SOP Test 11 - Passive avoidance test in adult zebrafish

1.0 Purpose

1.1 The passive avoidance test is a fear-based standard operating procedure (SOP) to measure learning and memory in adult zebrafish. This test uses an apparatus consisting of a tank separated into two distinct compartments. Two visual stimuli (either blue or green) can be switched on in both compartments. The animals can freely explore compartments of the chamber and a shock is delivered in one side of the compartment associated with one visual stimulus (either blue or green). Animals learn to associate a color with the shock. The latency to pass the gate in order to avoid the stimulus is used as an indicator of learning. The latency to avoid the stimulus associated with the shock 24hours after the training measures memory.

2.0 Scope:

2.1 This protocol is suitable for individuals who have been trained in zebrafish handling and care.

2.2. Any queries, comments or suggestions, either relating to this SOP in general, or to a specific problem encountered during the procedure should be addressed to the head of the AMATrace behaviour platform, Dr. Laure Bally-Cuif.

2.3. Any deviation from this protocol should be addressed to the head of the AMATrace behaviour platform, Dr. Laure Bally-Cuif.

2.4. All zebrafish should be kept, propagated and handled in accordance with the institutional guidelines on animal safety. Please also keep in mind the principle of replacement, refinement and reduction.

3.0. Safety Requirements

3.1. General laboratory safety procedures should be followed, which include: no eating, no drinking and no applying of cosmetics in the work area. Laboratory gloves must be worn at all times in the work area, unless the protocol specifically notes otherwise.

4.0. Associated Documents:

5.0 Notes:

5.1. This protocol is designed to compare animals that have been raised under similar conditions. Fish density, feeding regimes and age will play a significant role in modifying the level of adult locomotion.

5.2. Adult zebrafish do not show sex-specific difference in locomotion levels when measured using this protocol. Fish of both sexes can thus be combined in the experiment.

5.3. Environmental factors can play a significant role in changing locomotion levels. Behaviour should be recorded in a silent behavioural room with minimal experimenter disturbance. Lighting, temperature and time of day should be kept constant during testing.

5.4 Fish must be feed normally during the habituation period and the training sessions.

6.0 Quality Control:

6.1. A similar amount of water should be placed in the tank for each recording session.

6.3. Fish should be raised in groups of a defined number (20-25 fish in a group) from larval stages onwards.

6.4. Fish need to be habituated before analysis in the behavioural setup. At least 3 days of habituation in the set-up apparatus is recommended.

6.5 The cubicle (set-up apparatus) light must be cyclical , and adjusted to facility a day/night condition.

6.6. Care must be taken that the set-up apparatus light (lux illuminance) is similar to the light conditions used during fish raising.

6.7 The shocker parameters must be well set, at an Intensity of 75mV and a frequency of 2.5secs.

7.0 Equipment:

7.1 A cubicle (ViewPoint S.A) set-up connected to a core computer recording system that contains VideoTrack software from ViewPoint S.A.

7.2 The cubicle contains 8 standard small fish tanks (30x15x10cm) filled with 1L of water. These tanks are spatially split in two halves by a transparent barrier. However the fish can swim freely in the tank, passing under the barrier.

7.3 Homemade electronics device, controlling the shocker and the light system.

8.0. Supplies:

(Optional) drugs or chemicals to modify adult behaviour

9.0. Procedure:

9.1 ***habituation*****:** 3 days before testing, individual fish are placed singly into the cubicle setup using a standard fish net.

NB : make a first locomotion recording using the "training protocol" (9.3) before the training.

9.2 ***Training: protocol generation***. This protocol will be used only to allow the electric shock:

9.2.1 Switch on the computer and double-click on the ViewPoint space rocket icon. Launch the "Videotracking" option within the Videotrack menu.

9.2.2 Create a new protocol

--> "Parameter" > "generate protocol" > "protocol parameters" > second tab "time" > experiment time enter "3 hours" > in integration period enter "300secs"

9.2.3 Generate area

In order to shock the fish on the side associated with one light stimulus, areas need to be delineated using the Videotrack programme. Draw one area ("area11") that encompasses the entire tank. The area must be called "area 11" because this output generates an electric shock via the shocker. Do it for the 8 tanks of the cubicle.

Activate via the manual control the generator and the associated visual stimulus on the same side. On the opposite side, switch on the color stimulus. Then leave 15 minutes resting period. Repeat the experiment 13 times.

9.3 Testing: generate a training protocol.

9.3.1 Create a new protocol within the "Videotracking" software.

--> "Parameter" > "generate protocol" > "protocol parameters" > second tab "time" > experiment time enter "1 hour" > in integration period enter "300secs".

9.3.2 Generate area

In order to measure the time spent in both sides of the tanks, several areas need to be delineated. Draw different areas according to the different tank parts. Do it for the 8 tanks of the cubicle.

9.3.3 Select the appropriate threshold:

In the detection threshold menu, set animal color to black and detection threshold to 12 (this value may need to be calibrated for each new experimental setup)

9.3.4 Save the modified protocol

–> "file">"save

9.3.5 Start the recording

The Videotrack software should be started next. Choose "Execute" from the Experiment menu and input a name for the experiment – e.g. WT 1. Press the background and then start buttons.

–> "Execute" > "Experiment" > name your experiment > "background" > "start".

Results Analysis:

9.4 The results should be exported into Microsoft Excel and the data points analysed. The total distance spent in the conditioning area can be compared after and before training. Different latency periods may also indicate other learning and memory parameters.

10.0 Supporting Information:

11.0 History Review:

12.0 Emergency Procedures: