Natural preference of zebrafish (*Danio rerio*) for a dark environment

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Abstract

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The zebrafish (*Danio rerio*) has been used as a model in neuroscience but knowledge about its behavior is limited. The aim of this study was to determine the preference of this fish species for a dark or light environment. Initially we used a place preference test and in a second experiment we applied an exit latency test. A two-chamber aquarium was used for the preference test. The aquarium consisted of a black chamber and a white chamber. In the first experiment the animal was placed in the aquarium and the time spent in the two compartments was recorded for 10 min. More time was spent in the black compartment (Wilcoxon matched-pairs signed-rank test, $T = 7$, $N_1 = N_2 = 18$, $P = 0.0001$). In the second experiment the animal was placed in the black or white compartment and the time it took to go from the initial compartment to the opposite one was recorded. The test lasted a maximum of 10 min. The results showed that the animal spent more time to go from the black to the white compartment (Mann-Whitney rank sum test, $T = 48$, $N_1 = 9$, $N_2 = 8$, $P<0.0230$). These data suggest that this fish species has a natural preference for a dark environment and this characteristic can be very useful for the development of new behavioral paradigms for fish.

The zebrafish (*Danio rerio*) has been extensively used in research. In the late 1970's, investigators began to use it in neuroscience, studying the startle response and Mauthner cells. More recently, the zebrafish has been used as a model in vertebrate biology because many of its characteristics make it easier to study than some other vertebrate organisms (1). This species has been used in the study of reproductive (2), teratologic and developmental perturbations (3), and of environmental (4), genetic (5) and behavioral characteristics. In behavioral studies, the zebrafish has been used to determine the importance of innate and learned behavior in fish aggregation (6), the long- and short-term effects of isolation, color variation, back-ground color, physical and chemical characteristics of water, the effect of punished training, and the role of sight in schooling behavior. Granato et al. (7) studied the genes that modify zebrafish behavior with respect to touch response, motility and escape reflex. Li and Dowling (8) quantitatively analyzed the visual sensitivity of zebrafish, and suggested that this sensitivity is regulated by an endogenous circadian clock.

Although the zebrafish is being used in many kinds of experiments, now mainly in genetics, knowledge about its behavior is still limited. In our laboratory we are trying to develop an inhibitory avoidance test. For this purpose we needed to establish a possible natural environmental preference and/

or aversion for this species.

Thus, the aim of the present study was to determine the preference of this fish species for black or white compartments. We first used a place preference test and later an exit latency test.

Fifty-three experimentally naive, mature zebrafish (*Danio rerio*), approximately 6 months old and of undetermined sex, weighing 0.1 to 1.0 g were used. The animals were obtained from a single source (Cemusiquário, São Carlos, SP, Brazil) and maintained at 18-22ºC in a continuously filtered and aerated 4.5-liter aquarium. They were kept in the aquarium (2 fishes per aquarium) under a natural light cycle and fed five times a week with Nutrafish ration (Nutravit Comercial e Industrial Ltda., Campinas, SP, Brazil). There was a 3-week acclimation interval between the purchase of the fish and the beginning of the experiment.

A two-chamber aquarium was used for the preference test. The aquarium consisted of a dark chamber and a white chamber (15 cm high, 10 cm wide and 22.5 cm long each). Two sliding doors delimited a 5-cm passage area (start compartment) between the two compartments. When the sliding doors were removed, the animals had access to both compartments.

Experiment 1: This experiment was held on two consecutive days. On the first day, the animals were placed individually either in the white or in the black compartment and after 30 s the doors were removed and they had free access to the black and white compartments for 10 min. On the second day, the same animals were placed in the start compartment, the doors were removed after 30 s and the time spent in each compartment was recorded for 10 min.

Experiment 2: In this experiment half of the animals were released from the white compartment and the other half from the black compartment, delimited by one of the doors. They were placed in the compartment individually and after 30 s the sliding door was opened and they had free access to both compartments. The time the animals took to pass from the initial compartment to the opposite one was recorded. The maximum time allowed was 10 min.

Values are reported as medians and interquartile ranges. The Wilcoxon matched-pairs signed-rank test was used to determine the differences between the time spent in the black and white compartments in both experiments (dependent samples). Differences were considered significant if the probability of error was less than 5%.

The animals spent significantly more time in the black compartment than in the white compartment (Figure 1) (Wilcoxon matched-pairs signed-rank test, $T = 7$, $N_1 = N_2 = 18$, $P = 0.0001$).

A difference in latency was observed for the groups that started in different compartments (independent samples). The animals took longer to exit from the black to the

Figure 1 - Time spent in the black and white compartments during the preference test of zebrafish (Danio rerio). Data are reported as medians and interquartile ranges for 18 naive fish. *P<0.001 compared to the white compartment choice (Wilcoxon matched-pairs signed-rank test).
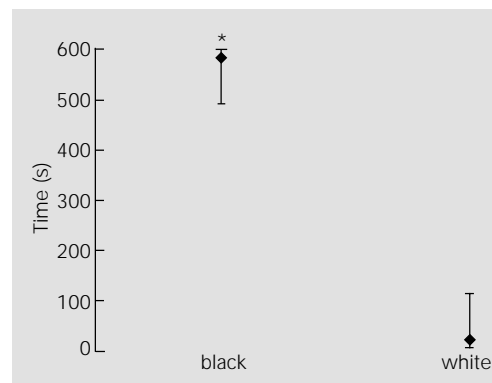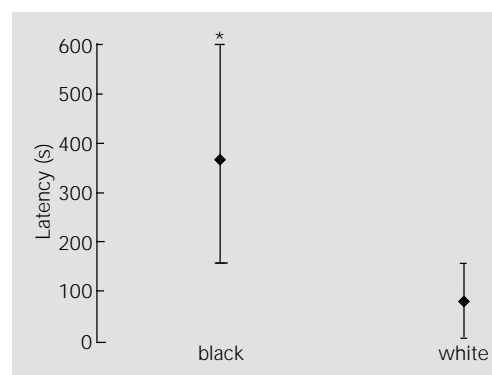

Figure 2 - Latency to exit from black and white compartments by zebrafish (Danio rerio). Data are reported as medians and interquartile ranges for 17 naive fish. *P<0.05 compared to the white compartment choice (Mann-Whitney rank sum test).

white compartment than from the white to the black compartment (Figure 2) (Mann-Whitney rank sum test, $T = 48$, $N_1 = 9$, $N_2 = 8$, $P<0.0230$). These data indicate that this fish species has a natural preference for the black compartment.

In neuroscience, some studies of reinforcement and learning processes use methodological procedures that include environmental discriminations, like dark and light environments. For example, the conditioning place preference test is used to determine the reinforcing effects of drugs. In this procedure the animals have to associate a specific compartment (black or white, dark or light) with the effects caused by drugs. A subsequent increase in the time spent in a given environment during a preference test is taken as evidence for the drug's positively reinforcing effects (9). In this kind of experiment the knowledge of the natural preference for one of the compartments is very important, because it can mask the results. The animals can spend more time in a compartment not because they were reinforced by the drug, but because they feel security in that compartment. This kind of environmental discrimination is also used in avoidance conditioning. In this case, the animals have to associate a specific compartment with an aversive stimulus.

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