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Original Article

Oral exposure to low-dose of nonylphenol impairs memory performance in Sprague-Dawley rats

Shinichiro Kawaguchi[1], Rika Kuwahara[2], Yumi Kohara[3], Yutaro Uchida[3], Yushi Oku[3] and Kimihiro Yamashita[4]

[1]Graduate School of Science and Technology, Nagasaki University, 1-14 Bunkyo-Machi, Nagasaki 852-8521, Japan
[2]Department of Agro-environmental Sciences, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
[3]Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, 1-14 Bunkyo-Machi, Nagasaki 852-8521, Japan
[4]Division of Environmental Chemistry and Ecotoxicology, Institute of Environmental Studies, Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, 1-14 Bunkyo-Machi, Nagasaki 852-8521, Japan

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ABSTRACT — Nonylphenol ethoxylate (NPE) is a non-ionic surfactant, that is degraded to short-chain NPE and 4-nonylphenol (NP) by bacteria in the environment. NP, one of the most common environmental endocrine disruptors, exhibits weak estrogen-like activity. In this study, we investigated whether oral administration of NP (at 0.5 and 5 mg/kg doses) affects spatial learning and memory, general activity, emotionality, and fear-motivated learning and memory in male and female Sprague-Dawley (SD) rats. SD rats of both sexes were evaluated using a battery of behavioral tests, including an appetite-motivated maze test (MAZE test) that was used to assess spatial learning and memory. In the MAZE test, the time required to reach the reward in male rats treated with 0.5 mg/kg NP group and female rats administered 5 mg/kg NP was significantly longer than that for control animals of the corresponding sex. In other behavioral tests, no significant differences were observed between the control group and either of the NP-treated groups of male rats. In female rats, inner and ambulation values for animals administered 0.5 mg/kg NP were significantly higher than those measured in control animals in open-field test, while the latency in the group treated with 5 mg/kg NP was significantly shorter compared to the control group in step-through passive avoidance test. This study indicates that oral administration of a low-dose of NP slightly impairs spatial learning and memory performance in male and female rats, and alters emotionality and fear-motivated learning and memory in female rats only.

Key words: Nonylphenol, Endocrine disruptors, Learning and memory, Behavior, Central nervous system, Rat

INTRODUCTION

4-Nonylphenol (NP), a metabolite of nonylphenol ethoxylate (NPE), is one of the most common environmental endocrine disruptors and has been shown to exhibit a weak estrogenic activity. NPE is a non-ionic surfactant that is used in industrial detergents, emulsifiers, and wetting agents, and is degraded to short-chain NPE and NP by bacteria in the environment (Ying *et al*., 2002).

A number of studies reported that NP has the potential to interfere with the reproductive, endocrine, and immune systems. For example, exposure to NP during the perinatal period or in adulthood was found to decrease ovarian weight and sperm count, and alter kidney structure (Nagao *et al.,* 2001; Chapin *et al.,* 1999; Lee, P.C. and Lee, W., 1996; Cunny *et al.,* 1997; Aly *et al.,* 2012). Furthermore, several reports have been published on the effects of NP on the central nervous system (CNS), as well as the reproductive function. Gestational exposure to NP impaired neurobehavioral development and memory performance in male rat offspring (Jie *et al.,* 2010). A study has shown that chronic application of NP can impair learning and memory and decrease exploratory activity in male mice (Mao *et al.,* 2010). In contrast, some pre-

Correspondence: Kimihiro Yamashita (E-mail: kimihiro@nagasaki-u.ac.jp)

viously performed studies did not show any effect on the reproductive tract after neonatal exposure to NP (Odum and Ashby, 2000; Noda *et al.,* 2006). Additionally, perinatal exposure to NP did not affect locomotor activity or learning and memory performance in rat offspring (Flynn *et al.,* 2002; Ferguson *et al.,* 2000; Negishi *et al.,* 2004). However, the effects of oral exposure to NP on CNS in adult male and female rats have not been reported and remain to be clarified.

Perinatal exposure to 0.1 mg/kg NP was shown to induce behavioral alterations, changing the responses to fear-provoking stimuli and affecting the monoaminergic neural pathways. The effective dose of NP, from a neurobehavioral standpoint, is much lower than the dosage associated with general toxicity (Negishi *et al.,* 2004). Our present study was, therefore, designed to evaluate the effects of oral exposure to low dose of NP on learning and memory performance, general activity levels, and emotionality in male and female Sprague-Dawley (SD) rats using a battery of behavioral tests, including a series of learning performance tests previously used in our laboratory (Kuwahara *et al.,* 2014). To our knowledge, this is the first study investigating the effects of oral NP administration on CNS in male and female rats.

MATERIALS AND METHODS

Animals

Male and female SD rats (5 weeks of age) were purchased from Kyudo Corp. (Saga, Japan). The rats were housed in plastic cages with stainless steel covers in an air-conditioned room at a 12:12-hr light-dark cycle (lights on from 07:00 to 19:00), temperature of 22 ± 2°C, and humidity of 55 ± 10%. The animals were acclimatized to the laboratory conditions for one week, with food and water were freely available for the first 7 days following their arrival. The animals in experiment were randomly housed (2-3 per cage) until 7 weeks of age. After the rats were assigned to three treatment groups, they were also group housed (2-3 per cage). In order to enhance their motivation for the rewards (20 g condensed milk in 100 mL water), food and water were restricted (male rats: food 12 g/day and water 33.3 mL/day; female rats: food 8.5 g/day and water 24 mL/day) from 6 weeks of age. At 7 weeks of age, the rats were acclimatized to the MAZE test apparatus and rewards, and assigned to three treatment groups. The animals were orally administered NP (0.5 or 5 mg/kg; Kanto Chemical Co., Inc., Tokyo, Japan) dissolved in corn oil, or vehicle alone (control; 1 mL/kg). Rats were lightly anesthetized using halothane (Fluothane, Takeda Pharmaceutical Co., Inc., Tokyo, Japan) prior to oral administration. The rats were orally administered NP or vehicle within 30 min after the MAZE test (training or test sessions) or training session of the step-through passive avoidance test. In the open-field test and the elevated plus-maze test, rats were treated with NP or vehicle on the day before test were conducted. 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.

MAZE test

The MAZE tests were performed to evaluate spatial learning and memory. The apparatus and the experimental procedures were adopted from our previous study (Kuwahara *et al.,* 2014). The apparatus (Fig. 1) consisted of a large compartment (90 × 90 × 50 cm), a goal compartment (15 × 15 × 50 cm) and partitions of various sizes (50 × 15 cm, 50 × 30 cm, 50 × 45 cm, 50 × 60 cm) which were inserted into the large compartment. Three 100-W bulbs, placed 100 cm above the floor, illuminated the apparatus. Four different cues were put on the walls. Three types of MAZE configurations with different levels of difficulty were used, and the complexity of the apparatus structure was increased in progressive MAZE test sessions [MAZE (A) → MAZE (B) → MAZE (C)]. At 7 weeks of the age, rats were acclimatized to the MAZE test apparatus and rewards over 3 consecutive days. MAZE (A) was performed at 8 weeks of age, MAZE (B) at 10 weeks of age, and MAZE (C) at 12 weeks of age. For every MAZE test, the rats were first trained on the correct approach to the goal using an apparatus with incorrect ways blocked off (training session). Subsequently, the rats were tested for 3 consecutive days, starting the day after the initial training using an apparatus with incorrect pathways not blocked off (testing session). Each rat was placed gently in the maze and allowed to find the goal and get the reward. For the training session, the rats underwent 3 trials in a single day with a 1-min inter-trial interval. During training trials, if a rat did not reach the goal within 180 sec, it was guided to the goal by the experimenter and got the reward. The testing was performed in 3 trials per day for 3 days with a 1-min inter-trial interval, and time-to-goal and error were recorded. During testing trials, if a rat did not reach the goal within 300 sec, it was guided to the goal by the experimenter and got the reward. Time-to-goal was defined as the latency required to reach the goal and start eating the reward, while error was defined as the number of entries into an incorrect area of the maze. Both parameters were used as measures of spatial learning and memory.

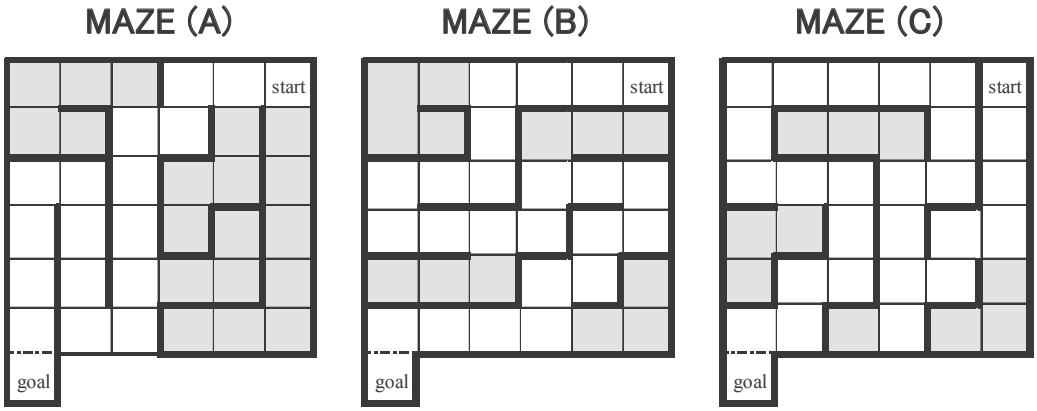

Fig. 1. MAZE apparatus. In consecutive MAZE tests, the route necessary to reach the goal was made more complicated than in the previous test (MAZE (A) → MAZE (B) → MAZE (C)). MAZE tests were performed to evaluate spatial learning and memory in male and female rats. White and gray segments represent the correct and incorrect path way, respectively.

Step-through passive avoidance test

Fear-motivated learning and memory was tested using the step-through passive avoidance test (Komatsu *et al.,* 2008). The passive avoidance apparatus (Shintecno Co., Ltd., Fukuoka, Japan) consisted of a light chamber (10 × 20 × 12 cm) and a dark chamber (30 × 30 × 30 cm) and a grid floor that could deliver an electric shock (1 mA for 5 sec) using a shock generator (MSG-001, Toyo Sangyo Co., Ltd., Toyama, Japan). The 2 chambers were connected with a path (8 × 8 cm), and separated by a guillotine door.

On day 1, each rat was allowed to enter the dark chamber for 90 sec, and was acclimatized to the apparatus. On day 2, each rat was gently placed in the light chamber and received an electric shock when they entered the dark chamber (training sessions). Acquisition time, defined as the latency to enter the dark chamber, was measured. On day 3, each rat was gently placed in the light chamber and the retention time, defined in this session as the latency to enter the dark chamber, was measured (test sessions). The maximum latency during the test period was 300 sec. The retention time was used as a measure of fear-motivated learning and memory. The step-through passive avoidance test was performed when rats were 13 weeks of age.

Open-field test

The general behavior and emotionality of the rats were measured using the open-field test, as described by Hall (Hall, 1934). The open-field 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 using black lines, and was divided into two regions: an outer ring (0-12 cm from the wall) and an inner ring (12-30 cm from the wall). Each rat was placed in the center of the floor and was observed for 3 min. The total number of sectors crossed by the rat (ambulation), the number of line crossings inside the inner circle (inner), and the frequency of times the rats stood on its hind legs (rearing) were recorded. General activity level, wariness behavior, and exploratory behavior were evaluated using ambulation, inner, and rearing respectively. Behavioral observations were performed 3 times at 2-hr intervals. The open-field test was performed at 8 weeks of age.

Elevated plus-maze test

Anxiety or fear of heights was observed using the elevated plus-maze test (Walf and Frye, 2007). A maze was constructed from black plastic in the shape of a plus sign, consisting of two open (50 × 10 cm) and two closed arms (50 × 10 × 60 cm) with all the arms connected by a central platform (14 × 14 cm). Closed arms were enclosed completely by 60 cm high walls, while open arms had no walls. Open and closed arms were opposed in each arm, and the elevated plus-maze was elevated 60 cm above the floor. The test was performed under bright light conditions. Each rat was placed in the center of the maze facing an open arm at the beginning of the test, and was observed for 5 min. Entries into and time spent in the open and closed arms were recorded. The time spent in the open arms and the numbers of entries into the open arms was used as measure 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.

Statistical analysis

All data are expressed as means ± S.E.M. Statistical significance between groups was analyzed using one or two-way analysis of variance (ANOVA) with a post-hoc Dunnett's multiple comparison tests (Stat View, SAS, Cary, NC, USA). One-way ANOVA was used to analyze the behavioral parameters in elevated plus-maze test and step-through passive avoidance test, and two-way ANOVA was used to assess the results of the MAZE test and open-field test. The threshold for statistical significance was set at $P < 0.05$.

RESULTS

Effect of oral NP administration on performance in the MAZE test

For control groups of both sexes, the time-to-goal and error tended to decrease with each consecutive day of testing in all MAZE test sessions, especially in the MAZE (C) test. These results indicate that the control groups of both sexes exhibit learning curve (Fig. 2).

In the male rats, time-to-goal for the animals treated with 0.5 mg/kg NP was longer than that for the control group on any of the 3 days in the MAZE (B) test and on Day 3 in the MAZE (C) test, with a significant difference observed on Day 3 in the MAZE (B) test ($P < 0.05$). The 5 mg/kg NP group had a longer time-to-goal compared to the control group on Day 3 at all three difficulty levels of the MAZE test; however, no significant differ-

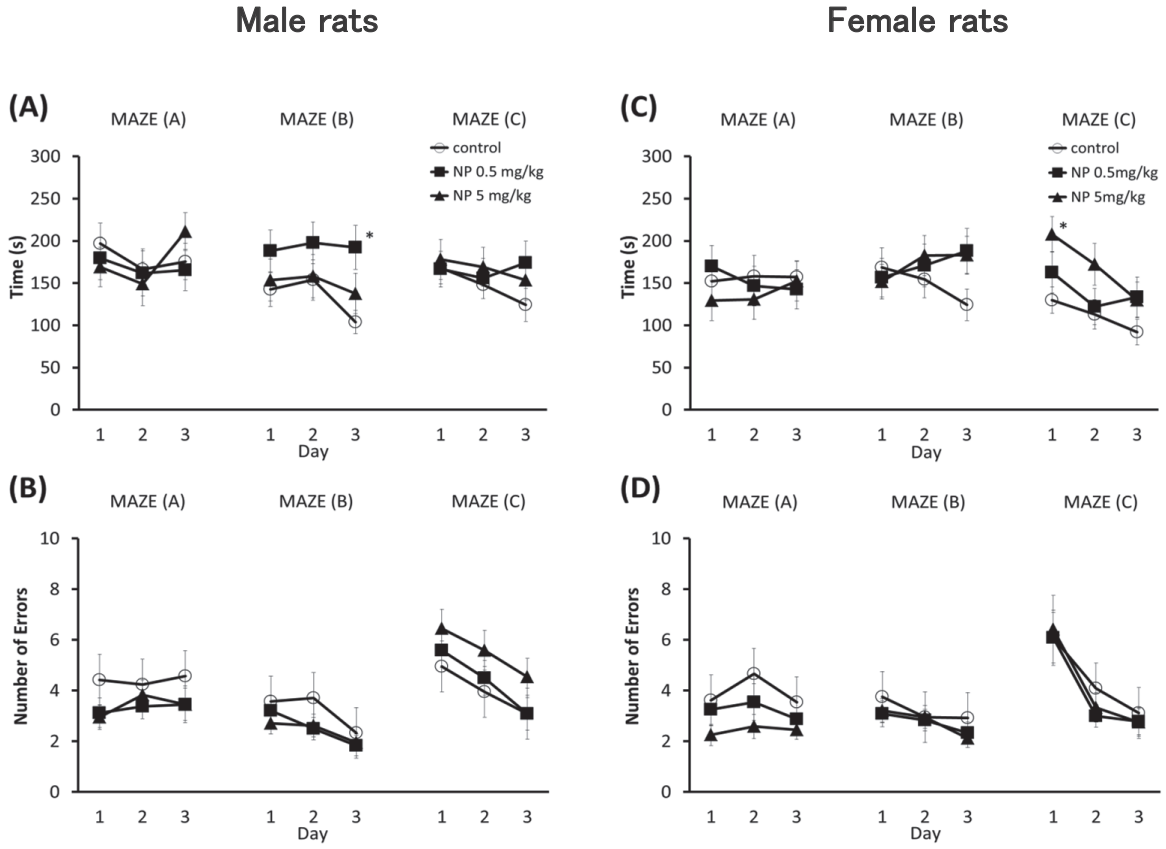

Fig. 2. The effects of oral NP administration on time-to-goal (A, C), defined as the latency required to reach the goal and start eating the reward, and error (B, D), defined as the number of entries into the incorrect area, in the MAZE test performed with 8-, 10-, and 12-week-old SD rats. Rats were orally administered NP (0.5 or 5 mg/kg) or vehicle within 30 min after the training or test session. The results are expressed as mean ± S.E.M. Controls: n = 7; NP groups: n = 8 male SD rats at each dose, n = 8 female SD rats at each dose. *$P < 0.05$ indicates significant differences compared to the control group of the same sex.

ences were observed between the groups (Fig. 2A). Error observed for the 0.5 mg/kg NP group was lower than that for the control group over the entire testing duration of the MAZE (A) test and on Day 2 of the MAZE (B) test. The 5 mg/kg NP group showed lower error compared to the control group on Days 1 and 3 of the MAZE (A) test, and Day 1 and 2 of the MAZE (B) test; in contrast, the 5 mg/kg NP group showed higher error compared to the male rats of the control group in the MAZE test (C). However, none of the differences were significant compared to the control group (Fig. 2B).

In the female rats, the time-to-goal for the 0.5 mg/kg NP group was longer than that observed in the control group on Day 3 of the MAZE (B) test, and on Days 1 and 3 of the MAZE (C) test; however, the differences between the groups were not significant. The 5 mg/kg NP group showed longer time-to-goal compared to the control group on Day 3 of the MAZE (B) test and any of the 3 days in the MAZE (C) test. Significant difference was observed on Day 1 of MAZE (C) test ($P < 0.05$) (Fig. 2C). Error measured in animals treated with 0.5 mg/kg NP was lower than that observed in the control group on Day 2 of the MAZE (A) and (C) tests. The 5 mg/kg NP group showed lower error compared to the control group for the entire testing duration in the MAZE (A) test and on Day 2 in the MAZE (C) test. However, none of these differences were significant compared to the control group (Fig. 2D).

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

Treatment of both sexes with NP did not alter behavioral parameters during the training session for step-through passive avoidance test, with no significant differences observed in comparison to the control group. When comparing measures of retention (assessed 24 hr after foot shock), NP-treated rats of both sexes showed shorter latencies compared to the control group of the same sex (Fig. 2). However, there were no significant differences when compared to the control group in male rats. The female rats treated with 5 mg/kg NP showed significantly shorter latencies compared to the control group ($P < 0.05$; Fig. 3B).

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

In the male rats, ambulation and inner values in the control group gradually decreased over time. In the 0.5 mg/kg NP group, ambulation and inner values were higher than those observed in the control group during later tests (2 and 4 hr); however, the differences between groups were not significant. In the 5 mg/kg NP group, ambulation and inner values were not significantly different compared to those in the control group (Fig. 4A, B). Rearing values in the control group decreased at 2 hr, and slightly increased at 4 hr. While both of the NP groups had lower rearing values compared to the control group during the initial test (0 hr), the differences were not statistically sig-

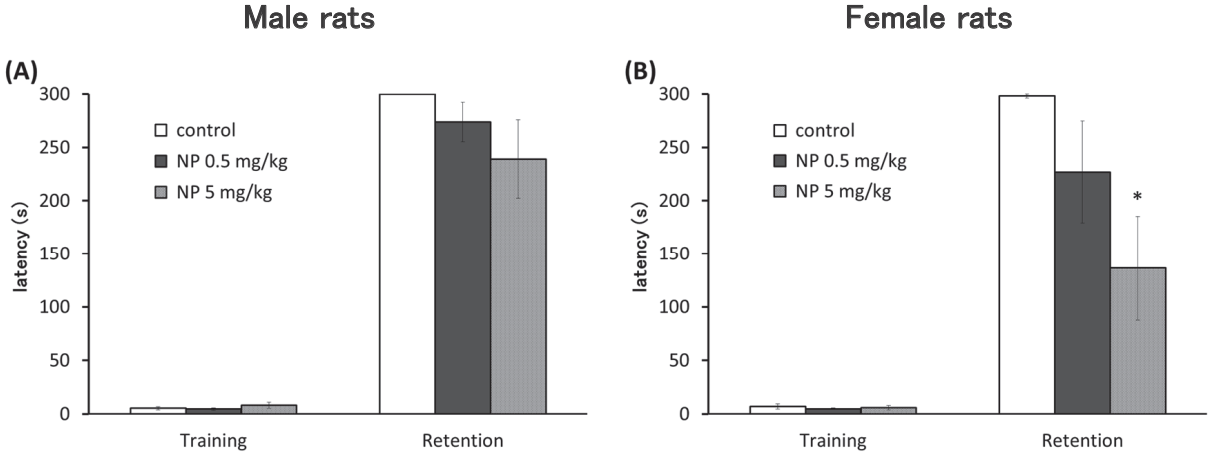

Fig. 3. The effects of oral NP administration on the latency to enter the dark chamber in the step-through passive avoidance test performed on 13-week-old SD rats. Rats were orally administered NP (0.5 or 5 mg/kg) or vehicle within 30 min following the training session. The results are expressed as mean ± S.E.M. Controls: n = 7; NP groups: n = 8 male SD rats at each dose, n = 8 female SD rats at each dose. *$P < 0.05$ indicates significant differences compared to the control group of the same sex.

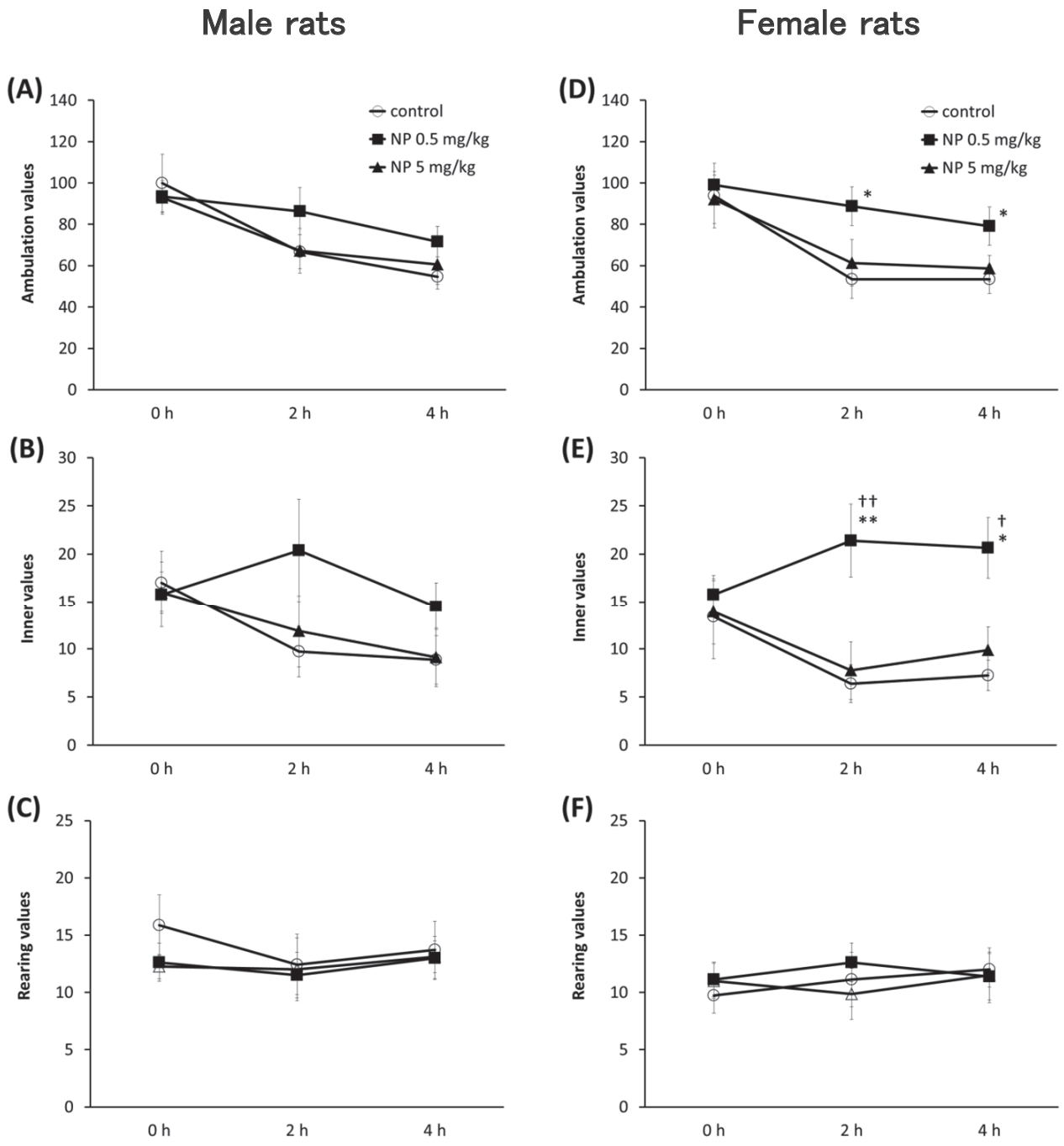

Fig. 4. The effects of oral NP administration on ambulation (the total number of sectors crossed in the arena; A, D), inner (the number of line crossings inside the inner circle; B, E), and rearing (the number of times the rats stood on their hind legs; C, F) during the open field test in 8-week-old SD rats. Rats were orally administered NP (0.5 or 5 mg/kg) or vehicle on the day before the test. Controls: n = 7; NP groups: n = 8 male SD rats at each dose, n = 8 female SD rats at each dose. $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant difference compared to the control animals of same sex, and $^{\dagger}P < 0.05$ and $^{\dagger\dagger}P < 0.01$ indicate significant difference compared to rats of same sex treated with 5 mg/kg NP.

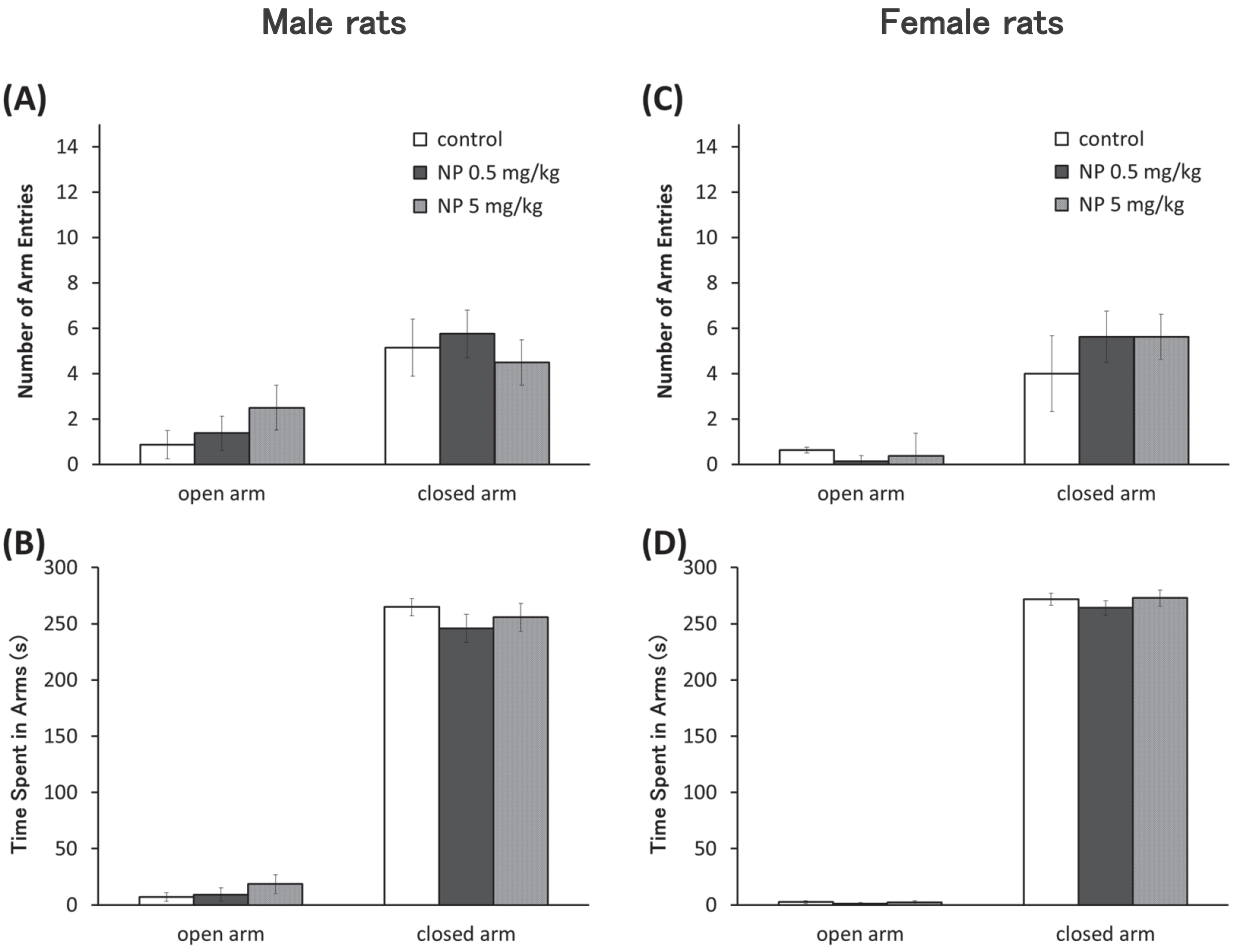

Fig. 5. The effects of oral NP administration on the number of entries to each arm of the elevated plus-maze (A, C) and the time spent in each arm (B, D) during the test performed on 10-week-old SD rats. Rats were orally administered NP (0.5 or 5 mg/kg) or vehicle on the day before the test. The results are expressed as mean ± S.E.M. Controls: n = 7; NP groups: n = 8 male SD rats at each dose, n = 8 female SD rats at each dose.

nificant (Fig. 4C).

In the female rats, ambulation and inner values in the control group greatly decreased at 2 hr, and did not change at 4 hr. The animals treated with 0.5 mg/kg NP showed significantly higher ambulation values compared to the control group during later tests ($P < 0.05$; Fig. 4D). Inner values in the 0.5 mg/kg NP group were significantly higher than those observed in the control group and in the female rats treated with 5 mg/kg NP during later tests ($P < 0.01$, $P < 0.05$; Fig. 4E). In the 5 mg/kg NP group, ambulation and inner values were not significantly different compared to those in the control group (Fig. 4D, E). Rearing in the control group gradually increased over time. In both NP-treated groups, rearing values were similar to those observed in the control group during the test period, and there were no significant differences when compared to the control group (Fig. 4F).

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

In the male rats, the number of entries into open arms and the time spent in open arms in both NP-treated groups slightly increased with increased dose, as compared to the values observed in the control group, but the increases were not significant (Fig. 5A, B). The number of entries into the closed arms and the time spent in closed arms in both NP-treated groups were similar to those observed in the control group, and there were no significant differenc-

es compared to the control group (Fig. 5A, B).

In the female rats, the number of entries into closed arms in both NP-treated groups was increased compared to that observed in the control group (Fig. 4C); however, the differences were not significant when compared to control group. In both NP-treated groups, the number of entries into open arms and the time spent in both arms were similar to those in the control group, with no significant differences observed between treated animals and the control group (Fig. 4C, D).

DISCUSSION

The present study investigated the effects of oral administration of a low-dose of NP on learning and memory performance, general activity levels, and emotionality in male and female SD rats. Nagao *et al*. (2001) have previously reported a no observed adverse effect level (NOAEL) of NP for general toxicity, as well as for reproduction (assessed in the next generation), to be 10 mg/kg/day in rats. Perinatal exposure to 0.1 mg/kg NP was shown to induce behavioral alterations in responses to fear-provoking stimuli and to affect monoaminergic neural pathways, with the effective dose of NP from a neurobehavioral standpoint being much lower than the dosage associated with general toxicity (Negishi *et al.,* 2004). On the basis of these findings, we postulated that CNS might be affected by low-dose NP that does not affect the reproductive system, and may therefore be more sensitive to NP exposure than the reproductive system. Therefore, this study used doses of 5 and 0.5 mg/kg NP, corresponding to 50 and 5% of the NOAEL dose, respectively. We evaluated the effects of oral exposure to low-dose NP on CNS in male and female SD rats.

Three types of MAZE tests with progressive levels of difficulty were used in this study. In male rats, time-to-goal for the 0.5 mg/kg NP group was significantly longer than that for the control group on Day 3 of the MAZE (B) test ($P < 0.05$; Fig. 2A). In contrast, oral administration of 5 mg/kg NP did not affect time-to-goal and error in any of the MAZE tests. Thus, our results showed that oral NP administration at 0.5 mg/kg slightly impaired spatial learning and memory performance in male rats, but the 5 mg/kg dose of NP elicited no effect. In female rats, animals treated with 0.5 mg/kg NP showed no significant effects on time-to-goal and error at any of the three difficulty levels of the MAZE test. However, time-to-goal for animals treated with 5 mg/kg NP was significantly longer than in the control group on Day 1 of the MAZE (C) test ($P < 0.05$; Fig. 1A). Therefore, our findings indicate that oral administration of 5 mg/kg NP slightly impaired spatial learning and memory performance in female rats. Taken together, our results show that oral administration of NP slightly impaired spatial memory consolidation in both sexes.

NP is known to exhibit a weak estrogenic activity, with some studies reporting that this activity, as well as the relative binding affinity of NP to the estrogen receptor (ER), are much lower than those of E_2 (Nishihara *et al.,* 2000; Blair *et al.,* 2000). A number of studies have reported that estrogen enhances spine and long-term potentiation (Phan *et al.,* 2012; McEwen *et al.,* 2001), has neuroprotective effects (McEwen and Alves, 1999; Dubal *et al.,* 1998; Goodman *et al.,* 1996), and improves learning and memory (Phan *et al.,* 2012; Frye *et al.,* 2005). In addition, it is known that a partial agonist like NP shows antagonistic effect to agonist like estrogen. Therefore, NP may inhibit the estrogenic effect on spatial learning and memory and, as a result, cause impairment in spatial memory consolidation.

Detectable levels of NP were reported in the rat brain 24 hr after the last treatment when NP is administered orally at 0.1 or 10 mg/kg doses for 4 consecutive days. The maximum NP concentrations in the rat brain induced by oral administration of 0.1 and 10 mg/kg NP were 0.12 and 1,183 ppb, respectively (Zalko *et al.,* 2003). Additionally, a study has shown that NP inhibits dendritic outgrowth in hippocampal neurons, with the NP-mediated outgrowth inhibition unaffected by ER antagonist ICI-182,786 (Matsunaga *et al.,* 2010). The hippocampal formation was proposed to be very important for the processing of certain aspects of spatial learning and memory (Morris *et al.,* 1990). NP, therefore, reaches the rat brain and the effects of NP on dendritic outgrowth in hippocampal neurons, which are unrelated to the estrogenic activity, may also contribute to elicit impairment in spatial learning and memory.

Oral administration of 0.5 mg/kg NP slightly impaired spatial learning and memory performance in male rats; in contrast, the 5 mg/kg dose of NP had no effect. Therefore, our results suggest a non-monotonic dose-response effect of NP on spatial learning and memory performance in male rats. In our previous studies, we evaluated the non-monotonic dose-response relationship between perinatal exposure to bisphenol A (BPA) and spatial learning and memory (Kuwahara *et al.,* 2013). BPA is also one of the most commonly encountered environmental endocrine disruptors with a very weak estrogenic activity. Hormones and agents acting as environmental endocrine disruptors including NP tend to display non-monotonic dose-response relationships that are represented by U-shaped or inverted U-shaped curves (Gao *et al.,* 2013;

Vandenberg *et al.,* 2012).

In step-through passive avoidance test, oral NP administration did not affect retention latencies in male rats. However, treatment with increasing doses of NP decreased the retention latencies in female rats. The female rats treated with 5 mg/kg NP showed significantly shorter retention latencies compared to the control group ($P < 0.05$; Fig. 2). Therefore, oral administration of 5 mg/kg NP altered fear-motivated learning and memory in female rats only. In the open-field test, there were no significant differences between the control group and either of the NP-treated groups in male rats (Fig. 3A-C). Oral administration of NP, therefore, did not affect general behavior and emotionality in male rats. However, in female rats, ambulation and inner values in animals treated with 0.5 mg/kg NP were significantly higher than those measured in the control group ($P < 0.01$ and $P < 0.05$, respectively; Fig. 3D, E). It is conceivable that the increase in inner values was accompanied by an increase in ambulation values. Animals treated with 5 mg/kg NP did not show significant differences compared to the control group in the open-field test (Fig. 3D-F). Therefore, although our results indicate that oral administration of 0.5 mg/kg NP alters emotionality in female rats, oral administration of 5 mg/kg NP did not affect locomotor activity.

In this study, oral NP administration altered fear-motivated learning and memory and emotionality in female rats. NP was previously reported to increase the concentrations of hydroxyl radicals, one of the reactive oxygen species, stimulating generation based on singlet oxygen production by an effect on the ER in the rat striatum (Obata and Kubota, 2000; Obata *et al.,* 2001). A number of studies indicated that the striatum might be involved in fear-motivated learning and memory and emotionality (Stefański *et al.,* 1993; Gong *et al.,* 1999). For example, a study has shown that the injection of an acetylcholine receptor antagonist into the caudate nucleus of the striatum impaired behavioral performance on passive avoidance test in rat (Prado-Alcalá *et al.,* 1985). Additionally, Swanson *et al*. (1997) have shown that the injections of D1 and D2 agonists into nucleus accumbens increase general activity levels in the experimental animals. It is well known that the brain is particularly sensitive to oxidative damage. Therefore, the impairment in fear-motivated learning and memory and altered emotionality may be consequences of NP-induced enhancement of hydroxyl radical generation in the striatum.

However, the significant effects of oral NP administration on step-through passive avoidance and open-field tests were observed in female rats only. Some studies reported that estrus cycle affect learning and memory, and emotionality (Frye *et al*., 2000; Marcondes *et al*., 2001; Pompili *et al*., 2010; van Goethem *et al*., 2012). Furthermore, a number of studies repoted that BPA inhibits the effect of estrogen which is an improvement in learning and memory, and the effects of BPA are dependent on endogenous estrogen (Xu *et al*., 2011; Inagaki *et al*., 2012). Our findings showing the effects of oral NP administration on fear-motivated learning and memory and emotionality in female rats may thus be related to the estrogen cycle. However, in order to evaluate the effects of oral NP administration on normal female rats without any estrogen-affecting treatment, female rats were not ovariectomized in this study.

In the elevated plus-maze test, treatment with NP did not alter behavioral parameters, as compared to the control groups in both sexes (Fig. 5). Our results therefore show that oral administration of NP does not affect anxiety or fear of heights in male and female rats. In female rats, oral NP administration altered emotionality in the open-field test, but did not affect anxiety or fear of heights in the elevated plus-maze test. General behavior and emotionality in a novel environment were measured using the open-field test; in contrast, the elevated plus-maze test was performed to evaluate anxiety or fear of heights. Therefore, we observed that oral NP administration altered emotionality but did not affect anxiety or fear of heights.

It was reported the mice of estrus show significant longer the time spent in open arm compared to the mice of diestrus in elevated plus-maze test (Galeeva and Tuohimaa, 2001). Walf and Frye (2005) have shown that administration of 17β-estradiol increase the time spent in open arm in ovariectomized rat. In contrast, it was reported that 17β-estradiol did not affect anti-anxiety-like behavior in ovariectomized rat (Morgan and Pfaff, 2001). These inconsistencies in the effects of estrogen on anti-anxiety-like behavior may be related to the dosage of estrogen used and the duration of estrogen treatment. Our results showed oral exposure to NP did not alter behavioral parameter in elevated plus-maze test in male and female rats, but the anti-anxiety-like behavior in female rats may be also affected depending on estrus cycle.

In conclusion, oral administration of a low-dose of NP slightly impaired spatial learning and memory in male and female rats. The oral NP administration affected fear-motivated learning and memory and emotionality in female rats only. Therefore, our data suggest that female rats are more susceptible to oral NP administration than male rats.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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