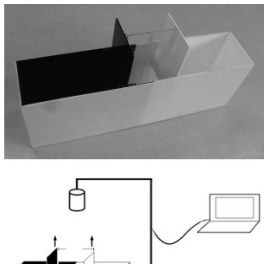
Aug 28, 2018 Version 2

Light/dark preference test for adult zebrafish (*Danio rerio*) V.2



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Fish behavior and physi... Medicinal Plants Southe...

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

The light/dark test is increasingly being adopted as one of the main behavioral assays in zebrafish neuroscience research. It is based on the innate preference of adult zebrafish for a black vs. a white compartment. Modifications and extensions of the protocol increased its breadth, but also its heterogeneity. The protocol presented here involves confining the animal in a central compartment for a initial 3-min acclimation period, followed by free exploration of an acryllic tank with a black and a white compartment. In addition to observing the spatial distribution of the animal, "ethological" variables (risk assessment, erratic swimming, freezing, thigmotaxis) are also manually scored in the white conpartment; these endpoints are selectively sensitive to drug treatments. The protocol is intended to measure anxiety-like behavior, with anxiogenic effects decreasing time on white and increasing risk assessment and thigmotaxis; some treatments also increase erratic swimming and freezing.

Guidelines

Standard guidelines for the ethical care of fish in research apply. Before beggining experiments, researchers should obtain all legally binding licenses, inclusing IACUC approval.

Troubleshooting

Safety warnings

The behavioral protocol is usually very innocuous for researchers. Care must be taken while preparing drugs for injection, following standard biosecurity practices. Moreover, care must be taken while capturing fish to not expose open wounds to tank water, reducing the risk of acquiring waterborne infections (cf. Murray et al., 2016, doi: 10.1089/zeb.2015.1206, for a primer on zebrafish biosecurity).

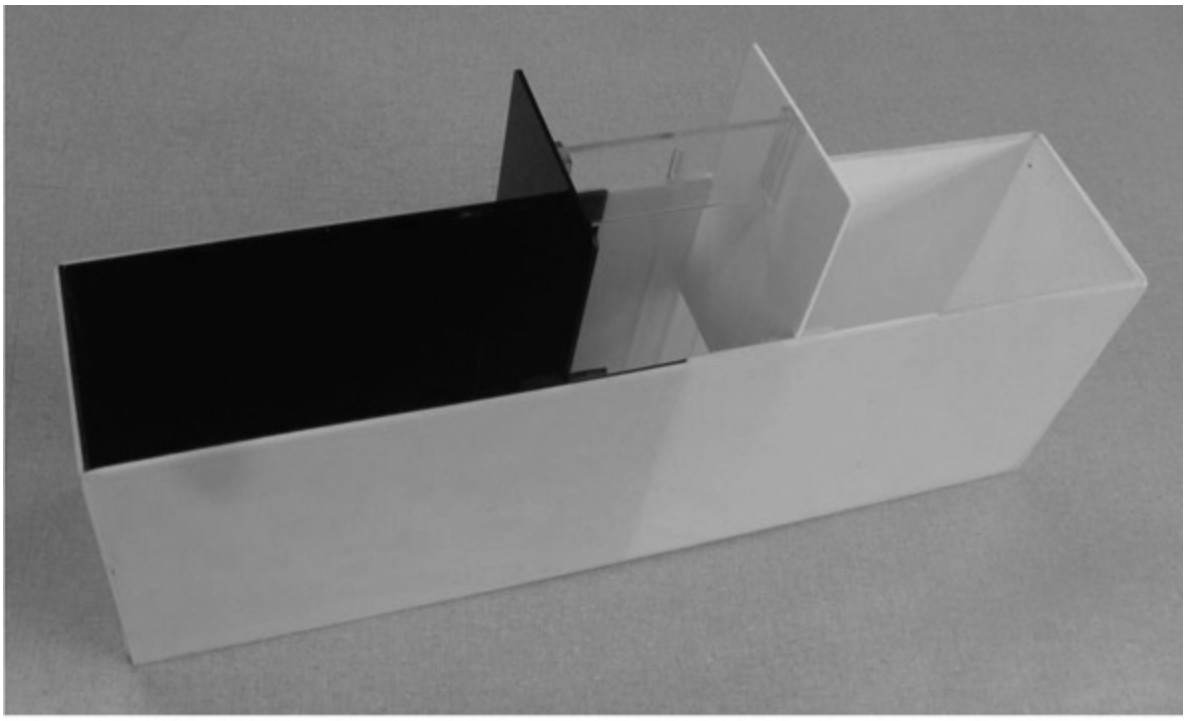
Before start

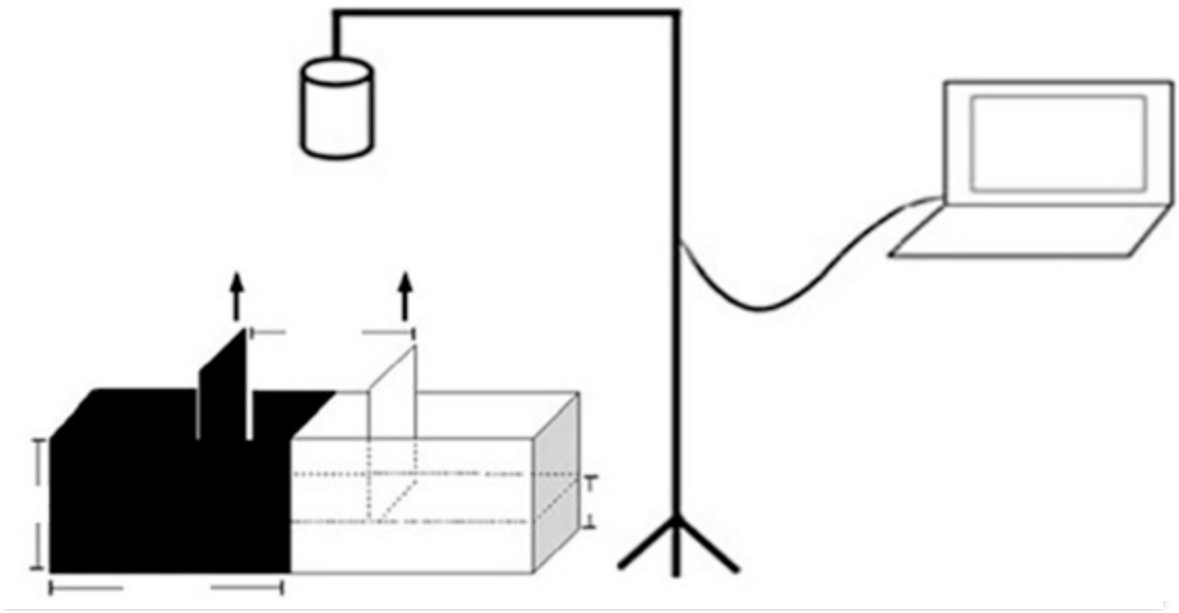
1. Room illumination is a critical variable, as it can alter fish behavior and the aversiveness of the white compartment. Before each trial, light levels should be measured; variations above or below 2 SDs from the average should be considered as too discrepant for an experiment. Ideally, lighting above the tank should be on the range of 500-600 lux.
2. Use Gaussian white noise throughout the experiment to cover unwarranted noise in the experimental room.
3. Bring the animal in a temporary transport recipient into the behavioral test room.

Apparatus

1

- An acrylic tank (15 cm × 10 cm × 45 cm height × width × length) is used that is divided equally into one-half black and one-half white.
- Walls and bottom are either black or white, so as to warrant uniform substrata for each compartment.
- The water column is kept at 10 cm, yielding a final volume of 4.5 liters.
- Water should be de-chlorinated.
- The colored material chosen should not be reflective, to avoid the tendency of those animals that present shoaling tendencies to behave in relation to their own reflection.
- The tank contains central sliding doors, colored with the same color of the aquarium side, thereby defining an uncolored central compartment measuring 15 cm × 10 cm × 10 cm.

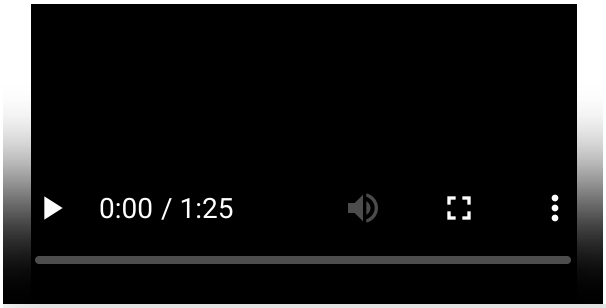




Preparation steps

2 Room illumination is a critical variable, as it can alter fish behavior and the aversiveness of the white compartment. 2. Before each trial, light levels should be measured; variations above or below 2 SDs from the average should be considered as too discrepant for an experiment. Ideally, lighting above the tank should be on the range of 500-600 lux.

3 Inject drug or vehicle in the animal, using a microsyringe (10 µL vol) and a wettened spong (see Kinkel et al., 2010; DOI: 10.3791/2126). Injection volume should be proportional to body weight (1 µL / 0.1 g), and the animal should be anesthetized with cold water (~ 12 ºC) or eugenol (60 ppm).



4 Use Gaussian white noise throughout the experiment to cover unwarranted noise in the experimental room.

5 Bring the animal in a temporary transport recipient into the behavioral test room.

Behavioral assay

6 Transfer the animal to the central compartment of the tank with a net. Allow the animal to acclimate to the tank for 3 min

00:03:00 Duration of acclimation

7 Remove the sliding doors, allowing the fish to swim freely in the apparatus for 15 min. Video recording is recommended.

00:15:00 Duration of observation

8 Transfer the animal to a discard tank with a net.

Endpoints

9 Videos should be analyzed blindly (i.e., the experimenter should not know the treatment/genotype of the animal) by two experienced researchers. An event/spatial distribution logger, such as X-Plo-Rat (https://github.com/lanec-unifesspa/x-plo-rat).

10 Variables to be recorded (and ZBC references) are:-Time spent in the white compartment (ZBC 1.137)-Latency to enter the white compartment-Number of entries in the white

compartment-Duration of entries in the white compartment-"Risk assessment" events (a fast (<1 s) entry in the white compartment followed by re-entry in the black compartment, or a partial entry in the white compartment (i.e., the pectoral fin does not cross the midline))-Duration and number of erratic swimming (ZBC 1.51): a zig-zag, fast, unpredictable course of swimming of short duration.-Duration and number of freezing events (ZBC 1.68): complete cessation of movements except eye and opercular movements.-Time on thigmotaxis (ZBC 1.173): swimming in a distance of 2 cm or less from the nearest wall.

11 If X-Plo-Rat is used, risk assessment, erratic swimming, freezing, and thigmotaxis are lassigned to keyboard keys (A, S, D, and F are a good choice). The I-maze pre-configuration can be used, with the "walled" squares ewuivalent to the black compartment.

Software

X-Plo-Rat	NAME
Windows	OS
Fernando Cardenas	DEVELOPER
https://github.com/lanec-unifesspa/x-plo-rat	REPOSITORY

Expected result

Under these conditions, adult zebrafish present preference for the dark compartment, spending about 80% of a 15 min session on that portion of the tank.
Drugs or treatments with an "anxiogenic"-like effect increase the preference, and can also increase risk assessment, thigmotaxis, erratic swimming, and/or freezing in the white compartment. Conversely, drugs with an "anxiolytic"-like effect reduce some or all of these endpoints.