Exploring object discrimination in zebrafish: behavioral performance and scopolamine-induced cognitive deficits at different retention intervals

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Abstract

The object discrimination test allows the testing of different memory retention periods. However, few behavioral endpoints have been measured in fish species such that retention is often assessed using a single parameter (time spent in object area). Here, we aimed to explore the object discrimination test in zebrafish by assessing their behavioral performance after 1 h or 24 h retention interval periods. To characterize putative interaction-like behaviors, fish were tested in the absence or presence of scopolamine (1 h before test session). Zebrafish were habituated for three consecutive days in the experimental tank and training session was performed for 10 min using two identical non-preferred objects (black cube or sphere). After the retention intervals, a familiar object was replaced by a novel object (test session, 10 min). Fish were also **exposed** to the novel tank diving test to assess locomotion and anxiety-like behaviors. At 1 h retention interval, animals performed more circular-like investigation near the familiar object, whereas 24 h after training session, a prominent rapid investigation was observed when animals explore the non-familiar object. Because scopolamine abolished these phenotypes, as well as **the increased time spent** in the novel object area during the test without changing locomotion and anxiety-related parameters, the behavioral responses described here may predictively reflect interaction-like behaviors involved in object discrimination memory in zebrafish models.

Keywords: object discrimination; interaction-like behaviors; zebrafish; memory retention; time intervals; scopolamine.

1. Introduction

The object discrimination task, also known as a spontaneous object recognition test [1], novel object recognition test [2] or novel preference recognition test [3], is used to evaluate the ability to recognize a new object in the environment, **which is one of the most popular paradigms used to assess memory** [4, 5]. The task consists of familiarization, retention, and test phases, in which animals are exposed initially to identical objects and after a retention interval, a novel object is placed with a familiar one [6]. In rodent models, for example, both ontogeny and retention period may alter the behavioral performance, thereby affecting the object recognition memory [7, 8]. **Nonetheless, it is important to investigate object recognition memory in a variety of organisms, including fish, which is only in its infancy.**

The zebrafish (*Danio rerio*) is a suitable model organism in behavioral neuroscience. This species has evolutionarily conserved genome when compared to the human counterpart (~70% of similarity) and well-characterized behaviors [9-11]. Although the organization of the zebrafish central nervous system (CNS) is simpler than those of mammals [12], various brain structures of teleost fish are structurally homologous to those of mammals [13], allowing the investigation of basic neural processes underlying complex behaviors (*e.g.*, aggression, anxiety, and fear) [14]. Moreover, studies involving learning and memory processing reveal the growing utility of zebrafish to assess aversive conditioning [15-17], spatial and aversive memories [18, 19], and positive reinforcement [20, 21].

Mounting evidence shows that zebrafish are able to discriminate objects [22-24]. **Unlike rodent data,** which show a clear interaction of animals with objects by directly touching and smelling [25], **the behavioral endpoint that reflects discrimination in zebrafish is, so far, restricted to the percentage of time spent in object areas** [3, 23]. Moreover, the use of different protocols, object shapes, sizes, and colors among

laboratories [22, 24, 26] associated with the lack of behavioral parameters to measure a proper interaction with objects, complicate the interpretation regarding preference or aversion to novel objects [27].

The involvement of cholinergic signaling **in** acquisition and consolidation has been extensively described [28-30]. Scopolamine is an antagonist of the muscarinic acetylcholine receptor, which is frequently used to evaluate the influence of cholinergic system on behavioral processes, particularly learning and memory, in various model organisms [31]. In rodents, scopolamine induces cognitive impairment [32] and negatively modulates memory formation in the object recognition test [33], elevated T-maze [34], among others. Zebrafish is also highly sensitive to scopolamine, which shows amnesic effects in passive avoidance, Y-maze, and visual attention tests [19, 35-37]. Thus, the use of scopolamine in experimental models help to improve their predictive validity, allowing a more precise identification of putative behaviors associated with learning and memory. We hypothesize that in addition to the time spent in object areas, other behavioral endpoints might reflect interaction with objects in zebrafish, which could differ according to the retention period. Thus, the goal of this study was to explore the behavioral repertoire of zebrafish in the object discrimination task after distinct retention times (1 h and 24 h) in the presence or absence of scopolamine, classically used to induce transient amnesia in experimental protocols.

2. Methods

2.1. Animals

Adult (4-6 months old) short-fin zebrafish (*Danio rerio*) of mixed genders (~50:50 male and female) were obtained from a local commercial supplier (Hobby Aquário, RS, Brazil). Animals were kept in 50 L tanks at a density of two animals per liter containing

non-chlorinated water (25 ± 2°C and pH 7.0–7.2), changed twice weekly and acclimated for 15 days before the experiments. Fish were kept under constant aeration and filtration with a light/dark illumination of 14/10h provided by fluorescent lamps. Feeding was provided twice daily with commercial fish flake food (Alcon BASIC ™, Alcon, Brazil). After the acclimatization period, animals were kept in housing tanks measuring 48 cm x 34 cm x 6 cm (lenght x width x height), which had equal divisions for each fish (6 cm x 6 cm x 6 cm – lenght x widht x height), and small perforations (0.5 cm diameter) to minimize the effects of isolation stress during 4 days. These perforated Plexiglas divisions were designed to allow free water circulation inside the tank [16, 17]. Fish were separated by transparent divisions to ensure the identification of each subject throughout the experimentation. Animals were maintained in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals. The protocols were approved by the Ethics Commission on Animals Use of the Federal University of Santa Maria (protocol number 2220181215).

2.2. Object discrimination test

Behavioral experiments were performed in a test tank measuring 20 cm x 20 cm x 15 cm (length x width x height) externally covered in the floor and walls with gray ethylene vinyl acetate paper to avoid reflexive areas as well as external interferences. The experimental apparatus was filled with non-chlorinated water at similar temperature and pH when compared to those of the housing tanks (25 ± 2°C, pH 7.0–7.2). Because zebrafish express a 3D swimming profile [38, 39], we used a lower water level (approximately 4 cm water column height) **that, though shallow, allows fish to swim freely in horizontal directions.** This strategy reduces their vertical activity and allows a

precise identification of the exploratory activity towards objects by using a single top-view camera. All tests were performed between 09:00 a.m. and 04:00 p.m.

In the habituation phase, animals were placed individually in the test tank for three consecutive days [24] in the absence of objects (10 min) to acclimate to the experimental apparatus and reduce the novelty stress [40]. As behavioral endpoints, we measured the distance traveled as a locomotor index and the number of erratic movements, characterized by sudden changes of direction and velocity of swimming that reflects an aversive behavior [11]. Distance traveled was measured using appropriate video-tracking system (Any-Maze™, Stoelting, CO, USA). Erratic movements were manually counted by two trained observers blinded to the experimental condition of fish (inter-rater reliability > 0.85).

On the fourth day, animals were exposed to the test tank with two identical objects for 10 min in the training session [3, 41]. Objects used were cube (side = 1.5 cm) and sphere (diameter = 3.0 cm), both of black color with defined sized and shapes to facilitate reproducibility among different laboratories. To minimize spatial bias, the sides of the objects were switched and their positions were also counterbalanced on the opposite side of the tank. The apparatus was divided virtually into 3 areas of similar dimensions: the proximal areas (which contained the objects), the central areas, and the distal areas (farthest areas from the objects) (see details in **Fig. 1**). We evaluated the time spent and the number of transitions to the proximal areas, locomotor-related behaviors, as well as potential behaviors suggestive of interaction with objects. We defined as circular-like investigation the behavior of zebrafish when they performed a contour near the object in a 4 x 4 cm area centered on the object. The rapid investigation was counted when fish swam towards the object (at a maximum distance of 2 cm) and then return rapidly to farthest areas (similar to a risk assessment episode). Both behaviors were expressed as

number of episodes and quantified manually by two trained experimenters (inter-rater reliability > 0.85) blinded to the experimental conditions. Other behaviors reflecting locomotion (distance traveled and absolute turn angle) were quantified using the Any-Maze™ software. Moreover, the number of erratic movements and immobile episodes were analyzed. Immobility was defined by a complete cessation of movements (≥ 2 s), except eyes and gills, at the bottom of the tank [11].

During the training session, animals that spent more than 70% of time exploring one of the objects in **the** proximal area **(a total of 6 fish from 170 subjects tested)**, as well as fish that did not enter in the object areas **(a total of 3 fish from 170 subjects tested)**, were excluded from the trial (exclusion criteria to minimize potential bias). These parameters were chosen aiming to avoid a strong preference for an object in a specific position of the apparatus and to minimize the effects of stress on **the** discrimination task as described for rodents [42].

After the training session, fish were placed in the housing habituation tanks with partitions. To evaluate the behavioral performance at different retention intervals, animals were tested after 1 h or 24 h, depending on the experimental group. One hour before testing, fish were exposed to non-chlorinated water (control) or 200 μM scopolamine [19, 35]. Fish were individually transferred to the test tank with a familiar and a novel object [3, 41]. The object discrimination test was performed for 10 min and all behaviors were recorded using a webcam connected to a laptop to further analysis using appropriate video-tracking system (Any-Maze™, Stoelting, CO, USA). Preference percentages were calculated as follows: [time of exploration of novel object/time of exploration of familiar object + time of exploration of novel object × 100] [24].

2.3. Scopolamine treatment

Scopolamine is a muscarinic antagonist that induces amnesic effects on various organisms, including zebrafish [36, 43]. One hour before behavioral tests, fish were individually placed in 500 mL tanks and exposed to 200 μM (−)-scopolamine hydrobromide trihydrate (Sigma-Aldrich, St. Louis, MO, USA) dissolved in non-chlorinated aerated water [19, 35]. Control group was handled in a similar manner, except that no scopolamine was placed in the tank. To minimize variables that could affect the behavior, scopolamine solution was made fresh and changed after each exposure. Figure 1 shows the experimental protocols used here to evaluate both retention intervals (1 h and 24 h), as well as the scopolamine exposure period.

2.4. Novel tank diving test

To investigate whether isolation and/or scopolamine could affect locomotion and anxiety-like behaviors, after each experimental set (1 h and 24 h retention intervals – in the absence or presence of scopolamine), animals were submitted to the novel tank diving test. This task is commonly used to evaluate the exploratory activity and habituation to novelty stress [44-46]. Zebrafish **(*n* = 8 per group)** were placed individually in the test apparatus (25 cm length x 15 cm height x 10 cm width) filled with 2.5 L non-chlorinated water and their behaviors were recorded for 6 min. Videos were analyzed using automated video-tracking system (Any-Maze™, Stoelting, CO, USA) at 30 frames/s. The apparatus was divided in two horizontal areas (top and bottom) and the following endpoints were measured: distance traveled, absolute turn angle, erratic movements, immobility, time spent in top area, transitions to top area, latency to enter the top area, and average duration in top area. Distance traveled and absolute turn angle reflect locomotor-related behaviors, while erratic movements and immobility are associated with aversive responses. The

other behavioral endpoints measured indicate vertical activity, which are associated with anxiety-like phenotypes in zebrafish [11].

2.5. Statistical analysis

Data normality and homogeneity of variances were analyzed by Kolmogorov-Smirnov and Bartlett's tests, respectively. Behavioral endpoints measured across the habituation phase were analyzed by repeated measures analysis of variance (ANOVA), while object preference and behaviors measured in the novel tank diving test were assessed by two-way ANOVA. Post hoc comparisons were made using Student-Newman-Keuls multiple range test when necessary. Object discrimination in each session was analyzed using Student's *t*-test and differences from 50% chance were calculated using one-sample *t*-test. Results were expressed as means ± S.E.M. and considered significant when $p < 0.05$.

3. Results

To minimize the novelty stress in the object discrimination test, we first investigated the habituation response of zebrafish to the test apparatus. Animals showed a significant reduction in both distance traveled ($F_{(34, 68)} = 14.18$; $p < 0.0001$) and erratic movements ($F_{(34, 68)} = 2.422$; $p = 0.001$) following a three-day trial **(Fig. 2)**.

Here, two types of interaction-like behaviors were observed in the presence of objects. One behavior was defined as 'rapid investigation', in which zebrafish swim towards the object and return rapidly. A second behavior was called 'circular-like investigation', in which fish perform a contour near the object **(Fig. 3A)**. **Figure 3B** shows a representative picture of the apparatus with objects, as well as the area in which interaction-like behaviors were counted. **The type of shape and the position of the**

shape within the tank had no effect on the interaction behaviors described here. Moreover, both transitions and time spent in the object areas were similar in the training session **(Fig. 3C)**. Furthermore, distance traveled, absolute turn angle, erratic movements, and immobility did not differ when distinct object shapes were tested **(Fig. 3D).**

Figure 4 shows the effects of the 1 h retention interval on the object discrimination test. In the test session, zebrafish spent more time near the novel object (t = 2.781, df = 46, p = 0.0078), which differed from 50% chance (t = 2.144, df = 23, p = 0.0428) **(Fig. 4A)**. Although the number of entries in the object areas and the rapid investigation behavior did not change in test session, a reduction in the circular-like investigation near the novel object was observed (t = 2.328, df = 46, p = 0.0244) **(Fig. 4B)**. Fish exposed to scopolamine showed a similar percentage of time spent near the objects and no differences from 50% chance were observed (t = 0.7027, df = 25, p = 0.4887) **(Fig. 4C).** Furthermore, the transitions to object areas and the interaction-like behaviors did not significantly differ in scopolamine-treated group **(Fig. 4D)**.

Figure 5 shows the effects of the 24 h retention interval on object discrimination test. In the test session, the zebrafish spent more time in the novel object area (t = 2.099, df = 36, p = 0.0429), showing a significant difference from 50% chance (t = 2.129, df = 18, p = 0.0473) **(Fig. 5A)**. The number of entries in object areas and the circular-like investigation in both familiar and novel objects did not differ. However, the rapid investigation behavior was markedly increased in the novel object when compared to the familiar one (t = 2.169, df = 36, p = 0.0368**) (Fig. 5B)**. **As expected, no differences from 50% chance were seen in scopolamine-treated fish** (t = 0.3152, df = 20, p = 0.7559), which showed a similar percentage of time spent near the objects **(Fig. 5C)**. Moreover, scopolamine abolished the differences observed in the rapid investigation at 24 h retention interval **(Fig. 5D)**.

Figure 6 shows the behavioral activity of zebrafish in the novel tank diving test. Locomotion, aversive behaviors, **(Fig. 6A)** and vertical exploration **(Fig. 6B)** did not alter in the absence or presence of scopolamine when both retention periods were tested (1 h and 24 h).

4. Discussion

In this report, we explored the object discrimination test in zebrafish, as well as the memory retention after different time intervals. To our knowledge, we describe for the first time how zebrafish interact with familiar and novel objects, by characterizing two main interaction-like behaviors when animals swim towards the objects, named here as circular-like investigation and rapid investigation. **Importantly, both exploration behaviors differ depending on the retention interval and on scopolamine treatment; scopolamine blocked changes in exploratory behavior as well as absolute time spent near the novel object.** Because locomotion and anxiety-like responses did not change in the presence or absence of scopolamine after 1 h and 24 h retention periods, the interaction-like behaviors described here may be associated with object discrimination memory.

The object discrimination task evaluates the ability in which animals interact with objects when a familiar one is replaced by a new object in the test session [5]. This task is widely used to assess recognition memory that may vary depending on the retention interval [47]. Zebrafish discriminate different object forms when stationary or moving 2D shapes are shown externally to the wall of the apparatus [37], as well as show a clear preference for novel objects at delay times ranging from 2 h to 24 h [48]. Conversely, zebrafish preferentially spent more time near familiar than novel objects when complex objects of different sizes (LEGO® figures) were used [3]. These inconsistencies may be

associated with differences in the experimental protocol used in zebrafish research, which are often complex and difficult to replicate since **there are a large number** of existing variables (*e.g.*, different object shapes, sizes and textures and/or even the presence or absence of habituation) [22, 24, 26, 27]. To circumvent these issues, we established a protocol aiming to reduce experimental variables **allowing precise data reliability.** We first habituated fish to the test tank in the absence of objects for three consecutive days to minimize the environmental stress, which could affect their performance in discrimination trials [24]. Here, zebrafish showed a gradual reduction in both distance traveled and erratic movements when exposed thrice to the apparatus, suggesting habituation to novelty stress [49]. Because zebrafish present the ability to discriminate **objects based on shapes** [22] and since different colors can directly influence behavioral activity [26], we chose two objects of simple shapes (sphere and cube) with similar color (black) as stimuli. Recent evidence also shows that zebrafish tend to explore the objects **independently of their location** [24] **and objects used here were placed at random positions** to allow a similar exploratory activity, corroborating with previous findings. Importantly, zebrafish behavior did not differ when sphere and cube were initially tested, thereby excluding a potential preference for the objects.

The main interaction-like behaviors varied depending on the retention period. At 1 h retention interval, zebrafish performed more circular-like investigations near the familiar object, but spent more time in the novel object area. Although these data seem controversial, the time spent in the object areas does not necessarily reflect a specific behavioral exploration pattern resembling a proper interaction with objects, which reinforce the importance of measuring different behavioral endpoints to investigate how zebrafish interact with familiar and non-familiar objects across time. Nonetheless, 24 h after training, zebrafish increased the number of rapid investigations **when exploring** the

novel object. The behavioral differences observed at distinct time intervals could be associated with isolation stress and/or a changes in exploratory pattern towards objects involved in discrimination. Mounting evidence shows the involvement of **the** cholinergic system in modulating memory formation after different retention intervals [19, 50, 51]. Scopolamine antagonizes muscarinic acethylcholine receptors and induces amnesia in experimental models of dementia [31]. Traditionally, scopolamine is used to characterize memory-related behaviors in various tasks, such as Y-maze test [19], inhibitory avoidance [52, 53], and object recognition test [37, 54, 55]. Scopolamine abolished the increased time spent in the novel object area, as well as changes in the interaction-like behaviors when animals were tested 1 h and 24 h after training session. Although scopolamine elicits antidepressant and antianxiety effects in humans [56], as well as anxiolytic-like responses in zebrafish [57], we did not observe changes in anxiety-related behaviors at the concentration tested. Similar to previous findings [43], we showed that 200 μM scopolamine induces amnesia, but does affect neither locomotion nor vertical activity of fish. Importantly, both locomotion and anxiety-like responses did not change in control fish tested at the different retention periods, excluding the involvement of isolation stress on interaction-like behaviors. Because scopolamine did not change transitions to the object areas, locomotion, and anxiety-related responses, the interaction-like behaviors described here may predictably reflect a distinct behavioral performance associated with object discrimination memory at 1 h and 24 h retention periods. However, the exact significance of such behaviors, as well as the neurochemical mechanisms involved in these responses still require further scrutiny.

In conclusion, we explored the behavioral performance of zebrafish in the object discrimination test. **In addition to corroborating previous findings** showing a persistent retention of object discrimination memory in zebrafish [37, 48], we described two novel

interaction-like behaviors, which are sensitive to scopolamine and differ depending on the memory retention interval. Because isolation and scopolamine do not affect locomotion and anxiety-related behaviors, the behaviors measured here predict potential temporal differences on the exploratory pattern of fish in the object discrimination test. Although more studies are needed to explore the neural mechanisms involved in both retention intervals (1 h and 24 h) in zebrafish, our data reinforce the increasing utility of this aquatic species as a model organism in learning and memory tasks.

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Conflict of interest

The authors declare that no competing interests exist.

References

1. Blaser R, Heyser C: Spontaneous object recognition: a promising approach to the comparative study of memory. Front Behav Neurosci. 2015;9:183.

2. Lucon-Xiccato T, Dadda M: Guppies Show Behavioural but Not Cognitive Sex Differences in a Novel Object Recognition Test. PLoS One. 2016;11:e0156589.

3. May Z, Morrill A, Holcombe A, Johnston T, Gallup J, Fouad K, et al.: Object recognition memory in zebrafish. Behav Brain Res. 2016;296:199-210.

4. Antunes M, Biala G: The novel object recognition memory: neurobiology, test procedure, and its modifications. Cogn Process. 2012;13:93-110.

5. 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.

6. Santos LC, Ruiz-Oliveira J, Oliveira JJ, Silva PF, Luchiari AC: Irish coffee: Effects of alcohol and caffeine on object discrimination in zebrafish. Pharmacol Biochem Behav. 2016;143:34-43.

7. Hatakeyama T, Sugita M, Yamada K, Ichitani Y: Temporal order memory of the rat in spontaneous object recognition: effects of number of items, exposure interval, and retention time. Learn Mem. 2018;25:574-9.

8. Reger ML, Hovda DA, Giza CC: Ontogeny of Rat Recognition Memory measured by the novel object recognition task. Dev Psychobiol. 2009;51:672-8.

9. Norton W, Bally-Cuif L: Adult zebrafish as a model organism for behavioural genetics. BMC Neurosci. 2010;11:90.

10. Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, et al.: The zebrafish reference genome sequence and its relationship to the human genome. Nature. 2013;496:498-503.

11. Kalueff AV, Gebhardt M, Stewart AM, Cachat JM, Brimmer M, Chawla JS, et al.: Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. Zebrafish. 2013;10:70-86.

12. Panula P, Chen YC, Priyadarshini M, Kudo H, Semenova S, Sundvik M, et al.: The comparative neuroanatomy and neurochemistry of zebrafish CNS systems of relevance to human neuropsychiatric diseases. Neurobiol Dis. 2010;40:46-57.

13. Mueller T, Dong Z, Berberoglu MA, Guo S: The dorsal pallium in zebrafish, *Danio rerio* (Cyprinidae, Teleostei). Brain Res. 2011;1381:95-105.

14. Fontana BD, Ziani PR, Canzian J, Mezzomo NJ, Muller TE, Dos Santos MM, et al.: Taurine Protects from Pentylenetetrazole-Induced Behavioral and Neurochemical Changes in Zebrafish. Mol Neurobiol. 2018.

15. Aoki T, Kinoshita M, Aoki R, Agetsuma M, Aizawa H, Yamazaki M, et al.: Imaging of neural ensemble for the retrieval of a learned behavioral program. Neuron. 2013;78:881-94.

16. Ziani PR, Muller TE, Stefanello FV, Fontana BD, Duarte T, Canzian J, et al.: Nicotine increases fear responses and brain acetylcholinesterase activity in a context-dependent manner in zebrafish. Pharmacol Biochem Behav. 2018;170:36-43.

17. Maximino C, Meinerz DL, Fontana BD, Mezzomo NJ, Stefanello FV, de SPA, et al.: Extending the analysis of zebrafish behavioral endophenotypes for modeling psychiatric disorders: Fear conditioning to conspecific alarm response. Behav Processes. 2018;149:35-42.

18. 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.

19. Cognato Gde P, 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;98:321-8.

20. Collier AD, Khan KM, Caramillo EM, Mohn RS, Echevarria DJ: Zebrafish and conditioned place preference: a translational model of drug reward. Prog Neuropsychopharmacol Biol Psychiatry. 2014;55:16-25.

21. Mathur P, Lau B, Guo S: Conditioned place preference behavior in zebrafish. Nat Protoc. 2011;6:338-45.

22. Oliveira J, Silveira M, Chacon D, Luchiari A: The zebrafish world of colors and shapes: preference and discrimination. Zebrafish. 2015;12:166-73.

23. Madeira N, Oliveira RF: Long-Term Social Recognition Memory in Zebrafish. Zebrafish. 2017;14:305-10.

24. Gaspary KV, Reolon GK, Gusso D, Bonan CD: Novel object recognition and object location tasks in zebrafish: Influence of habituation and NMDA receptor antagonism. Neurobiol Learn Mem. 2018;155:249-60.

25. Cordeira J, Kolluru SS, Rosenblatt H, Kry J, Strecker RE, McCarley RW: Learning and memory are impaired in the object recognition task during metestrus/diestrus and after sleep deprivation. Behav Brain Res. 2018;339:124-9.

26. Faillace MP, Pisera-Fuster A, Medrano MP, Bejarano AC, Bernabeu RO: Short- and long-term effects of nicotine and the histone deacetylase inhibitor phenylbutyrate on novel object recognition in zebrafish. Psychopharmacology (Berl). 2017;234:943-55.

27. Hamilton TJ: Chapter 9 - Object Novelty and Object Location Recognition Memory in Fish – Recent Advances. Handbook of Behavioral Neuroscience. 2018;27:151-61.

28. Agrawal R, Tyagi E, Saxena G, Nath C: Cholinergic influence on memory stages: A study on scopolamine amnesic mice. Indian J Pharmacol. 2009;41:192-6.

29. Jahanshahi M, Azami NS, Nickmahzar E: Effect of Scopolamine-based Amnesia on the Number of Astrocytes in the Rat's Hippocampus. Int J Morphol. 2012;30:388-93.

30. Pagnussat N, Almeida AS, Marques DM, Nunes F, Chenet GC, Botton PH, et al.: Adenosine A(2A) receptors are necessary and sufficient to trigger memory impairment in adult mice. Br J Pharmacol. 2015;172:3831-45.

31. Deiana S, Harrington CR, Wischik CM, Riedel G: Methylthioninium chloride reverses cognitive deficits induced by scopolamine: comparison with rivastigmine. Psychopharmacology (Berl). 2009;202:53-65.

32. Aydin E, Hritcu L, Dogan G, Hayta S, Bagci E: The Effects of Inhaled Pimpinella peregrina Essential Oil on Scopolamine-Induced Memory Impairment, Anxiety, and Depression in Laboratory Rats. Mol Neurobiol. 2016;53:6557-67.

33. Mitchnick KA, Wideman CE, Huff AE, Palmer D, McNaughton BL, Winters BD: Development of novel tasks for studying view-invariant object recognition in rodents: Sensitivity to scopolamine. Behav Brain Res. 2018;344:48-56.

34. Takahashi RN, Pamplona FA, Fernandes MS: The cannabinoid antagonist SR141716A facilitates memory acquisition and consolidation in the mouse elevated T-maze. Neurosci Lett. 2005;380:270-5.

35. Kim YH, Lee Y, Kim D, Jung MW, Lee CJ: Scopolamine-induced learning impairment reversed by physostigmine in zebrafish. Neurosci Res. 2010;67:156-61.

36. Richetti SK, Blank M, Capiotti KM, Piato AL, Bogo MR, Vianna MR, et al.: Quercetin and rutin prevent scopolamine-induced memory impairment in zebrafish. Behav Brain Res. 2011;217:10-5.

37. Braida D, Ponzoni L, Martucci R, Sala M: A new model to study visual attention in zebrafish. Prog Neuropsychopharmacol Biol Psychiatry. 2014;55:80-6.

38. Cachat J, Stewart A, Utterback E, Hart P, Gaikwad S, Wong K, et al.: Three-dimensional neurophenotyping of adult zebrafish behavior. PLoS One. 2011;6:e17597.

39. Rosemberg DB, Rico EP, Mussulini BH, Piato AL, Calcagnotto ME, Bonan CD, et al.: Differences in spatio-temporal behavior of zebrafish in the open tank paradigm after a short-period confinement into dark and bright environments. PLoS One. 2011;6:e19397.

40. Oliveira AM, Hawk JD, Abel T, Havekes R: Post-training reversible inactivation of the hippocampus enhances novel object recognition memory. Learn Mem. 2010;17:155-60.

41. Hamilton TJ, Myggland A, Duperreault E, May Z, Gallup J, Powell RA, et al.: Episodic-like memory in zebrafish. Anim Cogn. 2016;19:1071-9.

42. Leger M, Quiedeville A, Bouet V, Haelewyn B, Boulouard M, Schumann-Bard P, et al.: Object recognition test in mice. Nat Protoc. 2013;8:2531-7.

43. Bortolotto JW, Melo GM, Cognato Gde P, Vianna MR, Bonan CD: Modulation of adenosine signaling prevents scopolamine-induced cognitive impairment in zebrafish. Neurobiol Learn Mem. 2015;118:113-9.

44. Egan RJ, Bergner CL, Hart PC, Cachat JM, Canavello PR, Elegante MF, et al.: Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behav Brain Res. 2009;205:38-44.

45. Cachat J, Stewart A, Grossman L, Gaikwad S, Kadri F, Chung KM, et al.: Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. Nat Protoc. 2010;5:1786-99.

46. Mezzomo NJ, Silveira A, Giuliani GS, Quadros VA, Rosemberg DB: The role of taurine on anxiety-like behaviors in zebrafish: A comparative study using the novel tank and the light-dark tasks. Neurosci Lett. 2016;613:19-24.

47. Taglialatela G, Hogan D, Zhang WR, Dineley KT: Intermediate- and long-term recognition memory deficits in Tg2576 mice are reversed with acute calcineurin inhibition. Behav Brain Res. 2009;200:95-9.

48. Lucon-Xiccato T, Dadda M: Assessing memory in zebrafish using the one-trial test. Behav Processes. 2014;106:1-4.

49. Wong K, Elegante M, Bartels B, Elkhayat S, Tien D, Roy S, et al.: Analyzing habituation responses to novelty in zebrafish (*Danio rerio*). Behav Brain Res. 2010;208:450-7.

50. Agostini JF, Toe H, Vieira KM, Baldin SL, Costa NLF, Cruz CU, et al.: Cholinergic System and Oxidative Stress Changes in the Brain of a Zebrafish Model Chronically Exposed to Ethanol. Neurotox Res. 2018;33:749-58.

51. Vuaden FC, Savio LE, Piato AL, Pereira TC, Vianna MR, Bogo MR, et al.: Long-term methionine exposure induces memory impairment on inhibitory avoidance task and alters acetylcholinesterase activity and expression in zebrafish (*Danio rerio*). Neurochem Res. 2012;37:1545-53.

52. Roldan G, Cobos-Zapiain G, Quirarte GL, Prado-Alcala RA: Dose- and time-dependent scopolamine-induced recovery of an inhibitory avoidance response after its extinction in rats. Behav Brain Res. 2001;121:173-9.

53. Miranda MI, Bermudez-Rattoni F: Cholinergic activity in the insular cortex is necessary for acquisition and consolidation of contextual memory. Neurobiol Learn Mem. 2007;87:343-51.

54. Rutten K, Reneerkens OA, Hamers H, Sik A, McGregor IS, Prickaerts J, et al.: Automated scoring of novel object recognition in rats. J Neurosci Methods. 2008;171:72-7.

55. Rutten K, Prickaerts J, Blokland A: Rolipram reverses scopolamine-induced and time-dependent memory deficits in object recognition by different mechanisms of action. Neurobiol Learn Mem. 2006;85:132-8.

56. Furey ML, Khanna A, Hoffman EM, Drevets WC: Scopolamine produces larger antidepressant and antianxiety effects in women than in men. Neuropsychopharmacology. 2010;35:2479-88.

57. Hamilton TJ, Morrill A, Lucas K, Gallup J, Harris M, Healey M, et al.: Establishing zebrafish as a model to study the anxiolytic effects of scopolamine. Sci Rep. 2017;7:15081.

Figures

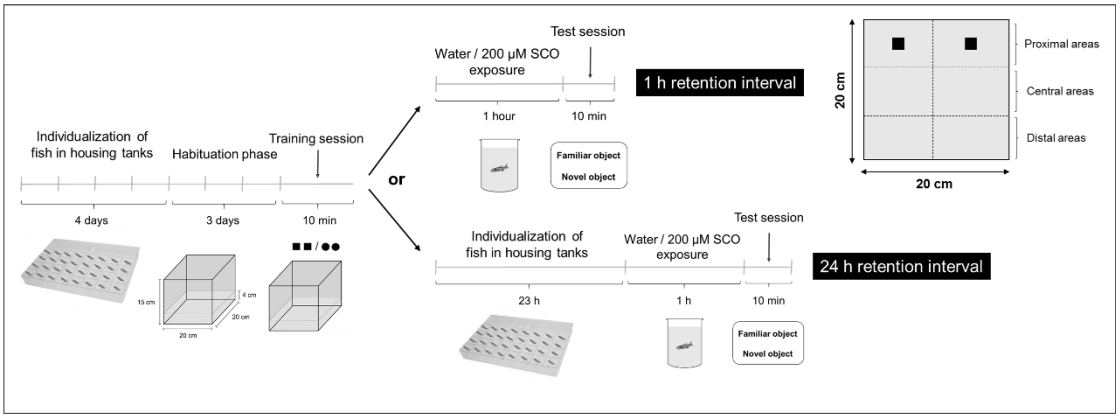

Fig. 1. Schematic representation of the experimental protocol. The figure demonstrates the previous individualization of fish, the habituation, training, and test session. Object discrimination test was assessed after 1 h or 24 h retention intervals. Fish were individually exposed to water (control) or 200 µM scopolamine 1 h before test session. The experimental tank with its respective dimensions, water column height, and virtual areas is shown.

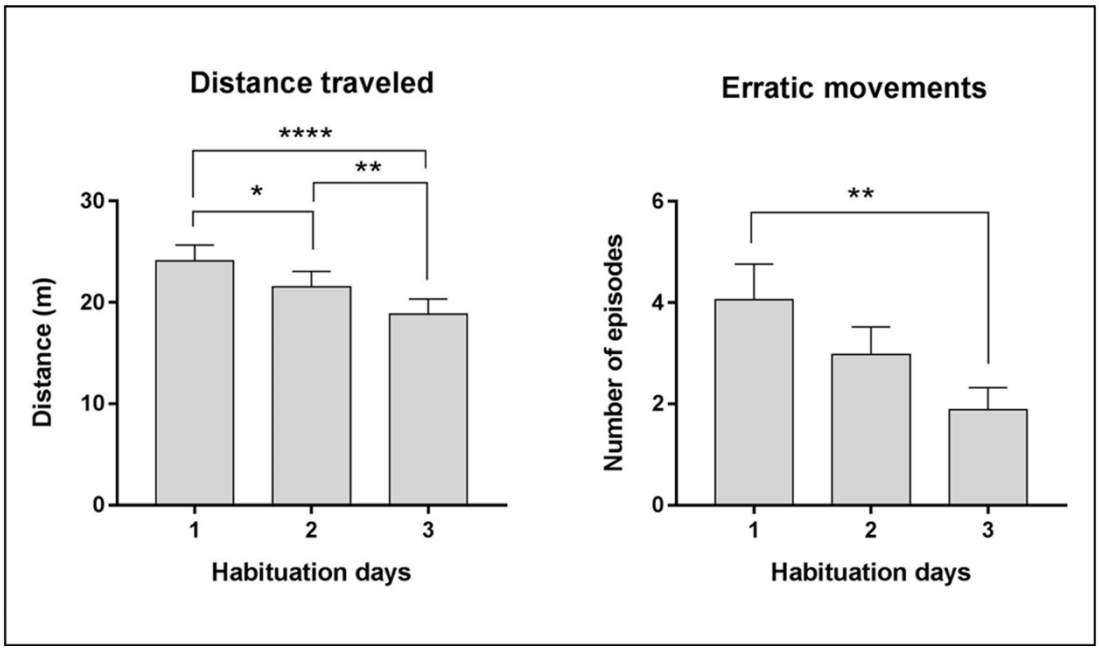

Fig. 2. Behavioral endpoints measured across the habituation phase. Data were expressed as means ± S.E.M. and analyzed by repeated measures analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple comparison test (* $p < 0.05$; ** $p < 0.01$, and **** $p < 0.0001$, $n = 35$).

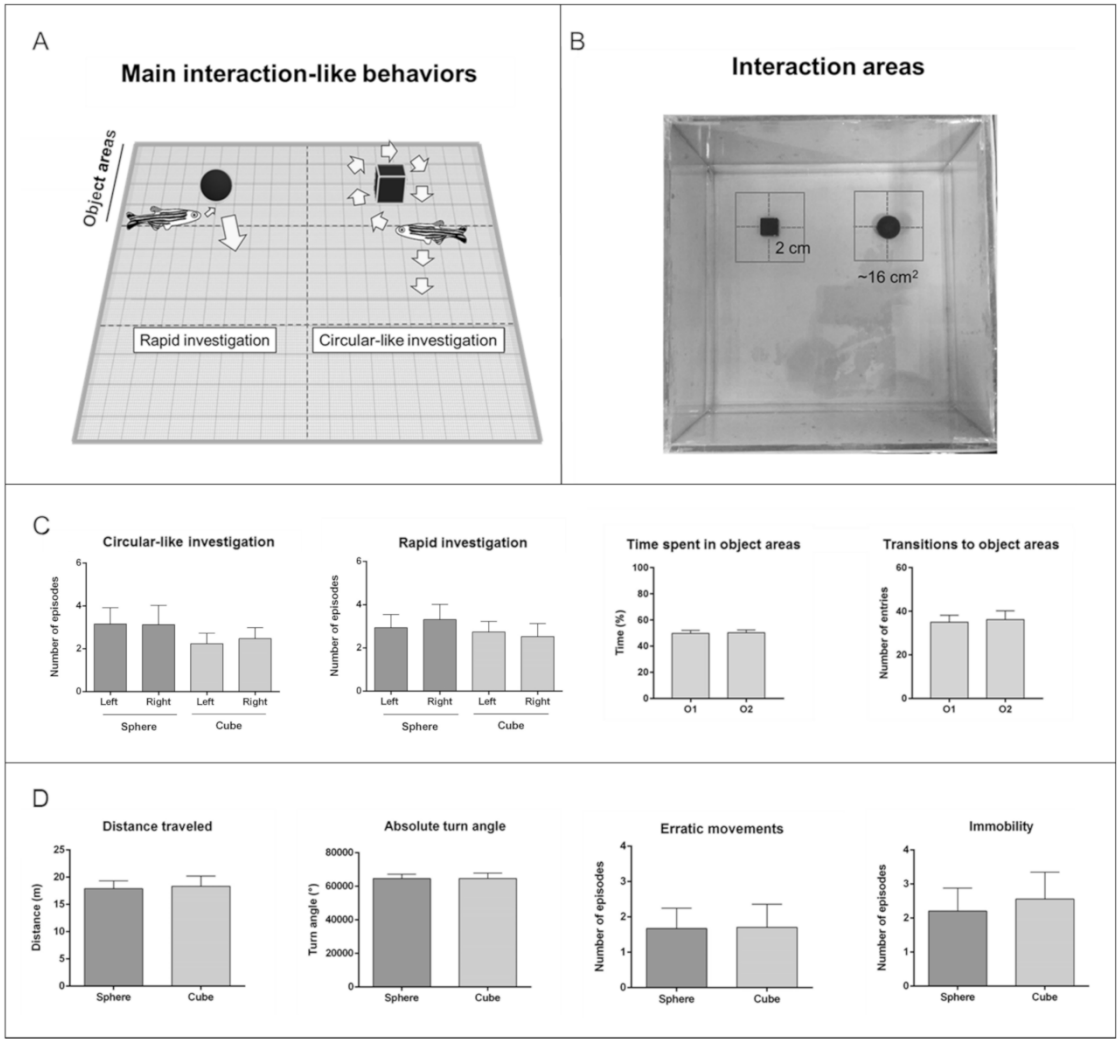

Fig. 3. Interaction-like behaviors, exploratory activity in the object areas and main locomotion-related endpoints. **(A)** Schematic representation of the main behaviors observed when zebrafish swim toward objects. **(B)** Representative picture showing the apparatus, objects and the interaction area (~16 cm^2). **(C)** Behavioral endpoints associated with interaction and exploration. **(D)** Locomotion-related behaviors when sphere or cube were used in the training session. Data were expressed as means ± S.E.M. and analyzed by two-way ANOVA (interaction-like behaviors) or Student's *t* test (exploration- and locomotion-related behaviors), $n = 33$ per group. O1: object 1; O2: object 2 (both similar objects).

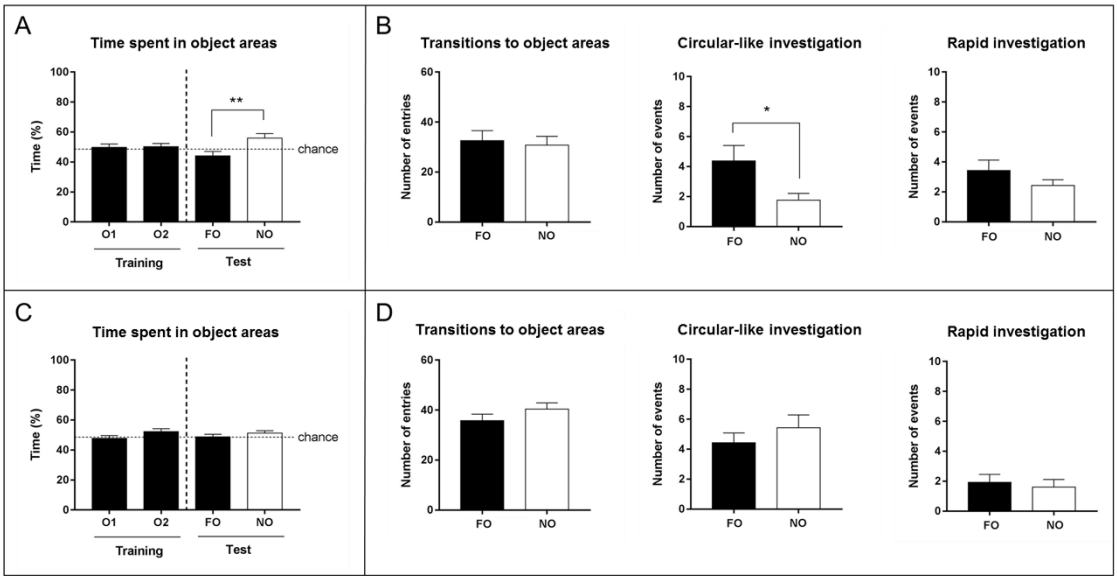

Fig. 4. Preference percentage 1 h after training session in the absence **(A and B)** or presence **(C and D)** of scopolamine. **A** and **C** show the percentage of time spent in object areas at training and test phases for control and scopolamine-treated groups, respectively. **B** and **D** show the exploratory and interaction-like activities for control and scopolamine-treated groups at test session, respectively. FO: familiar object; NO: novel object. Data were expressed as means ± S.E.M. and analyzed by Student's *t* test (* $p < 0.05$; ** $p < 0.01$, $n = 24$ for control and $n = 26$ for scopolamine-treated group).

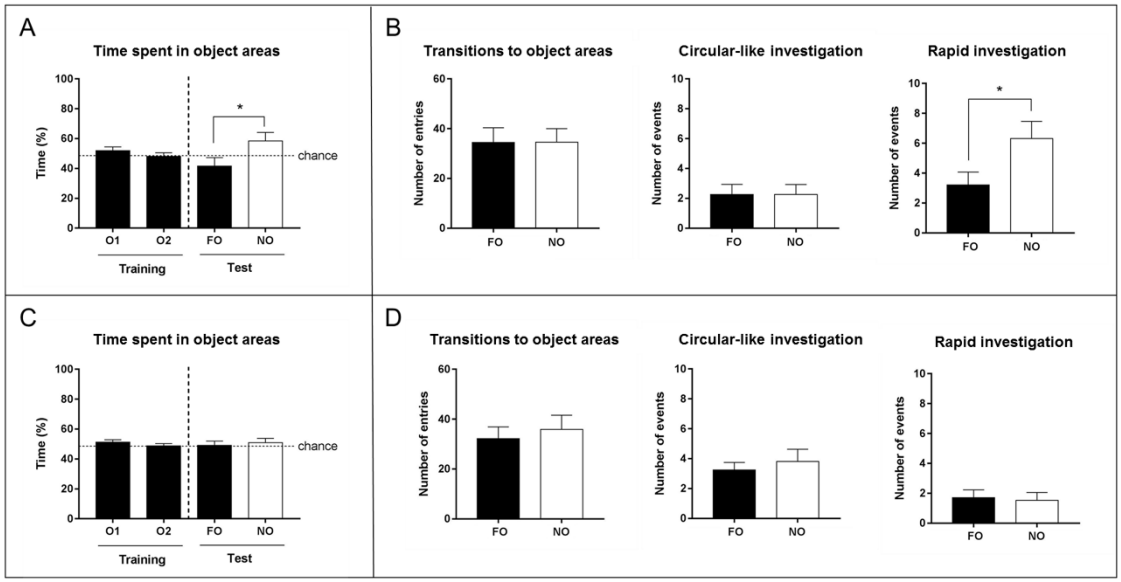

Fig. 5. Preference percentage 24 h after training session in the absence **(A and B)** or presence **(C and D)** of scopolamine. **A** and **C** show the percentage of time spent in object areas at training and test phases for control and scopolamine-treated groups, respectively. **B** and **D** show the exploratory and interaction-like activities for control and scopolamine-treated groups at test session, respectively. FO: familiar object; NO: novel object. Data were expressed as means ± S.E.M. and analyzed by Student's *t* test (* $p < 0.05$, $n = 19$ for control and $n = 26$ for scopolamine-treated group).

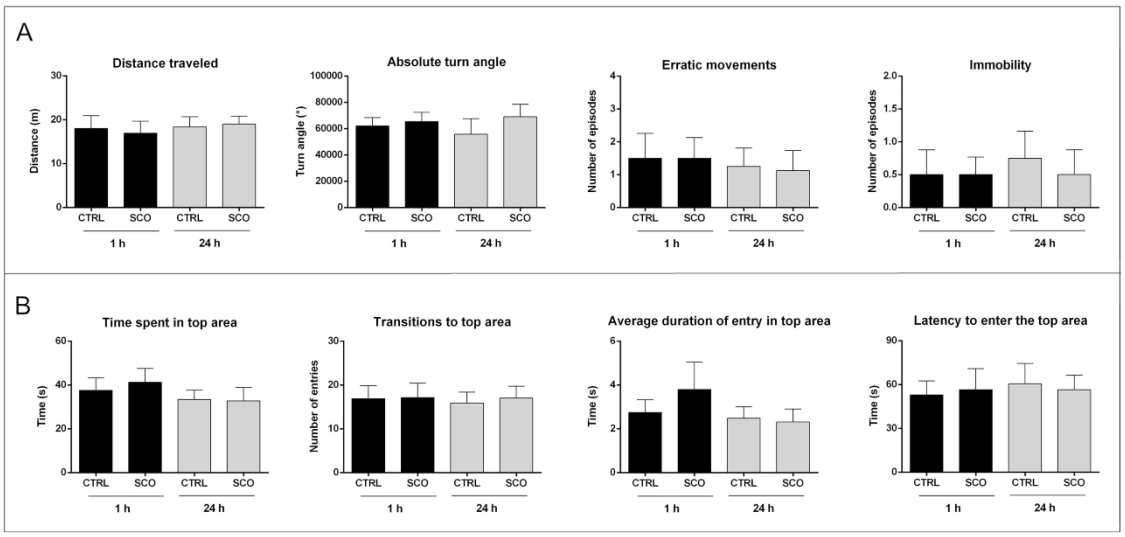

Fig. 6. Scopolamine did not alter behavioral parameters in the novel tank diving test when fish were analyzed 1 and 24 h after training session. **(A)** Locomotion-related endpoints (distance traveled and absolute turn angle) and aversive behaviors (erratic movements and immobility). **(B)** Vertical activity. Data were expressed as means ± S.E.M. and analyzed by two-way ANOVA, $n = 8$ per group. CTRL: control; SCO: scopolamine.