Novel object boldness (Adult zebrafish)

The aim of this experiment is to measure boldness by recording the amount of time spent close to a foreign object in the testing arena, a model predator.

Fish are placed singly into the setup, and the time spent within about one body length of the novel object is recorded. This area is defined using the VideoTrack programme from ViewPoint (although other software from other companies can also be used). As a control, the time spent in a comparable neutral area can also be recorded. Bold fish should spend more time near to the novel object (the predator model) compared to more timid fish.

Experimental setup

The novel object (supposed to look like a predator) is made by filling a 15ml Falcon tube with modelling clay. The first centimetre of the tube is yellow (head, conical) and the rest of the tube is filled with dark clay (dark blue). Two black eyespots are added to the head section of the model. The model has a length of wire protruding the centre of the dorsal side, allowing it to be suspended over one end of the tank. This is achieved by placing a plastic pipette across the tank to act as a beam.

The novel object is suspended over a standard clear plastic fish tank that measured 20 x 25 x 30cm and is filled with 10L system water at ambient room temperature (25C). The tank is lit from above with white light. All experiments were conducted in the afternoon (1pm onwards).

Experimental procedure

1) In this experiment, the amount of time spent within one body-length of the novel object is recorded using commercially available videotracking software. We have used Zebralab from ViewPoint, but other companies also provide suitable software.

2) There is no need to habituate fish to this setup. The test will thus also record the reaction of the fish to the novel arena. Results tend to be robust and repeatable.

3) A single adult fish is gently placed into the setup and its position is videotracked for 10 minutes. The fish is then removed and placed in a holding tank. This procedure is repeated for each fish in a group.

4) Any fish which does not swim for a significant period during the experiment (more than seconds) is removed from the analysis, since this would bias the amount of time spent close to the novel object.

5) The results are exported to Microsoft Excel for analysis. Parameters that can be compared include the percentage of time spent on either side of the tank, and the total distance swum during the experiment.

6) Appropriate statistical tests can be used to analyse the data. For a comparison of two genotypes, a T-test may be sufficient. For analysis of drug treatments in different genotypes, ANOVA followed by a posthoc. test may be needed.

Associated publications

For a published example of this experiment, refer to Norton et al., 2011 (J. Neurosci. 31(39):13796-13807). The protocol is modified from Modified from Wright et al., 2003 (Naturwissenschaften. 2003 Aug;90(8):374-7).