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Review Article

Appropriate maze methodology to study learning in fish

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Abstract

Using various sorts of mazes, researchers have gained much insight into the cognitive psychology of fish, particularly spatial and visual discrimination learning, along with the biochemical, morphological and ecological aspects of learning. Fish are known to orient themselves using landmarks, and, in some cases, create mental maps of geometric relationships using several landmarks. It is possible that by understanding the mapping mechanisms of fish we may one day uncover the possible evolutionary relationships in higher vertebrates. This is possible because the telencephalic structures became specialized early in the evolution of vertebrates for learning map-like representations of the environment. This review investigates the use of mazes (open-field, Y-maze, T-maze, radial, and multi-chamber) to learn more about spatial learning and memory in fish. To date, it has been shown that mazes are the most efficient way of studying the spatial capabilities of fish. Videographic experimentation in the natural environment would help to draw definitive conclusions on the cognitive capacities of fish.

Keywords: conditioning; fish; learning; mazes; telencephalon

Introduction

We owe the majority of our knowledge about the learning mechanisms in fish to controlled maze experiments conducted over the last decade. Spatial memory, which develops as an organism collects and processes sensory information from its surroundings, records one's spatial orientation in the environment. In the case of fish, it is used to help select cues that help them effectively perform a series of tasks, including navigating through unpredictable environments, foraging, avoiding predation and reproduction. Fish are known to orient themselves using single landmarks, and have the capacity to create mental maps of geometric relationships between numerous landmarks [1]. Depending on environmental conditions, fish develop preferences for specific sensory cues to maximize efficiency [2].

Spatial memory has been attributed to the telencephalon. This structure (constituting the anterior forebrain and the cerebral hemispheres) became highly-specialized early in the evolution of vertebrates. Its function is believed to have been refined through the use of map-like representations of the environment [3]. In fact, such use of map-based representations can help us uncover possible evolutionary relationship among higher vertebrates. Using various maze designs, researchers have gained insight into the cognitive psychology of fish: particularly the biochemical, morphological, and ecological aspects of spatial and visual discrimination learning.

Maze Set-up and Controlled Conditions

Experimental maze architecture is primarily categorized into five research models: the open-field maze, Y-maze, T-maze, radial maze and multi-chamber maze. The simplest structure is the open-field maze – a circular or rectangular arena lacking any barriers, but containing food rewards or landmarks depending on the task [4]. Y-mazes are simply a joining of three channels in the form of a Y, just as the T-maze is built in the form of a T. Connecting two T-mazes together forms a four-armed radial maze. Radial mazes consist of a central area branching off into as many as eight arms of equal length. Finally, a multiple chamber maze is an open-field maze sectioned off into several compartments by walls and doors, often built from opaque white Perspex® as opposed to transparent plastic that would allow vision through walls [5].

Learning behaviour is experimentally reinforced using food rewards. The food is administered using floating rings [6] or electronic feeders or tubes through which food is dropped [7]. Petri dishes containing Vaseline® are used on occasion to hold lodged bait that becomes visible when fish approach [4, 8].

Experimenters often use artificial illumination with fluorescent tubes in a twelve hour light-dark cycle when lighting the test room. However, light and dark conditions have been shown to affect learning [9], and must be accounted for.

Extra-maze cues are normally excluded by hanging curtains around the maze to rule out the effects of global cues in spatial learning when testing the significance of intra-maze cues [8]. In maze trials involving turn decision-making, fish will develop one of two strategies. An egocentric strategy entails learning spatial relationships relative to the organism, and is therefore easily disrupted by habitat alteration [10]. For instance, memorizing specific turns relative to one's body is a strategy susceptible to disruption by shifting silt or foliage in the environment. On the contrary, utilizing an allocentric strategy requires the fish to learn spatial relationships all around the maze environment, using physical landmarks or extra-maze cues [10]. Place and cue strategies are general terms that refer to allocentric strategies using extra-maze and intra-maze cues, respectively. At times, it is hard to distinguish between the strategies exercised by the fish. This is easily resolved by conducting probe tests, which manipulate available cues to observe how performance may be correspondingly disturbed.

Mazes uncover different learning mechanisms

Spatial learning

There is growing evidence that fish are capable of formulating cognitive maps to orient themselves within their respective environments. Open-field mazes are being used to understand the cognitive capabilities of fish based on environmental cues, since any kind of cue can be used by the fish in its spatial orientation – intra-maze, extra-maze and sensory cues. Saito and Watanabe confirm the advantages of an open-field maze in analyzing spatial learning [4]. In their experiment, goldfish were initially trained to find a particular baited hole among the 16 laid out throughout a circular maze containing a dead bloodworm food reward sunken in Vaseline®. Different situations were created to learn more about the utilization of different cues. These included changing the location of the baited hole (to analyze spatial learning), rotating the wall and floor (to examine for intra-maze cue use), putting up curtains (to exclude extra-maze cue use), and cutting of the olfactory tract or eye enucleation (to determine sensory cue use). It was found that goldfish relied mostly on extra-maze visual-sensory cues. An open-field maze was also used to study the blind Mexican cave fish and their ability to encode shape and size into a cognitive representation [5]. It was shown that blind Mexican cave fish can detect geographical changes, though this population warrants future study.

Schluessel and Bleckmann investigated the use of cognitive maps in stingrays using a radial maze [11]. Before proceeding with any trials, the fish underwent two controls within a T-maze. In the first, food was offered to the fish to confirm their knowledge of impending rewards, while the second assessed the stingrays' innate sense direction or place preferences by judging the frequency of left and right turns. Fish were then trained either egocentrically or allocentrically in a radial maze to locate a food reward at the end of an arm. Egocentric training simply used constant start and end positions. On the other hand, allocentric training involved setting up a constant end position with varying start positions in different arms to encourage the use of cues. The stingrays were subsequently put through four probe tests using novel start positions while the maze either remained stationary or moved within the room (thereby eliminating single or sets of extra-maze cues in case the fish rely on overall spatial arrangement or single cues). Extra-maze cues were eliminated using white sheets. The authors found that stingrays utilized visual information and constructed cognitive spatial maps using different navigating methods even within groups, perhaps simultaneously, to solve the spatial task [11].

Visual discrimination learning

Another important learning skill used by many fish species to navigate through the wild is the ability to discriminate visual cues. Visual learning is primarily tested using T-mazes and radial mazes. Colwill *et al*. demonstrated the ability of zebrafish to employ visual discrimination learning by conditioning the fish to enter the correct colour region of a T-maze with two different coloured arms in order to obtain a food reward [12]. Additional trials were conducted by swapping the colours. They found that zebrafish were capable of reversing their previously-learned discrimination by learning to choose a colour that was formerly incorrect or by ignoring an incorrect one that was previously rewarded.

Additionally, in two radial maze experiments, Hughes and Blight worked with corkwing wrasse and 15-spined sticklebacks in an effort to illustrate the use of visual association learning in foraging behaviour [13, 14]. In the second of these experiments, an eight-arm radial maze was used to investigate algorithmic behaviour and spatial memory employed in feeding [14]. First, all arms were loaded with a food source and experiments were conducted in the presence and absence of visual cues. Visual cues were presented with different colours marking each arm, and it was found that food rewards were discovered much faster compared to controls when coloration was used. The authors then restricted the consumption of food sources to three arms. Once all other arms were opened, the avoidance of previously-visited arms using spatial memory and visual cues was observed. A significant increase in arms revisited after repositioning of colour cues was also noted. However, when the maze was rotated while preserving the relative spatial configuration, there were no increases in revisited arms. Though promising, these results suggest that more research is required to determine the algorithmic behaviour and spatial memory in these and other fish.

Biochemical aspects of learning

To better understand the underlying mechanisms of learning in fish, researchers have studied the related neurotoxicology. The T-maze is most frequently used in such studies, an example of which is Creson *et al*'s study of black molly fish [15]. The study examined the correlation between chronic lithium treatment and spatial memory impairment. Previous work has shown that lithium treatment can have an adverse side effect on memory, and has warranted further study [15]. A place-learning task was given to four different dose regimen groups, including a control. A food goal was placed at one end of an arm. Start positions were rotated and a different arm of the four-armed maze was blocked each time in order to form a T-maze. The highest-dosage group took a significantly longer time to learn spatial task when compared to controls, suggesting that lithium treatment can have an adverse effect on spatial cognition.

Morphological aspects of learning

Studies investigating the morphological aspects of learning have been conducted using open-field and radial mazes. In the previously-described study by Saito and Watanabe, the goldfish were also tested using an open-field maze [8]. To compare the spatial learning functions of the dorsolateral and dorsomedial areas of the telencephalon, goldfish were first trained to locate a single baited hole in a circular tank of 16 sunken holes. The subjects were then given a lateral or medial telencephalon lesion and learning capabilities were examined. The first test analyzed the ability to use extra-maze cues to create a spatial map, while a second test assessed the subject's ability to use a landmark, in cases where the position of baited hole and landmark were fixed or varied (while maintaining the same distance relative to one another). Although no significant changes were observed between presurgical and postsurgical tasks, damage to the dorsomedial area significantly impaired spatial learning in the maze. The dorsomedial telencephalon, therefore, is crucial to the spatial learning capabilities of fish.

In a related study, Lopez *et. al,* looked at the effects of surgical excision of the telencephalon on place and cue learning [10]. Two groups of goldfish, one with intact telencephala and the other with telencephala removed, were trained to find a food reward in a T-maze that contained colourful visual cues. Two sets of experiments were performed. In the first, the four arms of the maze were blocked and intra-maze cues were altered in order to distinguish between place and cue strategies. In the second, extra-maze cue reliance was assessed by removing intra-maze cues and using novel start positions, while intra-maze cue reliance was examined by surrounding the maze with a curtain and using novel start positions. The results indicated that intact fish learn both place and cue strategies, whereas telencephalon-excised goldfish rely exclusively on a simple egocentric turn-strategy.

Ecological impacts on spatial learning

Research has shown that the type of spatial memories that fish use is significantly dependent on the environment in which a species lives. A recent study used a multi-chamber maze to study the influence of predation pressure and interspecific competition on spatial learning in poeciliid fish [6]. Four doors leading into equally-sized partitions were coloured using different markers. It was found that fish that experienced low predation pressure located the reward patch faster than highly-predated fish. The authors inferred that this was the result of relying on extra-maze cues and the coloured cues within the maze. It was also noted, however, that the close proximity of the sites from which the fish subjects were obtained for the study were not likely to cause differences in visual cue usage among the fish. The roles of predation pressure and interspecific competition in shaping spatial learning in fish of different habitats demand further scrutiny.

The multi-chamber maze has also been manipulated to compare the use of visual cues for orientation in fish from permanently turbid eutrophic ponds and clear water ponds [5]. A eutrophic pond is characterized by high mineral and nutrient content that promotes the growth of plant life, especially algae, resulting in a reduction in dissolved oxygen content. Once again, a maze with four compartments was used, with coloured tiles marking the doors leading into each. The subjects were required to find the contained shoal either with or without the landmarks. A shoal, or school of fish, is used as a reward due to the resulting social contact with other fish. The markers were then repositioned to generate test the use of intra-maze cues and algorithmic strategies. It was shown that fish from turbid environments followed global cues more effectively; however, both populations were adept at using visual landmarks in spatial tasks.

Use of the T-maze has also shown that habitat instability generates unreliable visual cues in the three-spined stickleback and other species [16]. River or pond habitats were used for sampling. River fish were found to more frequently employ a turn response in which the fish turned the same direction regardless of the changed landmark position.

Advantages of diverse maze utilization

Mazes are the most efficient way of studying the spatial capabilities of fish. The 60º arm-branching of Y-mazes have made them amenable to testing preference reactions. However, Hughes and Blight postulated that choosing a 4-armed starburst configuration instead of a Y-maze can offer distinctions between algorithmic alternations in foraging behaviour and behaviour guided by changing visual cues [13]. A starburst formation only has arms 1, 2, 3 and 6 open out of a radial 8, leaving arms 2 and 6 perfectly-aligned. It was also designed to allow experimenters to renew food sources unseen by the fish during tasks.

The T-maze is quite similar to the Y-maze in shape, but is popularly used to set controls prior to experimentation with more complex radial mazes. Such a maze can be used to familiarize the fish with a spatial task, confirm their knowledge of an impending award, and check for innate direction and place preferences [11].

Open-field mazes offer the advantage of enabling subjects to move freely in open space [8]. Spatial learning can thus occur due to multiple cues, and internal spatial maps can be formulated. It has been suggested that fish are less likely to form spatial maps in other kinds of mazes [8], such as radial mazes, where fish perceive goals as being fixed relative to extra-maze cues due to the identically-branching arms. In spite of this, radial mazes have been used successfully to test the complex behavior of cue memorization due to the number of arms and choices. One study used such a maze to assess foraging productivity – the memorizing of cues associated with quality of potential food sources at the end of the arms [14]. The maze formation allowed researchers to continually rotate the starting position. Overall, given that fish do not use the same strategy in different mazes, maze structure must be carefully selected according to investigational purpose.

Mazes are diverse in their abilities to challenge numerous types and aspects of spatial learning and memory. Each type of maze (open-field, Y-maze, T-maze, radial, and multi-chamber) is conventional in shape and investigational potential. Nevertheless, the structures are amenable to manipulation in order to create unique setups to optimize results and investigate a broader range of learning behaviour in fish. The results of learning studies have been largely inconsistent, making it difficult to draw definitive conclusions on the cognitive capacities of fish. Videography should be considered as a feasible next step. This would allow for more applied results pertaining to the daily living pressures on fish that make spatial learning indispensable.

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