Bisphenol A does not affect memory performance in adult male rats

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Abstract

Bisphenol A (BPA) is an estrogenic endocrine disruptor used for producing polycarbonate plastics and epoxy resins. This study investigated the effects of oral BPA administration on memory performance, general activity, and emotionality in adult male Sprague Dawley rats using a battery of behavioral tests, including an appetite-motivated maze test (MAZE test) used to assess spatial memory performance. In addition, in order to confirm the effects of BPA on spatial memory performance, we examined whether intra-hippocampal injection of BPA affects spatial memory consolidation. In the MAZE test, although oral BPA administration at 10 mg/kg significantly altered the number of entries into the incorrect area compared to those of vehicle-treated rats, male rats given BPA through either oral administration or intra-hippocampal injection failed to show significant differences in latencies to reach the reward. Also, oral BPA administration did not affect fear-motivated memory performance in the step-through passive avoidance test. Oral BPA administration at 0.05 mg/kg, the lowest dose used in this study, was correlated with a decrease in locomotor activity in the open-field test, whereas oral administration at 10 mg/kg, the highest dose used in this study, was correlated with a light anxiolytic effect in the elevated plus-maze test. The present study suggests that BPA in adulthood has little effect on spatial memory performance in male rats.

Keywords: Bisphenol A, Endocrine disruptors, Oral administration, Memory, Hippocampus

Introduction

Bisphenol A (BPA) is used to manufacture polycarbonate plastics, epoxy resins, dental composite resins, and the linings of food cans. BPA is one of the most common environmental endocrine disruptors with very weak estrogenic activity.

Many studies indicate that perinatal exposure to BPA affects the development and function of the central nervous system (CNS). For example, perinatal exposure to low-dose BPA abolished or inverted behavioral sex differences (Kubo et al. 2003), and induced hyperactivity and attention deficits in rats (Zhou et al. 2011). In addition, perinatal exposure to BPA impaired spatial learning and memory in rats (Poimenova et al. 2010; Gonçalves et al. 2010) and in mice (Xu et al. 2010). In contrast, Stump et al. (2010) observed no neurobehavioral changes, neuropathological changes, and brain morphometric changes in rats perinatally exposed to BPA. Thus, reports concerning the effects of perinatal BPA exposure on the CNS have been inconsistent. However, we recently concluded that perinatal exposure to low-dose of BPA non-monotonically impaired spatial learning and memory in male offspring rats (Kuwahara et al. 2013).

There have been many studies that examined the effects of BPA exposure during development on the CNS; however, there have been only a few studies that examined the effects of BPA when given to adult rats on the CNS. These few studies have revealed that

acute BPA administration did not influence place and visual memory consolidation in OVX rats (Inagaki et al. 2012), but significantly impaired both visual and spatial memory and decreased dendritic spine density in CA1 and the medial prefrontal cortex in adult male rats (Eilam-Stock et al. 2012). In contrast, Xu et al. (2011) reported that acute BPA administration enhanced passive avoidance memory in male rats. Many studies have indicated that estradiol improved cognitive learning and memory in rodents (Packard et al. 1998; Frye et al. 2005; Gresack et al. 2006; Harburger et al. 2007). However, it has not been confirmed whether BPA, which has estrogenic activity, as well as estradiol influences learning and memory in adult male rats. Therefore, the present study examined the effects of oral administration of BPA on memory performance, general activities, and emotionality in adult male Sprague Dawley (SD) rats using a battery of behavioral tests including a series of learning performance tests that we previously used. In addition, in order to confirm the effects of BPA on spatial memory performance, we examined whether BPA microinjections into the dorsal hippocampus, which is the area involved in spatial learning and memory, affect spatial memory consolidation in adult male SD rats.

Materials and Methods

Animals

Five-week-old male SD rats were purchased from Kyudo Corp. (Saga, Japan). The animals were maintained at a controlled temperature (22 ± 2°C) and humidity (55 ± 10%), with a 12:12 h light-dark cycle (lights on from 07:00 to 19:00). Food and water were freely available for 7 days after their arrival. After that, in order to enhance the motivation for rewards used in the MAZE test, they were restricted to 12 g/day food and 33.3 mL/day water. In the microinjection experiment, rats were restricted to 12 g/day food and 33.3 mL/day water except during the periods of stereotaxic surgery and recovery from surgery. Animal care and experimental procedures were performed in accordance with the Guidelines for Animal Experimentation of Nagasaki University, with the approval of the Institutional Animal Care and Use Committee.

Stereotaxic surgery

For the microinjections into the dorsal hippocampus, guide cannulae (0.7 mm diameter) were implanted bilaterally at a point 1 mm above the dorsal hippocampus in 8-week-old rats anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The coordinates determined

from a stereotactic atlas (König et al. 1967) were anterior (A) from the bregma, lateral (L) to the middle and horizontal (H) below the dura, and were as follows: A, –2.6; L, ±1.5; H, 1.9. The microinjection experiment commenced at least 9 days after cannulae implantation.

Drug administration

Rats were treated with oral BPA administrations at 0.05, 1, or 10 mg/kg (Wako Pure Chemical Industries, Ltd., Osaka, Japan), or were treated with vehicle. BPA was dissolved in ethanol and then diluted in corn oil (Sigma-Aldrich Co., St. Louis, MO, USA). The final ethanol concentration was 1% in the BPA 0.05 and 1 mg/kg groups, and 9% in the BPA 10 mg/kg group. The control group for BPA 0.05 and 1 mg/kg groups, or BPA 10 mg/kg group received oral administrations of 1% ethanol/corn oil or 9% ethanol/corn oil, respectively. All drugs were administered in a volume of 1 mL/kg of body weight. Oral administrations were performed under light anesthesia with halothane (Fluothane, Takeda Pharmaceutical Co. Ltd., Tokyo, Japan).

In the microinjection experiment, BPA was injected into the dorsal hippocampus using 11 mm injection cannulae (0.35 mm diameter) that was inserted through the guide cannulae implanted into bilaterally. The microinjections were performed with

microsyringes (Hamilton, USA) connected to the injection cannulae through polyethylene tubes. Two μL of saline, vehicle (40% propylene glycol), or 20 μg/2 μL of BPA dissolved in 40% propylene glycol was injected bilaterally in the dorsal hippocampus over a 2 min period, and the injection cannulae were left in the place for 1 min to allow for adequate solution diffusion. The locations of the cannula tips were confirmed histologically at the termination of the experiments. Rats in which both cannula tips were located outside of the dorsal hippocampus were excluded from the analysis.

In the MAZE test, drugs were administered within 30 min after training or test sessions. In the open-field test and the elevated plus-maze test, drugs were administered the day before the tests were conducted. In the step-through passive avoidance test, drugs were administered within 30 min after the training session.

MAZE test

Spatial learning and memory was observed using the MAZE test. The apparatus consisted of a large compartment (90 × 90 × 50 cm) and a goal compartment (15 × 15 × 50 cm). The maze was constructed by inserting partitions of various sizes (50 × 15 cm, 50 × 30 cm, 50 × 45 cm, 50 × 60 cm) into the large compartment. Two 100 W bulbs located 100 cm above the floor illuminated the apparatus. Visual cues (four different stickers) to

remember the route to the goal compartment were placed on the walls. The rats were habituated to the apparatus and the reward (condensed milk 20 g/100 mL water) at 7 weeks of age.

Four types of MAZE with different levels of difficulty were used (Fig. 1). The route required to reach the goal in each MAZE test was more complicated as the mazes were advanced [MAZE (A) → MAZE (B) → MAZE (C) → MAZE (D)]. In the oral administration experiment, MAZE (A) was performed at 8 weeks of age, MAZE (B) was performed at 10 weeks of age, MAZE (C) was performed at 12 weeks of age, and MAZE (D) was performed at 14 weeks of age. MAZE (D) was used only for the BPA 10 mg/kg group and the vehicle control group (9% ethanol/corn oil). In the microinjection experiment, MAZE (A) was performed at 10 weeks of age, MAZE (B) was performed at 12 weeks of age, and MAZE (C) was performed at 14 weeks of age. In each MAZE test, the rats were first trained and learned the correct approach using an apparatus that only had the correct approach (Training). The rats were subsequently tested for 3 consecutive days from the day after Training (Testing). Each rat was placed gently in the maze and allowed to find the goal and get the reward. The goal of the MAZE test was to reach a dish containing 300 μL of milk located in the goal compartment.

For Training, the rats underwent 3 trials in a single day with a 1 min inter-trial interval. During Training, if the rat did not reach the goal within 180 s, an experimenter guided

the rat to the goal. For Testing, the rats underwent 3 trials every day for 3 days, with a 1 min inter-trial interval. During Testing, if the rat did not reach the goal within 300 s, an experimenter guided the rat to the goal. Time-to-goal was defined as the latency required to reach the goal and start consuming the reward. Error was defined as the number of entries into the incorrect area of the maze.

Open-field test

General behavior and emotionality of the rats was assessed using the open-field test, as described by Hall (Hall et al. 1934). The apparatus consisted of a circular floor 60 cm in diameter enclosed with a 50 cm high wall. The open-field was illuminated by a 100-W bulb placed 80-cm above the center of the floor. The floor was divided into 19 equivalent sectors, which were categorized into 2 regions: an outer ring (0–12 cm from the wall) and an inner ring (12–30 cm from the wall). The total number of sectors crossed by the rat (Ambulation), the number of line crossings inside the inner circle (Inner), and the frequency of rearing (Rearing) were counted for 3 min. Ambulation is a general measure of activity level, while Inner is used as a measure of wariness behavior. Rearing is used to measure exploratory behavior. Behavioral observations were performed 3 times at 2 h intervals. The open-field test was performed at 8 weeks of age.

Elevated plus-maze test

Emotional behavior was observed using the elevated plus-maze test (Walf et al. 2007). A maze constructed from black plastic in the shape of a plus sign (each arm was 50 × 10 cm and the central platform was 14 × 14 cm) was elevated 60 cm. One set of opposing arms was enclosed completely by 60 cm high walls (closed arms), while the other set of opposing arms had no walls (open arms). Entries and time spent in the open and closed arms were measured for 5 min. The time spent in the open arms and the number of entries into the open arms were used as measures of anti-anxiety-like behavior. In this task, higher values indicate lower levels of anxiety. The elevated plus-maze test was performed at 10 weeks of age.

Step-through passive avoidance test

Fear-motivated learning and memory was observed using the step-through passive avoidance test (Komatsu et al. 2008). The apparatus (Shintecno Co. Ltd., Fukuoka) consisted of a small illuminated platform (10 × 20 × 12 cm) and a larger dark chamber (30 × 30 × 30 cm) connected with a path (8 × 8 cm). On Day 1, each rat was habituated to

the apparatus. On Day 2, each rat was given an electric shock (1 mA) for 5 s through the grid floor when it entered the dark chamber. On Day 3, each rat was again gently placed on the platform, and the latency to enter the dark chamber was measured. The upper time limit for entry was set at 300 s. The step-through passive avoidance test was performed at 13 weeks of age.

Statistical analyses

All results were expressed as mean ± SEM. Statistically significant differences among 3 groups were assessed using two-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests (StatView, SAS, Cary, NC, USA). Statistically significant differences between 2 groups were assessed using the Student's *t*-test (StatView, SAS, Cary, NC, USA). The threshold for statistical significance was set at $P < 0.05$.

Results

Effect of oral BPA administration on performance in the MAZE test

In the MAZE (A) test, the time-to-goal and Error for the vehicle control rats (1% ethanol/corn oil) increased on Day 2, but decreased on Day 3 (Fig. 2a and c). For both BPA groups, the time-to-goal and Error did not differ appreciably from those of the control group on any of the 3 days. The time-to-goal and Error for the vehicle control rats (9% ethanol/corn oil) decreased slightly on Day 2, but increased on Day 3 (Fig. 2b and d). The 10 mg/kg BPA group showed a longer time-to-goal than the control group on Day 2, but the difference was not significant. Error for the 10 mg/kg BPA group did not differ appreciably from that of the control group on any of the 3 days.

In the MAZE (B) test, the time-to-goal and Error for the vehicle control rats (1% ethanol/corn oil) did not vary appreciably during the 3 days of testing (Fig. 2a and c). For both BPA groups, the time-to-goal and Error did not differ appreciably from those of the control group on any of the 3 days. The time-to-goal for the control group (9% ethanol/corn oil) increased with each consecutive day of testing (Fig. 2b). In contrast, the time-to-goal for the 10 mg/kg BPA group decreased with each consecutive day of testing. Although the 10 mg/kg BPA group showed a longer time-to-goal than the control group on Day 1, the difference was not significant. For the control group and the 10 mg/kg BPA group, Error decreased with each consecutive day of testing (Fig. 2d). For the 10 mg/kg BPA group, Error was significantly higher than that for the control group on Day 2 ($P < 0.05$).

In the MAZE (C) test, the time-to-goal and Error for the vehicle control rats (1% ethanol/corn oil) decreased with each consecutive day of testing (Fig. 2a and c). For both BPA groups, the time-to-goal and Error did not differ appreciably from those of the control group on any of the 3 days. The time-to-goal and Error for the vehicle control rats (9% ethanol/corn oil) did not vary appreciably during the 3 days of testing (Fig. 2b and d). In contrast, the time-to-goal and Error for the 10 mg/kg BPA group decreased with each consecutive day of testing. For the 10 mg/kg BPA group, the time-to-goal was slightly shorter than that for the control group on Days 2 and 3, and Error tended to be lower than that for the control group on Day 3, but these differences were not significant.

In the MAZE (D) test, the time-to-goal and Error for the vehicle control rats (9% ethanol/corn oil) did not vary appreciably during the 3 days of testing (Fig. 2b and d). For the 10 mg/kg BPA group, the time-to-goal was slightly shorter than that for the control group during the test period, but these differences were not significant. Compared to the control group, the 10 mg/kg BPA group showed a significantly lower Error ($P < 0.01$) on Day 3.

Effect of oral BPA administration on performance in the open-field test

For control groups (1% ethanol/corn oil and 9% ethanol/corn oil), Ambulation gradually

decreased over time (Fig. 3a and b). For the 0.05 mg/kg BPA group, Ambulation was lower[I1] than that for the control group during the test period, and the difference was significant at the initial test (0 h, $P < 0.05$). In the test conducted at 2 h, Ambulation decreased in the 1 mg/kg BPA group, but the change was not significant when compared to the Ambulation values for the control group. In the 10 mg/kg BPA group, Ambulation was similar to that of the control group during the test period, and there was no significant difference when compared to the control group.

For the control rats (1% ethanol/corn oil), Rearing and Inner did not change during the test period (Fig. 3c and e). For the 0.05 mg/kg BPA group, Rearing and Inner were lower than those for the control group during the test period, but there were no significant differences when compared to the control group. In the 1 mg/kg BPA group, Rearing and Inner did not change during the test period, and the Rearing and Inner values for this group were not significantly different from those for the control group. For the control rats (9% ethanol/corn oil), Rearing gradually increased over time (Fig. 3d). In contrast, Rearing gradually decreased over time for the 10 mg/kg BPA group, and compared to the control group, tended to be higher in the initial test (0 h) and lower in the test conducted at 4 h, but these differences were not significant. For the control rats (9% ethanol/corn oil), Inner gradually decreased over time (Fig. 3f). For the 10 mg/kg BPA group, Inner tended to be higher, but this was not significantly different from that of the control

group.

Effect of oral BPA administration on performance in the elevated plus-maze test

Oral BPA administration at 0.05 or 1 mg/kg did not significantly alter any parameter tested with the elevated plus-maze test (Fig. 4a and c).

The number of open arm entries and the time spent in open arms tended to increase in the 10 mg/kg BPA group compared to the control group (9% ethanol/corn oil), but this was not significant (Fig. 4b and d). The number of closed arm entries significantly increased ($P < 0.05$) and the time spent in the closed arms significantly decreased ($P < 0.05$) in the 10 mg/kg BPA group compared to the control group.

Effect of oral BPA administration on performance in the step-through passive avoidance test

Oral BPA administration did not significantly alter the latencies during the training session for the step-through passive avoidance test (Fig. 5a and b). Compared to the control groups, the 0.05 and 10 mg/kg BPA groups showed slightly longer latencies; however, oral BPA administration had no significant effect on retention (24 h after foot

shock).

Effect of BPA microinjection on performance in the MAZE test

In the MAZE (A) test, the time-to-goal for saline group and vehicle group decreased with each consecutive day of testing (Fig. 6a). In contrast, the time-to-goal for the BPA group increased with each consecutive day of testing. However, there were no significant differences among the groups. For all 3 groups, Error did not vary appreciably during the 3 days of testing, and Error for the BPA group did not differ appreciably from those for other groups on any of the 3 days (Fig. 6b).

In the MAZE (B) test, the time-to-goal for the saline group was stable during the 3 days of testing (Fig. 6a). For the vehicle group, the time-to-goal was similar to that for the saline group on Days 1 and 3, whereas the BPA group showed a slightly shorter time-to-goal than other groups on Days 1 and 3. However, there were no significant differences among the groups. For the saline and vehicle groups, Error decreased with each consecutive day of testing (Fig. 6b). For the BPA group, Error was lower than those for the other groups on Day 1, but the difference was not significant.

In the MAZE (C) test, the time-to-goal and Error for the saline group and vehicle group decreased with each consecutive day of testing (Fig. 6a and b). The time-to-goal for the

BPA group did not vary appreciably during the 3 days of testing, but there were no significant differences among the groups. Error for BPA group was slightly lower than those of other groups on Day 2, but did not differ significantly from other groups on any of the 3 days.

Discussion

In the present study, we investigated whether oral BPA administration affects memory performance, general activities, and emotionality in adult male SD rats. We administered BPA orally in this study because the primary route of environmental BPA exposure is through dietary intake. There are reports that a single oral administration of BPA at 200 mg/kg induced the maximum BPA concentration in the rat brain (19.70 ppb) within 60 min of administration and the peak BPA concentration in rat plasma (1.6 ppm) at 30 min after administration (Sun et al. 2002), and a nanomolar dose of BPA affected spinogenesis in adult hippocampal neurons (Tanabe et al. 2012). Therefore, it is likely that BPA crosses the blood-brain barrier and affects the CNS.

At first, we evaluated the behavioral effects of BPA using doses of 0.05 mg/kg (the established value of tolerable daily intake [TDI] for BPA in humans), which is the dosage

that we previously found the significant effects of perinatal BPA exposure on spatial learning and memory (Kuwahara et al. 2013), and 1 mg/kg (20 times higher than the TDI). Although perinatal exposure to low-dose BPA impaired the spatial learning and memory in male offspring rats, oral BPA administration at 0.05 and 1 mg/kg did not affect the spatial memory consolidation in adult male rats (Fig. 4a and c). These results suggest that BPA exposure during fetal and neonatal stages affects the development of brain and the sexual differentiation of brain in male rats, however, BPA exposure in adulthood does not affect spatial memory performance in mature male rats. Therefore, the fetal and neonatal stages might be more vulnerable to BPA neurotoxicity than later stages.

To confirm this result, we furthermore evaluated the behavioral effects of BPA using a higher dose of 10 mg/kg (200 times higher than the TDI). In this case, to investigate more precisely, we used the MAZE (D) test as an additional MAZE test following the MAZE (A), (B) and (C) tests. The 10 mg/kg BPA group tended to have a longer time-to-goal than the control group in the MAZE (A) and (B) tests (Fig. 2b), and had a significantly higher Error than the control group in the MAZE (B) test ($P < 0.05$; Fig. 2d). In contrast, the 10 mg/kg BPA group had a slightly shorter time-to-goal than the control group in the MAZE (C) and (D) tests (Fig. 2b), and showed a significantly lower Error than the control group in the MAZE (D) test ($P < 0.01$; Fig. 2d). These results might suggest that oral

administration of 10 mg/kg of BPA slightly impaired and then slightly enhanced spatial memory consolidation in early and late test phases, respectively, in the MAZE test. However, the primary measure of spatial memory performance in the MAZE test is the time-to-goal, and we found no significant differences in this variable. Thus, we consider oral BPA administration had little effect on spatial memory consolidation. In contrast, Jain et al. (2011) reported that chronic exposure to BPA at 2 and 20 μg/kg for 28 days during adulthood results in impaired performance on the Morris water maze (MWM) test in adult male rats. The discrepancy between that report and our results might be attributed to differences in the behavioral objectives and lengths of treatment; performance in the MWM test is motivated by escaping from the water, while in the MAZE test, performance is motivated by obtaining a reward. Hikida et al. (2010) reported that different dopaminergic neural pathways are involved in reward and aversive learning. In addition, although Jain et al. (2011) investigated the effects of chronic exposure to low BPA doses, we examined the effects of short-term BPA exposure with a wide range of doses.

In the open-field test, Ambulation for the 0.05 mg/kg BPA group was significantly lower than that of the control group at the initial test (0 h; $P < 0.05$; Fig. 3a). In contrast, the 1 and 10 mg/kg BPA groups showed no significant effects on Ambulation and Rearing (Fig. 3a–d). These results suggest that although oral BPA administration at 0.05 mg/kg, which

is the lowest dose of BPA we used in this study, decreased locomotor activity, oral BPA administrations at1 and 10 mg/kg did not affect general activities.

In the elevated plus-maze test, the time spent in the open arms and the number of entries into the open arms were used as measures of anti-anxiety-like behavior. In this task, higher values indicated lower levels of anxiety. Although there were no significant differences, we observed slight increase in the number of open arm entries for the 1 and 10 mg/kg BPA groups (Fig. 4a and b), and the time spent in the open arms for the 10 mg/kg BPA group (Fig. 4d). Furthermore, the 10 mg/kg BPA group showed significant increases in the number of closed arm entries and decreases on the time spent in closed arms ($P < 0.05$; Fig. 4b and d). Therefore, these results suggest that oral BPA administrations at low doses, such as 0.05 and 1 mg/kg, do not affect emotionality, but a higher BPA dose (10 mg/kg) has a light anxiolytic effect.

The step-through passive avoidance test measures learning and memory in rats, and is based on their natural aversion to well-lit places. Though the 0.05 and 10 mg/kg BPA groups had longer latencies in the retention trial, there were no significant differences compared to the control group (Fig. 5). These results suggest that oral BPA administration does not affect fear-motivated memory performance in adult male rats. However, Jain et al. (2011) reported that chronic exposure to BPA 2 and 20 μg/kg for 28 days during adulthood impairs performance on the passive avoidance tasks in adult male

rats. On the other hand, Xu et al. (2011) showed that acute exposure to BPA enhanced the passive avoidance memory of male rats, which is similar to the effects of estradiol benzoate. Although Xu et al. (2011) investigated the effects of BPA on short-term passive avoidance memory 60 min after training in young male rats on postnatal day 18; we evaluated the effects of BPA on long-term passive avoidance memory 24 h after training in 13-week-old adult male rats. These differences might explain the discrepancy between that report and our results. Thus, the reports concerning the effects of BPA on fear-motivated learning and memory have been inconsistent. Further investigation is needed to clarify the effects of BPA exposure in adulthood on fear-motivated learning and memory.

It was proposed that hippocampal formation is very important for processing certain aspects of spatial learning and memory (Morris et al. 1990). Several studies demonstrated that intra-hippocampal infusion of estradiol enhanced object recognition and place learning in female mice (Fernandez et al. 2008) and rats (Zurkovsky et al. 2007), respectively. In addition, previous *in vitro* studies have shown that BPA significantly enhanced long-term depression in both CA1 and CA3, and increased spine density in CA1 of the hippocampus as well as estradiol (Ogiue-Ikeda et al. 2008), and spinogenesis was significantly enhanced by BPA within 2 h in adult hippocampal neurons (Tanabe et al. 2012). In contrast, Eilam-Stock et al. (2012) reported that acute

BPA administration significantly decreased dendritic spine density on CA1 pyramidal cells. These reports suggest the hippocampal formation is a primary target for BPA in case BPA affects spatial learning and memory. Thus, in order to confirm the effects of BPA on spatial memory performance, we investigated the effects of microinjection of BPA directly into the dorsal hippocampus in adult male rats. We dissolved BPA in 40% propylene glycol and the concentration of the propylene glycol in the vehicle was high. Therefore, the saline group was established to eliminate the effect of the vehicle on spatial memory performance. In the MAZE test, no significant differences were observed among groups that received intra-hippocampal injections (Fig. 6). These results suggest that intra-hippocampal injection of BPA, as well as oral administration of BPA, does not affect spatial memory consolidation in adult male rats.

In conclusion, oral administration of a lower dose of BPA like 0.05 mg/kg decreased the locomotor activity and a higher dose of BPA like 10 mg/kg had a light anxiolytic effect, respectively, in adult male rats. Our results also suggest that BPA treatment in adulthood had little effect on spatial memory performance in male rats. This is different from the effect of perinatal BPA exposure, which impaired spatial learning and memory in male rats. Therefore, our study emphasizes the need to pay attention to BPA exposure during fetal and neonatal stages rather than during adulthood.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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Figure legends

Fig. 1 MAZE apparatus. White and gray segments represent the correct approach and error areas, respectively. Four different marks placed on the walls show visual cues to remember the route to the goal compartment. These types of MAZE with different levels of difficulty [MAZE (A) → MAZE (B) → MAZE (C) → MAZE (D)] were used to assess spatial memory performance in rats

Fig. 2 The effects of oral Bisphenol A (BPA) administration on time-to-goal (a, b), defined as the latency required to reach the goal and start eating the reward, and Error (c, d), defined as the number of times the rats entered the error area, in the MAZE test in 8-, 10-, 12-, and 14-week-old male rats. MAZE test (D) was applied only to the 10 mg/kg BPA group and the vehicle control. They were treated with oral BPA administrations (0.05, 1, or 10 mg/kg) or each vehicle (1% ethanol/corn oil or 9% ethanol/corn oil) within 30 min after training or test sessions. The results are expressed as mean ± SEM. Controls, 0.05 mg/kg BPA group, and 10 mg/kg BPA group (n = 8 for each group); 1 mg/kg BPA group (n = 9). $*P < 0.05$, and $**P < 0.01$ indicate significant differences from control rats

Fig. 3 The effects of oral BPA administration on Ambulation (the total number of crossings) (a, b), Rearing (frequency of rearing) (c, d), and Inner (the number of crossings inside the inner circle) (e, f) during the open-field test in 8-week-old male rats. They were treated with oral administrations of BPA (0.05, 1, or 10 mg/kg) or each vehicle (1% ethanol/corn oil or 9% ethanol/corn oil) the day before the test was conducted. The results are expressed as mean ± SEM. Controls, 0.05 mg/kg BPA group, and 10 mg/kg BPA group (n = 8 for each group); 1 mg/kg BPA group (n = 9). $*P < 0.05$ indicates a significant difference from control rats

Fig. 4 The effects of oral BPA administration on the number of entries to an arm (a, b) and the time spent in each arm (c, d) during the elevated plus-maze test in 10-week-old male rats. They were treated with oral administrations of BPA (0.05, 1, or 10 mg/kg) or each vehicle (1% ethanol/corn oil or 9% ethanol/corn oil) the day before the test was conducted. The results are expressed as mean ± SEM. Controls, 0.05 mg/kg BPA group, and 10 mg/kg BPA group (n = 8 for each group); 1 mg/kg BPA group (n = 9). $*P < 0.05$ indicates a significant difference from control rats

Fig. 5 The effects of oral BPA administration on the latency to enter the dark chamber in the step-through passive avoidance test in 13-week-old male rats. They were treated

with oral administrations of BPA (0.05, 1, or 10 mg/kg) or each vehicle (1% ethanol/corn oil or 9% ethanol/corn oil) within 30 min after the training session. The results are expressed as mean ± SEM. Controls, 0.05 mg/kg BPA group, and 10 mg/kg BPA group (n = 8 for each group); 1 mg/kg BPA group (n = 9)

Fig. 6 The effects of microinjection of BPA on time-to-goal (a), defined as the latency required to reach the goal and start eating the reward, and Error (b), defined as the number of times the rats entered the error area, in the MAZE test in 10-, 12-, and 14-week-old male rats. They received microinjections bilaterally into the dorsal hippocampus of BPA (20 μg/2 μL/side), saline, or vehicle within 30 min after training or test sessions. The results are expressed as mean ± SEM. Saline group: n = 8 in the MAZE (A) and (B) tests, and n = 4 in the MAZE (C) test; Vehicle group: n = 6 each MAZE test; BPA group: n = 10 in the MAZE (A) test, n = 9 in the MAZE (B) test and n = 8 in the MAZE (C) test

Fig. 1

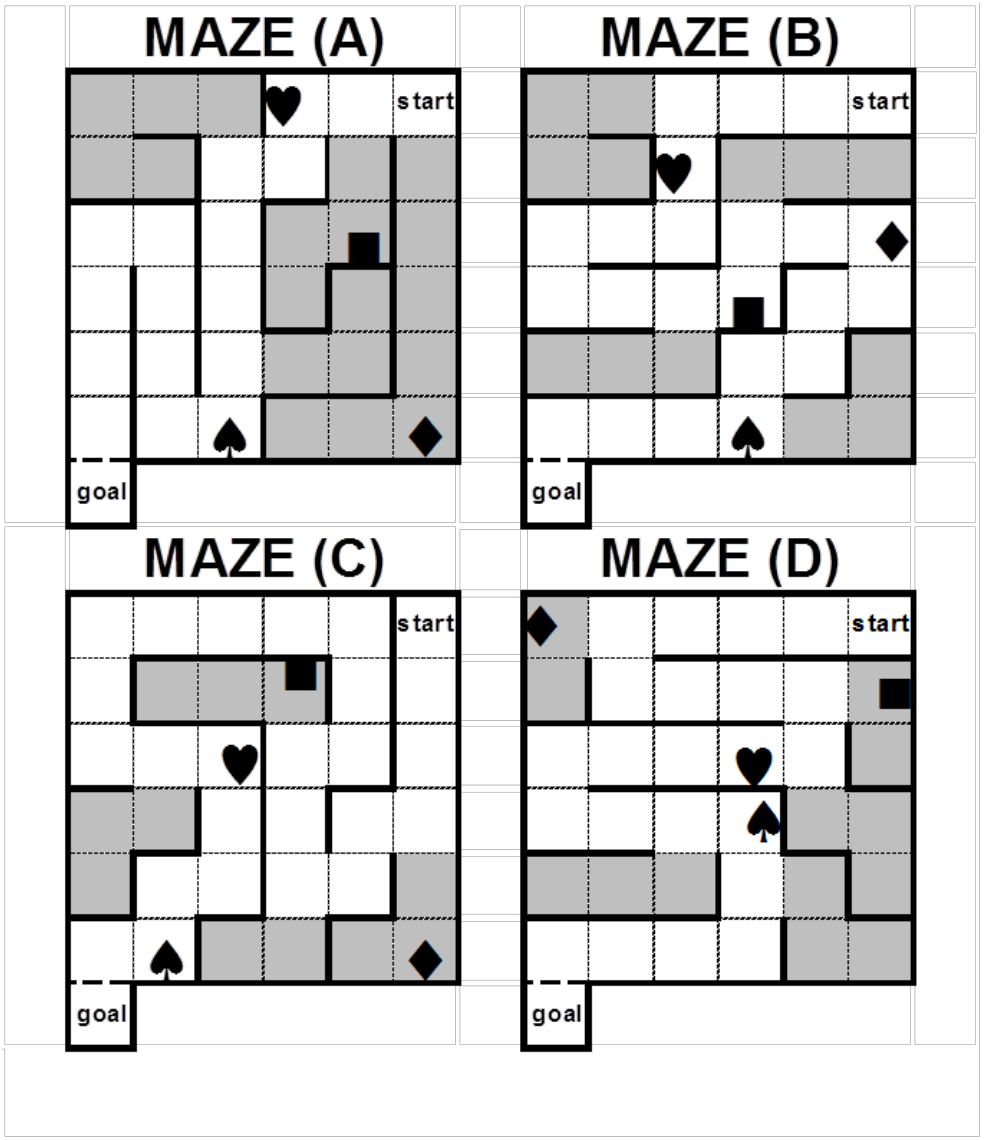

MAZE (A)
start
goal
MAZE (B)
start
goal
MAZE (C)
start
goal
MAZE (D)
start
goal

Fig. 2

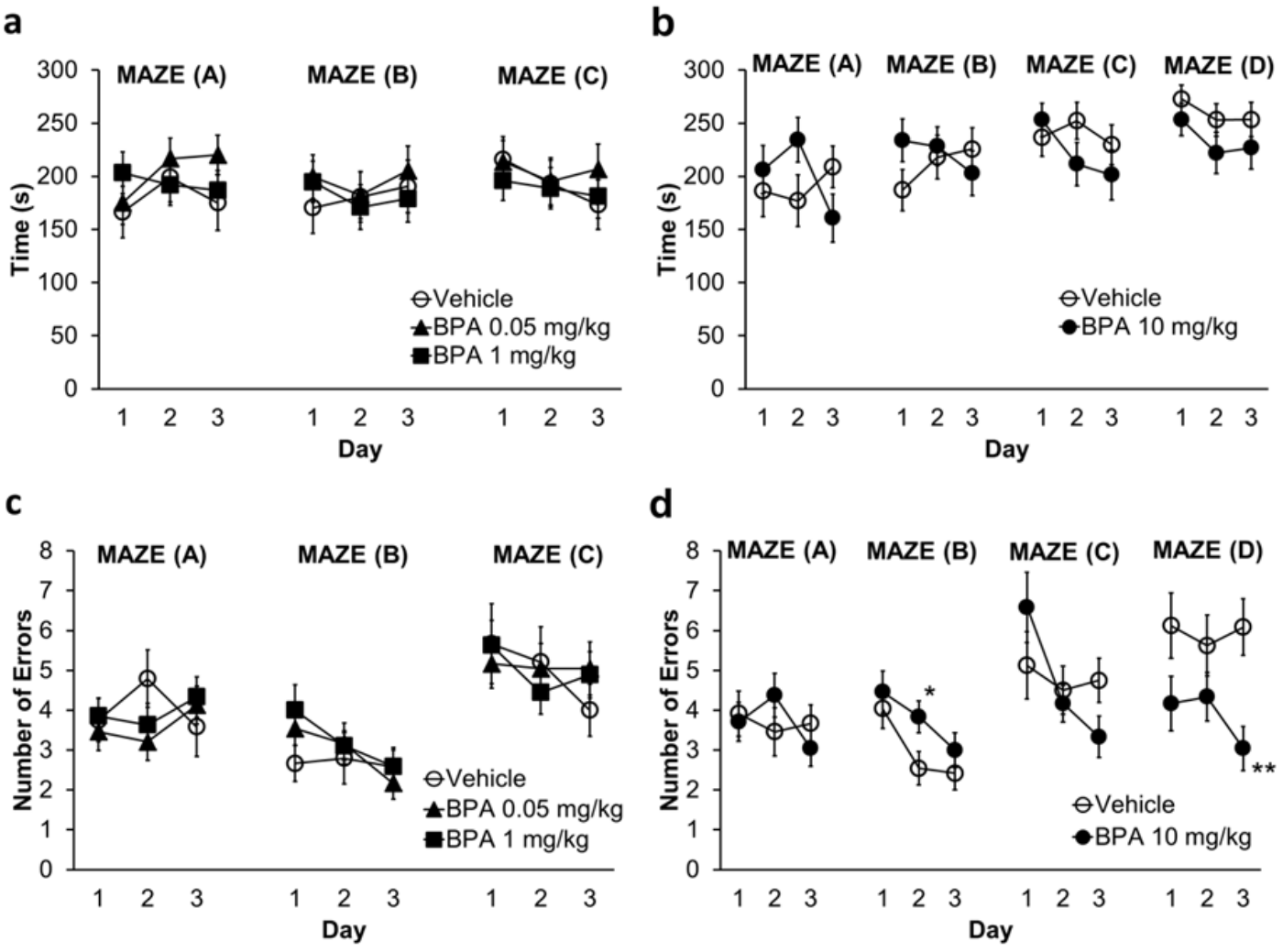

a
MAZE (A)
MAZE (B)
MAZE (C)
Time (s)
Day
Vehicle
BPA 0.05 mg/kg
BPA 1 mg/kg
b
MAZE (A)
MAZE (B)
MAZE (C)
MAZE (D)
Time (s)
Day
Vehicle
BPA 10 mg/kg
c
MAZE (A)
MAZE (B)
MAZE (C)
Number of Errors
Day
Vehicle
BPA 0.05 mg/kg
BPA 1 mg/kg
d
MAZE (A)
MAZE (B)
MAZE (C)
MAZE (D)
Number of Errors
Day
Vehicle
BPA 10 mg/kg
*
**

Fig. 3

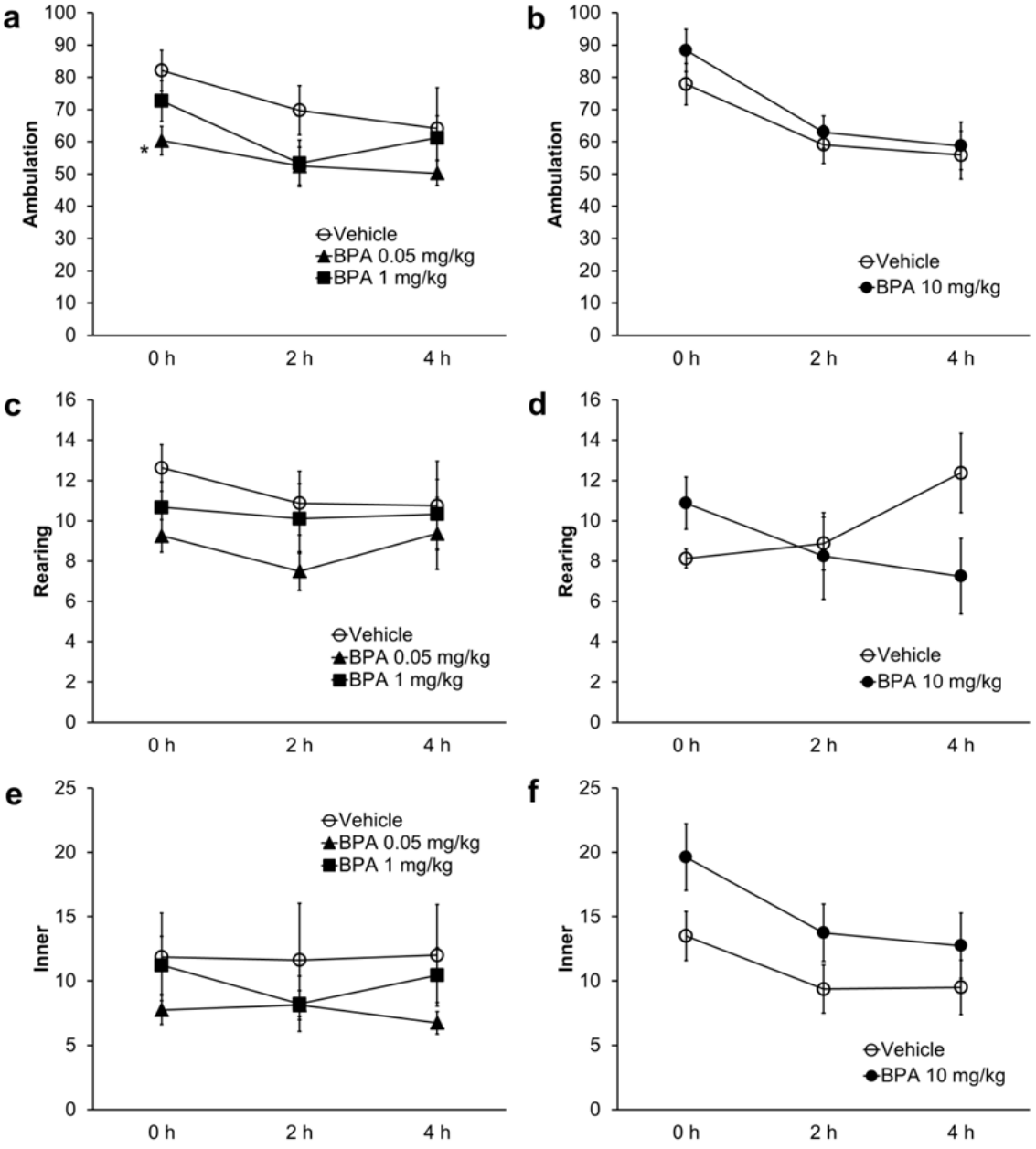

a
Ambulation
100
90
80
70
60
50
40
30
20
10
0
*
Vehicle
BPA 0.05 mg/kg
BPA 1 mg/kg
0 h
2 h
4 h
b
Ambulation
Vehicle
BPA 10 mg/kg
c
Rearing
16
14
12
10
8
6
4
2
d
Rearing
e
Inner
25
20
15
5
f
Inner

Fig. 4

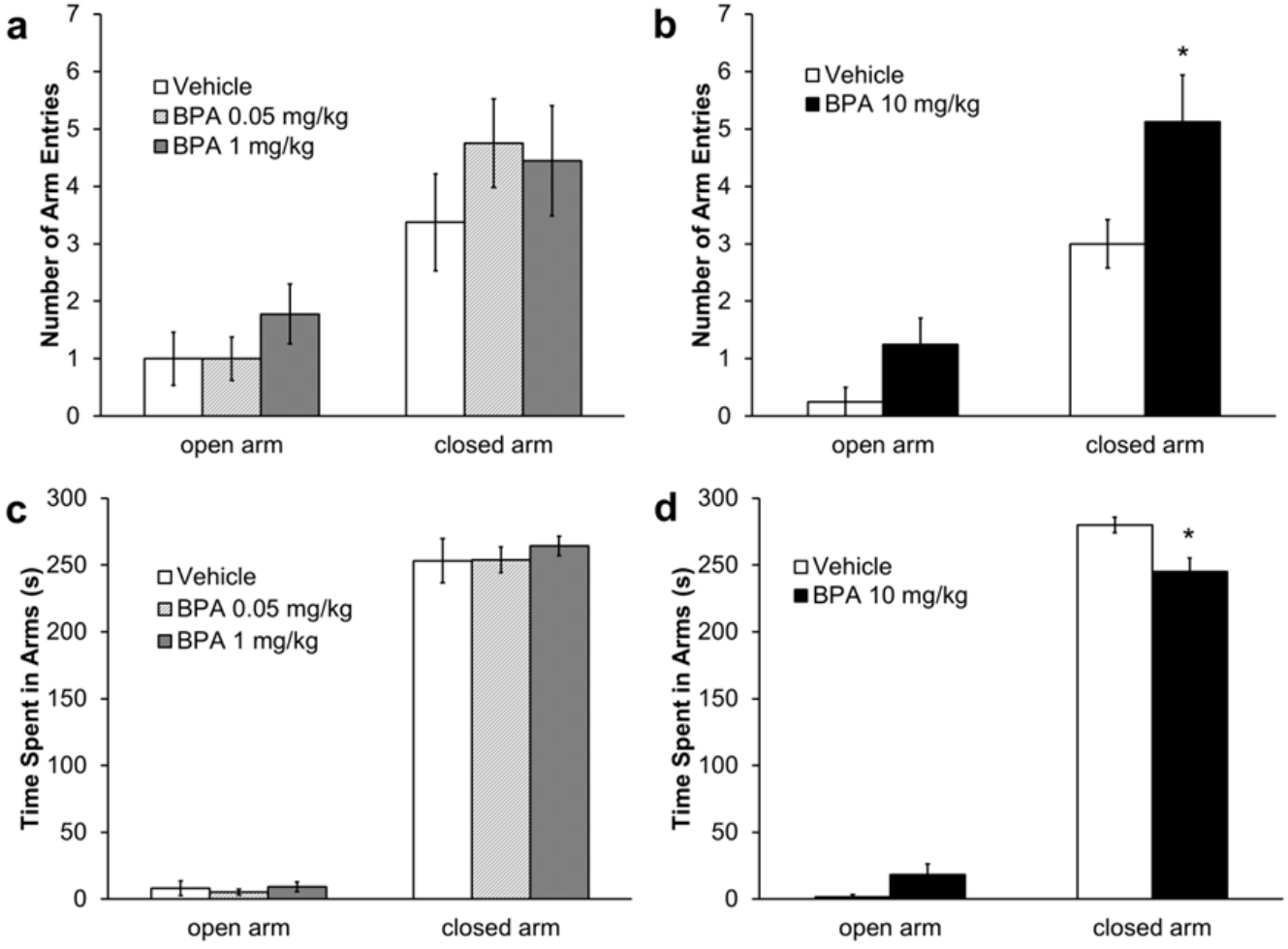

a
Number of Arm Entries
Vehicle
BPA 0.05 mg/kg
BPA 1 mg/kg
open arm
closed arm
b
Number of Arm Entries
Vehicle
BPA 10 mg/kg
*
open arm
closed arm
c
Time Spent in Arms (s)
Vehicle
BPA 0.05 mg/kg
BPA 1 mg/kg
open arm
closed arm
d
Time Spent in Arms (s)
Vehicle
BPA 10 mg/kg
*
open arm
closed arm

Fig. 5

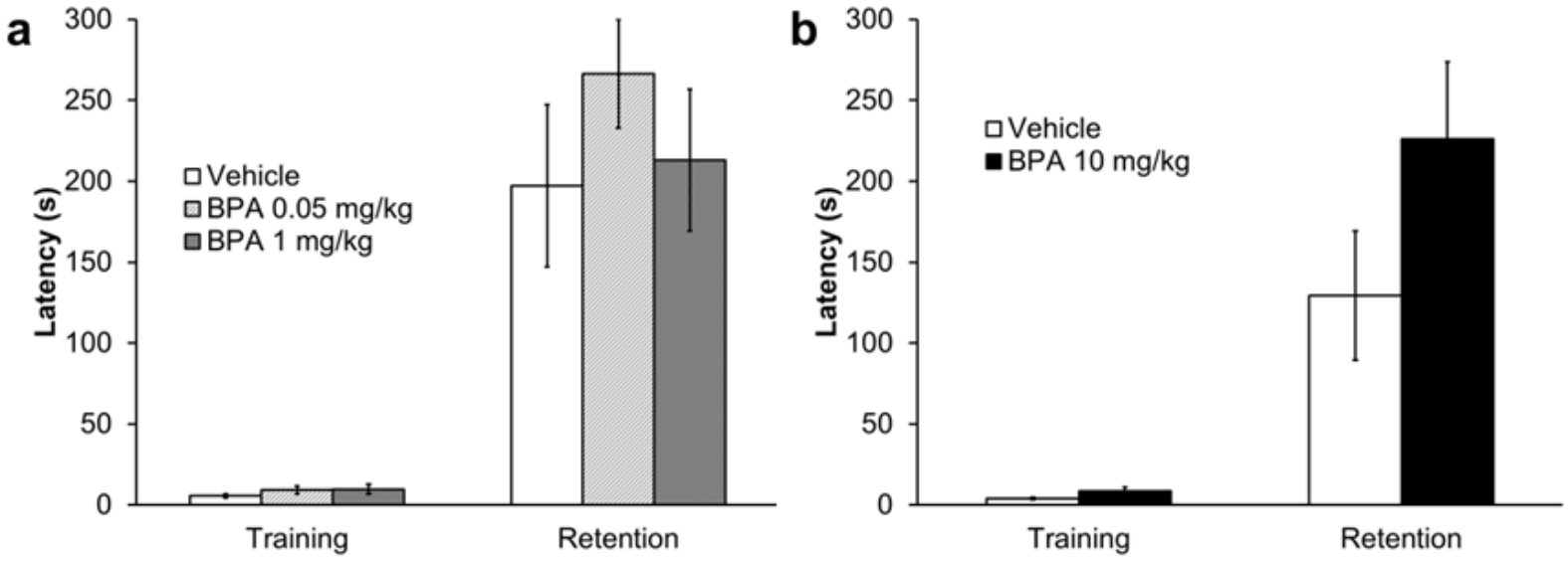
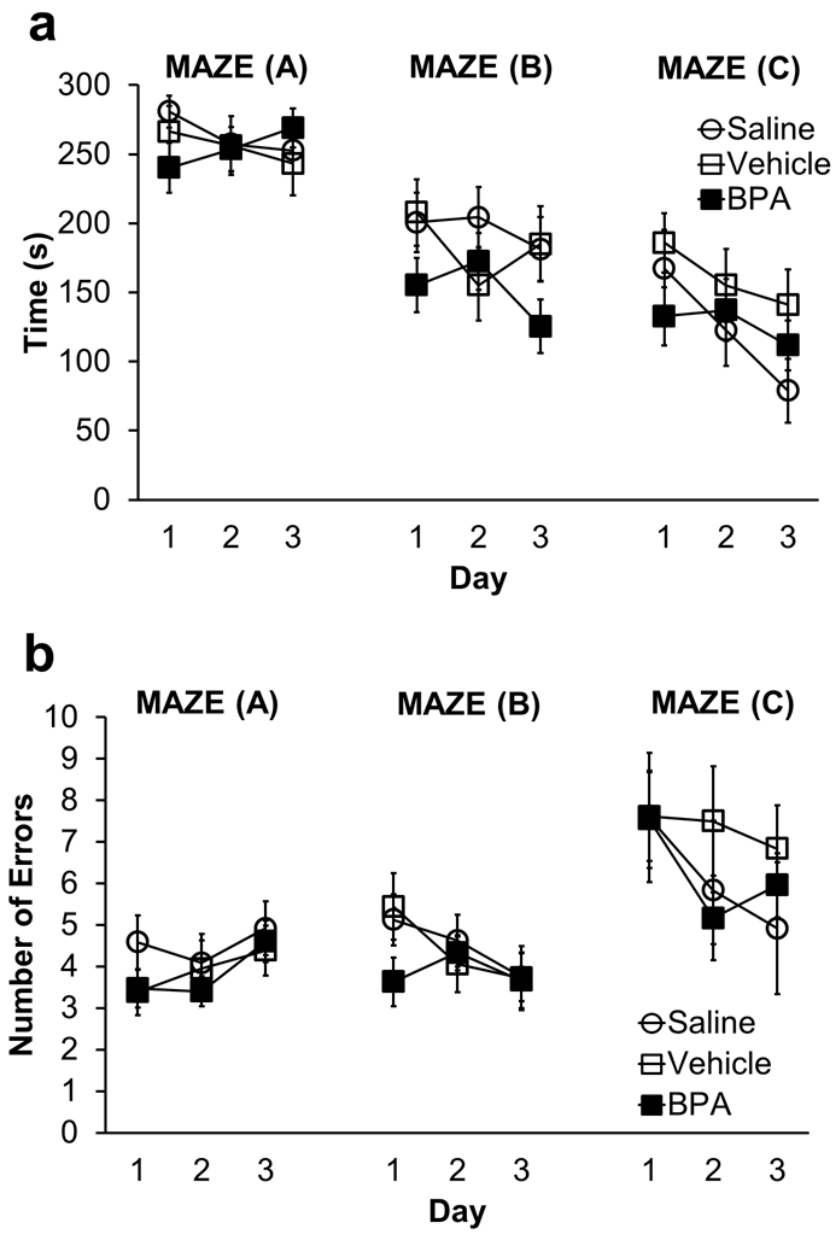

a
300
250
200
150
100
50
0
Latency (s)
Vehicle
BPA 0.05 mg/kg
BPA 1 mg/kg
Training
Retention
b
300
250
200
150
100
50
0
Latency (s)
Vehicle
BPA 10 mg/kg
Training
Retention

Fig. 6


a
MAZE (A)
MAZE (B)
MAZE (C)
Saline
Vehicle
BPA
Time (s)
300
250
200
150
100
50
0
1 2 3 1 2 3 1 2 3
Day
b
MAZE (A)
MAZE (B)
MAZE (C)
Number of Errors
10
9
8
7
6
5
4
3
2
1
0
Saline
Vehicle
BPA
1 2 3 1 2 3 1 2 3
Day