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Research Article

DESIGN AND DEVELOPMENT OF A NOVEL COLOR BIAS AVOIDED AND APPETITE CONDITIONING T-MAZE MODEL FOR EVALUATING THE MEMORY ENHANCING DRUGS IN ZEBRA FISH

Vemula Pranav Kumar, B. Vinoth Kumar, Battula Ramakrishna, Karthik Maddula*
Bharat Institute of Technology, Mangalpally (V), Ibrahimpatnam (M), Ranga Reddy (D), Andhra Pradesh, India
*Corresponding Author Email: karthikmaddula@gmail.com

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ABSTRACT
Zebrafish is being used as a model for preclinical studies because of its unique innate physiological and behavioral aspects that almost correlate to the mammals including human. Many behavioral models have been developed based on visual stimuli, appetite conditioning, colour preference ability of zebrafish. In the present study, we used two different colors (Red and Green) which are the equal preference of zebrafish to avoid the bias and food was placed in one of the colored short arms (Green) only during the training sessions. In the test session, food was not placed in the green arm and animals were evaluated for its learning and memory in finding out the food. For evaluating the newly designed T-Maze, we employed two drugs, Scopolamine, that induce memory impairment and Rivastigmine that ameliorates the effects of scopolamine. In the test session, animals treated only with scopolamine spent less time in the green arm and found to have more number of total entries unlike the animals pre-treated with Rivastigmine one hour before scopolamine treatment. Results showed that Rivastigmine reduced the memory impairment effects of scopolamine. The findings from the present study suggest that the newly designed color bias avoided and appetite conditioning T-Maze was found to be useful for evaluating the memory enhancing drugs effectively.
Keywords: Alzheimer's disease, Color preference, Scopolamine, Rivastigmine, learning and memory.

INTRODUCTION

In the preclinical studies, though rodent models were successful, they are uneconomical and require more effort and time to maintain and breed. Therefore, they must be replaced with any other model which can be effective, economical and easy to maintain and possess characteristics that can be correlated to humans. Zebra fish model is the one which has become an upcoming model recently for preclinical investigations that can fulfill the above needs. Zebra fish has been used as a vertebrate pharmacological model in various fields like reproductive biology, teratology, developmental biology, genetics, and behavioral analysis[1]. Various beneficial aspects of zebra fish like fully characterized genome, significant physiological similarity to mammals including humans[2], rapid development, longer lifespan, similar design and connectivity to CNS with human[3], short duration of 3 months to get sexual maturity, higher reproductive rate, see-through embryos, and external fertilization led its usage in research and development. They also exhibit social behavior like conditioning reflexes, shoaling preferences, aggression, dominance, exploratory behavior and also memory and learning which enabled as an effective tool for studying behavior in neuroscience. Zebra fish were found to be very advantageous as they are easy to maintain, very economical than rodents, large numbers can be easily accommodated and suitable for large screening on one go[4]. Till now various behavioral evaluation tests were established, majority of which include color preference tests like T Maze[5], Plus maze[6], Y maze[7], Inhibitory avoidance task[8], conditioning place preference task[9,10]. Some others include appetite conditioning task like Shuttle box appetitive conditioning[11], Appetitive choice discrimination task[12]. In case of T maze, Plus maze and Y maze contain three compartments where two compartments are provided with different colors. In case of Inhibitory avoidance task, 2 compartments one is white colored and the other is dark colored compartment are used. The major convincing factor to pick out zebra fish model for carrying out behavioral tests where color discrimination is involved is that zebra fish has a retinal anatomy and physiology similar to that of other vertebrates including humans[13]. Many studies have been established based on the color discrimination using rodent models[14,15]. These experimental studies in rats and mice provide significant evidence that color preference play a pivotal role in behavioral analysis. The rationale of our present study is to establish a new model where appetite conditioning is created in a color based learning and memory task where the colors, green and red used in the experiments are the equal preferences of zebra fish. In the present study, we have used scopolamine and Rivastigmine, former drug induces the memory impairment and the latter reduces the memory impairment. Our aim is to find whether the model designed by us is beneficial for studying behavioral aspects like memory and learning.

MATERIALS AND METHODS

Animals

Adult Male Zebra fishes were obtained from a local aquarium pet store. Animals were placed in RO treated water in housing tanks of 30 cm X 15 cm X 15 cm dimensions at a density of 5 animals per liter[16]. 14-10 h light and dark photo period was maintained and aerated continuously. Dry food pellets were given as a feed which were bought from the same pet store to which the fishes got habituated to eat. Before the beginning of experiment, animals were kept for acclimatization for 10 days to the laboratory conditions.

Supplier

Zebra Fishes were obtained from Sri Sai Aquarium Stores, Hyderabad, Andhra Pradesh, India.

Chemicals

Scopolamine (Gift sample from IICT, Hyderabad, Andhra Pradesh, India) and Rivastigmine (RIVAMER capsules Marketed by Sun Pharma were used).

Equipments

Housing tank Aquarium (Dimensions 30cmX15cmX15cm), Animal feed [Dry food containing crude protein (30 %), crude fat (4 %), crude fiber (5 %), crude ash (12 %), moisture (10 %)], Aerator (Sobo aquarium air pump-SB-548A), Micropipette (Thermo scientific finnpipette), T-maze test apparatus.

T-Maze Test

Apparatus

T Maze apparatus was fabricated using transparent acrylic glass sheet. Dimensions of the maze were long arm of 50 cm X 10 cm X 10 cm and two short arms of 20 cm X 10 cm X 10 cm and start box of 10 cm X 10 cm X 10 cm at the foot of the stem [9]. The two short arms, left and right arms are covered with green and red colored sleeves respectively. Water in the maze was filled up to 6 cm height and temperature of water was maintained at 28^{o}C throughout the experiment.

Design of New Procedure

Based on the study by Avdesh *et al*. (2010), we preferred Red and Green colors. The reason for selecting Red and Green colors is that zebra fishes showed equal preference towards both the colors hence the bias towards color can be avoided. Food is placed in any one of the two arms (Green) only during the training session. The time spent in the food containing green arm and Total Number of entries into short arms is noted down to evaluate the learning and memory in finding out food.

Animal Grouping

According to S.K. Richetti *et al*. (2011) animals were exposed to 200 µM scopolamine solution 1 hour prior to the experiment. According to Bejar C *et al*. (1999) animals were treated with 1.5 mg/kg of Rivastigmine 2 hours before the commencement of experiment.

Hence the animals were grouped into four groups each containing 5 fishes as following:

- Group I normal group animals were treated only with water.
- Group II animals were exposed to 200 µM scopolamine solution 1 hour prior to the experiment.
- Group III animals were treated only with rivastigmine (1.5 mg/kg)
- Group IV animals were treated with 1.5 mg/kg of rivastigmine 2 hours before the commencement of experiment.

Procedure

Initially, animals were placed in the T Maze without any colored sleeves to habituate the animals before beginning of the experiment. This evaluation test consists of training and test sessions. Training session was carried out for 6 consecutive days and the following day is test session i.e. on 7th day. In the training session food was placed in green arm before beginning the experiment. The overnight fasted animal is placed in the start box for 1 minute. Then, sliding door was opened and closed after the exit of fish. Fish was then allowed to enter the short arms, once it entered any of the arms, another sliding door at the junction of the long arm and two short arms was closed. Now, fish was allowed to swim and observed for 4 minutes in the short arms. The total number of entries into the short arms and the time spent in the green arm were evaluated. After 6 days of training, on 7th day, the test session was carried out with the same procedure as that of training session by depriving food in the green arm.

Statistical Methods

Data were presented as Mean ± SEM and analysis was performed using One-Way Anova. Comparison of groups was performed using Dunnet's test. Differences between groups were considered statistically significant at $P < 0.05$.

RESULTS

Comparison of normal and scopolamine groups in training (TR) and test (TE) sessions using time spent as a parameter

In order to determine the memory impairing property of scopolamine effectively, we compared the normal and scopolamine groups in both the training and test sessions. Time spent in the food containing green arm by the Group I animals (normal group animals) in the training session (NTR) was found to be 107 seconds and whereas in the test session (NTE) was 103 seconds. In the Group II animals (only scopolamine treated animals), time spent in the food containing green arm in the training session (STR) was 71 seconds and in the test session (STE) was 68 seconds. These results indicated that scopolamine group showed hindrance in memory retention ($P < 0.05$ and $P = 0.0374$). (Figure 2)

Effect of Rivastigmine on memory impairment by Scopolamine in test (TE) session using time spent in green arm as a parameter

The time spent in the food containing green arm by the only scopolamine treated animals (STE) was 71 seconds and whereas by the rivastigmine pre-treated scopolamine group (SRTE) was found to be 137 seconds and rivastigmine treated water group was found to be 129 seconds (Mean ± SEM of SRTE 137 ± 16.7 Vs STE 71.4 ± 7.13, unpaired t-test, $t = 3.62$, $P = 0.0068$, $F = 5.50$) and (Mean ± SEM of RTE 129 ± 40.67 Vs STE 71.4 ± 7.13, unpaired t-test, $t = 1.858$, $P = 0.1126$, $F = 19.51$). (Figure 3)

Comparison of normal and scopolamine groups in t maze paradigm using total number of entries into short arms as a parameter

In the comparison of normal and scopolamine groups, the average number of entries (rounded off to the nearest whole number) ± SD by normal group in the training session (NTR) was 23 and in the test session (NTE) was found to be 15. The average number of entries into the arms by the only scopolamine treated animals in the training session (STR) was found to be 33 and in the test session (STE) was 17 ($P < 0.05$ and $P = 0.0126$). (Figure 4)

Effect of Rivastigmine on memory impairment by Scopolamine in T-maze paradigm using total number of entries into short arms as a parameter

The average number of entries (rounded off to the nearest whole number) ± SD into the short arms of the only scopolamine treated animals (STE) in the test session was found to be 17 and that of the Rivastigmine pre-treated scopolamine groups (SRTE) in the test session was found to be 8 (Mean ± SEM of SRTE 8 ± 1.72 Vs STE 17.4 ± 1.25, unpaired t-test, $t = 4.70$, $P = 0.0015$, $F = 1.90$) and (Mean ± SEM of RTE 6.333 ± 3.528 Vs STE 17.4 ± 1.25, unpaired t-test, $t = 3.608$, $P = 0.0113$, $F = 4.786$) (Figure 4)

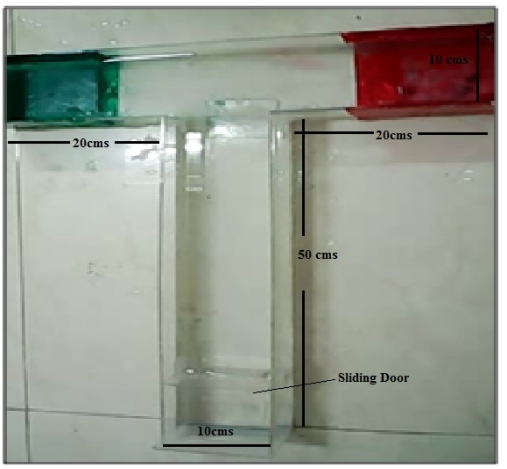

Figure 1: T-Maze apparatus with dimensions

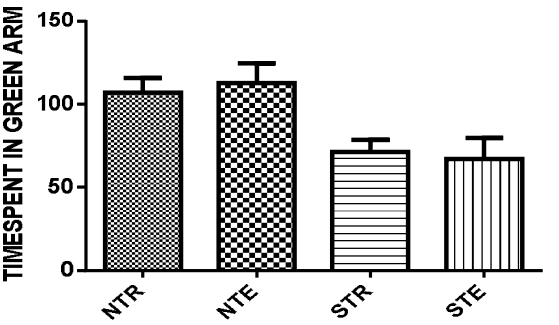

Figure 2: Comparison of animals in Normal group and only scopolamine treated group

Time spent in the food containing arm i.e. Green arm by Normal (water treated) test group (NTE) was found to be 107 seconds. Whereas the scopolamine treated test group (STE) was found to 68 seconds. This reduction (63.5 %) in the time spent reveals that the learning and memory got decreased after exposing the animals with scopolamine. These results indicates that scopolamine group showed hindrance in memory retention ($P < 0.05$ and $P = 0.0374$). Data are presented as mean ± SEM, pooled from five animals.

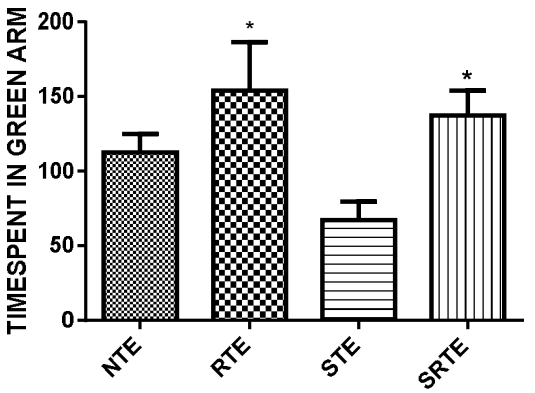

Figure 3: Comparison among normal, rivastigmine, only scopolamine treated and rivastigmine scopolamine treated groups

Time spent in the food containing arm i.e. Green arm by by the only scopolamine treated animals (STE) was 71 seconds. In comparison with scopolamine the time spent by rivastigmine treated water (RTE) group was found to be 154 seconds (54 % increase). This reveals that the learning and memory got improved after the administration of rivastigmine. In comparison with scopolamine the time spent by Rivastigmine pre-treated scopolamine group (SRTE) was found to be 137 seconds (43% increase). This increase in the time spent reveals that the rivastigmine ameliorated the effects of scopolamine. Data are presented as mean ± SEM, pooled from five animals. Asterisk indicates a statistically significant difference (*$P < 0.05$ from the STE value).

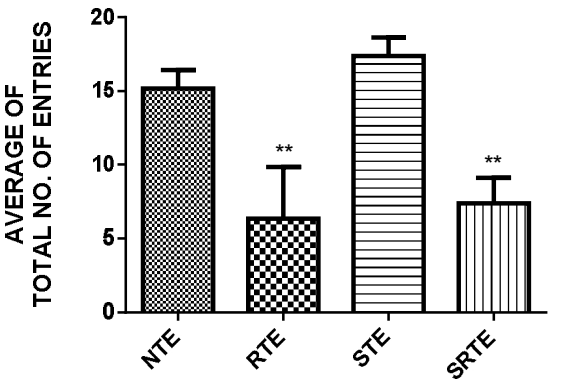

Figure 4: Comparison of Total Number of Entries of normal, rivastigmine, only scopolamine and rivastigmine-scopolamine treated test group animals

The average number of entries (rounded off to the nearest whole number) ± SD into the short arms of the only scopolamine treated animals (STE) in the test session was found to be 17 and that of the Rivastigmine pre-treated scopolamine groups (SRTE) in the test session was found to be 8 (Mean ± SEM of SRTE 8 ± 1.72 Vs STE 17.4 ± 1.25, unpaired t-test, $t = 4.70$, $P = 0.0015$, $F = 1.90$) and (Mean ± SEM of RTE 6.333 ± 3.528 Vs STE 17.4 ± 1.25, unpaired t-test, $t = 3.608$, $P = 0.0113$, $F = 4.786$)

DISCUSSION

Let us consider Light and Dark chamber method of evaluation, zebrafish enters the dark compartment undoubtedly as the natural preference of it is dark chamber. Similarly, if different colors were used, then similar confound could arise, as the zebrafish have different preference for different colors. Therefore, Green and Red combination was used as they are equally preferred hence bias towards color is avoided. In addition to the color we used appetite condition where we placed food to the overnight fasted animal in the green colored arm only in training sessions and checked for its memory retention in the test sessions. In the comparison of animals in Normal group and only scopolamine treated group (Figure 2), Time spent in the food containing arm i.e. Green arm by Normal (water treated) groups (NTR and NTE) were found to be higher than the scopolamine groups in training and test sessions (STR and STE). This comparison indicated that scopolamine group showed hindrance in memory retention. To find the effect of rivastigmine and in turn effectiveness of our newly designed T-maze, the comparison (Figure 3) among normal test (NTE), rivastigmine treated test (RTE), scopolamine test and scopolamine rivastigmine treated test groups (STE and SRTE), was carried out. Only scopolamine treated test (STE) was found to spend less time compared to scopolamine rivastigmine treated test group (SRTE) and rivastigmine treated water group (RTE) which indicates that there was a hindrance in memory in case of former group whereas the

latter group showed retention in memory. To find the effectiveness of T-maze, Total Number of entries into the short arms is another parameter used for evaluation. In the present study, total number of entries into green arm, Red arm and Blank (Middle portion) was considered. In the comparison of normal (Only water treated) (NTR and NTE) and only scopolamine treated groups (STR and STE) (Figure 4), only Scopolamine treated group in both training (STR) and test sessions (STE) was found have more number of entries into all arms than the Normal group in training (NTR) and test sessions (NTE). This observation indicates that scopolamine showed declined memory retention than (only water treated) normal group. Among normal test (NTE), rivastigmine treated test (RTE) and scopolamine treated test groups (STE and SRTE), (Figure 4) comparison was carried out among only scopolamine treated group (STE) and rivastigmine groups (RTE and SRTE). Only scopolamine treated group (STE) was found to show more total number of entries than the rivastigmine groups (RTE and SRTE). This indicates that latter groups showed retention in memory.

CONCLUSION

Although many zebra fish models available for evaluating the memory enhancing drugs, we made an attempt to develop a new model of T maze with colors of equal preference and appetite conditioning. We used scopolamine to impair the memory and rivastigmine to ameliorate the effects of scopolamine. The time spent in the green arm and the total number of entries into the short arms was the two parameters used to find out the effectiveness of our model. We found that in the test session the overnight fasted animal when treated with scopolamine alone spent less time in the green arm and showed more total number of entries. But when they were treated with rivasigmine 1 hour before the scopolamine treatment they spent more time in the green arm and showed less total number of entries. The increase in time spent in the green arm and the decrease in the total number of entries reveals that the animal remembered the arm in which food was placed in the training session. With this effect of rivastigmine in ameliorating the effects of scopolamine, we conclude that our newly designed color bias avoided and appetite conditioning T maze can be used as an effective model to evaluate the memory enhancing property of drugs.

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Abbreviations

SEM, Standard mean error; NTR, Group 1 animals in the training session; NTE, Group 2 animals in the test session; STR, Group 2 animals in the training session; STE, Group 2 animals in the test session; RTE, Group 3 animals in the training session; SRTE, Group 4 animals in the test session.

REFERENCES

1. Serra EL, Medalhaand CC, Mattioli R. Natural preference of zebra fish (*Danio rerio*) for a dark environment. Braz. J. Med. Biol. Res 1999; 32(12): 1551-53. http://dx.doi.org/10.1590/S0100-879X 1999001200016
2. Barbazuk WB, Korf I, Kadavi C, Heyen J, Tate S, Wun E, Bedell JA, Mc Pherson JD and Johnson SL. The Syntenic Relationship of the Zebrafish and Human Genomes. Genome. Res 2000; 10: 1351-58. http://dx.doi.org/10.1101/gr.144700
3. Guo S. Linking genes to brain, behavior and neurological diseases: what can we learn from zebrafish? Genes. Brain. Behav 2004; 3: 63-74. http://dx.doi.org/10.1046/j.1601-183X.2003.00053.x
4. Spence R, Gerlach G, Lawrence C and Smith C. The behavior and ecology of the zebrafish, *Danio rerio*. Biol. Rev 2008; 83(1): 1-102.
5. Colwill RM, Raymond MP, Ferreira L, Escudero H. Visual discrimination learning in zebrafish (*Danio rerio*). Behav. Process 2005; 70(1): 19–31. http://dx.doi.org/10.1016/j.beproc.2005.03.001
6. Sison M and Gerlai R. Associative learning in zebra fish (*Danio rerio*) the plus maze. Behav. Brain. Res 2010; 207(1): 99. http://dx.doi.org/10.1016/j.bbr.2009.09.043
7. Grella SL, Kapur N and Gerlai R. A Y-maze Choice Task Fails to Detect Alcohol Avoidance or Alcohol Preference in Zebrafish. Int. J. Comp. Psychol 2010; 23: 26-42.
8. 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: 529-34. http://dx.doi.org/10.1016/j.nlm.2009.07.001
9. Avdesh A, Martin Iverson MT, Mondal A, Chen M, Askraba S, Morgan N, Lardelli M, Groth DM, Verdile G and Martins RN. Evaluation of color preference in zebra fish for learning and memory. J. Alzheimers. Dis 2010; 28(2): 459-69.
10. Swain HA, Sigstad C, Scalzo F. Effects of dizocilpine (MK-801) on circling behavior, swimming activity and place preference in zebrafish (*Danio rerio*). Neurotoxicol. Teratol 2004; 26: 725-29. http://dx .doi.org/10.1016/j.ntt.2004.06.009
11. Pather S and Gerlai R. Shuttle box learning in zebra fish (*Danio rerio*). Behav. Brain. Res 2009; 196: 323-27. http://dx.doi.org/ 10.1016/j.bbr.2008.09.013
12. Bilotta J, Risner ML, Davis EC, Haggbloom SJ. Assessing appetitive choice discrimination learning in zebra fish. Zebrafish 2005; 2: 259-68. http://dx.doi.org/10.1089/zeb.2005.2.259
13. Thornberry T, Risner M and Haggbloom SJ. Wavelength Discrimination in the Zebrafish, (*Danio rerio*): Evidence for Functional Color Vision. Electronic Journal of Integrative Biosciences 2008; 5(1): 1-10.
14. Thomas BB, Samant DM, Seiler MJ, Aramant RB, Sheikholeslami S, Zhang K, Chen Z and Sadda SR. Behavioral Evaluation of Visual Function of Rats Using a Visual Discrimination Apparatus. J. Neurosci. Meth 2007; 162(1-2): 84–90. http://dx.doi.org/ 10.1016/j.jneumeth.2006.12.010
15. Broadbent NJ, Squire LR and Clark. RE. Rats depend on habit memory for discrimination learning and retention. Learn. Memory 2007; 14: 145-51. http://dx.doi.org/10.1101/lm.455607
16. Richetti SK, Blank M, Capiotti KM, Piato AL, Bogo MR, Vianna MR, Bonan CD. Quercetin and rutin prevent scopolamine-induced memory impairment in Zebrafish. Behav. Brain. Res 2011; 217: 10–15. http://dx.doi.org/10.1016/j.bbr.2010.09.027

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