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A place preference test in the fish Nile tilapia

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Abstract

The Nile tilapia fish (*Oreochromis niloticus*) has a high potential to be used as a model in neuroscience studies. In the present study, the preference of the Nile tilapia between a gravel-enriched (GEE), a shelter-enriched (SEE) or a non-enriched (NEE) environment was determined, for developing a place preference model. Nile tilapia had an initial preference for GEE, but after 1 day of observation, the fish stabilized their frequency of visits among compartments. Hence, any stimulus motivating tilapia increase in compartment visiting indicates a positively reinforcing effect. This feature is very useful for the development of new behavioural paradigms for fish in tests using environmental discrimination, such as the conditioning place preference test.

Keywords: Conditioning place preference; Behaviour; *Oreochromis niloticus*; Nile tilapia; Fish

Introduction

In neuroscience, some studies of reinforcement and learning processes use methodological procedures that include environmental discriminations (Serra et al., 1999). Accordingly, we may highlight the conditioning place preference test, which is

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usually used to determine the reinforcing effects of drugs (Hasenohrl et al., 1989). Commonly, this place-preference test paradigm uses two compartments as choice possibilities (Coelho et al., 2001), where the animal has to associate a specific place with a stimulus. An increase in the time spent in a specific environment during preference test indicates a positively reinforcing stimulus effect (Hasenohrl et al., 1989; Mattioli et al., 1998; Coelho et al., 2001). However, an animal may spend more time in a compartment than in another, in a specific environment, because it is feeling safe instead of having positively reinforcing effect (Serra et al., 1999). Therefore, to know a natural preference for one out of the other compartments is very important to avoid wrong interpretations of the results.

The conditioning place preference test has been largely applied in experiments that use mammalian models, since they are considered awareness animals (Griffin and Speck, 2004). Nevertheless, other animals may be considered aware to take choices of action they likely get what they want, or avoid what they dislike, suffer or fear, such as fish (Chandroo et al., 2004; Conte, 2004). In this way, recently, the conditioning place preference model has been successfully applied to fish (for example, see Mattioli et al., 1998; Medalha et al., 2000; Coelho et al., 2001; Darland and Dowling, 2001; Faganello et al., 2003). However, these studies focusing on fish, as far as we know, comprise only two fish species: the goldfish (*Carassius auratus*), and the zebrafish (*Danio rerio*). Fish represents one of the most diverse taxon of vertebrates (Pough et al., 2001), living in a wide variety of habitats. Consequently, this variety of species and environmental locations is a source of biological matter useful in different areas of biological research, including neuroscience and behaviour (Bolis et al., 2001). According to above statements, the advantage of the animal model is represented by the fact that a peculiar intraspecific characteristic makes them especially useful for addressing specific questions. Thus, we consider Nile tilapia a good candidate as a model to elaborate a place preference test due to some special features of them as pointed out below.

This species is a territorial fish that builds nests on gravel during reproduction (Gonçalves-de-Freitas and Nishida, 1998; Barreto et al., 2003b; Volpato et al., 2004) and they may use shelters as territories, contributing, for instance, to protect themselves against predator attacks by hiding (Kolding, 1993). Hence, both gravel and shelter are important resources for Nile tilapia life. Thus, in the present study, the preference of the Nile tilapia fish (*Oreochromis niloticus*) between a gravel-enriched (GEE) or a shelter-enriched (SEE) environment was tested, for developing a place preference model. Our test faces an environment that offers protection for the fish with one another representing an essential resource for their reproduction. Thus, this test model allows us to distinguish Nile tilapia preference between two important resources for them. Moreover, Nile tilapia fish were chosen because it is easy to maintain, in fact it is the most cultured cichlid of the world, and it has been used in studies addressing some themes of neuroscience and behaviour, such as stress (Volpato and Barreto, 2001; Corrêa et al., 2003; Barreto and Volpato, 2004; Moreira and Volpato, 2004); anxiety, emotionality and/or defence (Ide and Hoffmann, 2002; Barreto et al., 2003a); and drugs affecting feeding behaviour (Delicio and Vicentini-Paulino, 1993).

Materials and methods

Fish and holding conditions

Nile tilapia (~12 cm), *O. niloticus* (Linnaeus, 1759), of both sex and approximately 6 months old maintained for about 1 month in an indoor 1200 L plastic tank (~1 fish/12 L; holding density ~2 g/L) were used as our stock population. During this time, the water temperature was ~24 °C, with continuous aeration and recirculating system through a biological filter, and supplied with a constant flow of dechlorinated water. The photoperiod was set from 06:00 to 18:00 h, controlled by a timer, in a room equipped with artificial illumination: a fluorescent light (daylight), with a light intensity of ~350 lux. The fish were daily fed ad libitum with tropical fish chow (Purina Ltda, Campinas, SP, Brazil) with protein concentration of 38%. The animals used here were experimentally naïve. For the tests we used only males because they build nests (Gonçalves-de-Freitas and Nishida, 1998; Barreto et al., 2003b), thus gravel is probably a more important resource for them than for the females.

Experimental design

The basic design of the present study was done to evaluate fish place choice, assessing the preference of Nile tilapia for one of three possibilities: a GEE, a SEE or a non-enriched (NEE) environment.

The experiment was conducted in aquaria (100 × 30 × 30 cm) equally divided into three compartments (~33,3 cm each) by using opaque plastic dividers with a window (10 × 10 cm) in the bottom centre (Fig. 1). Only the two lateral compartments were enriched with one out of two items, gravel or shelter. The middle compartment was never supplied with any resource (gravel or shelter) and was used as the start compartment, where each fish was initially placed during seven days for adjustment. During this period, the windows were blocked by an opaque partition avoiding fish accessing the lateral compartments.

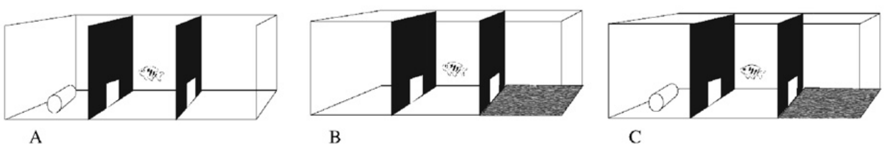

Fig. 1. Aquaria used for the preference test model. Each compartment was ~33 cm long. A is the control aquarium SEE versus NEE, B is the control aquarium GEE versus NEE, and C is the test aquarium SEE versus GEE. It was used a white pipe (15 cm) as a shelter. The middle compartment was never enriched and used as the start compartment. The compartments were isolated from each other by an opaque plastic partition with a window (10 × 10 cm) in the bottom centre. SEE and GEE compartments were chosen by choice. SEE = shelter-enriched environment; GEE = gravel-enriched environment; NEE = non-enriched environment.

We tested only one fish per aquarium. After 7 days, windows were unblocked allowing fish to achieve either GEE or SEE; at that point, preference test was started. The test was performed on 4 consecutives days, and the frequency of place choice was measured every 1 min, during 10 min four times a day (08:00, 11:00, 14:00 and 17:00 h).

Two other tests using similar procedures were conducted as controls. In these tests, we have offered one-resource enriched aquarium for assessing fish preference between SEE versus NEE and GEE versus NEE. These control experiments allowed us to assess if fish choices for a specific environment were merely aimless. The fish were daily fed, and food was always dropped in the middle compartment. All tests were conducted at the same time and during day light periods, since Nile tilapia is a daytime species. These three experiments had $n = 6-7$ fish each.

Experimental aquaria conditions

The experimental aquaria were supplied with continuous aeration, and equipped with a biological filter (recirculating water system). During experimentation, the water temperature was ~24 °C, pH ranged from 6.2 to 6.5, and oxygen concentrations were near saturation (7.5 mg/L), nitrite and ammonia were 0.0 ppm. Photoperiod and illumination were as in the holding condition, but light was about 480 lux, instead of 350 lux. The shelter was a 15 cm length white PVC pipe (diameter = ~10 cm) and the gravel used was neutral stream pebble (2 kg/tank).

Statistical analyses

For data analyses, we used the total frequency of place choice per day. We compared the fish preference among the three compartment of the tank in each day and the preference for a specific compartment throughout the 4 consecutives days of the test. For these analyses, we used Friedman ANOVA test followed by Tukey HSD test of sums of ranks when necessary (Zar, 1999). Differences were considered significant, if the probability of error was less than 5%.

Results

Control tests

Control test results are expressed in Fig. 2. In the SEE versus NEE test, we observed a significant increase in the mean frequency of fish's visit in the SEE in the 2nd day, remaining at a similar rate until the 4th day (Friedman ANOVA test; $P<0.02$). Concomitantly, we observed a decrease in the mean frequency of visits on NEE after the 2nd day, maintaining it at a similar level until the last day (Friedman ANOVA test; $P<0.04$). Moreover, we observed a higher frequency of visits on SEE

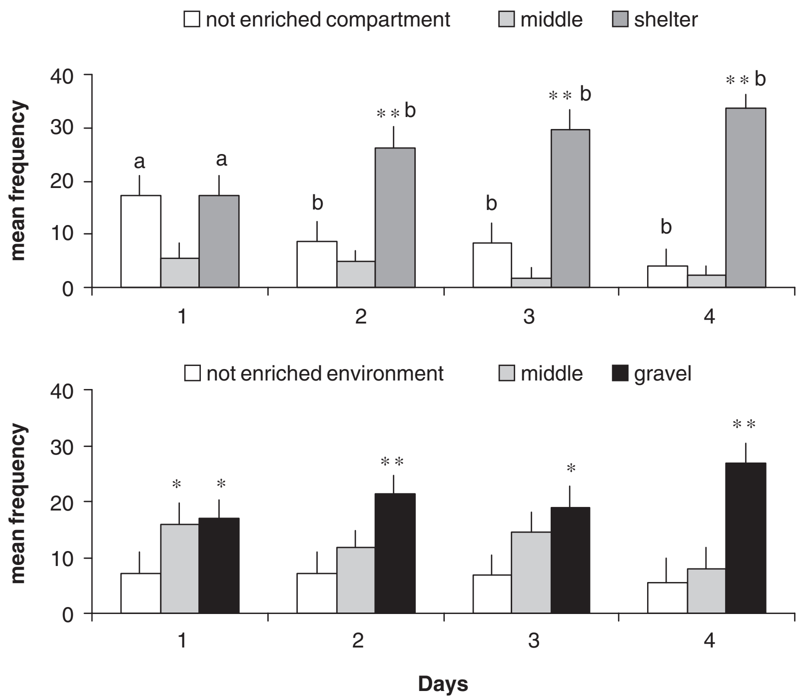

Fig. 2. Control experiments of place preference in Nile tilapia: (a) SEE versus NEE and (b) GEE versus NEE. Data represent the mean frequencies ($\pm$SE; $n = 7$) of fish visit in each environment and they were compared by using Friedman ANOVA test followed by Tukey HSD test of sums of ranks with $\alpha = 0.05$. Letters compare the overtime visit frequency for each compartment, and the mean frequencies that do not share a same letter are statistically different. *Frequency statistically different from NEE, and **different from both middle and NEE. SEE = shelter-enriched environment; GEE = gravel-enriched environment; NEE = non-enriched environment.

from 2nd to 4th day when compared with the NEE and the middle compartment (Friedman ANOVA test; 2nd day, $P<0.05$; 3rd day, $P<0.02$; 4th day, $P<0.01$).

In the GEE versus NEE test, no difference was observed in the overtime visit rate considering the three compartments separately (Friedman ANOVA test; lower P value = 0.09). However, the visits to GEE was higher than the NEE and middle in the 2nd and in the 4th days of the test (Friedman ANOVA test; 2nd day, $P<0.05$; and 4th day, $P<0.03$). In the 1st day GEE frequency was similar to middle one, and both higher than NEE (Friedman ANOVA test; $P<0.04$) and in the 3rd day the GEE frequency was only higher than the NEE (Friedman ANOVA test; $P<0.01$).

SEE versus GEE test

In this test (Fig. 3), we only observed a significant higher frequency of visit to GEE compared to SEE and middle compartment in the 1st day (Friedman ANOVA test;

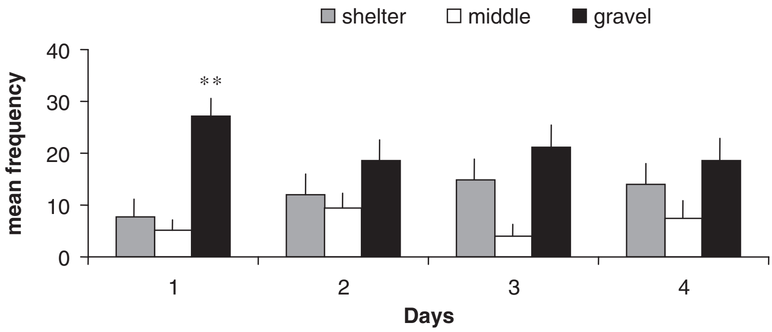

Fig. 3. The SEE versus GEE experiment of place preference in Nile tilapia. Data represent the mean frequencies ($\pm$SE; $n = 6$) of fish visit in each environment and they were compared by using Friedman ANOVA test followed by Tukey HSD test of sums of ranks $\alpha = 0.05$. **GEE visit frequency is statistically higher than both middle and SEE. SEE = shelter-enriched environment; GEE = gravel-enriched environment.

1st day, $P < 0.04$; 2nd day, $P = 0.70$; 3rd day, $P = 0.21$; 4th day, $P = 0.61$). Also, no difference in the overtime visit rate was observed for any compartment (Friedman ANOVA test; lower P value $= 0.23$).

Discussion

Firstly, we analysed the two control experiments. The control experiments compared the possibility of choice among three compartments with only one of them enriched. In both experiments, tilapia tested have visited the enriched compartments more frequently than the NEE (see Fig. 2). Thus, we can state that Nile tilapia in fact prefers an enriched aquarium environment than one with only water. This conclusion is important for the following experiment, because it indicates that any preference reported is not merely by chance.

In the SEE versus GEE experiment, we observed that Nile tilapia frequency of visits was higher in the GEE than both SEE and middle compartment only in the 1st day of observation. Naturally, a novel environment causes a necessity to look for a safe place. Hence, the behaviour to hide or keep oneself near a refuge might be an expected solution at the first time in a place. However, fish chose the GEE instead of SEE in the 1st day. This choice might be explained by a fish perceiving no predators or conspecific competitors; hence there would be no reason to hide. Therefore, SEE did not represent an important resource in this situation. On the other hand, the higher frequency of visits in the GEE might indicate fish has a chance of building a nest for a possible reproduction. However, after the 1st day, the frequency of visits stabilized among compartments. This behaviour may be due to the absence of a female, which would represent a reward for Nile tilapia males execute their reproductive tasks, including nest building on the gravel. Therefore, the lack of a specific stimulus abolished fish preference for gravel or shelter. We suggest that the presence of an extra stimulus (predator, female, food, etc.) is necessary to motivate individuals facing a situation of choice between two enriched environments. Differently, for the control experiments, one compartment was enriched only and, thus, it was preferred instead of a place without any resource.

Accordingly, this preference test may indicate the best environment an animal might choose in a specific situation, once any stimulus able to redirect tilapia's choice implies a reinforcing effect. Consequently, this kind of environmental discrimination can be used to determine the reinforcing effects of a stimulus in which the fish has to connect a specific place with the effects caused by the stimulus. Thus, if the time spent in a given enriched environment (SEE or GEE) increases, it indicates a positively reinforcing effect of the stimulus. On the other hand, this kind of environmental discrimination may be also used in avoidance conditioning, by associating a specific compartment with an aversive stimulus.

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