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Short communication

Associative learning in the multichamber tank: A new learning paradigm for zebrafish

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HIGHLIGHTS

- A simple, easy to manufacture learning apparatus was designed.
- Visual discrimination based associative learning was studied.
- Zebrafish were found to exhibit procedural learning and memory.
- Zebrafish were found to exhibit acquisition of CS-US association.
- Maze is argued to be simple enough for high throughput applications.

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ABSTRACT

The zebrafish has been gaining prominence in the field of behavioural brain research as this species offers a good balance between system complexity and practical simplicity. While the number of studies examining the behaviour of zebrafish has exponentially increased over the past decade, the need is still substantial for paradigms capable of assessing cognitive and mnemonic characteristics of this species. Here we describe and utilize a novel visual discrimination task with which we evaluated acquisition of CS (colour)-US (sight of conspecifics) association in adult zebrafish. We report significant acquisition of CS-US association indicated by the increased time the fish spent in and the increased frequency of visits of the target chamber during a probe trial in the absence of reward. Given the simplicity of the apparatus and procedure, we conclude that the new task may be employed to assay learning and memory in adult zebrafish in an efficient manner.

1. Introduction

Despite concerted efforts by a large number of laboratories, the mechanisms of learning and memory are still not clearly understood. For example, the number of genes and gene product known to be involved in neuronal plasticity ([1]) is a small fraction of the number of genes found expressed in the vertebrate brain including the zebrafish brain (e.g [2]). Notably, a large proportion of these expressed genes are not yet functionally annotated, but are suspected to be involved in mnemonic or cognitive processes. For these reasons, laboratory species that would aid gene identification via large-scale mutagenesis screens have been proposed [3]. The zebrafish is one such species. Over the past few decades substantial amount of genetic information has been collected and numerous recombinant DNA methods have been developed for this species [4].

The bottleneck in zebrafish behaviour genetics research has been the behavioural characterization of this species and the development of appropriate behavioural testing procedures and apparati [3,5]. Although these questions are still relatively underexplored, the number of studies attempting to address them has been rapidly increasing over the past few years. For example, studies of learning and memory in zebrafish now show that zebrafish are capable of active avoidance [6], simple CS-US associative learning as well as complex latent spatial learning [7]. [also reviewed in 8]. Furthermore various apparati including the plus-maze (+) [9,10], the T-maze [11], and the shuttle-box [12] have been developed. In addition, different stimuli including animated images presented on a computer screen have been explored for zebrafish learning paradigms [13,14]. Despite these recent developments, the number

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of learning paradigms available for zebrafish is significantly smaller compared to those developed for traditional vertebrate laboratory organisms including the rat or the mouse [3]. Proper characterization of changes in mnemonic and/or cognitive processes induced by mutations or drugs may require multiple behavioural tasks that tap into similar central nervous system functions while having different performance demands [15]. Given the complex nature of learning and memory and the relative paucity of test paradigms that can measure these processes in the zebrafish, there is a substantial need for the development of efficient and robust learning and memory tasks as well as for the characterization of these processes in zebrafish.

In the current study, we developed and tested a new apparatus that we employed to analyze visual discrimination, an associative learning task performed in a cheap aquarium retrofitted with acrylic inserts. Although not novel in its conceptual features, the task has some advantages and/or complementary features to currently existing paradigms. For example, compared to the T-maze it allows the investigator to study the subjects' choices between more than two options. Compared to the plus or radial mazes, the test apparatus has a smaller physical footprint, and it is also easier to construct. Last, although simple in terms of experimental set up and easy to conduct, the shuttle box paradigm developed for zebrafish was found to lead to less than robust acquisition of memory [12].

In summary, the purpose of the current study is to determine whether this simple maze-design in which zebrafish are required to associate a visual cue (CS) with a reward (also a visual stimulus, the sight of conspecifics) may be employed efficiently to induce and quantify associative learning in zebrafish.

2. Methods

2.1. Animals and housing

Sixteen zebrafish (Danio rerio) of the AB strain were used in this experiment. All fish tested were sexually mature, 6–10 months old, young adults (males and females 50–50%). The fish were bred and raised at the University of Toronto Mississauga (UTM) vivarium. They were housed in high-density racks (Aquaneering Inc., San Diego, CA, USA) equipped with multistage filtration that included a mechanical filter, fluidized glass bed biological filter, an activated carbon filter, as well as a fluorescent UV light sterilizing unit. Ten percent of the water was replaced daily with deionized water supplemented with 60 mg/l Instant Ocean Sea Salt (Big Al's Pet Store, Mississauga, ON).

During the habituation phase, fish were housed in groups of 8–10, in 2.8 l rearing tanks (Aquaneering Inc.) placed in a high-density rack system. After habituation fish were individually housed for identification purposes, in 1 l tanks that were part of a high-density rack system (Aquaneering Inc.). The water temperature was maintained at 26 ± 2 ◦ C. Illumination was provided by fluorescent light tubes from the ceiling with lights turned on at 07:00 h and off at 19:00 h. Fish were fed a mixture of ground flake food (4 parts, Tetramin Tropical Flakes, Tetra, USA) and powdered spirulina (1 part, Jehmco Inc., Lambertville, NJ, USA).

In the current paradigm we allowed the experimental fish to interact with a group of conspecific called the stimulus fish. We used five zebrafish as stimulus. In nature zebrafish have been found to form shoals ranging in size between only a few to hundreds of members. In the laboratory, varying the number of members of animated shoals between 3–8 was also found not to have a significant effect on the strength of the shoaling response [16], but in numerous past studies we employed 5 stimulus fish [13,14,17,18], and thus we decided to use the same number of fish here as well. To distinguish "stimulus fish" from the experimental subject, we injected the former with 0.05 μl of blue marking tissue dye (Sigma Aldrich) near the caudal fin as described before [19].

All behavioural experiments were video-recorded from an overhead camera (JVC Everio GZ-MG500, Yokohama, Japan), and later replayed for observation-based quantification using Observer Colour Pro XT (Noldus Info Tech., Wageningen, The Netherlands).

2.2. Experimental maze

The new maze (Fig. 1) was made of Plexiglas, rectangularly shaped, measuring 60 cm × 47 cm × 25 cm (length × width × height). While designing the maze, we kept simplicity in mind. For example, the interior construction of the maze utilizes acrylic inserts that are rectangular and do not have to be glued in complicated shape or angles. Furthermore, these inserts may be lowered into a standard 40 l pet store variety tank made of glass and thus unlike in Y or T-mazes do not require precision water-tight manufacturing. Along the length of the maze, on either side were four chambers (15 cm × 10.5 cm × 25 cm), which were separated by an open compartment (60 cm × 25 cm × 25 cm). One side contained the four start chambers, labeled one through four. Directly opposite the four start chambers were the four target chambers. Each target chamber had one coloured (yellow, blue, red, or green) removable cue card. The choice of cue colour was based on previous research [20]. The colour cue marking the chamber where the stimulus fish were placed we designated as CS_+, while the other colour cues that mark target chambers without the US are referred to as CS_0. The target chambers were accessible from the open area through a 9 cm tube that connected each target chamber to the open compartment. The opening between the tube and the target chamber was closed by a transparent door, which allowed the experimental subject to see the stimulus fish (the unconditioned stimulus, US) and the cue while preventing the stimulus fish from leaving the target chamber. At the tube entrance, which connected to the open compartment was a slot for a second door. The second door was placed at the entrance, once the experimental subject entered the tube.

2.3. Procedure

The experimental procedure had three phases: habituation, training, and the probe. During the habituation phase the experimental fish were placed in the maze in groups, and the size of these groups gradually decreased from 16 (first day, one 20 min long exploration session), to 8 (second day, two separate 10 min exploration sessions), to 4 (third day, two separate 10 min exploration sessions), to 2 (fourth day, four exploration session of 5 min each) to 1 (fifth day, four 5 min long exploration sessions). For each habituation trial, the fish were/was placed in a different start chamber. During the habituation/exploration sessions, only the start chamber from which the fish were released remained open, while the other three were closed. All reward chambers were open.

Following the habituation, experimental fish entered the training phase. Experimental subjects were housed in the behavioural testing room. A black metal divider (120 cm × 180 cm, width × height) blocked the view of the maze from the holding tanks. Fish were netted from their holding tank, the net with the fish was placed into a 500 ml beaker, and the fish were transported while immersed into the beaker with the net to the experimental tank. The experimental fish was placed singly into the start chamber of the test apparatus and was released by raising the guillotine door. All target chambers were open and accessible to the experimental fish. One of the target chambers contained a stimulus shoal, i.e. the reward (US). A transparent door prevented these fish from leaving the target chamber. The particular colour cue (CS_+) marking the location of the target chamber containing the stimulus fish