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Original Article

The Zebrafish World of Colors and Shapes: Preference and Discrimination

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Abstract

Natural environment imposes many challenges to animals, which have to use cognitive abilities to cope with and exploit it to enhance their fitness. Since zebrafish is a well-established model for cognitive studies and high-throughput screening for drugs and diseases that affect cognition, we tested their ability for ambient color preference and 3D objects discrimination to establish a protocol for memory evaluation. For the color preference test, zebrafish were observed in a multiple-chamber tank with different environmental color options. Zebrafish showed preference for blue and green, and avoided yellow and red. For the 3D objects discrimination, zebrafish were allowed to explore two equal objects and then observed in a one-trial test in which a new color, size, or shape of the object was presented. Zebrafish showed discrimination for color, shape, and color + shape combined, but not size. These results imply that zebrafish seem to use some categorical system to discriminate items, and distracters affect their ability for discrimination. The type of variables available (color and shape) may favor zebrafish objects perception and facilitate discrimination processing. We suggest that this easy and simple memory test could serve as a useful screening tool for cognitive dysfunction and neurotoxicological studies.

Introduction

Color and shape perception allows the animal's visual environment discrimination and brings advantages for feeding, defense, life in groups, migration, and mate choice.[1,2] In fact, fishes are able to see color from blue to infrared[3] and can discriminate a variety of geometrical forms.[4] The advantage of visual cues recognition is the straight signal source, while olfactory and auditory signals show scattered routes of dispersion.[5]

Color perception and/or preference directly affect fish learning,[6,7] memory formation,[8] and decision-making.[9] Many studies show ambient color and hint color influence on fish navigation,[1,10] spatial location,[4,11] and welfare.[12–14] However, studies approaching the fish's ability to discriminate different colors and shapes are still lacking and may indicate an animal's cognitive faculty, plus allowing neural throughput screening studies, such as neurological diseases or neural disabilities caused by drugs.[15,16]

Memory formation is an important feature for animals living in a stable environment, in which remembering items, places, and routes from experience bring advantages for the animal's fitness.[17] The zebrafish has been successfully used to study memory in many different paradigms, such as aversive experiences,[18,19] appetitive stimulus,[20,21] spatial memory formation,[7,10,22,23] and appetitive choice discrimination.[24] The studies on appetitive reinforcement use multiple exposure to the stimulus to ensure memory consolidation, while those on aversive reinforce use a single aversive stimulus, shown to promote memory formation due to the increased emotional response (fear, anxiety) and the highly adaptive characteristic of avoiding a deleterious event.[25] However, we propose to investigate memory using a paradigm of a single exposure to a neutral stimulus, to avoid appetitive or aversive emotional response and test a type of memory that is much more vulnerable than that from multiple events.[19,26] Thus, we have chosen to use a one-trial recognition test to address fish's cognition in this study.

Among the fish species studied in cognitive research, zebrafish *Danio rerio* has gained increasing popularity in behavioral brain research due to its practical simplicity and elaborated brain structure[27,28] and neurochemistry[29] that offers translational relevance to humans.[30] In addition, the zebrafish shares many molecular pathways, genes, and protein products with mammals,[31–39] which makes it an ideal model organism for embryology, development, and disease studies.[7] A large number of genetic tools have been produced for the zebrafish and genetic knowledge has been accumulated. These materials have been successfully used for the examination of brain function and the development of brain diseases, and the zebrafish has been accepted as one of the best research animals for high-throughput screening in many

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areas of study. In the past decade, many studies approached the genetics of behavior and brain function of the zebrafish, but only a few attempted to study memory processes based on visual signals. The zebrafish visual system comprehend retinas with cones sensible to red, green, blue, and ultraviolet[40] and they are diurnal animals, hence an ideal model to developing research on cognitive response based on visual signals.[41] Therefore, we aimed to (1) determine zebrafish environmental color preference, (2) investigate the fish ability to distinguish objects in one-trial memory test, and (3) test fish discrimination for color, size, and shape.

Materials and Methods

Subjects and holding conditions

The present study used adult zebrafish (*D. rerio*) of both sexes, obtained from a local fish farm (Natal, Brazil) and kept in storage tanks (50 × 40 × 30 cm, 50 L, one fish/L) with aerated and filtered water in the Laboratory of Fish (Department of Physiology UFRN, Natal, Brazil). Four 50-L tanks formed a stock unit in a closed recirculation system with mechanical, biological, and chemical filtration and UV disinfection, which maintained water at 28°C ± 1°C, pH 7.2, and low levels of ammonia and nitrite. Water was changed in 30% every 10 days to ensure quality. Illumination was set on a 12-h light/12-h dark cycle and light intensity was around 250 ± 30 lx.

Fish used for the tests were 9 months old (3.53 ± 0.05 cm and 0.55 ± 0.08 g). They were brought from the fish farm at the age of 75 days and kept under the conditions described above for 6–7 months. During this period, the animals were fed once a day in excess with a commercial diet (60% protein and 15% fat until the age of 6 months and 38% protein and 4% fat after 6 months; Nutricom Pet). All animal procedures were performed with the permission of the Ethics Committee for Animal Use of the Universidade Federal do Rio Grande do Norte (CEUA 010/2013).

Color preference

For the color preference test, six 50-cm-diameter transparent plastic tanks were divided into four lateral compartments of similar size with a 7-cm hole in the central region to allow the fish to move between compartments (Fig. 1a). The water depth was 10 cm. The laboratory was illuminated with fluorescent tubes set at the 12-h light/12-h dark cycle. Different environmental colors were randomly chosen for each compartment by covering the chosen compartment with a gelatin filter (LeeFilters; blue—λ_{max} 435 nm, green—λ_{max} 494 nm, yellow—λ_{max} 546 nm, red—λ_{max} 610 nm). Light intensity was set around 60 lx for each compartment by adding layers of the same gelatin filter; the number of gelatin layers had no effect on the wavelengths mentioned above. From the six tanks used, each one showed a different order of compartment colors that were chosen in random.

Color preference was observed individually (1 fish/tank) for a period of 5 days ($n = 12$). Each fish was introduced in the experimental tank 1 day before observations started for acclimation to the apparatus set. Twenty-four hours after fish introduction into the tank, the observation period started. On the first testing day, fish were observed without colors on the compartments. At the end of the first day, after the lights in the room were turned off, the gelatin filters were placed on the top and around the tanks. When the lights were turned on the day after, color compartments were available for the fish. On the second, third, and fourth testing days (compartments set with different colors), fish were observed for color preference. On the fourth day, after the lights turned off, the gelatin filters were removed from the top and lateral of the tanks. On the fifth day, fish were observed again without colors on the compartments. During the first and fifth days, fish visit frequency in each compartment was observed to check possible preference between noncolored compartments. Visiting frequency was observed throughout the five days and data were collected every 2 min for a 20-min period at 08:00, 11:00, 14:00, and 17:00 h, making a total of

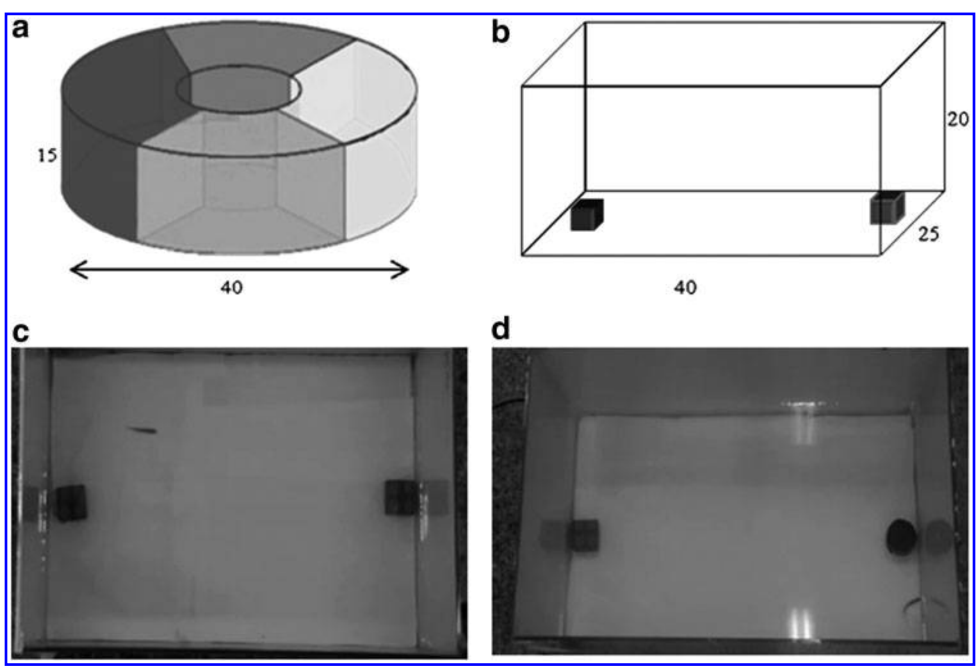

FIG. 1. **(a, b)** The schematic view of the experimental tanks used for the color preference test and the objects discrimination test, respectively. The numbers show the dimensions of the tanks in cm. Tanks were filled to **(a)** 10 cm and **(b)** 18 cm water depth. **(c, d)** The *upper view* of the experimental tanks used for the objects discrimination test on the memorization day (both objects show same characteristics) and discrimination day (a different shape object replaced one of the two objects; i.e., objects shape discrimination group), respectively.

40 observations per day. Food was not offered during the experimental days to prevent the fish from choosing one specific compartment due to a driving force/stimulus other than light color. A starvation period of five days is not long enough to affect fish survival or well-being.[42] Since we had six tanks for the preference test, the experiment was done twice to achieve $n = 12$. The whole test lasted 10 subsequent days.

For statistical analysis, the nonparametric procedure of Friedman ANOVA was used for multiple group analyses of visit frequency. The Friedman test was used because fish preference for one compartment instead of the others provides dependent data. In cases where the Friedman test was significant ($\alpha < 0.05$), the nonparametric Dunn's *post hoc* test was used to determine significant differences among compartments.

Objects discrimination

For the objects discrimination test, 48 zebrafish from the stock population were separated into four groups ($n = 12$ in each group): (1) objects color discrimination, (2) objects size discrimination, (3) objects shape discrimination, and (4) objects shape + color discrimination. Each group underwent three experimental phases: acclimation in the aquaria (5 days), objects memorization (1 day) and objects discrimination (1 day). For these 7 days, fish were kept isolated in 1-L (10 × 10 × 10 cm) glass tanks (home tank), with visual contact between them to avoid social isolation stress. During this period, 30% of water was changed every other day using system water. The experimental phases (acclimation, memorization, and discrimination) occurred in 15-L glass tanks (40 × 20 × 25 cm) with all walls covered with white paper to avoid external interferences (Fig. 1).

The acclimation phase lasted 5 days. Each fish was individually introduced in the tank for 15 min per day, without objects. It was done to make fish familiar with the experimental arena. After the 15-min period, each fish was removed to its home tank.

The objects memorization phase occurred on the sixth day, following the acclimation phase. Two identical objects were introduced in the tank, each one positioned next to each smaller wall, thus the two objects (named A and B) were around 34 cm away from one another (Fig. 1c). Each fish was introduced in the tank for 30 min to explore the objects. After the first 5 min, fish behavior was recorded from above during 3 intervals of 5 min using a handycam (Sony Digital Video Camera Recorder; DCR-SX45). Between the 5-min records, a period of 5 min was left until a total of 30 min was completed. Thus, each fish was recorded for 15 min (5 + 5 + 5 min), according to the methodology proposed by Callaghan *et al.*[43] After this period, fish returned to its home tank.

On the next day (seventh), fish were submitted to the objects discrimination phase. For that, one out of the two objects used in the memorization phase was changed for a new object (Table 1). Object B was replaced for object C, differing in color, size, shape, or color and shape, depending on the group (see Materials and Methods section). For the objects color discrimination group, a new object with different color but the same size and shape was used. For the objects size discrimination group, the new object was twice the size of the former but of the same color and shape. For the objects shape discrimination, the new object had a different shape but same

Table 1. Summary of Stimuli Used

Test	*Memorization day*	*Discrimination day*
Objects color discrimination	 vs. 	 vs. 
Objects size discrimination	 vs. 	 vs. 
Objects shape discrimination	 vs. 	 vs. 
Objects shape + color discrimination	 vs. 	 vs. 

Three-dimensional stimuli used for objects discrimination test. All objects share similar volumes (except the bigger cube used on the size discrimination group). Cube: side = 2.7 cm, height = 2.7 cm; cylinder: diameter = 3.0 cm, height = 2.8 cm; triangle: side = 3.5 cm, height = 3.0 cm.

color and size. Moreover, for the objects color + shape, the new one was different in shape and color; for example, instead of two 3D blue squares, it was offered 1 blue square and 1 red triangle (Table 1; Fig. 1d). Fish were left with the new objects for 30 min and the behavior was recorded in 5-min intervals, as on the sixth day (memorization phase).

The procedure used describes a one-trial memory test because it does not allow the learning of a pattern (more than one trial for association). Instead of repeated exposure to a condition, the fish memorization phase occurred only once and fish recognition of the new object was based on the spontaneous exploratory behavior of the objects.

All recorded data were analyzed using the software Any-Maze™ Video Tracking System. The time spent was registered around each object to estimate objects exploration. The exploration area was established by increasing once the size of the object area; thus, we considered exploration when the fish were up to 3 cm far from each side of the object. The total exploring area, including the object (placed in the middle of the area), was 54 cm^2, on each side of the tank (6.75% of the total area). The time the fish spent in the exploration area of each object was statistically compared using paired Student's t test, since all data passed the normality and equal variance tests. In all cases, $p < 0.05$ was used as a reference value.

Results

Color preference

During the first observation day of the experiment (compartments without colors), zebrafish showed equal distribution among the compartments (Friedman ANOVA, $X12 = 4.42$, $p = 0.22$); fish visited all the compartments similarly. When the colored gelatin filters were placed over the compartments (days 2, 3, and 4), the average visit frequency in the blue and green compartments was significantly higher than in the yellow and red compartments (Friedman ANOVA day 2: $X12 = 34.5$, $p < 0.001$; day 3: $X12 = 26.7$, $p < 0.001$; day 4: $X12 = 43.0$, $p < 0.001$; Fig. 2). On the fifth day (colored gelatin filters were removed the previous evening), the fish showed preference for the previously blue and green compartments (Friedman ANOVA: $X12 = 11.49$, $p = 0.003$; Fig. 2d).

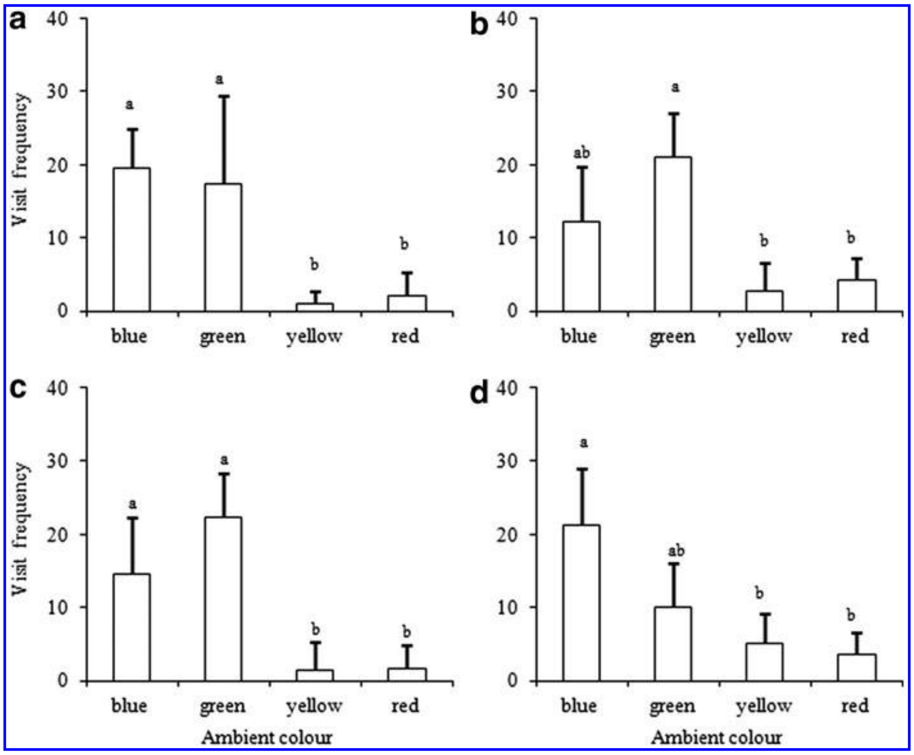

FIG. 2. Preference of zebrafish for *blue*, *green*, *yellow*, and *red* ambient ($n=12$). Bars represent the mean visit frequency ± SD in each compartment of the tank. On the second **(a)**, third **(b)**, and fourth **(c)** day, compartments were covered with gelatin filters: *blue* (435 nm), *green* (494 nm), *yellow* (546 nm), and *red* (610 nm). On the fifth day **(d)**, the tank was not covered with gelatin colors. Each day, fish was observed at 8:00, 11:00, 14:00, and 17:00 h, 20 min each period. Statistical difference between fish visit frequency in each compartment is indicated by different letters (Friedman ANOVA, $p<0.05$).

Objects discrimination

During the objects memorization phase (sixth day), neither group showed statistical differences between objects A and B exploration time (Student's t test: objects color discrimination group: $t=-1.68$, $p=0.12$; objects size discrimination group: $t=1.59$, $p=0.14$; objects shape discrimination group: $t=1.06$, $p=0.31$; and objects color + shape discrimination group: $t=1.18$, $p=0.26$; Fig. 3).

The objects color discrimination group showed similar exploration of the object A between the memorization and discrimination phases (sixth vs. seventh day; Student's t test: $t=-1.05$, $p=0.32$). However, there were differences in exploration time between object B at sixth day and object C at seventh day (Student's t test: $t=-2.69$, $p=0.02$) and this group spent significantly more time exploring object C than A in the discrimination phase (seventh day; Student's t test: $t=-3.59$, $p=0.004$; Fig. 3a).

For the objects size discrimination group, there were no differences in exploration between objects A from the memorization and discrimination phases (sixth vs. seventh day; Student's t test: $t=1.14$, $p=0.28$). This group showed similar exploration of object B in the memorization phase and object C in the discrimination phase (Student's t test: $t=-2.05$, $p=0.06$) and also similar exploration of the objects (A vs. C) in the discrimination phase (Student's t test: $t=-1.16$, $p=0.27$; Fig. 3b).

The objects shape discrimination group showed similar exploration of object A during the sixth and seventh day (Student's t test: $t=1.49$, $p=0.16$). However, fish showed high exploration of object C on the seventh day than object B on the sixth day (Student's t test: $t=-3.52$, $p=0.004$). This group also showed significant higher exploration of object C than A in the discrimination phase (Student's t test: $t=-2.96$, $p=0.01$; Fig. 3c).

For the objects color + shape discrimination group, there were no differences between object A exploration in the memorization and discrimination phases (Student's t test: $t=0.07$, $p=0.95$). Fish showed similar exploration of objects A and C in the discrimination phase (Student's t test: $t=-1.90$, $p=0.07$), but fish spent more time near object C on the seventh day than near object B on the sixth day (Student's t test: $t=-3.65$, $p=0.004$; Fig. 3d).

Discussion

Our results corroborate that zebrafish show blue/green color preference[6,9] and also indicate that zebrafish can discriminate 3D objects based on color and shape, but not size. In an experimental series, zebrafish were tested for ambient color preference as well as stimuli discrimination (3D objects color, size, and shape). This type of cognitive skill is best known for mammals and birds, but was recently shown in fish when food reward is associated.[1,5,10,11,44,45] Our study does not use food reinforcement or test memory based on an event repetition. Meanwhile, we present a rapid and effective memory protocol in a unique trial memory test. Memory from a single episode that does not include an emotional response (pleasure or fear/anxiety) is much more vulnerable than that based on repetition,[20,25] and thus more representative for studies of amnesic syndromes.

The first part of our study approached the zebrafish color preference in a multiple-choice tank. Other studies have already reported the presence of four cone types in zebrafish retina: red (570 nm), green (480 nm), blue (415 nm), and ultraviolet (362 nm).[40,46–48] This type of retina composition allows for color vision, and our data confirm zebrafish behavioral responsiveness to colors. The color preference is mainly related to the ambient where fish achieve visual comfort.[13,42,49] According to Munz[50] and Loew and Lythgoe,[51] the

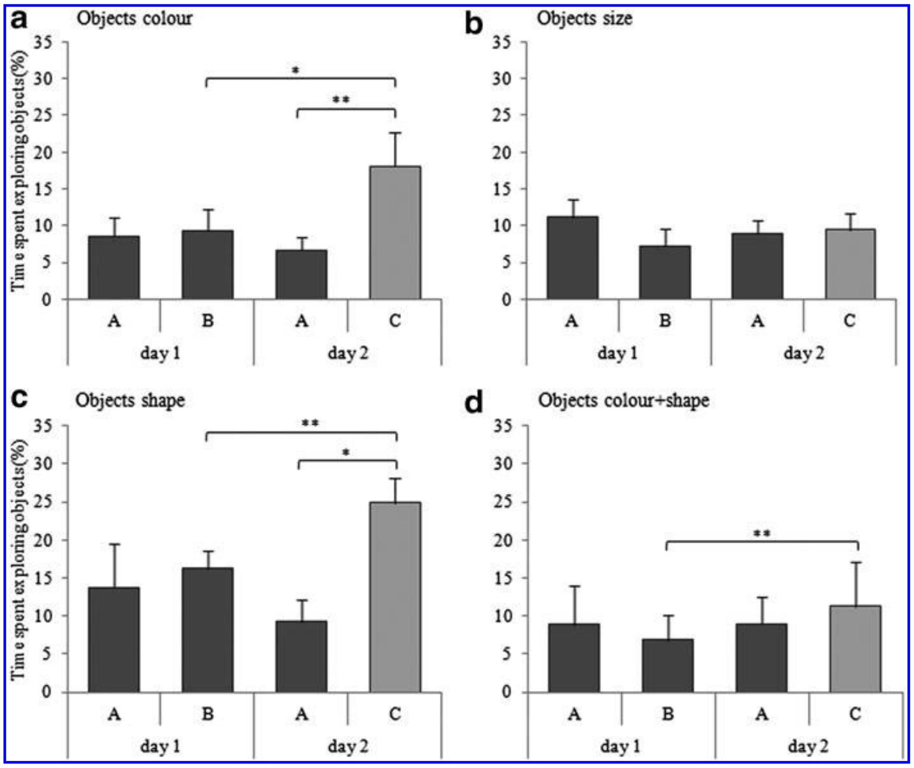

FIG. 3. Zebrafish objects discrimination in a one-trial memory test: day 1 was memorization phase and day 2 was discrimination test. Three different treatments were performed: **(a)** objects changing only in color, but not size and shape (color discrimination, $n=12$), **(b)** objects changing in size, but not color and shape (size discrimination, $n=12$), **(c)** objects changing in shape, but not color and size (shape discrimination, $n=12$), and **(d)** objects changing in color and shape, but not size (color + shape discrimination = 12). After 5 acclimation days, on day 6, equal objects A and B were introduced for memorization. On day 7, object A was the same seen before and object B was changed for C, differing in the aspects cited above. Bars represent the percentage of time spent around each object. Each day, fish was observed for 15 min after being introduced in the tank with the objects. Statistical difference between fish objects exploration is indicated by * (Student's t test, $p<0.05$) or ** (Student's t test, $p<0.01$).

vision sensitivity is adjusted to the spectral quality of the ambient light where a population evolved. These authors suggest that the natural ambient light spectrum enables fish to catch the greatest number of photons available and enhance vision to permit better detection of predators and preys. Thus, zebrafish visual pigments may better match the wavelengths present in the blue/green environment, which could have driven the fish preference for these light colors.

During the ambient color preference test, zebrafish explored all compartments and showed preference for blue and green areas. We also observed that fish seemed to avoid the yellow and red environment, as the visit frequency to these compartments was very low. For zebrafish, the longest pigment wavelength absorbance is 570 nm,[40,46–48] so the yellow and red environments in which light wavelength was around 546 and 610 nm, respectively, may restrict vision, as photons are not well captured by the cones. The blue and green environment may have offered visual comfort in a way that on the fifth preference day (without colors), zebrafish showed conditioned preference for the areas kept in blue and green. It seems that the fish was motivated to visit areas previously kept in blue and green due to its experience on days 2, 3, and 4. This behavior may indicate that zebrafish recognized the place and revisited it expecting to find the blue/green environmental colors, which characterizes conditioned preference according to Tzschentke.[52] As an inhabitant of streams, canals, ditches, and shallow ponds, zebrafish feeds on insects, crustaceans, algae, and detritus[53] and may not need specialized visual sensitivity for foraging on these items. Shallow water bodies allow mainly short wavelength light penetration[54] and the presence of short cones improves visibility in its natural environment, which may have driven zebrafish increased exploration of the blue and green ambient (Fig. 2).

Although the color preference result is not new (zebrafish color preference was already shown[6,9,12]), it reinforces zebrafish color exploration. In addition, testing color preference was important to properly establish the protocol for the objects discrimination test, since we could avoid offering cues that could attract the fish due to some preference for a specific color. Thus, the color preference test supported our choice for the objects used in the discrimination test. In nature, exploration is a key animal behavior in response to the environment and its novelties,[55–57] and we used the knowledge from the color preference test to ensure that the fish would be attracted by the novelty, but not the color. Zebrafish is naturally an exploring animal and new features of the ambient are investigated to obtain information of potential sources of food, mate, and shelter.[58,59] In our study on objects discrimination, two equal objects were first presented (A and B) and fish explored them in a similar manner (memorization phase, Fig. 3). However, when a known object was replaced by a new one, fish increased exploration of the new color, new shape, and new color + shape objects, but not the new size object (Fig. 3).

The natural and spontaneous preference for the novelty was already studied in several different ways for rodents and primates.[26,60–64] In our test, fish could show the same tendency for choosing the new, in which important points were taken into account; fish had to show mnemonic feature for the objects instead of the ambient, and discrimination was based on the time spent checking each object instead of first choice. Moreover, although we did not estimate fish physical interaction with the objects, we noticed that fish used to bite the new object during the first exploration sections of the color and shape discrimination tests.

The new color object may have been recognized as a novelty due to the zebrafish improved color vision.[65–67] As presented above, these fish show morphological and physiological substrate for color perception[3,40,46–48,67] and, thus, they mostly use this ability for the ambient exploration. However, not only color was an important cue for objects discrimination but also shape (Fig. 3a, c). It is known that food and shelter identification and heterospecific and predator recognition are all based on visual discrimination. According to Ennaceur and Delacour,[26] this mnemonic capacity can be considered a pure working memory. The zebrafish discrimination showed here may be a useful tool for pharmacological and neurological studies on memory.

We did not observe zebrafish objects discrimination based on size. It seems that fish cannot accurately discriminate a continuous variable when a variation is not notable. As stated by Agrillo *et al*.[68] fish discriminates discrete and continuous variables, such as volume, brightness, and movement, yet discrete variables are more difficult to realize than continuous. In agreement to this, our study showed that objects twice bigger, but same color and shape, were not distinguished as a novel item. In addition, the study by Schluessel *et al*.[45] suggests that object and figure discrimination is based on categorization. These authors propose that an animal creates discrete mental units for items in the ambient and treats some equivalent and others different. In accordance to this idea, zebrafish may have included objects that shared color and shape in the same category, thus not distinguishing smaller differences in size.

On the other hand, it seems that salient stimuli as color or shape are easier to notice, and multiple cues may not improve the object discrimination (color + shape, Fig. 3d), but hamper it. According to Wilkie,[69] Fitzgeorge *et al.*,[70] Buckolz *et al.*,[71] the inclusion of an extra cue of different category may have acted as a distracter, and thus, fish was forced to perform conjunctive search,[72] in which accuracy is lower due to the need of increased time to compare items of each category. Evidence suggests that animals exposed to a to-be-remembered stimulus hold it in memory and respond faster during a probe when no other stimuli are present.[69,73] However, an apparently contradictory result was reported by Ohnishi,[44] which showed that goldfish quickly learns to find food when the cues include color and shape variation, but learning performance decreased when the cue varied in only color or only shape. In fact, food is a vital item to be distinguished and also plays a reward role. When memory for the environmental item feature is tested in a single-trial experiment (without a reward), it seems that multiple variables do not favor discrimination (Fig. 3). Our results suggest that for the one-trial memory test, the more the variables available, the harder the distinction. However, long period experience may favor learning of multiple features, and deserves more investigation for extensive understanding.

The ability to distinguish items in the environment in an organized form is useful for animal behavior such as solving problems, communicating in groups, and obtaining information.[74–76] Although the cognitive mechanisms differ, this skill was observed in many mammals,[77–79] birds,[80,81] and lizards.[82] Since we showed a similar ability in fish, this phylogenetically old skill may indicate a base predating the divergence of the main vertebrate classes.

In summary, our data show that zebrafish uses cognitive abilities to discriminate and categorize environmental items to better evaluate the ambient, while vision is a key feature in exploring the ambient elements. Most importantly, the data indicate similarities between zebrafish and mammal cognitive behavior. As the characterization of the molecular machinery underlying memory and learning needs consistent behavioral paradigms, we suggest the present one-trial memory test for future studies on neurodegenerative diseases or neurotoxic drugs that affect memory and perception. This study ultimately contributes to improve our current knowledge on the zebrafish behavioral repertory, which has been widely investigated for learning and memory mechanisms.

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Disclosure Statement

No competing financial interests exist.

References

1. Hughes RN, Blight CM. Two intertidal fish species use visual association learning to track the status of food patches in a radial maze. Anim Behav 2000;59:613–621.
2. Blackiston D, Briscoe AD, Weiss MR. Color vision and learning in the monarch butterfly, *Danaus plexippus* (Nymphaelidae). J Exp Biol 2011;214:509–520.
3. Levine JS, MacNichol EJ Jr. Color vision in fishes. Sci Am 1982;216:108–117.
4. Petrazzini MEM, Agrillo C, Piffer L, Dadda M, Bisazza A. Development and application of a new method to investigate cognition in newborn guppies. Behav Brain Res 2012; 233:443–449.
5. Siebeck UE, Litherland A, Wallis G. Shape learning and discrimination in reef fish. J Exp Biol 2009;212:2113–2119.
6. Spence R, Smith C. Innate and learned colour preference in the zebrafish, *Danio rerio.* Ethology 2008;114:582–588.
7. Sison M, Gerlai R. Associative learning in zebrafish (*Danio rerio*) in the plusmaze. Behav Brain Res 2010;207:99–104.
8. Colwill RM, Raymond MP, Ferreira L, Escudero H. Visual discrimination learning in zebrafish (*Danio rerio*). Behav Proc 2005;70:19–31.
9. Avdesh A, Martin-Iverson MT, Mondal A, Chen M, Verdille G, Martins RN. Natural colour preference in the zebrafish (*Danio rerio*). P Meas Behav 2010;24:155–157.

10. Cognato GP, Bortolotto JW, Blazina AR, Christoff RR, Lara DR, Vianna MR, *et al.* Y-Maze memory task in zebrafish (*Danio rerio*): the role of glutamatergic and cholinergic systems on the acquisition and consolidation periods. Neurobiol Learn Mem 2012;98:321–328.
11. Parker MO, Gaviria J, Haigh A, Millington ME, Brown VJ, Combe FJ, *et al.* Discrimination reversal and attentional sets in zebrafish (*Danio rerio*). Behav Brain Res 2012;232: 264–268.
12. Serra EL, Medalha CC, Mattioli R. Natural preference of zebrafish (*Danio rerio*) or a dark environment. Braz J Med Biol Res 1999;32:1551–1553.
13. Luchiari AC, Duarte CRA, Freire FAM, Nissinen K. Hierarchical status and colour preference in Nile tilapia (*Oreochromis niloticus*). J Ethol 2007;25:169–175.
14. Luchiari AC, Marques AO, Freire FAM. Effects of substrate colour preference on growth of the shrimp *Litopenaeus vannamei* (Boone, 1931) (Decapoda, Penaeoidea). Crustaceana 2012;85:789–800.
15. White RE. High-throughput screening in drug metabolism and pharmacokinetic support of drug discovery. Annu Rev Pharmacol Toxicol 2000;40:133–157.
16. Gerlai R. High-throughput behavioral screens: the first step towards finding genes involved in vertebrate brain function using zebrafish. Molecules 2010;15:2609–2622.
17. Johnston TD. Selective costs and benefits in the evolution of learning. Adv Study Behav 1982;12:65–106.
18. Xu X, Scott-Scheiern T, Kempker L, Simons K. Active avoidance conditioning in zebrafish (*Danio rerio*). Neurobiol Learn Mem 2007;87:72–77.
19. Blank M, Guerim LD, Cordeiro RF, Vianna MR. A one-trial inhibitory avoidance task to zebrafish: rapid acquisition of an NMDA-dependent long-term memory. Neurobiol Learn Mem 2009;92:529–534.
20. Pather S, Gerlai R. Shuttle box learning in zebrafish. Behav Brain Res 2009;196:323–327.
21. Luchiari AC, Chacon DMM. Physical exercise improves learning in zebrafish, *Danio rerio*. Behav Proc 2013;100: 44–47.
22. Al-Imari L, Gerlai R. Conspecifics as reward in associative learning tasks for zebrafish (*Danio rerio*). Behav Brain Res 2008;189:216–219.
23. Eddins D, Petro A, Williams P, Cerutti DT, Levin ED. Nicotine effects on learning in zebrafish: the role of dopaminergic systems. Psychopharmacology 2009;202: 103–109.
24. Bilotta J, Risner ML, Davis EC, Haggbloom SJ. Assessing appetitive choice discrimination learning in zebrafish. Zebrafish 2005;2:259–268.
25. McGaugh JL. The Amygdala modulates the consolidation ofd memories of emotionally arousing experiences. Annu Rev Neurosci 2004;27:1–28.
26. Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav Brain Res 1988;31:47–59.
27. Kolb B, Whishaw IQ. Brain plasticity and behavior. Annu Rev Psychol 1998;49:43–64.
28. Klee EW, Schneider H, Clark KJ, Cousin MA, Ebbert JO, Hooten WM, *et al.* Zebrafish: a model for the study of addiction genetics. Hum Genet 2012;131:SI977–SI1008.
29. Chatterjee D, Gerlai R. High precision liquid chromatography analysis of dopaminergic and serotoninergic responses to acute alcohol exposure in zebrafish. Behav Brain Res 2009;200:208–213.
30. Crollius H, Weissenbach J. Fish genomics and biology. Gen Res 2005;15:1675–1682.
31. Kaslin J, Panula P. Comparative anatomy of the histaminergic and other aminergic systems in zebrafish (*Danio rerio*). J Comp Neurol 2001;440:342–377.
32. Holzschuh J, Ryu S, Aberger F, Driever W. Dopamine transporter expression distinguishes dopaminergic neurons from other catecholaminergic neurons in the developing zebrafish embryo. Mech Dev 2001;101:237–243.
33. Teraoka H, Russell C, Regan C, Chandrasekhar A, Concha ML, Yokoyama R, *et al.* Hedgehog and Fgf signaling pathways regulate the development of tphR-expressing serotonergic raphe neurons in zebrafish embryos. J Neurobiol 2004;60:275–288.
34. Kaslin J, Nystedt JM, Ostergard M, Peitsaro N, Panula P. The orexin/hypocretin system in zebrafish is connected to the aminergic and cholinergic systems. J Neurosci 2004;24: 2678–2689.
35. Mclean DL, Fetcho JR. Ontogeny and innervation patterns of dopaminergic, noradrenergic, and serotonergic neurons in larval zebrafish. J Comp Neurol 2004;480:38–56.
36. Mueller T, Vernier P, Wullimann MF. The adult central nervous cholinergic system of a neurogenetic model animal, the zebrafish *Danio rerio*. Brain Res 2004;1011: 156–169.
37. Faraco JH, Appelbaum L, Marin W, Gaus SE, Mourrain P, Mignot E. Regulation of hypocretin (orexin) expression in embryonic zebrafish. J Biol Chem 2006;281:29753–29761.
38. Prober DA, Rihel J, Onah AA, Sung RJ, Schier AF. Hypocretin/orexin overexpression induces an insomnia-like phenotype in zebrafish. J Neurosci 2006;26:13400–13410.
39. Yokogawa T, Marin W, Faraco J, Pézeron G, Appelbaum L, Zhang J, *et al.* Characterization of sleep in zebrafish and insomnia in hypocretin receptor mutants. PLoS Biol 2007; 10:2379–2397.
40. Robinson J, Schmitt EA, Hárosi FI, Reece RJ, Dowling JE. Zebrafish ultraviolet visual pigment: absorption spectrum, sequence, and localization. Proc Nat Acad Sci U S A 1993;90:6009–6012.
41. Fadool JM, Dowling JE. Zebrafish: a model system for the study of eye genetics. Prog Ret Eye Res 2008;27:89–110.
42. Luchiari AC, Pirhonen J. Effects of ambient colour on colour preference and growth of juvenile rainbow trout Oncorhynchus mykiss (Walbaum). J Fish Biol 2008;72: 1504–1514.
43. Callaghan, RMO, Ohle R, Kelly AM. The effects of forced exercise on hippocampal plasticity in the rat: a comparison of LTP, spatial- and non-spatial learning. Behav Brain Res 2007;176:362–366.
44. Ohnishi K. Goldfish's visual information processing patterns in food-reinforced discrimination learning between compound visual stimuli. J Comp Physiol A 1991;168:581–589.
45. Schluessel V, Fricke G, Bleckmann H. Visual discrimination and object categorization in the cichlid *Pseudotropheus sp*. Anim Cogn 2012;15:525–537.
46. Nawrocki L, BreMiller R, Streisinger G, Kaplan M. Larval and adult visualpigments of the zebrafish, *Brachydanio rerio*. Vis Res 1985;25:1569–1576.
47. Cameron DA. Mapping absorbance spectra, cone fractions, and neuronal mechanisms to photopic spectral sensitivity in the zebrafish. Vis Neurosci 2002;19:365–372.
48. Allison WT, Haimberger TJ, Hawryshyn CW, Temple SE. Visual pigment composition in zebrafish: evidence for a

rhodopsin-porphyropsin interchange system. Vis Neurosci 2004;21:945–952.
49. Luchiari AC, Freire FAM. Effects of environmental colour on growth of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), maintained individually or in groups. J Appl Ichthyol 2009;25:162–167.
50. Munz FW. The photosensitive retinal pigments of fishes from relatively turbid coastal waters. J Gen Physiol 1958; 42:445–459.
51. Loew ER, Lythgoe JN. The ecology of cone in teleost fishes. Vis Res 1978;18:715–722.
52. Tzschentke T. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addict Biol 2007;12:227–462.
53. McClure MM, McIntyre PB, McCune AR. Notes on the natural diet and habitat of eight danionin fishes, including the zebrafish *Danio rerio*. J Fish Biol 2006;68:1–18.
54. Kageyama CJ: What Fish See. Frank Amato Publications, New York, 1999.
55. Kalueff AV, Zimbardo PG. Behavioral neuroscience, exploration, and K.C. Montgomery's legacy. Brain Res Rev 2007;53:328–331.
56. Kim D, Chae S, Lee J, Yang H, Shin HS. Variations in the behaviors to novel objects among five inbred strains of mice. Gen Brain Behav 2005;4:302–306.
57. Kliethermes CL, Crabbe JC. Pharmacological and genetic influences on hole-board behaviors in mice. Pharmacol Biochem Behav 2006;85:57–65.
58. von Krogh K, Sorensen C, Nilsson GE, Oyvind O. Forebrain cell proliferation, behavior, and physiology of zebrafish, *Danio rerio*, kept in enriched or barren environments. Physiol Behav 2010;101:32–39.
59. Avdesh A, Martin-Iverson MT, Mondal A, Chen M, Askraba S, Morgan N, *et al.* Evaluation of color preference in zebrafish for learning and memory. J Alzheimers Dis 2012;28:459–469.
60. Parkinson JK, Murray EA, Mishkin M. A selective mnemonic role for the hippocampus in monkeys: memory for the location of objects. J Neurosci 1988;8:4159–4167.
61. Lee I, Kesner RP. Differential contribution of NMDA receptors in hippocampal subregions to spatial working memory. Nat Neurosci 2002;5:162–168.
62. Nakazawa K, Sun LD, Quirk MC, Rondi-Reig L, Wilson MA, Tonegawa S. Hippocampal CA3 NMDA receptors are crucial for memory acquisition of one-time experience. Neuron 2003;38:305–315.
63. Bevins RA, Besheer J. Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. Nat Prot 2006;1:1306–1311.
64. Miyashita T, Kubik S, Haghighi N, Steward O, Guzowski JF. Rapid activation of plasticity-associated gene transcription in hippocampal neurons provides a mechanism for encoding of one-trial experience. J Neurosci 2009;29:898–906.
65. Clark DT: Visual Responses in the Developing Zebrafish (*Brachydanio rerio*). [Ph.D. Thesis]. University of Oregon Press, Eugene, OR, 1981.
66. Chinen A, Hamaoka T, Yamada Y, Kawamura S. Gene duplication and spectral diversification of cone visual pigments of zebrafish. Genetics 2003;163:663–675.
67. Neuhauss SCF: Zebrafish vision: structure and function of the zebrafish visual system. In Zebrafish. Perry SF, Ekker M, Farrell AP, and Brauner CJ (eds), pp 81–122, Academic Press, London, UK, 2010.
68. Agrillo C, Dadda M, Serena G, Piffer L, Bisazza A. Fish can use numerical information when discriminating between small discrete quantities. Anim Cogn 2009;15:2414–2419.
69. Wilkie DM. Pigeons' spatial memory: III. Effect of distractors on delayed matching of key location. J Exp Anal Behav 1983;40:143–151.
70. Fitzgeorge L, Buckolz E, Khan M. Recently inhibited responses are avoided for both masked and nonmasked primes in a spatial negative priming task. Atten Percept Psychol 2011;73:1435–1452.
71. Buckolz E, Lok M, Kajaste B, Edgar C, Khan M. The preservation of response inhibition after-effects in a location-based spatial negative priming task: younger versus older adults. Psychol Res 2015;79:120–133.
72. Zentall TR, Wasserman EA, Lazareva OF, Thompson RRK, Rattermann MJ. Concept learning in animals. Comp Cogn Behav Rev 2008;3:13–45.
73. Shettleworth SJ, Westwood RP. Divided attention, memory, and spatial discrimination in food-storing and non-storing birds, black-capped chickadees (*Poecile atricapilla*) and dark-eyed juncos (*Junco hyemalis*). J Exp Psychol Anim Behav Proc 2002;28:227–241.
74. Agrillo C, Piffer L, Bisazza A. Number versus continuous quantity in numerosity judgments by fish. Cognition 2011; 119:281–287.
75. Smith EE, Medin DI: Categories and Concepts. Harvard University Press, Cambridge, England, 1981.
76. Spinozzi G. Categorization in monkeys and chimpanzees. Behav Brain Res 1996;74:17–24.
77. Hothersall B, Gale EV, Harris P, Nicol CJ. Cue use by foals (*Equus caballus*) in a discrimination learning task. Anim Cogn 2010;13:63–74.
78. Savarese S, Fei-Fei L. 3D generic object categorization, localization and pose estimation. 11th International Conference on Computer Vision 2007;1–8.
79. Sigala N, Logothetis NK. Visual categorization shapes feature selectivity in the primate temporal cortex. Nature 2002;415:318–320.
80. Watanabe S. Object-picture equivalence in the pigeon—an analysis with natural concept and pseudo concept discriminations. Behav Proc 1993;30:225–231.
81. Cook RG, Qadri MAJ, Kieres A, Commons-Miller N. Shape from shading in pigeons. Cognition 2012;124:284–303.
82. Day LB, Ismail N, Wilczynski W. Use of position and feature cues in discrimination learning by the whiptail lizard (*Cnemidophorus inornatus*). J Comp Psychol 2003;117:440–448.

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