SOP Test 6 - Measuring Exploration in Adult Zebrafish

1.0 Purpose:

1.1 The purpose of this standard operating procedure (SOP) is to measure the exporation level of mature adult zebrafish by recording the amount of time needed to explore a novel environment, a T-shaped aquarium.

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 expression of exploration.

5.2. Adult zebrafish do not show sex-specific difference in exploration 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 the expression of boldness. 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.

6.0 Quality Control:

6.1. The T-shaped exploration tank should cleaned with both 70% ethanol and then fresh system water before starting the experiment. A similar amount of water should be placed in the tank for each recording session.

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

6.4. Fish need do not need to be habituated before analysis in the behavioural setup. Fish can be brought to the testing room in their home tanks immediately before analysis begins.

6.5. Care must be taken that the tank is illuminated evenly without shadows or reduced light at the corners. The observer should remain out of sight of the fish in the tank throughout the duration of the experiment.

6.6. Fish which freeze and do not swim for long periods should be removed from the final experimental analysis. Including such fish would skew behavioural results, since it would necessarily increase the amount of time needed to explore the T-shaped aquarium.

7.0 Equipment:

7.1. The only piece of equipment needed to measure exploration is a large plastic T-shaped aquarium (home-made). The tank is made out of clear Plexiglas and has a white bottom. Each arm of the tank is 80cm long and 10 cm high. A blue start-line is marked on one arm of the tank using an indelible marker pen.

7.2. (Optional) The exploration pattern of adult zebrafish in the tank can be recorded using a video camera and tracking software (such as VideoTrack from ViewPoint S.A.). Alternatively, the behaviour of the animals can be recorded live using a stopwatch or timer.

8.0. Supplies:

Zebrafish for analysis, 12 – 15 for each genotype or treatment group.

(Optional) drugs or chemicals to modify adult behaviour,

System water to fill the setup.

9.0. Procedure:

9.1. Adult fish are raised to adulthood in groups of 15 or more. Immediately before testing, fish are transported to the behaviour room (ideally in their home tank, or if not possible then in a 10L plastic mouse cage).

9.2. The exploration tank is filled with system water up to a depth of 10cm. The observation tank is lit from above by a circular white-light light bulb. If live observation is used to record the fish's behaviour, the T-shaped aquarium should be arranged so that fish can be watched without disturbing their behaviour.

9.3. If the VideoTrack programme is being used to make films of behaviour, it needs to be started at this point. Switch on the computer and double-click on the ViewPoint space rocket icon. Launch the "Videotracking" option within the VideoTrack menu. In the detection threshold menu, set animal colour to black and detection threshold to 12 (this value may need to be calibrated for each new experimental setup). Make sure that the programme is able to track the fish smoothly, without either miss-tracking or losing the animal.

9.4. In order to measure track the fish within the tank, define one large rectangular area (area 1) that covers the whole extent of the T-shaped aquarium.

Measurement Process:

9.5. Fish are placed singly into the central arm of the T-shaped exploration tank using a standard fish net. The amount of time needed to fully explore the tank (by swimming to within one body-length of the end of each arm) after crossing the initial start line is recorded – either by live observation using a stopwatch to record time, or by using Videotracking software.

9.6. At the end of the experiment fish are then placed in a holding tank, or back in their home tank following testing.

9.7. If the the VideoTrack software is being used, choose "Execute" from the Experiment menu and input an name for the experiment – e.g. WT 1. Press the background and then start buttons.

Results Analysis:

9.8. The results should be manually stored in a Microsoft Excel spreadsheet and the data points analysed. For exploration, the mean time needed to fully explore the T-shaped aquarium can be compared for each genotype or treatment group.

9.9. Plot the data as a histogram, and use appropriate statistical tests to compare the different groups (either a Student's *t*-test, or ANOVA followed by an appropriate post hoc test).

10.0 Supporting Information:

11.0 History Review:

12.0 Emergency Procedures: