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Development of a Y-Maze Task for Studying Cognitive Function Using Teleost Zebrafish (*Danio Rerio*) and Cavefish (*Astyanax mexicanus*)

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Abstract: *The usage of zebrafish in cognitive test by responses to visual cues has been established in the past two decades. In contrast to zebra fish, cavefish is a blindfish that uses hydrodynamic to gather information about its surroundings and make cognitive interpretations.Thus, the aim of this study is to compare the cognitive functions of both fish using Y-maze test.Adult male zebrafish and cavefish were used to assess their capabilities to respond towards a new environment provided by a Y-maze test.There is no significant difference on cavefish enters and time spent in the visual cues test, but inconsistent on the novel arm performance. However, numbers of entries and time spent in the novel arm were significantly higher in the zebra fish both in the visual cues and arms performance. Zebrafish have higher exploratory behavior and are able to recognize the preferential cues after several intervals hours. Nevertheless, the cognitive functions of cavefish are based on their mechanosensory lateral line and olfactory buds, which are highly sensitive to water movements and helps them to discover novelty in complete darkness. Thus, the advantage of Y-maze and the characteristics of these teleosts can be a good bio model for studying olfactory and neuro function.*

Keywords*:* *zebrafish, cavefish, Y-maze, cognitive function*

1. Introduction

The zebrafish is a tropical freshwater fish and the most widely ornamental species used in biomedical areas. The physiological similarity to mammals, ease of genetic manipulations, low cost of maintenance and ease to breed contributes to the developing zebrafish as an animal model. Recently, the zebrafish is rapidly gaining popular and useful as an attractive test model organism for pharmacological [1, 2] and diabetes study [3]. In fact, this teleost has been recognized as suitable vertebrate model in translational neuroscience and behavioral area. As an example, in experiments regarding learning and memory, zebrafish is a very good model for testing social cognition [4],rearing environment [5]and olfactory stimulus[6].All these characteristics prove that this teleost is very sensitive to acquire information about their surrounding environment. Thus, this suggested that the zebrafish is a unique animal model for experimental social cognitive functions.

The vision typically allows animals to gather information and control their behavior with respect to other animals. However, having this sense is not very useful in dark environment. In this condition, some animals generate their own stimulus energy such as sound or body sensory to investigate the spatial elements of the surrounding area [7]. The cavefish is a unique fish because it does not have visual and proper eyes. This fish moves and find their way around by the lateral lines, which are highly sensitive to fluctuating water pressure [8].It has been reported that the cavefish is a powerful subject for studying eyes evolution and offer some unique advantages for studying its mechanism [9]. Indeed, the cavefish offers an opportunity to study the genetic and their mechanism about how eyes pigments were lost during cavefish evolution.

However, the wayof cavefish adapt to life in darkness is poorly understood in terms of evolutionary biology. Therefore, the study of development of Y-maze test on cognitive function using cavefish have provided new information regarding the visuospatial memory. Thus, this study will provide a solid foundation on the cavefish representing one of the best examples of regression evolution and offer some unique advantages for studying cognitive function and the mechanisms. This study could indicate that the cavefish have their own cognition in their blind condition making it a perfect model for comparison of the cognitive function with the zebrafish.

The existing procedures to learn about visual and cognitive function in teleost are generally depends on their willingness or capability to explore new environments in the novel arm of the Y-maze test. A two-step of cognitive procedure in a Y-maze test has been previously developed to study learning and memory function on third generation rats [10, 11]. In fact, it was established that several conditioning memory tasks including Morris water maze [12]and radial arm maze[13]are associated with brain cognitive function in rodents. However, there are little scientific reports regarding the Y-maze test on teleost cognitive function performance [4]. Therefore, the aim of this experiment is to contrast the performance of two teleost, which is the zebrafish (*Danio rerio*) and cavefish (*Astyanax mexicanus*) on cognitive functions using the Y-maze test. Interestingly, the cavefish is well known to lack of visual systems, thus this study is to see how their cognition works in the blind state. While memory is the capacity to recall previously experienced sensations, information and ideas, the Y-maze test also would help to measures the process of memory of visionless cavefish.

Clarifying the differences in cognitive function between these teleosts are likely would improve our understanding of the factors contributing to differences in cognitive functions due to advantage the characteristic of zebrafish and cavefish. With its small size and active model organism, these teleosts provides an ideal system to apply novel tools for imaging targeted subsets of their senses and manipulating their activity. Therefore, the research on ecology and behavior of these teleosts will have profound implication in relation to the suitability of this animal as a biological model for studies on cognition development, behavioral and evolutionary ecology. In addition, uunderstanding the natural behavior and biology of experimental laboratory fish is crucial for improving animal welfare and the quality of scientific research.

2. Materials and Methods

2.1. Subjects

Adult male wild type zebrafish *(Danio rerio)* (n=10; 4.5 cm) and cavefish *(Astyanax mexicanus)* (n=10; 5.5 cm) were purchased from a local dealer (Three Aquatics, Bandar BaruBangi, Selangor, Malaysia) and kept in Neurobehavioral Room, Physiology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Both species were housed separately in plastic aquarium tanks (25 cm length x 30 cm width x 30 cm height) and filled with dechlorinated tap water (20 L) and aerated with air pump. The water temperature was set at room temperature of 25-26°C and filters were installed to remove organic toxins from the tanks. The water quality were monitored daily and maintained at pH 6.0 to 7.0, conductivity at 400–600 lS and temperature at 25–28 °C. They were fed daily with TetraMin Tropical Flake Fish (Germany). The fish were acclimatized for at least one week before the experiments. Animals handlingand techniques were approved by the Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia (IACUC/UPM/FPV.026).

2.2. The Y-Maze Apparatus

The cognitive and memory function of animal model was tested using the Y-maze which has been described previously [10]. The Y-maze plastic aquarium (Figure 1) consisting of 3 identical arms of equal size (25 cm long, 8 cm wide and 15 cm high). The first arm is start arm where the animals is first placed. The second arm is familiar arm and the third arm is novel arm, which was blocked during the first trial but open during the second trail. Three different visual cues such as square, circle and triangle were placed on the external maze walls and were removed during arms performance test. The remaining external walls was covered with black paper and the floor was covered with white paper to create a contrast between fish and maze as well as to facilitate the recording analysis. The same water approximately 1L from the home aquarium tank was used in the Y-maze arm.

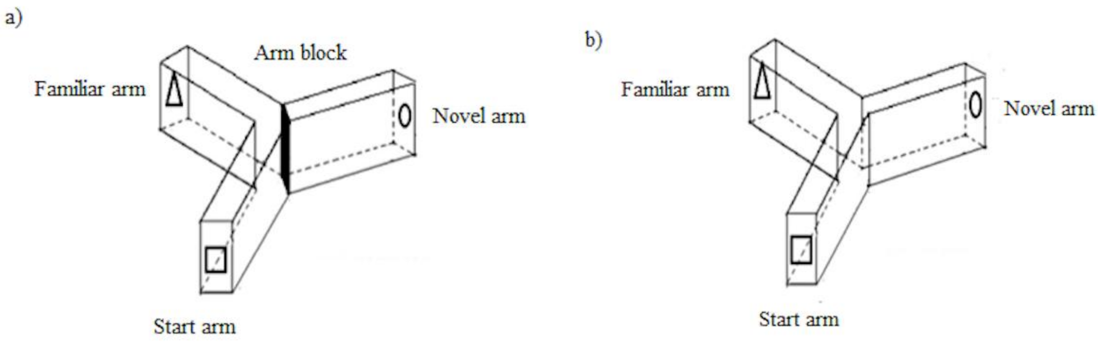

Figure1. *Schematic representation of the Y-maze test with (a) the novel arm blocked with a sliding partition during the first trial, but open during the second trial (b). Different visual cues (square, circle and triangle) were placed on the external maze walls.*

2.3. The Y-maze Test

The Y-maze testing consisted of 2 trials, which have been described previously in rodent's examination [10, 11]. The first trial was 10 min of duration and fish were allowed to explore only 2 arms (the start and familiar arms) in the maze, with the third arm (novel arm) was blocked. The center of the maze was not calculated in the analysis. The second trial was conducted after different interval hours (1h, 3h and 6h) [4],to assess the capabilities of the fish to respond towards new environments of the novel arm[10]. During the second trial, the fish were placed back in the same starting arm, with free access to all three arms for 5 min. Fish were placed in different arms as starting points and the maze was rotated in every different interval hours in order to randomize the maze cues and arms. The capabilities of the fish to respond towards the novel arm were recorded by using a ceiling-mounted camera. Recordings were then examined to count the time spent (in seconds) and the number of entries in all arms.

2.4. Statistical Analysis

Data obtained from the studies were analyzed with Statistica (StatSoft, Tulsa, USA) using one-way analysis of variance (ANOVA) with repeated measures followed by post hoc least significant difference test.All data are reported as mean ± SEM and statistical significance was described as a p value of less than 0.05

3. RESULTS

3.1. Visual Cues of Y-Maze Performance

One-way ANOVA test indicated a significant interaction ($F_{3,10}$=3.653, p<0.05)between different visual cues and time spent in all arms of zebrafish. One hour after the second trial with triangle cue as a starting point, the zebrafish spent more time in the circle cue (63.7 ± 14.2 s; p<0.05) than in the triangle and square cues (29.3 ± 13.8 s and 26.6 ± 13.1 s). Three hours after second trial with square cue as a starting point, the zebrafish spent 41.1 ± 12.0 s of the total time in the circle cue (p<0.05) compared to31.2 ± 13.4 s and 29.6 ± 13.5s in the square and triangle cues, respectively. Six hours after second trial with circle cue as a starting point, the zebrafish spent significantly higher total time in the circle cue (70.5 ± 18.8 s; p<0.05) compared to the triangle and square cues (24.1± 12.7 s and 19.9 ± 13.1 s).

Similarly, one-way ANOVA test indicated a significant interaction ($F_{3,10}$=3.553, p<0.05) between different visual cues and number of entries in all arms of zebrafish. An hour after the second trial with triangle cue as a starting point, the zebrafish had significantly higher number of entries in the circle cue (70.7 ± 14.8; p<0.05) than 42.1 ± 12.6 and 39.5 ± 15.3number of entries in the triangle and square cues, respectively. Three hours after second trial with square cue as a starting point, the zebrafish had 52.2 ± 11.7number of entries in the circle cue (p<0.05), and 53.6 ± 17.9 and 49.2 ± 13.5 number of entries in the square and triangle cues, respectively. Six hours after second trial with circle cue as a starting point, the zebrafish had 69.3 ± 9.8 number of entries in the circle cue (p<0.05) compared to 44.3 ± 15.9 and 28.8 ± 12.6 number of entries in the triangle and square cues, respectively.

However, there is no significant different on total time spent and number of entries in the visual cues performance of cavefish (data are not shown).

3.2. The Y-Maze Arms Performance

In zebrafish, one-way ANOVA indicated a significant interaction ($F_{3,10}$=3.862, p<0.05) between different arms and their time spent in all arms. An hour after second trial with start arm as a starting point, the zebrafish spent more time in the novel arm (73.6 ± 19.4 s; p<0.05), than in the start and familiar arms (42.4 ± 12.6 s and 35.7 ± 10.9 s). Three hours after second trial with familiar arm as a starting point, the zebrafish spent 52.3 ± 16.6 s in the novel arm (p<0.05),compared to the start and familiar arms (38.7± 13.4 s and 41.6 ± 18.5 s). Six hours after second trial with the novel arm as a starting point, the zebrafish spent 61.4 ± 17.6 s in the novel arm (p<0.05), and35.1 ± 12.1 s and 39.6 ± 13.7 s in the start and familiar arms, respectively.

Likewise, one-way ANOVA indicated a significant interaction ($F_{3,10}$=3.765, p<0.05) between different arms and number of entries on zebrafish. An hour after second trial with start arm as a starting point, the zebrafish had significantly higher number of entries in the novel arm (71.2 ± 12.9 entries; p<0.05) than in the start and familiar arms (39.3 ± 11.6 and 41.6 ± 15.4entries). Three hours after second trial with familiar arm as a starting point, the zebrafish had66.2 ± 11.1 number of entries in the novel arm (p<0.05) compared to the start and familiar arms (48.9 ± 13.2 and 31.6 ± 16.1entries). Six hours after second trial with the novel arm as a starting point, the zebrafish had 81.5 ± 17.3number of entries in the novel arm (p<0.05), and 32.8 ± 13.6 and 29.8 ± 10.4number of entries in the start and familiar arms, respectively.

However, the total time spent and numbers of novel arm entries in the cavefish were inconsistent. An hour after second trial with the start arm as a starting point, the cavefish spent more time in the novel arm (60.8 ± 14.4 s; p<0.05) compared to the start and familiar arms (44.6 ± 10.4 s and 36.6 ± 15.8 s). Three hours after second trial with the familiar arm as a starting point, the cavefish spent equally in all arms (p>0.05). Six hours after second trial with the novel arm as a starting point, the cavefish spent more time in the novel arm (58.2 ± 11.4 s; p<0.05) compared to the start and familiar arms (44.2 ± 13.1 s and 40.7 ± 11.1 s).Moreover, an hour after second trial with the start arm as a starting point, the cavefish had 77.5 ± 15.4number of entries in the novel arm (p<0.05), compared to the start and familiar arms (62.3 ± 13.6 and 56.4 ± 12.2 entries).However, the cavefish had equally in all arms (p>0.05) after three and six hours with the familiar and novel arms as a starting point, respectively during the second trial of the Y-maze test.

4. DISCUSSION

The current study shows that the cognitive function is associated with a tendency of the zebrafish to explore more the novel arm than other arms on the Y-maze test. Indeed, the higher number of entries and time spent in the novel arm are indicator of successfully spatial working memory [10, 11, 14]. The zebrafish had higher exploratory behavior to the novel arm demonstrating that this fish have a good learning in memorizing the visual cue arms. It has been shown when preferential exploration of novelty is established, they memorizes the novel arm and keeps on exploring the arm even after several hours [15]. The result shows that the differences of visual cues performed significantly improved an exploration the zebrafish in the novel arm. This showing that the animals could recognize the new cues, which represent the circle cues located in the novel arm after second trial in the Y-maze test. This was supported bya previous study showed that the zebrafish spent less time in the Y-maze arm with cross and triangle cues, but spent more time in circles cues which located in the novel arm[4]. Thus, this could suggest that the visual cues at the end of the arm will help the animals guide itself to explore the novel arm in the Y-maze test.

The cavefish showed time spent and number of entries equally in all visual cues arm, and performed inconsistent in the novel arm performance. This could be due to cavefish was exploring the novel arm based on their mechanosensory lateral line and olfactory sensory bulb making it not significant in the visual cues arm performance. Indeed, themechanosensory line and olfactory bulb are more useful for finding food in the complete darkness. Previous studies reported that they have better olfactory sense by having olfactory buds all over its head, which help them find food more quickly in complete darkness [16]. In fact, they are very sensitive to fluctuating water movement, pressure and they will detect the vibration by their prey and will move towards the source of direction for feeding [8]. In this study, no stimulus such as vibration or smell was initiated to attract the cavefish to a certain area. As this fish did not have visual cues and they swim following the arm wall, we postulated that they do not even know the difference of each arm in the Y-maze task. This can be due to the blind state of the cavefish, making them unable to differentiate between cues but able to explore and travel the novel arm than others arms. Interestingly, a study has stated that cavefish respond to novel environment by increasing their swimming speed [7] which will produces a flow-field around itself [17]. Clearly, the time spent and numbers of entries in all arms are indicating that cavefish use hydrodynamic water by increasing swimming speed to sense their surroundings.

The important benefits of a quick and non-invasive behavioral observation is well documented from the capability of zebrafish absorb minute molecules in water rapidly and efficiently as compared to other model organism especially cavefish. All the advantages of the Y-maze procedures and the mechanism of neuro-systems related to memory processes in zebrafish indicates that this teleost can be a good animal biomodel for the study of visual and cognitive functions. Indeed, the Y-maze memory test for zebrafish is a novel, rapid and appropriate for the study of memory and vision in this teleost. From this study, we can conclude that there are differences in zebrafish and cavefish pertaining of its cognitive function as zebrafish has a higher exploratory behavior indicating that this fish has a good cognitive and memory functions. However, cognitive function on the cavefish is depends only on its olfactory sensory and mechanosensory lateral line. Future work can be done by giving treatment to these teleosts to see the effects on cognitive function by the Y-maze performance. It was established that dietary omega-3 fatty acid improved cognitive function in rodents up to three generation [10]. In fact, a molecular study of the brain gene expression can be performed to give more insight especially the cognitive mechanisms on these teleosts.

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REFERENCES

[1] Dittmar, F., Seyfried, S., Kaever, V. and Seifer, R.,Zebrafish as model organism for cNMP research. BMC Pharmacology and Toxicology. 16: (Suppl 1):A45(2015).

[2] Maximino, C., da Silva, A. W. B., Gouveia Jr, A. and Herculano, A. M., Pharmacological analysis of zebrafish (Danio rerio) scototaxis. Progress in Neuro-Psychopharmacology&BiologicalPsychiatry.35: 624–631 (2011).

[3] Kimmel, R. A., Dobler, S., Schmitner, N., Walsen, T., Freudenblum, J. and Meyer, D..Diabetic pdx1-mutant zebrafish show conserved responses to nutrient overload and antiglycemic treatment. Scientific Reports. 5: 14241 (2015).

[4] Cognato, G. D. P., Bortolotto, J. W., Blazina, A. R., Christoff, R. R., Lara, D. R., Vianna, M. R. and Bonan, C. D., Y-Maze memory task in zebrafish (Danio rerio): The role of glutamatergic and cholinergic systems on the acquisition and consolidation periods. Neurobiology of Learning and Memory. 98: 321–328 (2012).

[5] Spence, R.,Magurran, A. E. andSmith, C., Spatial cognition in zebrafish: The role of strain and rearing environment. AnimCogn. 14:607–612 (2011).

[6] Maaswinkel, H. and Li, L., Olfactory input increases visual sensitivity in zebrafish: A possible function for the terminal nerve and dopaminergic interplexiform cells.J Exp Biol. 206 (Pt 13):2201-9 (2003).

[7] Kulpa, M., Bak-Coleman, J. and Coombs, S., The lateral line is necessary for blind cavefish rheotaxis in non-uniform flow. The Journal of Experimental Biology. 218:1603-1612, (2015).

[8] Yoshizawa, M., Jeffery, W. R., van Netten, S. M. and McHenry, M. J., The sensitivity of lateral line receptors and their role in the behavior of Mexican blind cavefish (Astyanax mexicanus). The Journal of Experimental Biology. 217: 886-895 (2014).

[9] Rohner, N., Jarosz, D. F., Kowalko, J. E., Yoshizawa,M., Jeffery, W. R., Borowsky, R. L., Lindquist, S. andTabin, C. J. 2013. Cryptic Variation in Morphological Evolution: HSP90 as a Capacitor for Loss of Eyes in Cavefish. Science. 342 (6164): 1372-1375 (2003).

[10] Hafandi, A., Begg, D. P., Premaratna, S. D., Sinclair, A. J., Jois, M. and Weisinger, R. S., Dietary repletion with ω3 fatty acid or with COX inhibition reverses cognitive effects in F3 ω3 fatty-acid–deficient mice. American Association for Laboratory Animal Science. 64(2): 106–109 (2014).

[11] Ahmad Sopian, N. F., Ajat, M., Shafie, N. I., Mohd Noor, M. H., Ebrahimi, M., Rajion, M. A., Meng, G. Y. and Hafandi, A.,Does short-term dietary omega-3 fatty acid supplementation influence brain hippocampus gene expression of zinc transporter-3?.Int. J. Mol. Sci. 16:15800-15810 (2015).

[12] Morris, R., Developments of a water-maze procedure for studying spatial learning in the rat. Journal of Neuroscience Methods. 11(1):47-60 (1984).

[13] Olton, D. S. and Samuelson, R. J., Remembrance of placed passed: spatial memory in rats. Journal of Experimental Psychology: Animal Behavior Proceedings. 2: 97-116 (1976).

[14] Onaolapo, O. J., Onaolapo, A. Y., Mosaku, T. J., Akanji, O. O. and Abiodun, O. R., Elevated plus maze and y-maze behavioral effects of subchronic, oral low dose monosodium glutamate in Swiss albino mice. J Pharmacy Biol Sc. 3:21–27 (2012).

[15] Al-Imari, L. and Gerlai, R., Sight of conspecifics as reward in associative learning in zebrafish (Danio rerio). Behav Brain Res.189 (1): 216-9 (2008).

[16] Yoshizawa, M., Goricki, S., Soares, D. and Jeffery, W. R., Evolution of behavioural shift mediated by superficial neuromasts helps cavefish find food in darkness. Current Biology. 20:1631-1636 (2010).

[17] Windsor, S. P., Tan, D. and Montgomery, J. C.,Swimming kinematics and hydrodynamic imaging in the blind Mexican cave fish (Astyanax fasciatus). The Journal of Experimental Biology. 211, 2950-2959 (2008).

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