Supplementary Information 2

Laboratory instructor preparation:

9-16 days prior to lab:
Set up new fly cultures for students. Each lab group will need 1 vial of wildtype flies. Cultures started 9-16 days ahead of the lab (with 5-10 flies/new culture) will ensure many larvae will be present in each vial.

Day of lab:
Transfer flies into new vials or discard, so that students receive larvae-only vials.

Sample Laboratory Handout:

Objective:

To examine the molecular basis of temperature-sensing in animals.

Background:

The proper detection of temperature is critical to the survival of many organisms. Body temperatures must be maintained within a narrow range so that metabolic processes operate efficiently. In addition, extremes of temperature can irreparably damage biological tissues(1).

Many organisms, from flies to humans, sense temperature by means of thermosensory ion channels that are expressed in sensory neurons. These channels--which are permeable to sodium, calcium, and other cations--open in response to their preferred temperature range. Once open, the resulting influx of positive charges depolarizes the sensory neuron and initiates an action potential(1). In this way, temperatures are detected in the periphery, and the resulting information is relayed to the central nervous system.

In the fruit fly (*Drosophila melanogaster*), one ion channel responsible for heat-sensing has been suggested to be dTRPA1(2,3). In this exercise, we will examine the temperature preference of wildtype fruit flies. After establishing what is "normal" thermotactic behavior, we will examine the temperature preference of transgenic flies in which dTRPA1 has been rendered non-functional.

Materials:

Fruit flies (in vial with fly food) (fly supplies obtained from Carolina Biological Supply)
Petri dish (10cm, 3/group)
agarose (Fisher Scientific)
graduated cylinder, 100 mL screwcap bottle to prepare agarose
small paint brush (for moving larvae around)
Beaker with distilled water
Cheesecloth (Walmart)
Beaker for waste materials
Metal spatula
Two-temperature preference assay chamber

Procedure:

Details will be provided during class. An outline is as follows:

1) Coat 2 petri dishes in agarose (use 20 mL of 2% agarose per dish).

2) Isolate 10-20 2^{nd} and 3^{rd} instar larvae (hint: they are the biggest!) using a metal spatula and small paint brush. You may find it helpful to scoop out the larvae/fly food mixture from your vial into your unused petri dish and spread it out.

3) Rinse larvae briefly with distilled water using cheesecloth. This removes the blue fly food.

4) Set plates at desired temperature. Compare each of the following:

25ºC vs. 25ºC; 25 vs. 35ºC; 25 vs. 45ºC; 25ºC vs. 15ºC; 25ºC vs. 5ºC (4 trials for each)

5) Place an agarose plate spanning the two sides of the assay chamber, with lid on, for 5 minutes.

6) Align 10-20 larvae along the midline of the plate, replace lid, and wait 5 minutes.

7) Observe locations of larvae, counting the number on each side of the plate. Record your data as a table in your laboratory notebook.

8) Clean lab supplies and work area

Questions:

1) What are some of the differences between the mouse TRPV1, mouse TRPA1, and *Drosophila* TRPA1 channels? Compare both the DNA sequence and the amino acid sequence. Which genes/proteins are most similar?

2) What is known about the ability of dTRPA1 to be activated by heat? Describe the techniques used to identify its role in heat-sensing?

3) Why do we examine the thermal preferences of flies when both plates are set at 25ºC? What is significant about this temperature?

4) Why don't we use 1st instar larvae?

5) Why would we use the assay chamber to keep plate temperatures constant at 25ºC, even if we planned to examine chemotactic behaviors rather than thermotactic behaviors?

Write-up:

There will be a write-up associated with this laboratory exercise. In this write-up, give appropriate background information for the topic, state a hypothesis, describe the methods used in your experiments, and provide the data collected both in written form and with tables and graphs. Use appropriate statistical analyses to compare your experimental groups. Your discussion of this exercise should describe how your work relates to previous research in this field and whether or not your hypothesis was supported. More information will be provided by the instructor during class.

References:

1. Temperature Sensing Across Species (McKemy, 2007).

2. The Drosophila ortholog of vertebrate TRPA1 regulates thermotaxis (Rosenzweig et al., 2005).

3. An internal thermal sensor controlling temperature preference in Drosophila (Hamada et al., 2008).