Natural Colour Preference in the Zebrafish (*Danio rerio*)

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ABSTRACT

The zebrafish (*Danio rerio*), traditionally used in genetics is now becoming increasingly popular in behavioural neuroscience. Zebrafish is a small teleost (bony) freshwater fish originating from northern India, used worldwide in research in most stages of development from embryo to adult. While, a couple of papers on zebrafish related to visual colour discrimination and learning have been published [1, 2], its natural colour preference has received little attention [2]. Of particular interest is the receiver bias towards a specific colour. Natural colour preference towards a specific colour may lead to changes in visual discrimination learning, memory and decision making of an animal. Zebrafish has been shown to have colour vision with peak absorbance in ultraviolet (362nm), blue (415nm), green (480nm) and red (570nm) [2]. In the present study we used four different colours (red, yellow, green and blue) to test the natural colour preference of zebrafish. A conditioned place preference (CPP) apparatus using six different colour combinations of gravels was used to measure the time spent in each coloured compartment. This study might be helpful in developing further colour based learning and memory paradigms in zebrafish.

Author Keywords

Zebrafish, natural colour preference, learning and memory.

INTRODUCTION

Zebrafish (*Danio rerio*) is now becoming popular in behavioural neuroscience [5]; although it's natural colour preference has received little attention. In the present study we have used four different colours (red, yellow, green and blue) to test natural colour preference of the zebrafish. To carry out the investigations, fish were divided into six groups (six colour combinations) and tested for a place preference in a conditioned place preference apparatus. In our laboratory we are trying to develop novel learning and memory paradigms for zebrafish. For this purpose we needed to study the probable natural preference and/ or aversion for a specific colour.

MATERIALS AND METHODS

The fish were tested in a two-chambered conditioned place preference (CPP) box as described previously [3] with slight modifications shown in Figure 1. Briefly, the test apparatus was 23cmX15cmX15cm and was filled with water up to 12cm from bottom to minimize stress. The apparatus was divided into two equal halves with a perforated wall that allows complete albeit impeded movement. System water maintained at 28^{o}C and pH of 7.3 (maintained by sodium bicarbonate) was used for the experiments. The apparatus used in the experiment is shown in Figure1A and B.

Animal Care and Maintenance

The principles and procedures were conducted in accordance with National Health and Medical Research Council, Australia (NHMRC), Guidelines for the Care and Use of Laboratory Animals.72 mixed sex wild type (AB) zebrafish were used for the experiment. Zebrafish were housed in an environmentally controlled room with 14:10h

A

B

Figure 1. Conditioned place preference apparatus with colored gravels (A) and with a perforated wall (B) that allows complete, albeit impeded, movement of fish from one compartment to another.

light: dark cycle in aquaculture research facility. Fish were fed twice daily with live brine shrimp in the morning and dry food during evening. Red ocean salt was added to the system water at a concentration of 0.3 mg/ml. Other maintenance conditions were followed as described previously [4]. Fish were separated in 3 litres tanks (12 fish in a tank) two days prior to habituation in the testing apparatus. All the fish tested were 25 weeks old and were F3 generations of AB strain.

Procedure

All the fish were habituated in groups in the testing apparatus for four days. Since zebrafish is a shoaling fish, the number of fish per group was slowly reduced in subsequent habituation trials. On the first day, fish were given 5 minutes to fully explore the apparatus. In the next two consecutive days, fish were restricted to each side for 4 minutes using a transparent divider. On the fourth day, the apparatus was partitioned into two equal halves with a perforated wall that allows complete, albeit impeded, movement from one chamber to another. Fish were allowed to explore for 4 minutes. On the following day, the apparatus was equally filled with two different colours. Each fish was tested individually (n=12 per group). Since four colours were used, six different colour combinations were tested (red + yellow, red + green, red + blue, yellow + green, yellow + blue, green + blue). Each fish was placed in one side of CPP box and time spent in each compartment was recorded. The position and side of entry of each fish was counterbalanced. Number of entries into each compartment and time spent into each compartment was recorded.

Reflectance spectra of these different coloured gravels were also measured using a spectrometer (Ocean optics, USB, 2000 with a halogen lamp light source. This was done to measure the wavelengths of the coloured gravels to compare the wavelengths range with the photo pigment spectral sensitivity of zebrafish.

Analysis

Data were analysed with a two-tailed t-test for the preference of one colour over another in each combination using R.2.10 software.

RESULTS

We have found a strong aversion of the blue colour relative to all other colours (red, yellow and green) when tested in

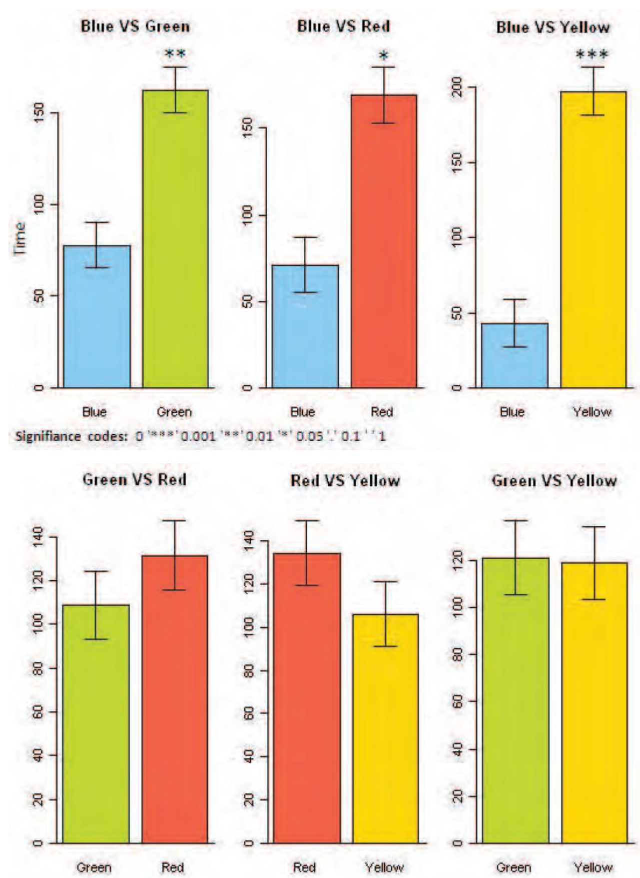

Figure 2. Results showing the time spent (seconds) in the coloured compartments when fish were tested for a combination of colours in CPP.

combinations. No preferences were found amongst the other red, yellow and green. Results are shown in Figure 2.

CONCLUSION

The present results are helpful in choosing colours to use in future colour-based learning and memory paradigms by considering natural colour preferences and aversions of zebrafish. Reds, yellows and greens apparently are equally pleasant or aversive, and are good choices for appetitive experiments. Blues are more aversive than all others, and might be useful for experiments involving aversion, anxiety or fear.

Ethical Statement

All the experimental procedures have been approved by Edith Cowan University Animal Research Ethics Committee.

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