Assaying thermotaxis behavior in *Drosophila* 3rd instar larvae using a two-way choice test

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Introduction

When given a choice between two temperatures, *Drosophila* larvae will select the preferred temperature. This protocol outlines a step-by-step procedure for performing a two-way choice test on Drosophila larvae, to identify the preferred temperature.

Procedure

Rearing 3rd instar larvae

1) Tap adult flies over to bottles or vials containing fresh food and yeast granules. Use healthy flies that had not been exposed to CO_2 for ≥3 days, and do not expose the flies to CO_2 during the transfer.

2) After 2—3 days, remove the flies from the bottles by gentle tapping. Do not use CO_2.

3) Allow the larvae to grow for an additional 2—3 days. Add H_2O as needed to ensure that the fly food remains moist.

Collection of larvae from the food

1) Scoop out food containing the larvae (~3—6 ml) into 40 ml of a 15% sucrose solution in a 50 ml tube. The food will sink and the larvae will float.

2) After a brief incubation (~30—60 seconds), transfer the larvae (using a cut off P1000 pipet tip; diameter opening 5—8 mm) to a fresh 50 ml tube and wash the larvae with 15% sucrose until all remaining food debris is removed (usually 1—3 times).

3) Transfer the larvae to a new tube, wash them with H_2O at least two additional times to remove the sucrose. Be sure that all pupae and dead flies are removed.

4) (Optional) If the collected larvae include more than 5% of 1st and/or 2nd instar larvae, transfer the larvae to a bacterial plate with moisturized 2% agarose, and remove the early stage instar larvae by aspiration.

5) Before initiating the behavioral assays, keep the collected larvae for 15—30 minutes at room temperature (~22 °C) in a 35 x 10 mm Petri dish or in the cap from a 50 ml tube under a dim light with adequate moisture to ensure that they do not become desiccated.

Set-up for thermotaxis assays

1) The apparatus for performing the thermotaxis assay consists of two adjacent aluminum blocks

containing temperature controlled circulating H_2O (Thomas Scientific, 9106). The two blocks are separated by a thin insulator (X-ray film) and held together in a plexiglass tray.

2) Monitor the temperatures on the test plates using microprobe thermometers (BAT-12, Physitemp Instruments) and flexible implantable probes (IT-21, Physitemp Instruments) placed at the center of each side

of the test plates. The temperatures deviate ±0.2 °C over the whole area on each side of the test plate.

3) The test plates used to perform the thermotaxis assays are plexiglass covers from 6 x 12 mini trays (Nunc 136528) coated with 7 ml of 2% agarose.

4) Establish the temperatures on each side of the test plates by placing the cover on the apparatus.

5) To prevent drying, lightly spray H_2O onto the agarose surface of the test plate.

The Behavioral test

1) Transfer 40—100 larvae (≥95% 3rd instar) from the Petri dish or cap described above to the middle of the two sides of the thermotaxis plate, and conduct the experiment in complete darkness.

2) After 15 min, photograph the test plate, tabulate the number of larvae on each side of the plate, and calculate the preference index (PI) according to the following formula:

PI = [(no. of larvae on the 18 ºC side) – (no. of larvae on the side with the variable temperature)]/(total no. of larvae on both sides of the test plate)

Figures

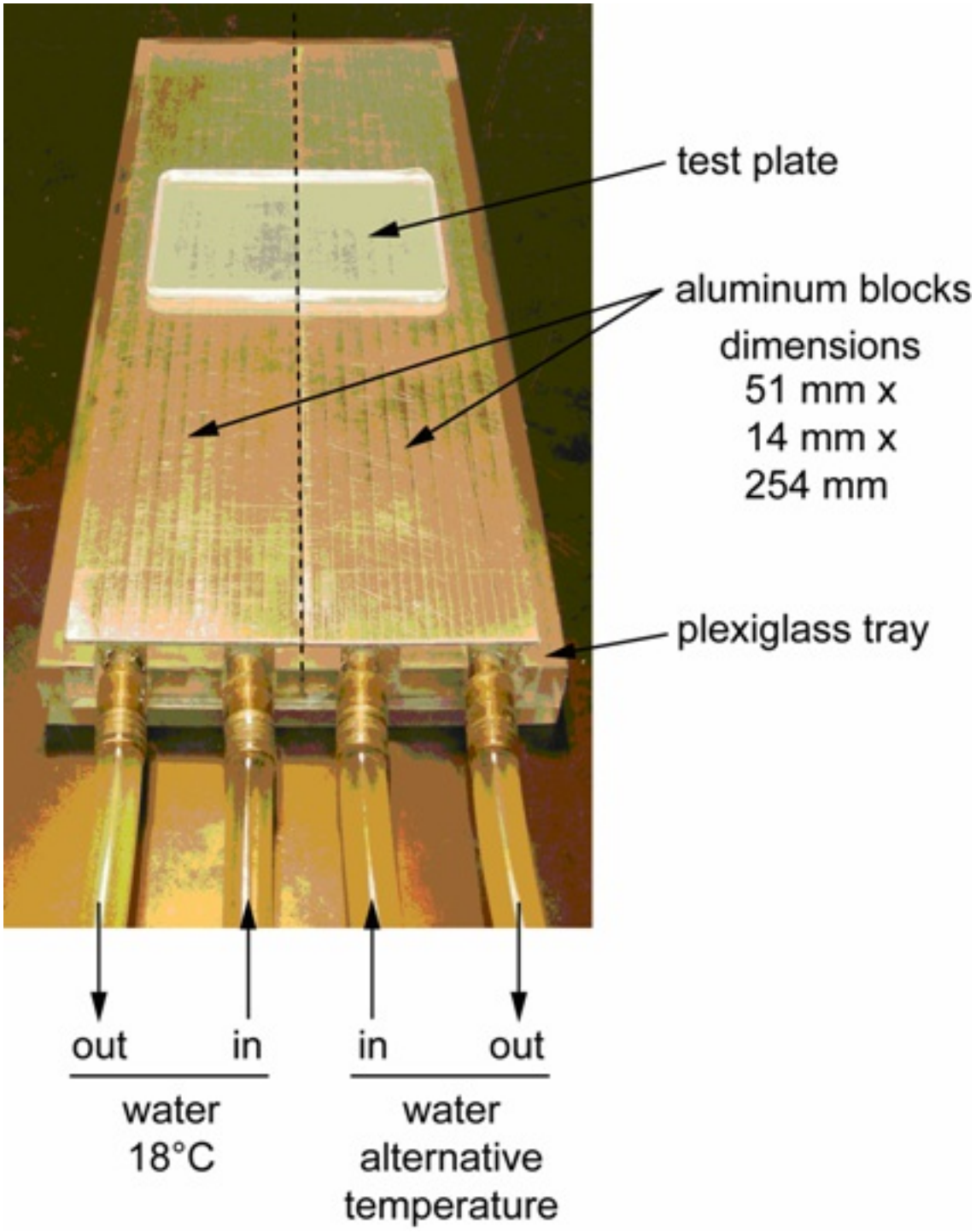

Figure 1

Set-up for thermotaxis assays

Control of thermotactic behavior via coupling of a TRP channel to a phospholipase C signaling cascade

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