Perinatal exposure to low-dose bisphenol A impairs spatial learning and memory in 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 perinatal BPA exposure on learning and memory, general activity, and emotionality in male Sprague Dawley rats using a battery of behavioral tests, including an appetite-motivated maze test (MAZE test) used to assess spatial learning and memory. Mother rats were orally administered BPA (50 or 500 μg・kg^{-1}/day) or vehicle (1 ml・kg^{-1}/day) from gestational day 10 to postnatal day 14. In the MAZE test, compared to the offspring of vehicle-treated rat mothers, male offspring of mothers exposed to 50 μg・kg^{-1}/day of BPA, but not those of mothers exposed to 500 μg・kg^{-1}/day of BPA, needed significantly more time to reach the reward. Although male offspring of mothers exposed to 50 μg・kg^{-1}/day of BPA showed an increase in a behavioral measure of wariness after repeated testing in the open-field test, no significant effects were observed in locomotor activities. No significant differences were observed in any other behavioral test including the elevated plus-maze test. The present study suggests that perinatal exposure to low-dose BPA specifically and non-monotonically impairs spatial learning and memory in male offspring rats.

Keywords: Bisphenol A, Endocrine disruptors, Perinatal exposure, Learning and memory, Behavior

Introduction

Bisphenol A (BPA) is one of the most common environmental endocrine disruptors with very weak estrogenic activity. BPA is used to manufacture polycarbonate plastics, epoxy resins, dental composite resins, and the linings of food cans. A number of studies have reported the effects of BPA on the reproductive system, and their results are contradictory. For example, perinatal BPA exposure led to a dose-dependent decline in the reproductive capacity of female mice (1) and altered the size of reproductive organs and decreased daily sperm production in male mice (2). In rats, prenatal and perinatal exposure to BPA perturbed mammary gland histoarchitecture (3) and decreased testicular testosterone levels (4), respectively. In contrast, several studies demonstrated that perinatal BPA exposure did not adversely affect the sexual development and reproductive functions in rats (5–7).

Many studies have also reported BPA effects on the development and function of the central nervous system (CNS). For example, perinatal exposure to low-dose BPA abolished and inverted behavioral sex differences and decreased the volume of the locus coeruleus in rats (8). In mice between adolescence and young adulthood, long-term exposure to low-dose BPA abolished sex differences in exploration,

anxiety, and spatial learning and memory (9). In addition, perinatal BPA exposure impaired spatial learning and memory in rats (10) and in mice (11). Rats exposed to BPA perinatally showed not only impaired learning and memory but also decreased exploratory behavior (12). Perinatal BPA exposure also induced hyperactivity and deficits in attention in rats (13). In contrast, Stump et al. (14) reported no neurobehavioral changes induced by BPA. Similarly, they found no neuropathological evidence or evidence of an effect on brain morphometry in rats perinatally exposed to BPA, despite investigating a wide range of BPA doses (including doses used in the above-mentioned studies) and despite the use of Sprague Dawley (SD) rats (previously shown to be sensitive to BPA). Therefore, the effects of perinatal exposure to BPA are still debatable.

The present study examined the effects of perinatal exposure to BPA on learning and memory function, general activities, and emotionality in male SD rats, using a battery of behavioral tests.

Materials and Methods

Animals and treatments

Pregnant SD rats, at gestational day (GD) 6, were purchased from Kyudo Corp. (Saga). The animals were maintained at 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. Rats were treated with oral administrations of BPA (50 or 500 μg·kg^{-1}/day; Wako Pure Chemical Industries, Ltd., Osaka) or vehicle (1 ml·kg^{-1}/day) from GD 10 to postnatal day (PND) 14. Oral administrations were performed under light anesthesia with halothane (Fluothane, Takeda Pharmaceutical Co. Ltd., Tokyo). BPA was dissolved in ethanol and then diluted in corn oil to a final ethanol concentration of 1%.

The pups were counted on PND 1 (the day of birth) and randomly culled to 10 pups on PND 3. Offspring were weaned on PND 20 and housed with a same-sex sibling after 4 weeks of age. On the basis of perinatal exposure, the pups either belonged to the control, 50 μg/kg BPA, or 500 μg/kg BPA group. Twelve male offspring from each group were used in the MAZE test, and the other 12 male offspring from each group were used in the open-field, elevated plus-maze, Morris water maze (MWM), and step-through passive avoidance tests. In order to enhance the motivation for rewards used in the MAZE test, male offspring used in this task

were restricted to 12 g/day food and 33.3 ml/day water from 6 weeks of age. 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.

Open-field test

General behavior and emotionality of the rats was assessed using the open-field test, as described by Hall (15). The apparatus consisted of a circular floor 60 cm in diameter enclosed with a 50 cm high wall. The floor was divided with black lines into 19 equivalent sectors. The open-field was illuminated by a 100 W bulb placed 80 cm above the center of the floor. The open-field was divided 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 7 weeks of age.

Elevated plus-maze test

Emotional behavior was observed using the elevated plus-maze test (16). A maze constructed from black plastic in the shape of a plus sign, was used (each arm was 50 × 10 cm and the central platform was 14 × 14 cm). One set of opposing arms was enclosed completely by 60 cm high walls, while the other set of opposing arms had no walls. The entire maze was elevated 60 cm. Rats were individually tested in the maze for 5 min. At the beginning of the test, each rat was placed in the center of the maze facing an open arm. Entries and time spent in the open and closed arms were measured. 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 7 weeks of age.

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 placed 100 cm above the floor illuminated the apparatus. Four different cues were put 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.

Three types of MAZE with different levels of difficulty were used (Fig. 1). In each MAZE test, the route necessary to reach the goal was more complicated as the mazes were advanced [MAZE (A) → MAZE (B) → MAZE (C)]. MAZE (A) was performed at 8 weeks of age, MAZE (B) was performed at 10 weeks of age, and MAZE (C) was performed at 12 weeks of age. For every 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.

MWM test

Spatial learning and memory was also observed using the MWM test. The apparatus consisted of a blue circular pool (diameter: 120 cm, height: 55 cm) filled with water (depth: 29 cm, temperature: 23 ± 1°C) and a transparent escape platform (diameter: 10 cm). Cues were fixed at specific locations surrounding the maze and were visible to the rats. Prior to water maze testing, the rats were habituated to swimming and were taught to escape from water by climbing onto a platform.

During testing, the escape platform was submerged 2 cm below water surface. The escape platform was positioned at the center of 1 of the 4 quadrants of the pool, and the location of platform was randomly changed each day. The rats underwent 3 trials per day for 3 consecutive days.

From a random start position, which varied each day, each rat was placed in water facing the edge of the pool. For each trial, the rat was allowed 60 s to escape

to the platform. If the rat did not escape within 60 s, an experimenter gently guided the rat to the platform, and it was allowed to remain on the platform for 10 s. Then the rat was removed from the pool and dried with a towel for an inter-trial interval of 50 s. The water maze test was performed at 9 weeks of age.

Step-through passive avoidance test

Learning and memory was also observed using the step-through passive avoidance test (17). The passive avoidance 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). The 2 chambers were separated by a guillotine door.

On Day 1, each rat was gently placed on an illuminated platform and allowed to enter the dark chamber for 90 s (Acclimation). On Day 2, each rat was gently placed on the platform and given an electric shock (1 mA) for 5 s through the grid floor. The shock was delivered when it entered the dark chamber using a shock generator (MSG-001, Toyo Sangyo Co. Ltd., Toyama). Acquisition time was defined as the latency to enter the dark chamber. On Day 3, each rat was again gently placed on the platform, and the retention time, defined as 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 11 weeks of age.

Statistical analyses

All results were expressed as means ± S.E.M. Statistical significance was assessed using two-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests (StatView, SAS, Cary, NC, USA). The threshold for statistical significance was set at $P < 0.05$.

Results

Effect of perinatal BPA exposure on performance in the open-field test

For control rats, Ambulation, Rearing, and Inner gradually decreased over time (Fig. 2). For the 50 μg/kg BPA group, Ambulation did not change during the test period, and the Ambulation values for this group were not significantly different from those for the control group (Fig. 2A). In the 500 μg/kg BPA group, Ambulation tended to be higher than that in the control group during the initial test (0 h), but it

was not significantly different from that of the control group during the later tests (2 h and 4 h). In the test conducted at 2 h, Rearing decreased in the 50 μg/kg BPA group and slightly increased in the 500 μg/kg BPA group (Fig. 2B). However, these changes were not significant when compared to the Rearing values for the control group. For the 50 μg/kg BPA group, Inner gradually increased and was significantly higher than that for the control group in the test conducted at 4 h ($P < 0.05$; Fig. 2C).

Effect of perinatal BPA exposure on performance in the elevated plus-maze test

Although the number of both open and closed arm entries slightly increased in the 500 μg/kg BPA group compared to the control group (Fig. 3), BPA exposure did not significantly alter any parameter tested with the elevated plus-maze test.

Effect of perinatal BPA exposure on performance in the MAZE test

For control rats, the time-to-goal decreased with each consecutive day of testing in MAZE (A), (B), and (C) tests.

In the MAZE (A) test, the 50 μg/kg BPA group had slightly longer times-to-goal than the control group on Day 2 and Day 3 (Fig. 4A). For the 500 μg/kg BPA group,

the time-to-goal did not vary appreciably during the 3 days of testing. For the control group, Error increased on Day 2 and decreased on Day 3 (Fig. 4B). Error for both the BPA groups did not differ appreciably from that for the control group on any of the 3 days (Fig. 4B).

In the MAZE (B) test, both the BPA groups showed slightly longer times-to-goal than the control group on Days 1 and 3; however, these differences were not significant (Fig. 4A). For all 3 groups, Error decreased with each consecutive day of testing (Fig. 4B), while on Days 2 and 3, Error for both the BPA groups was slightly higher than that for the control group (Fig. 4B).

In the MAZE (C) test, the time-to-goal for the 50 μg/kg BPA group was significantly longer than that for the control group on Days 1 ($P < 0.01$) and 3 ($P < 0.05$; Fig. 4A). Compared to the control group, the 500 μg/kg BPA group showed slightly longer time-to-goal on Days 1 and 2, but the difference was not significant (Fig. 4A). For all 3 groups, Error decreased with each consecutive day of testing (Fig. 4B). Compared to the control group, the 50 μg/kg BPA group had higher Error on all 3 days, but the difference was not significant (Fig. 4B). Similarly, on Days 2 and 3, Error for the 500 μg/kg BPA group was higher than that for the control group (Fig. 4B).

Effect of perinatal BPA exposure on performance in the MWM test

In the MWM test, the time taken by the control group to reach the escape platform slightly decreased on Day 2, then slightly increased on Day 3 (Fig. 5). On Day 2, compared to the control group, both the BPA groups took slightly longer time to reach the escape platform, but the differences were not significant (Fig. 5).

Effect of perinatal BPA exposure on performance in the step-through passive avoidance test

During the training session for the step-through passive avoidance test, the 50 μg/kg BPA group showed significantly longer latencies ($P < 0.05$) than the control group (Fig. 6). Compared to the control group, the 50 μg/kg and 500 μg/kg BPA groups showed slightly longer and slightly shorter latencies, respectively; however, despite this, rats perinatally exposed to BPA did not show any significant alteration in retention (24 h after foot shock; Fig. 6).

Discussion

This study investigated the effects of perinatal BPA exposure on learning and memory, general activities, and emotionality in SD male rat offspring. BPA was administered orally to dams from GD 10 to PND 14, which is a critical and sensitive period for brain development in the offspring. In order to evaluate the effects of BPA, doses of 50 μg・kg^{-1}/day, (the established value of tolerable daily intake [TDI] for BPA in humans) and 500 μg・kg^{-1}/day (10 times higher than the TDI) were administered.

Oral administration of BPA was used in this study because the primary route of environmental BPA exposure is through dietary intake. One study reported that BPA administered orally to pregnant rats easily passed through the placental barrier and reached the fetus (18). In addition, the aglycone form of BPA was detected in milk from lactating dams treated with daily gavage of BPA (19). Doerge et al. (20) demonstrated that, despite a higher concentration of aglycone BPA in the brain than in either the liver or serum of fetuses following a single intravenous administration to dams, a single oral administration of the same dose did not produce measurable levels of aglycone BPA in the fetal brain. However, in the current study, BPA would be likely transferred into the brains of offspring due to

chronic BPA administration to mother rats throughout the gestation period and into the lactation period.

The present study demonstrates that perinatal exposure to low-dose (50 μg·kg^{-1}/day) BPA impaired spatial learning and memory in male rat offspring (Fig. 4). This impairment of spatial learning and memory was considered specific, because locomotor activities (measured in the open-field test) and emotionality (measured in the elevated plus-maze test) were not affected in these offspring (Figs. 2–3).

Three types of MAZE test with progressive levels of difficulty were used in this study (Fig. 1). The time-to-goal of the control group decreased with each consecutive day, not only within each MAZE test, but also as the MAZE tests progressed. These results indicate that control offspring are capable of learning the task well. In contrast, the 50 μg/kg BPA group did not demonstrate the same pattern of decreased time-to-goal with the progression of the MAZE tests. This low learning performance in the 50 μg/kg BPA group resulted in significant differences in time-to-goal performance on the MAZE (C) test, when compared to the control group (Fig. 4A). These results suggest that in order to evaluate the impairments in spatial learning and memory resulting from low-dose BPA exposure, a series of learning performance tests like the ones used in the present study are needed. In the

open-field test, Ambulation and Inner of the control group decreased with time, likely due to habituation to the novel circumstance. In contrast, the 50 μg/kg BPA group did not show any appreciable change in Ambulation during the test (Fig. 2A). In addition, Inner of the 50 μg/kg BPA group gradually increased and was significantly higher than that of the control group in the test conducted at 4 h ($P < 0.05$; Fig. 2C). This lack of a habituation effect in the 50 μg/kg BPA group may also reflect an impairment of learning performance. Kunz et al. (21) reported that exposure to low-dose BPA (approximately 70 $\mu g \cdot kg^{-1}$/day) during gestation and lactation led to significant changes in the Glu/Asp ratio in the hippocampus, which may reflect impaired mitochondrial functioning and result in neuronal and glial developmental alterations. In addition, a study showed that 10–100 nM BPA significantly enhanced long-term depression in both CA1 and CA3 of the hippocampus (22). It has been proposed that hippocampal formation is very important for processing certain aspects of spatial learning and memory (23). Therefore, it is likely that exposure to low-dose BPA during critical periods of development could affect spatial learning and memory functions in the offspring.

Several lines of evidence have reported that perinatal BPA exposure does not affect learning and memory abilities in rodents (14, 24). The results from the MWM

test in this study also suggest that BPA does not affect spatial learning and memory in male offspring (Fig. 5). In contrast, our MAZE test results indicate that perinatal exposure to low-dose BPA impairs spatial learning and memory in male offspring (Fig. 4A). The discrepancy between the results of these tests might be caused by a difference in behavioral objectives; performance in the MAZE test is motivated by obtaining a reward, while in the MWM test, it is motivated by escaping from the water. Hikida et al. (25) reported that different dopaminergic neural pathways are involved in reward and aversive learning. In addition, we determined reference memory by the MAZE test, while the MWM test used in this study determined working memory. Ros-Simó et al. (26) reported that brain regions involved in these 2 types of memory are different. Spatial reference memory involves the hippocampus, but spatial working memory is more dependent on the prefrontal cortex. Perinatal exposure to BPA has previously been observed to alter synaptic structural modification (27) and the serotonergic system (28) in the hippocampus. Thus, perinatal BPA exposure may specifically affect hippocampus-dependent spatial reference memory.

In the MAZE test, the 500 μg/kg BPA group did not demonstrate any significant changes in the time-to-goal and in Error, compared to the control group (Fig. 4).

These results suggest that the dose-dependent effects of BPA on spatial learning and memory could not be detected in this experiment. Similarly, in the open-field test, Ambulation and Rearing tended to increase for the 500 μg/kg BPA group compared to the control group (Fig. 2), and in the elevated plus-maze test, the number of entries to open arms and closed arms also increased slightly in the 500 μg/kg BPA group (Fig. 3). This increase in activity in the 500 μg/kg BPA group might have masked the impairments in spatial learning and memory. Hormones and agents like environmental endocrine disruptors tend to display non-monotonic dose-response relationships like those indicated by U-shaped or inverted U-shaped curves (29–33). Previous studies have shown that lower, but not higher, doses of BPA can advance puberty (34). The current results are consistent with these reports. The non-monotonic dose-response relationship between spatial learning and memory and perinatal BPA exposure might be caused by the difference between low and high doses of BPA in receptor selectivity. It is suggested that at low doses, BPA almost exclusively binds to the estrogen receptor, but at high doses, it can also bind weakly to other hormone receptors such as the androgen receptor and thyroid hormone receptor (35). This property of BPA might contribute to the non-monotonic dose-response relationship between spatial learning and memory and perinatal BPA

exposure observed in our study. For BPA, the 50 mg·kg^{-1}/day dose is the currently accepted lowest adverse effect level and was used to calculate the Environmental Protection Agency Reference Dose of 50 µg·kg^{-1}/day (33). However, impairments were detected in this study after maternal exposure to this low dose. Therefore, these results support the suggestion of Welshons et al. (36) that a new risk assessment for BPA is required.

The step-through passive avoidance test measures learning and memory of rats, based on their natural aversion to well-lit places. A shorter latency before entering the dark chamber in the retention trial suggests impairment of fear-motivated learning and memory. Males exposed to low-dose BPA had significantly longer latencies ($P < 0.05$) than control rats in the acquisition trial (Fig. 6), while latencies in the retention trial were not significantly affected by perinatal BPA exposure (Fig. 6). These results suggest that perinatal exposure to low-dose BPA increases wariness but does not affect fear-motivated learning and memory in male offspring. Gonçalves et al. (12) reported that perinatal exposure to low-dose BPA impaired inhibitory avoidance memory in male rats; however, our results support other reports showing that perinatal BPA exposure does not affect inhibitory avoidance memory in male mice (11) and male rats (37).

In conclusion, perinatal exposure to low-dose BPA, but not to high-dose BPA, impaired spatial learning and memory, without significantly changing the locomotor activity level of male offspring. Our results also suggest a non-monotonic dose-response relationship for spatial learning and memory impairment and perinatal BPA exposure. Further experiments are needed to elucidate the mechanisms underlying BPA-induced impairment in spatial learning and memory in male offspring.

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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. Three types of MAZE with different levels of difficulty [MAZE (A) → MAZE (B) → MAZE (C)] were used to assess spatial learning and memory of rats.

Fig. 2. The effects of perinatal BPA exposure on (A) Ambulation (the total number of crossings), (B) Rearing (frequency of rearing), and (C) Inner (the number of crossings inside the inner circle) during the open-field test in 7-week-old male rat offspring. Mother rats were treated with oral administration of BPA (50 or 500 μg・kg^{-1}/day) or vehicle from GD 10 to PND 14. The results are expressed as mean ± S.E.M. n = 12 per group. *$P < 0.05$ indicates a significant difference from control rats.

Fig. 3. The effects of perinatal BPA exposure on (A) the number of entries to an arm and (B) the time spent in each arm during the elevated plus-maze test in 7-week-old male rat offspring. Mother rats were treated with oral administration of

BPA (50 or 500 μg･kg^{-1}/day) or vehicle from GD 10 to PND 14. The results are expressed as mean ± S.E.M. Controls and 50 μg･kg^{-1}/day BPA group: n = 12 each; 500 μg･kg^{-1}/day BPA group: n = 11.

Fig. 4. The effects of perinatal BPA exposure on (A) Time-to-goal, defined as the latency required to reach the goal and start eating the reward, and (B) Error, defined as the number of times the rats entered the error area, in the MAZE test in 8-, 10-, and 12-week-old male rat offspring. Mother rats were treated with oral administration of BPA (50 or 500 μg･kg^{-1}/day) or vehicle from GD 10 to PND 14. The results are expressed as mean ± S.E.M. n = 12 per group. *P< 0.05 and **P< 0.01 indicate significant differences from control rats.

Fig. 5. The effects of perinatal BPA exposure on the latency to find the platform in the Morris water maze test in 9-week-old male rat offspring. Mother rats were treated with oral administration of BPA (50 or 500 μg･kg^{-1}/day) or vehicle from GD 10 to PND 14. The results are expressed as mean ± S.E.M. n = 12 per group.

Fig. 6. The effects of perinatal BPA exposure on the latency to enter the dark

chamber in the step-through passive avoidance test in 11-week-old male rat offspring. Mother rats were treated with oral administration of BPA (50 or 500 μg・kg^{-1}/day) or vehicle from GD 10 to PND 14. The results are expressed as mean ± S.E.M. Controls: n = 11; BPA groups: n = 12 each. *$P < 0.05$ indicates a significant difference from control rats.

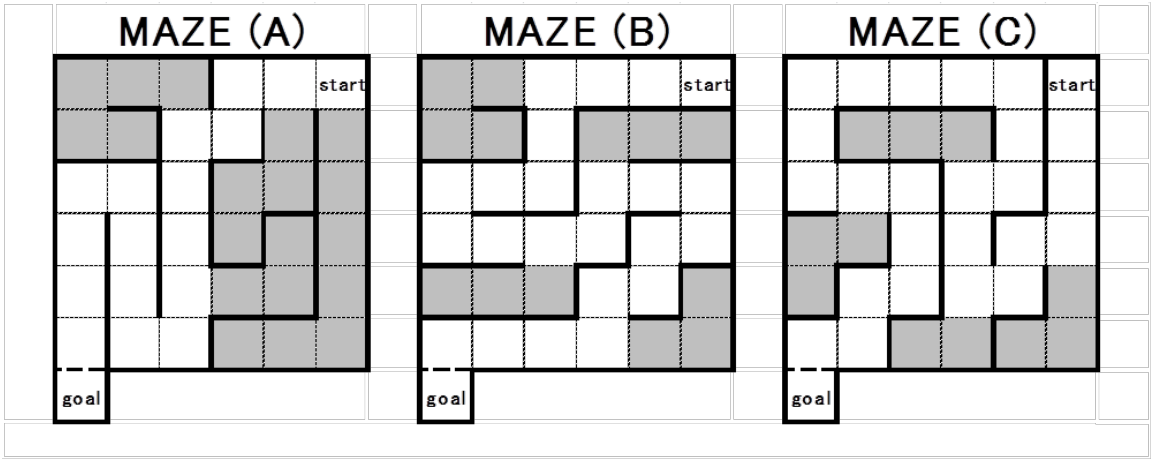

Fig. 1

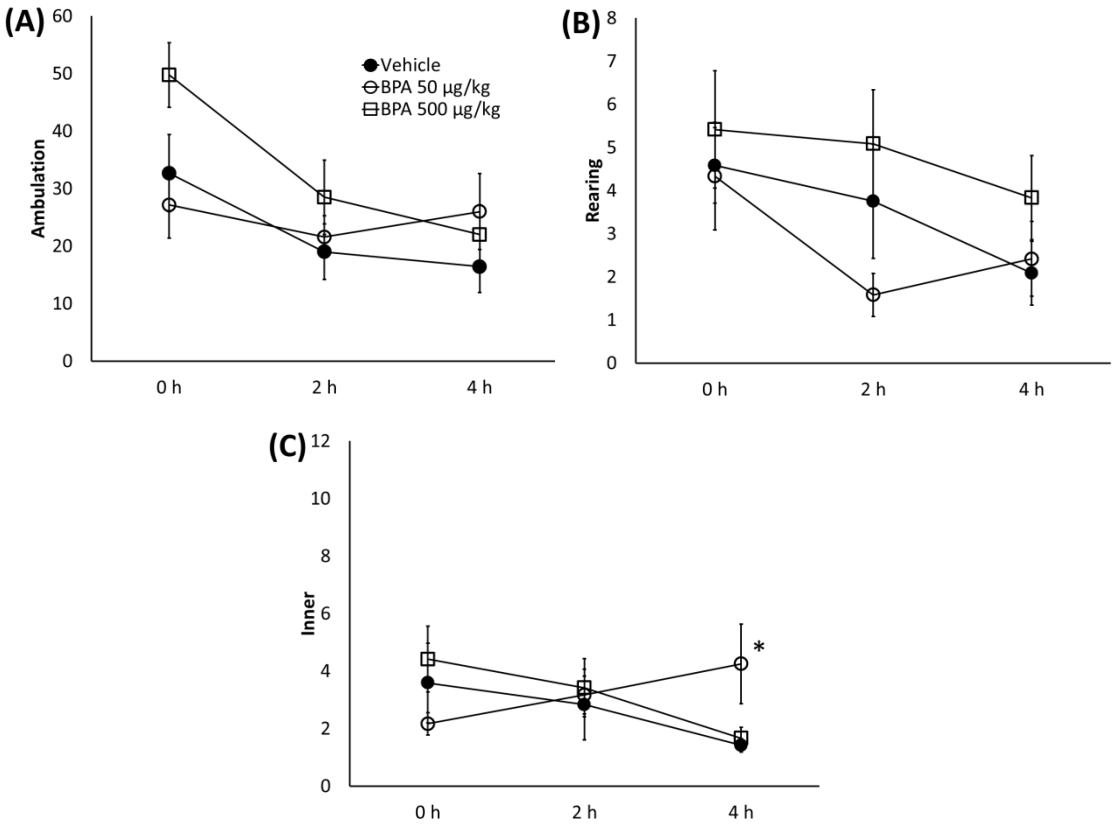
(A)
Ambulation
60
50
40
30
20
10
0
Vehicle
BPA 50 µg/kg
BPA 500 µg/kg
0 h
2 h
4 h
(B)
Rearing
8
7
6
5
4
3
2
1
0
0 h
2 h
4 h
(C)
Inner
12
10
8
6
4
2
0
*
0 h
2 h
4 h

Fig. 2

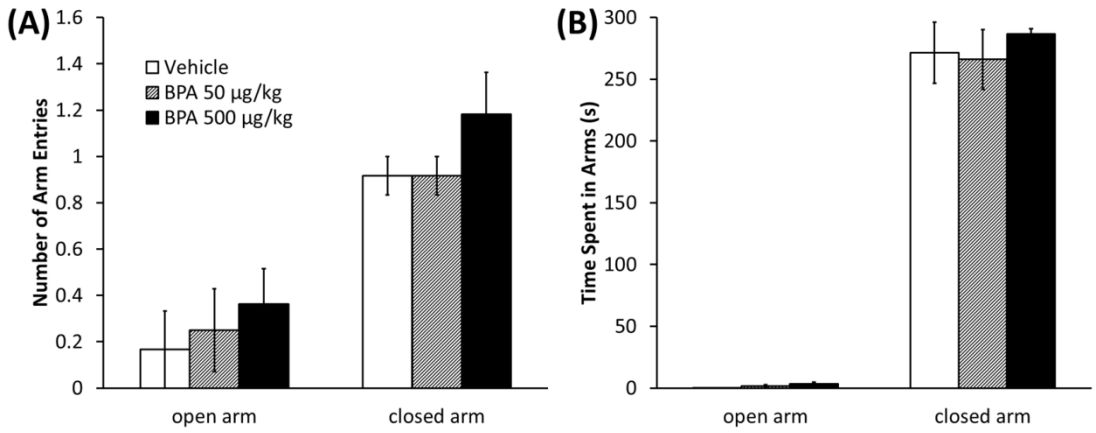
(A)
Number of Arm Entries
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Vehicle
BPA 50 µg/kg
BPA 500 µg/kg
open arm
closed arm
(B)
Time Spent in Arms (s)
300
250
200
150
100
50
0
open arm
closed arm

Fig. 3

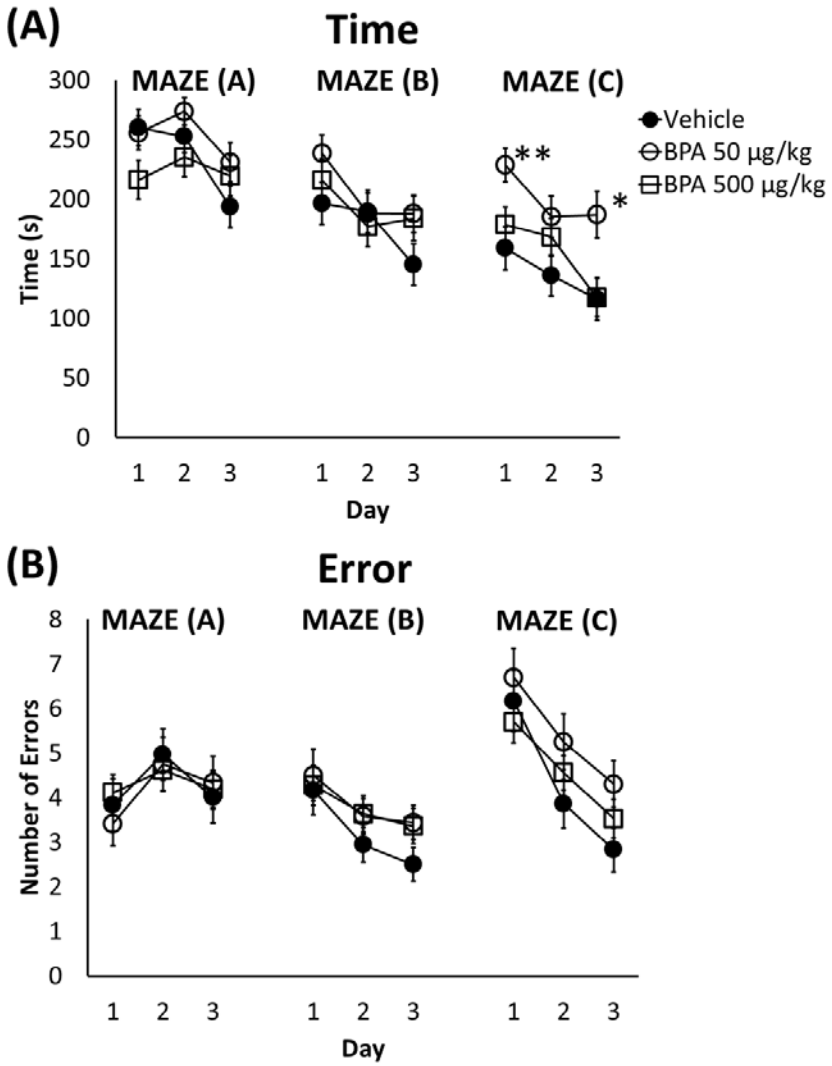
(A)
Time
MAZE (A)
MAZE (B)
MAZE (C)
Vehicle
BPA 50 µg/kg
BPA 500 µg/kg
Time (s)
300
250
200
150
100
50
0
**
*
1 2 3 1 2 3 1 2 3
Day
(B)
Error
MAZE (A)
MAZE (B)
MAZE (C)
Number of Errors
8
7
6
5
4
3
2
1
0
1 2 3 1 2 3 1 2 3
Day

Fig. 4

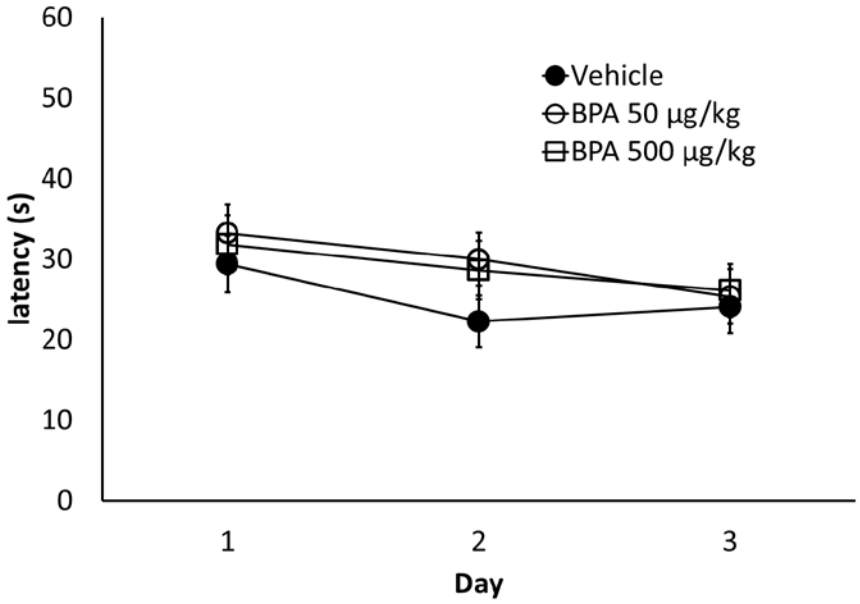
60
50
40
30
20
10
0
latency (s)
Vehicle
BPA 50 µg/kg
BPA 500 µg/kg
1
2
3
Day

Fig. 5

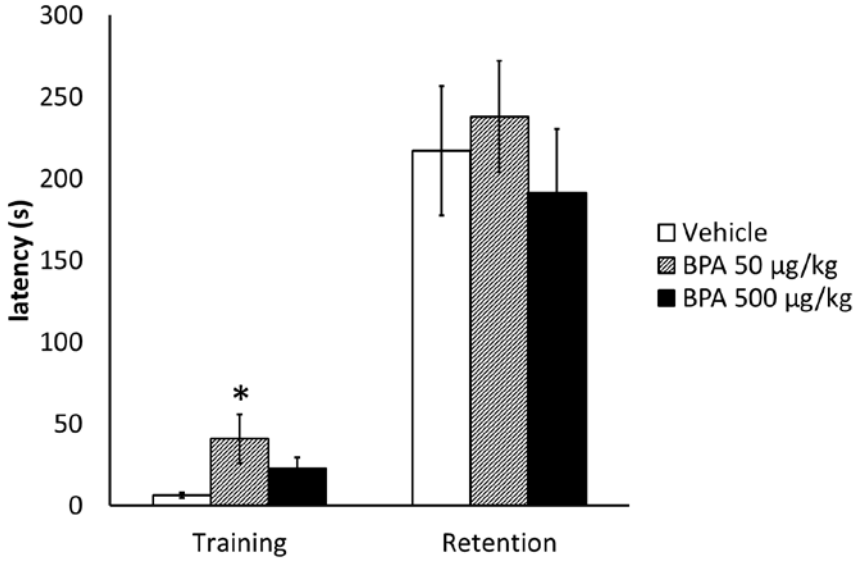
300
250
200
150
100
50
0
latency (s)
*
Training
Retention
Vehicle
BPA 50 µg/kg
BPA 500 µg/kg

Fig. 6