in Shiraiwa and Carlson, 2007

1. Twist a 6 mm wide strip of Kimwipe into a thread, and pull apart into small pieces of cone-like shaped wick (< 1 cm).

2. Dip small wicks into chemical tastant/ water.

Performing positive and negative controls:

Scoring the response: no extension - 0, full extension - 1, partial extension - 0.5 (partial response is recorded for any extension between a full, robust extension and no extension). Note down the score for each fly. ***Note:** Helpful to create/print a worksheet to record data uniformly between experiments.

1. Negative control: Perform before testing any tastants, but following satiation. Contact the labellum with water. If the fly responds to the water, re-satiate the fly and retest controls. If the fly continues responding to water, discard the fly. The fly must show PER=0 in order to proceed with experiment.

2. Positive control: Before testing any tastants, dip wick into a high and very attractive concentration of sucrose (100 mM) and present the wick gently and briefly (~1s) to the fly's proboscis. Discard any fly that did not respond. The fly must show PER=1 in order to proceed with experiment.

3. Following your experiment, re-test these controls. If the fly fails controls do not count the results from the individual fly, discard the fly.

Experiment

Sample size: Test 12 flies per genotype per day to reach a minimum of 60 flies tested over 5 separate days. Each individual fly is considered an n=1.
Depending on the effect size you may need to test a larger number of flies. If so, maintain this scheme by expanding the number of days rather than the number of flies tested per day.

Statistics: As the data will not have a normal distribution you should perform a Kruskal-Wallis non-parametric test ANOVA to test significance for groups of 3 or more. For groups of 2 you may use the Mann-Whitney non-parametric u test.

Design: The experimental design depends heavily on the kind of behavior you seek to test as well as the flies you will be comparing. Before beginning your experiment, you should perform preliminary pilot experiments to:

1) Determine the appropriate starvation time and regimen for your behavior.

2) Determine at which time of day you will perform your assay. Keep in mind circadian rhythm and natural feeding time. Most people perform behavior assays either in the morning when flies tend to have a feeding peak or in the evening when flies have high locomotor behavior. Once you select your time of assay you should perform all future assays at the same time.

3) Determine which sex you will assay. You can assay either males, females or both. This will depend on your behavior. If you choose one sex over another, you should justify your choice and/or show your phenotype is not dependent on sex.

4) Determine the best concentration of chemical to use by testing a range of concentrations and generating a concentration curve

5) Determine your effect size and the number of total flies required to attain a statistical power >80%.

If you wish to test PER using the tarsi, see methods in Masek and Keene, 2013.

Cited protocol:

Shiraiwa, T., Carlson, J.R. Proboscis Extension Response (PER) Assay in Drosophila. J. Vis. Exp. (3), e193, doi:10.3791/193 (2007)