SOP Test 7 – Conditioned Place Preference (CPP) test for adult zebrafish

1.0 Purpose:

1.1 The purpose of this standard operating procedure (SOP) is to provide a protocol that describes how to carry out a conditioned place preference (CPP) test using adult (3-months or older) zebrafish. During the test the fishes swimming paths are monitored and recorded allowing for the subsequent analysis of a number of behavioural variables.

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:

6.0 Quality Control:

6.1. Adult zebrafish are measured in clean specialized tanks that are described below. The tanks are reused, cleaned, washed, and dried in between each experiment.

7.0 Equipment:

7.1 The experimental setup of the conditioned place preference (CPP) test consists of two main parts:

a) 15 large retangular fish tanks (33 x 14,5 x 13 cm) are each filled with 2 litres of water. The tanks are divided into two equally spaced compartments: the preferred compartment is coloured in brown, whereas the non-preferred compartment is coloured in white. Within the tanks and during the behavioural assay the fishes are thus visually isolated from each other.

b) A computer recording system that contains Videotrack software from ViewPoint (Champagne-au-Mont-d'Or, France) that allows to monitor and record the fishes behaviour and swimming paths.

8.0 Supplies:

8.1 Adult (3-month or older) zebrafish for analysis, 15 for each genotype or treatment group.

8.2 15 large retangular fish tanks (33 x 14,5 x 13 cm) that are divided in an equally spaced white and brown compartment.

8.3 A balance that can measure differences between 10 to 1000 mg.

8.4 a) 40 mM D-Amphetamine hemisulfate salt (Sigma 5880) solution dissolved in 110 mM NaCl; b) 110 mM NaCl solution.

8.5 Fish nets.

9.0. Procedure:

9.1 Habituation

Day 1

9.1.1 The weight of all fishes is measured in order to determine the future dose of the injection (1 µl=100 mg body weight).

9.1.2 The 15 adult (3-months or older) zebrafish are then placed separately into the large rectangular tanks.

9.1.3 The fish are allowed to swim freely for 2 hours in their tanks and to get accustomed to the new environment. Following this habituation process, individual fish are placed separately into mouse cages for at least 1 hour. The procedure is repeated at least once before the fishes are placed separately into mouse cages overnight.

Day 2

9.1.4 The habituation procedure as it is described for day 1 is repeated and the fishes are placed separately into mouse cages overnight.

9.3 Measurements, injection scheme, and analysis

Day 3

9.3.1 The 15 individual fish are placed separately into the corresponding rectangular tanks and allowed to swim freely for 2 hours. During this time interval, their activity is recorded using the Videotrack software. The preferred compartment is subsequently defined as the compartment in which a fish spends most of its time. The place preference (PP) is calculated as the percentage of time that the fish spends in the preferred compartment.

9.3.2 The fishes are removed from the tanks and are placed separately into mouse cages for at least 1 hour. After the removal of the fishes a separation wall is placed in the tanks separating the white from the brown compartment.

9.3.3 10 fishes are intraperitoneal injected with 40 mM of amphetamine in 110 mM of NaCl (1 µl=100 mg body weight) and are placed separately into the white, non-preferred compartment of their corresponding tanks for 2 hours; similarly, 5 control fishes are injected with 110 mM NaCl and are also placed into the white, non-preferred compartment of their corresponding tanks for 2 hours.

9.3.4 Following the conditioning the fish are removed from the white compartments and are placed separately into mouse cages overnight.

Day 4

9.3.5 All 15 fishes are intraperitoneal injected with 110 mM of NaCl (1 µl=100 mg) and placed separately into the brown, preferred compartment of their corresponding tanks for 2 hours.

9.3.6 The fishes are removed from the brown tank compartment and placed separately into mouse cages overnight.

Day 5

9.3.7 The intraperitoneal amphetamine and saline control injections, as described in 3.3 for day 3, are repeated.

9.3.8 Following the conditioning the fish are removed from the white compartments
and are placed separately into mouse cages overnight.

Day 6

9.3.9 The intraperitoneal saline injections and the confinement of the fish to the preferred brown compartment, as described in 3.5 for day 4, is repeated.

9.3.10 The fishes are removed from the brown tank compartment and placed separately into mouse cages overnight.

Day 7

9.3.11 The intraperitoneal amphetamine and saline control injections, as described in 3.3 for day 3, are repeated.

9.3.12 Following the conditioning the fish are removed from the white compartments and are placed separately into mouse cages overnight.

Day 8

9.3.13 The measurement of the place preference (PP) as determined on day 3 is repeated. The 10 amphetamine and 5 saline control injected fish are placed separately into the corresponding rectangular tanks and allowed to swim freely for 2 hours. During this time interval, their activity is recorded using the Videotrack software.

9.3.14 The change in place preference seen in amphetamine- but not in

$$\%\,\text{of change} = \frac{(PP - CPP)}{PP}$$

control-injected fish can thus be calculated as follows:
where %of change is the relative change in place preference, PP is the place preference before treatment, and CPP is the place preference after the amphetamine.

10.0 Supporting Information:

See publications:

K.J. Webb, W.H.J. Norton, D. Trümbach, A.H. Meijer, J. Ninkovic, S. Topp, D. Heck, C. Marr, W. Wurst, F.J. Theis, H.P. Spaink and L. Bally-Cuif. Zebrafish reward mutants reveal novel transcripts mediating the behavioral effects of amphetamine. Genome Biology 10: R81 (2009).

J. Ninkovic, A. Folchert, Y.V. Makhankov, S.C.F. Neuhauss, I. Sillaber, U. Straehle and L. Bally-Cuif. Genetic identification of AChE as a positive modulator of addiction to the psychostimulant D-amphetamine in zebrafish. J. Neurobiol. 66:463-475 (2006).

J. Ninkovic and L. Bally-Cuif. The zebrafish as a model for assessing the reinforcing properties of drugs of abuse. In Methods, the Zebrafish; S. Burgess Ed., Elsevier Press. Vol. 39, pp. 262-274 (2006).

11.0 History Review:

12.0 Emergency Procedures: