Video Article

Proboscis Extension Response (PER) Assay in Drosophila

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Abstract

Proboscis extension response (PER) is a taste behavior assay that has been used in flies as well as in honeybees.

On the surface of the fly's mouth (labellum), there are hair-like structures called sensilla which houses taste neurons. When an attractive substance makes contact to the labellum, the fly extends its proboscis to consume the material. Proboscis Extension Response (PER) assay measures this taste behavior response, and it is a useful method to learn about food preferences in a single fly. Solutions of various sugars, such as sucrose, glucose and fructose, are very attractive to the fly. The effect of aversive substances can also be tested as reduction of PER when mixed in a sweet solution.

Despite the simplicity of the basic procedure, there are many things that can prevent it from working. One of the factors that requires attention is the fly's responsive state. The required starvation time to bring the fly to the proper responsive state varies drastically from 36 to 72 hours. We established a series of controls to evaluate the fly's state and which allows screening out of non-responsive or hyper-responsive individual animals. Another important factor is the impact level and the position of the contact to the labellum, which would be difficult to describe by words. This video presentation demonstrates all these together with several other improvements that would increase the reproducibility of this method.

Video Link

The video component of this article can be found at https://www.jove.com/video/193/

Protocol

Starvation

1. First, starve the fly.
2. Put a small piece of Kim-wipe soaked with water in an empty vial.
3. Transfer 20-30 well-fed flies to the empty vial.
4. Check every 12 -24 hours to make sure the paper is moist.
5. Starve the fly for 36-72 hours.

Fix the fly

1. Take one fly in an aspirator.
2. Place a 200 µl yellow tip over the opening. Simultaneously flipping and blowing on it, you can squeeze the fly up into the yellow tip.
3. Gently apply some air to push the fly up to a point where it cannot move.
4. By using a razor, put a mark approximately at the fly's eye, then suck the fly back and cut off the tip.
5. Gently blow the fly back to position, but just before reaching the very end, make a slant cut at the position where the proboscis is going to be located. You can do without this slant cut, but it would definitely make the following process easier.

Prepare sucrose wicks

1. Twist a 6 mm wide strip of Kimwipes® paper into a thread, and pull apart into small pieces of cone-like shaped wick (< 1 cm).
2. These small wicks were dipped into sucrose solutions or water and presented to the proboscis by making contact.

Prepare controls and experimental animals

1. Before testing any tastants, give a 4% sucrose solution to the fly. This acts as a positive control. Discard any fly that did not respond.
2. Next, test dH_20 on the fly. This act as a negative control. Discard the fly if it did respond.
3. Test these controls after testing your tastants.

Discussion

In this presentation, we've gone through all the steps for Proboscis extension response assay. The method sounds pretty straightforward but as in many other behavior assays, many small things can prevent the assay from working. We have gone through many such steps that need careful attention. Here, I reemphasize some of the most important factors.

One such factor is the shape of the wick. I have shown some of the very good examples, but of course, in reality everything would be a continuous spectrum between very good and very bad. In the beginning, you might be using things close to the very good side, but day-by-day your standard might start to shift bit by bit to the not-so-good range. Human perception of so called "good" can very easily shift unconsciously. One way to prevent this is to take a picture of the wick you used in your experiments, and compare all the pictures side by side. Or, at least take one picture of the wicks that worked very well, and stare at it for a while before testing PERs.

Another important thing is the impact level of the contact. This is easier to deal with because the goal is to make the contact as gentle as possible. If you don't see the fly's head move at all, that is the best.

The starvation condition is the toughest factor to control. In many taste or olfactory behavioral assays, a starvation time of 24 hours is used very often. 24 hours might be very convenient for people to do the experiment, but if you rear your flies in a very good condition, this is a rather short period. But even if you used a rich fly food, and control the density precisely, the window for testing PER can vary from 36 hours to 72 hours. So, as an experiment, I have to say it is very inconvenient for people. There is another factor that make it more difficult for experimenters. As a matter of fact, there would be many batches, where the window for the experiment might be very short, or might not even exist at all.

So there are some major hurdles you have to go through, but the most important thing is the positive and negative control I mentioned. By keeping to those standard very strictly, you will be able to collect reliable data sets.

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References

1. Dethier, V. G. The Hungry Fly (Harvard University Press, Cambridge, 1976).

