Comparing the Role of Dopamine D1 and D3 Receptors in Mediating Aversive Learning in Zebrafish

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Abstract. Dopamine is a key neuromodulator in the vertebrate brain. Despite accumulating evidence of dopamine's involvement in learning and memory regulation, the roles of individual receptor subtypes remain unclear. This study investigates the effects of SCH-23390 and SB-27011-A, selective antagonists of the D1 and D3 dopamine receptors on learning and memory in zebrafish. Using an associative learning task in a T-maze with electric shocks as stimuli, we assessed the impact of these drugs on zebrafish performance. Our results show that SCH-23390 at both 1 mg/L and 5 mg/L and SB-27011-A at 5 mg/L altered behaviors. However, the effects observed at 5 mg/L were confounded by impaired locomotion, suggesting that reliable cognitive assessments should focus on lower doses. Although no significant differences were observed in percentage of correct arm entries, the SCH-23390 group at 1 mg/L showed a significantly lower proportion of fish with correct first arm entries and significantly more time spent in the wrong arm, whereas no such effects were seen in the SB-27011-A group. These findings suggest that D1 receptors, but not D3 receptors, play a crucial role in associative learning involving aversive stimuli. Further research is necessary to clarify how dopamine receptor subtypes contribute to different learning paradigms.

1 Introduction

Cognition is a complex mental process that enables individuals to acquire, retain, and utilize information [1]. Cognitive deficits have long been associated with various neuropsychiatric disorders, including schizophrenia, bipolar disorder, depression, chronic stress, and panic disorder [2, 3]. Among the many cognitive domains, learning and memory performance is one of the most extensively studied, as it provides essential insights into how information is processed [4]. Understanding the role of neuromodulatory systems in learning and memory is therefore crucial for advancing therapeutic strategies.

Dopamine is a key neuromodulator that regulates learning and memory [5, 6]. Its effects are mediated through five receptor subtypes, categorized into two families—D1-like (D1, D5) and D2-like (D2, D3, D4). In humans, these receptors are widely distributed across

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various brain regions, including the prefrontal cortex, hippocampus, amygdala, and striatum [7]. A growing body of evidence suggests that the dopamine receptors play distinct functional roles in different aspects of learning and memory [8]. However, considerable variability remains in how these receptors function, highlighting the need for further studies to elucidate their specific roles in various learning contexts.

Zebrafish (*Danio rerio*) have emerged as a highly valuable vertebrate model in neurobiological studies. Their highly conserved neuroanatomy and neurochemistry compared to humans [9] offer a significant advantage for translational research. Zebrafish also demonstrate robust performance across various paradigms, including social [10] and motor learning [11], as well as associative (reward-based and aversive) [12, 13] and non-associative learning tasks [14], facilitating the analysis of vertebrate learning and memory. These behavioral characteristics, combined with the feasibility of pharmacological manipulations, make zebrafish an attractive model for investigating the roles of neuromodulatory systems involved in learning and memory tasks. In this study, we tested the effects of SCH-23390 and SB-27011-A, selective antagonists of the D1 and D3 receptors in zebrafish to further characterize their role in associative learning tasks involving aversive stimuli.

2 Materials and Methods

2.1 Chemicals

R(+)-SCH-23390 hydrochloride (D054) and SB-27011-A hydrochloride hydrate (S4326) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Both compounds were dissolved in water and diluted to final concentrations of 1 mg/L and 5 mg/L, respectively, prior to the start of the experiments.

2.2 Fish Husbandry

Three-month-old wild-type zebrafish were sourced from local breeders. The fish were housed in tanks measuring 900 mm × 445 mm × 455 mm and maintained at 26–28 °C under a 14-hour light/10-hour dark cycle. They were fed twice daily with commercial fish feed. All experimental procedures were approved by the UTAR Scientific and Ethical Review Committee (U/SERC/58-18/2024).

2.3 Apparatus

A T-maze was constructed from acrylic sheets and equipped with an electric shock device, as previously described [13]. The maze consisted of a central intersection leading to two lateral arms, enabling fish to make choices and form associations between a visual cue (red LED; conditioned stimulus, CS) and potential outcomes (electric shock, unconditioned stimulus, US). To further enhance the CS, the designated CS arm was covered with green material (Fig. 1).

2.4 Associative Learning Assay

The experimental procedure was adapted from [15]. Prior to the training and testing phases, each fish was treated with the respective drugs for 30 minutes and allowed 15 minutes to explore the maze to acclimate to isolation. On the following day, each fish underwent the same drug treatment and acclimatization period before being gently lifted and placed in the

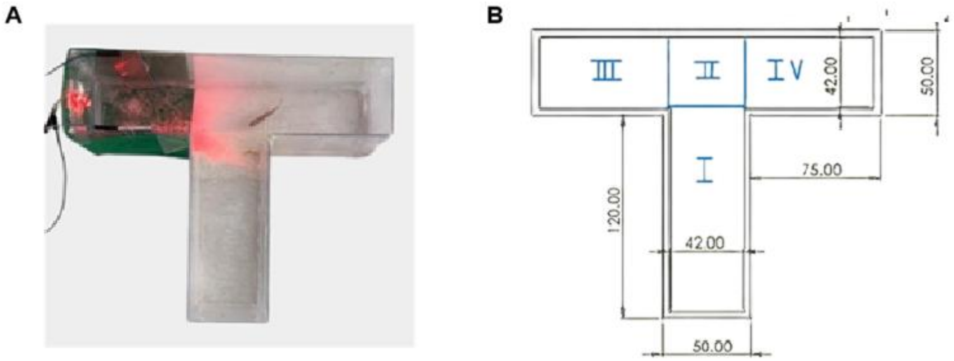

Fig. 1. T-maze apparatus. (A) Photograph of the T-maze. The green cue was alternated between the left and right arms across different fish. The maze is equipped with an electric shock circuit to deliver shocks (not shown). (B) Schematic drawing illustrating the maze dimensions. All measurements are in millimeters. The maze is divided into four zones: I: Start, II: Middle, III: Left (designated as wrong arm in this case), and IV: Right (designated as correct arm in this case).

starting arm behind a barrier for one minute. Upon removal of the barrier, the fish was allowed to swim freely toward the lateral arms. Whenever the fish entered the arm containing the green cue and red LED, it received an electric shock (7–8 V, 0.1–0.2 mA, 80 ms ON–OFF duration) delivered via electrodes embedded in the T-maze. This procedure continued until the fish had received three shocks. On the third day, the procedure from day 2 was repeated, but only in the presence of the CS in one arm. The testing phase lasted two minutes, during which the swimming activity of each fish was recorded. The experimental timeline is depicted in Fig. 2.

Control zebrafish were subjected to the same procedure but were exposed to tank water instead of the drug solution. Only male zebrafish were used in this study to avoid confounding effects of sexual dimorphism [13]. All recordings were captured at 60 frames per second at a resolution of 1280 × 720 pixels using a phone camera (iPhone 6S) positioned 51 cm above the T-maze. Zebrafish behavior was analyzed using SMART V3.0.05 tracking software (Pan Lab, Harvard Apparatus).

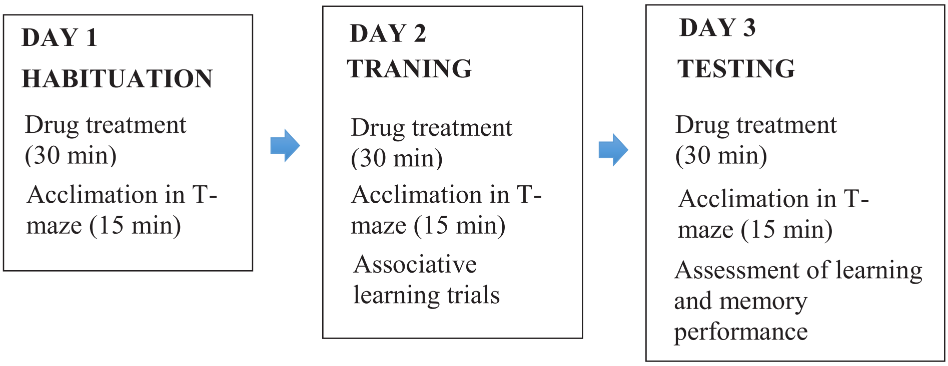

Fig. 2. Experimental timeline showing the procedure followed across three days.

2.5 Statistical Analysis

Data are presented as mean ± SEM. Group differences in categorical outcomes were assessed using the Fisher Exact test followed by post hoc z-tests with Bonferroni correction. Continuous variables were analyzed using one-way ANOVA followed by Tukey's post-hoc test. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS, version 29.0), with $p < 0.05$ considered statistically significant.

3 Results and Discussion

Previous studies have suggested that zebrafish exhibit innate color preferences, particularly toward green cues [15-17]. To validate this, we conducted control experiments in a T-maze under four conditions: (i) both arms transparent, (ii) both arms with green cues, (iii) green cue on the left arm, and (iv) green cue on the right arm. Our results demonstrated a strong innate preference for green, with 90% of zebrafish entering the arm containing the green cue (Fig. 3). Importantly, no left-right bias was observed, indicating that the color cues, rather than spatial orientation, primarily influenced the zebrafish's arm choice in the T-maze. These findings support the use of the green cue to enhance the CS in our avoidance learning assay.

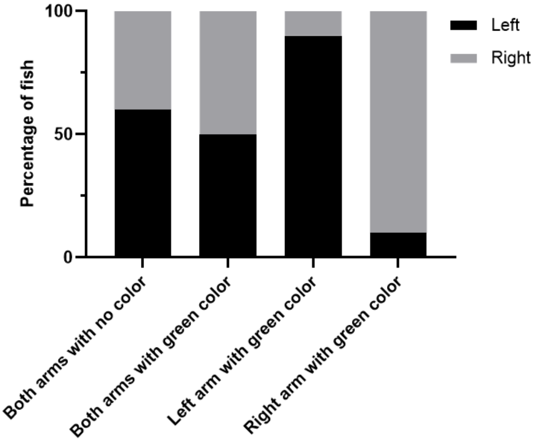

Fig. 3. Assessment of fish's innate preference for green color cue.

Fig. 4 shows the effects of the dopamine receptor antagonists SCH-23390 and SB-27011-A on zebrafish performance in the T-maze. First arm choice differed significantly among groups ($p < 0.001$; Fig. 4A). Post-hoc comparisons revealed that the 1 mg/L and 5 mg/L SCH-23390 groups, as well as the 5 mg/L SB-27011-A group, had a significantly lower proportion of fish with correct first arm entries compared to controls (55.17%, 3.45%, and 50.00% vs. 100.00%, respectively; all $p < 0.05$). Treatment also significantly affected the overall percentage of correct arm entries for both antagonists (F(4,122) = 83.906, $p < 0.001$), with differences restricted to the 5 mg/L groups (SCH-23390: $p < 0.001$; SB-27011-A: $p = 0.007$; Fig. 4B). Similarly, the time zebrafish spent in the wrong arm was significantly influenced by the treatment (F(4,130) = 70.296, $p < 0.001$; Fig. 4C), with the largest increase observed in the 5 mg/L SCH-23390 group ($p < 0.001$) and smaller but significant increases in the 1 mg/L SCH-23390 ($p = 0.005$) and 5 mg/L SB-27011-A groups ($p < 0.001$).

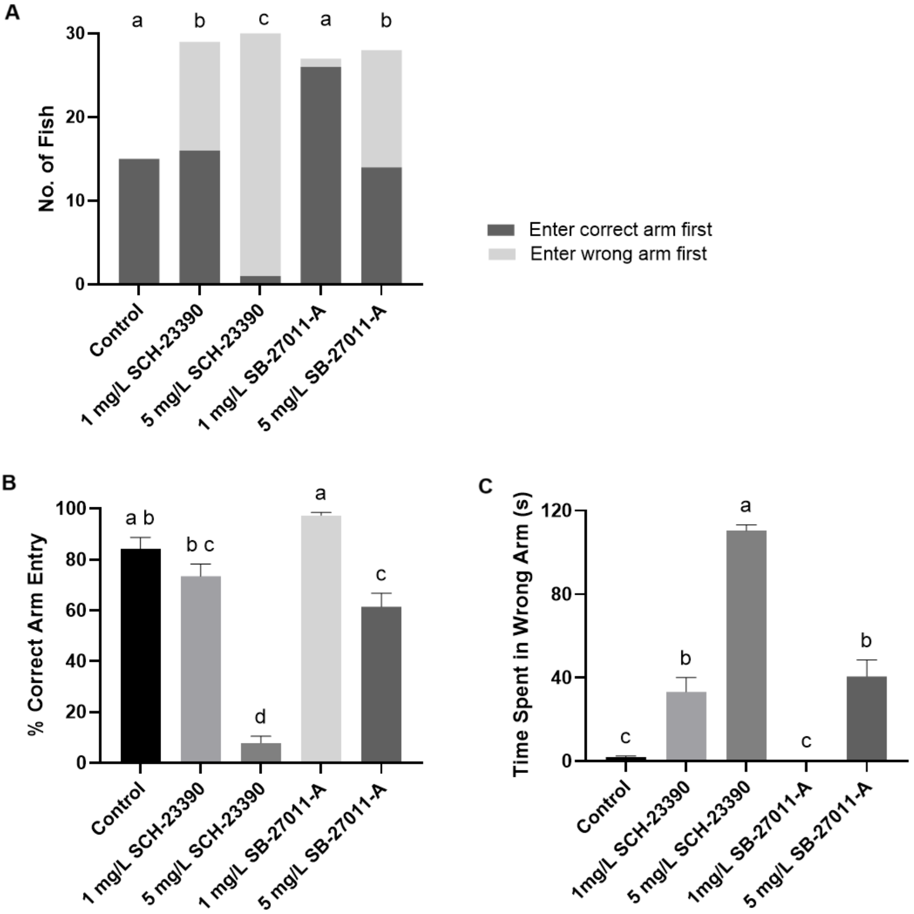

Fig. 4. Effect of SCH-23390 and SB-27011-A on learning and memory in zebrafish in the T-maze. (A) First arm choice, (B) Percentage of correct arm entry, and (C) Time spent in wrong arm. Data are expressed as mean ± SEM (n=15 for control and n = 30 for each treatment group). Different letters indicate statistically significant differences ($p < 0.05$).

The dopaminergic system is a key modulator of locomotor activity [18]. To determine whether the observed changes were attributable to locomotor impairment, we analysed total distance travelled and mean swimming speed. Treatment significantly affected both distance (F(4,130) = 21.199, $p < 0.001$; Fig. 5A) and speed (F(4,130) = 22.874, $p < 0.001$; Fig. 5B). Post-hoc analyses revealed significant reductions in the 5 mg/L groups for both measures (all $p < 0.001$), whereas the 1 mg/L groups did not differ from controls. These findings strongly suggest that the cognitive effects of dopaminergic antagonism are most reliably assessed at lower doses (e.g. 1 mg/L), where locomotion remains unaffected, whereas higher doses confound interpretation due to motor deficits.

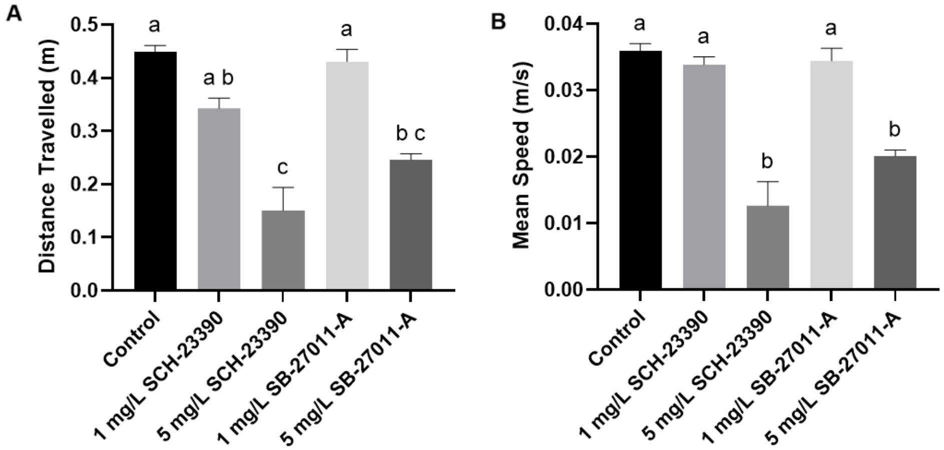

Fig. 5. Locomotor activity of zebrafish. (A) Distance travelled and (B) Mean speed. Data are expressed as mean ± SEM (n=15 for control and n = 30 for each treatment group). Different letters indicate statistically significant differences ($p < 0.05$).

The observed impairment in memory and learning performance aligns with findings in rodents, where D1 receptor antagonism has been shown to disrupt memory retention and punishment-related associative learning [19]. However, this contrasts with the previous zebrafish study using SCH-23390 at 1 mg/L, which reported enhanced learning and memory in reward-based paradigms [20]. In another study on working memory, Cleal et al. [21] reported significant deficits in zebrafish administered with 1.5 mg/L SCH-23390. These varying outcomes across paradigms suggest that the effects of dopamine antagonism may be task-dependent. Given that dopaminergic neurons are widely distributed throughout the zebrafish brain, it is plausible that distinct dopaminergic pathways or subpopulations are differentially recruited depending on the learning paradigm, as has been demonstrated in mammals [22, 23].

Several studies have examined the involvement of D3 receptors in zebrafish learning and memory, but often in the context of broader dopaminergic modulation rather than D3-specific effects. Naderi et al. [20] showed that activation of D2/D3 receptors with quinpirole enhanced memory acquisition, but blockade with eticlopride impaired learning. More recently, Nabinger et al. [18] found that quinpirole impaired avoidance learning in a dose-dependent manner. In our study, SB-27011-A, a selective D3 antagonist did not significantly affect performance at 1 mg/L, suggesting that D3 receptor antagonism has a limited role in avoidance learning. This may be due to the relatively low expression of D3 receptors in brain regions critical for memory encoding and synaptic plasticity, but also to their more specific involvement in emotional and motivational processes rather than general memory functions [24]. Further studies that directly target D3 receptors will be essential for clarifying their role across different learning paradigms and cognitive domains.

Siew-Ying Mok advised the experiment design, analyzed the data, and wrote the manuscript. Pek-Yee Tang advised the experiment design, analyzed the data, and reviewed the manuscript. Zhai-Yong Wong and Hong-Yao Lai designed the experiment, conducted the experiment, collected data, and analyzed the data.

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