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Effects of prenatal and postnatal exposure to a low dose of bisphenol A on behavior and memory in rats

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ABSTRACT

Endocrine disruptors (EDs) are increasingly common chemicals in the environment. Bisphenol A (BPA), used to manufacture polycarbonate plastics, is an ED recognized for its estrogenic, anti-estrogenic, and anti-androgenic effects. Behavior is considered a vital characteristic for an animal's life cycle. This study evaluated the effect of exposure to low doses of BPA during pregnancy and/or lactation on several aspects of rat behavior, including memory, locomotion, and the exploratory instinct. Pups at 16 weeks of age (females and males) were divided into groups according to the mother's exposure to BPA (40 μg/kg/day): CON (vehicle only); PRE (during pregnancy); LAC (during lactation); PRE-LAC (during both pregnancy and lactation). In the PRE-LAC group, exposure to BPA impaired both short-term (STM) and long-term memory (LTM) in inhibitory avoidance and the object recognition task, and also affected locomotor activity and spatial memory. Some sex-specific behavioral characteristics disappeared in the LAC group. Sex-specific memory and behavior impairment were caused by BPA exposure during brain organogenesis and differentiation.

1. Introduction

Exposure to gonadal steroid hormones during intrauterine and neonatal life plays a pivotal role in the development of brain circuits, and this differentiation persists, determining behavior patterns throughout life (Kubo et al., 2001; Patisaul and Polston, 2008). Estrogens cause proliferation and differentiation of nervous system cells and synaptic plasticity. Estrogens could act through genomic (nuclear-receptor) or non-genomic (membrane receptors) pathways. Estrogen receptors (ERs), ERα and ERβ, are estrogen-regulated, are expressed in various regions of the central nervous system (CNS) including the hippocampus, and have an important direct and indirect influence on higher cognitive functions (Prange-Kiel and Rune, 2006).

Endocrine disruptors (EDs) are synthetic or natural substances that are able to affect the action of endogenous hormones. EDs mimic or block endogenous hormones and may modify the synthesis, transport, metabolism, or elimination of natural hormones (Mlynarcıkova et al., 2005; Hanet et al., 2008). These chemicals influence the development and function of the CNS and the reproductive system (Kubo et al., 2001; Kubo et al., 2003; Fujimoto et al., 2007). Bisphenol A (BPA) is a well-known ED, and is recognized as having both estrogenic and anti-estrogenic effects, in addition to anti-androgenic and anti-thyroid effects (Zoeller et al., 2005; Fujimoto et al., 2007; Gonçalves et al., unpublished). About 3.0 million tons of BPA are produced annually, and it is used in the manufacture of polycarbonate, epoxy resins, polysulfone, polyester, and polyether ketones, and also as an antioxidant and flame retardant. BPA has been shown to leach from these materials, and in this way has become ubiquitous in the environment. Suspected of being hazardous to humans, BPA has been found in human urine (Calafat et al., 2005), milk, and plasma samples, and also in placental tissues (Schönfelder et al., 2002). Despite hepatic BPA metabolism (mainly glucurodation) be fast, BPA presence in human fluids indicates a constant exposure.

Low dose of BPA increases ERα mRNA and ERβ mRNA brain levels (see review in Vom Saal and Hughes, 2005). Therefore, BPA has been linked as a potential cause of alteration in sexual dimorphisms of the CNS and behavioral impairment in rats (Kubo et al., 2001).

For BPA, the lowest-observed-adverse-effect-level (LOAEL) was 50 mg/kg/day, and was used to calculate a reference dose of 50 μg/kg/day based on experiments conducted in the 1980s (IRIS, 1988). Exposure to a low dose (low compared to the reference dose) of BPA during the fetal and suckling periods in mice affected

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their exploratory emotional behavior, decreasing the normal sexual differences in behavior (Kubo et al., 2003). Rats exposed to a low dose of BPA showed behavioral defeminization in females and demasculinization in males (Kubo et al., 2001; Gioiosa et al., 2007).

Taking into consideration the widespread presence of BPA in the environment, and considering its weak estrogen activity and experimental limitations, some authors have affirmed (Vom Saal and Hughes, 2005) and others have doubted (Dekant and Völkel, 2008) the real risk of human exposure to BPA.

Concerning experimental condition, via of administration deserves some consideration: while the oral route is the main pathway in BPA exposure, parenteral administration avoids BPA first-pass in the liver (glucuronide conjugation). On the other hand, the toxicological core "the dose makes the poison" is confronted with hormesis mechanisms (i.e., low dose effect) or non-monotonic dose response curves (Vandenberg et al., 2009). Current human BPA exposure occurs in low dosage. Thus, low dose BPA effect in a single dose is interesting to observe, under adequate experimental conditions.

Independently of dosage, exposure period is crucial. Organisms exposed to EDs such as BPA during embryonic, fetal (free BPA increase for deconjugation β-glucuronidase-mediated in placental and fetal tissue) or neonatal (hepatic immaturity in glucuronidation capacity) life would present predisposition to dysfunctions observed in puberty or adulthood. Therefore, an important experimental approach is to study the effect of different BPA exposure times, at the same dosage, on memory and behavioral tasks.

This study evaluated the effects of a low dose of BPA (40 μg/kg/day, administered orally) on litters of female and male Wistar rats during the pregnancy and/or lactation periods, with respect to conditioning and spatial memory, locomotion, exploratory activity, and emotional state.

2. Materials and methods

All the procedures involving animal subjects were reviewed and approved by the Institutional Research Ethics Committee of the Universidade Federal do Rio Grande, Rio Grande, Rio Grande do Sul (CEPAS-Approval: 15/2008).

Female and male Wistar rats (*Rattus norvegicus*) at 16 weeks of age obtained from the Universidade Federal do Rio Grande breeding colony were housed in standard cages. After confirmed copulation, females were exposed to one of the following treatments: corn oil-treated, with BPA, control (CON, $n = 5$); BPA-treated during the pregnancy period (20 days), prenatal group (PRE, $n = 6$); BPA-treated during the lactation period (21 days), postnatal group (LAC, $n = 4$) and BPA-treated during the pregnancy and lactation periods (41 days), pre- and postnatal group (PRE-LAC, $n = 3$). BPA (Sigma–Aldrich, USA) was dissolved in corn oil (20 μg/ml), and each animal received their treatment daily in the morning (BPA 40 μg/kg/day) by oral gavage. Each female received 1 ml per gavage. Pregnant dams were maintained in home cages under a 12-h light/dark cycle, temperature 21 ± 2 °C, with water and food ad libitum (soy-free rat chow, BioBase Bio-tec, Águas Frias, SC, Brazil). Each litters was standardized on the day after birth to 8–11 pups, with similar numbers of females and males. The CON, PRE, LAC and PRE-LAC treatments were composed respectively of 24, 29, 19, and 12 male pups and 29, 30, 16 and 14 female pups. In sum, 173 pups were analyzed in this study. After weaning on postnatal day 21, all offspring were housed by same-sex and same-litter group and maintained in the same conditions as the dams. Behavioral and memory studies were initiated at 16 weeks of age with both female and male rats following the order presented in materials and methods.

2.1. Step-down inhibitory avoidance task (IAT)

The IAT is a behavioral aversive hippocampus-dependent task that allows evaluation of short-term memory (STM) and long-term memory (LTM) (Medina and Izquierdo, 1995; Izquierdo et al., 1998). The task was carried out in two phases: training and test. The apparatus was a 50 cm × 25 cm × 25 cm acrylic box, with a floor consisting of parallel 1.0 mm diameter stainless steel bars spaced 1.0 cm apart. A 7.0 cm wide, 2.5 cm high, and 25.0 cm long platform occupied the left end of the floor. Briefly, in a training session, immediately after stepping down and placing all four paws on the grid, the animals received a 0.4 mA shock for 2 s. In the test sessions, no shock was administered, and the step-down latency was used as a measure of retention (to a ceiling of 180 s). STM was tested 60 min after training, and LTM 24 h after the training session. All animals tested for STM were also tested for LTM; this double test procedure does not affect LTM performance (Izquierdo et al., 1998).

2.2. Open field test (OF)

This procedure allows estimation of the locomotor activity levels (number of squares crossed), exploratory activity (rearing frequency), and emotional state (numbers of fecal boli and grooming events) (Küçük et al., 2008). The open field was a 50 cm × 40 cm linoleum floor divided into 12 equal squares by black lines, surrounded by 60 cm high walls. The front wall was made of Plexiglas® and the other walls were made of plywood. Animals were gently placed individually in the rear left rectangle and left to explore the field freely for 5 min (Barros et al., 2000; Barros et al., 2001).

2.3. Object recognition test (OR)

In the first session, two identical objects were presented to the animal and it was allowed to explore them for 5 min. After 90 min, one object was replaced by a new object and the animal was again allowed to explore both new and old objects, in order to evaluate the STM. Twenty-four hours later, the new object from the STM was replaced by a third object, to evaluate LTM. If the time spent to explore the old objects is longer than for the new object, this can be interpreted as impairment in operational memory. This kind of memory is associated with hippocampus activity (Friedman and Goldman-Rakic, 1988). The results were expressed as recognition indexes (RI), calculated using the exploration time for each object: RI = time for the novel object/(time for the novel + time for the known), as described by Aguiar et al. (2006).

2.4. Morris water maze (MWM)

A modification of the spatial version described by Morris (1984) was used. This behavioral test was developed to evaluate spatial memory, and is employed to assess the function of the hippocampus in the development of this type of memory. The MWM consisted of a circular dark tank (168 cm diameter and 70 cm depth) filled with cloudy water and divided into four quadrants: Northwest (NW), Northeast (NE), Southeast (SE), and Southwest (SW). A hidden platform was fixed in one of the four virtual quadrants for all of the training sessions. The water was kept at 24 ± 1 °C. Visual cues were placed in the water maze room. The training took place for 5 days, and in each training session, 5 trials of 120 s were performed at 70-s intervals. For each trial, the animals started from a different position in the water maze. In the training sessions, the learning progress was evaluated as the delay in reaching the hidden platform (escape); the path length and velocity were also recorded. On the sixth day, the retention test was executed: the platform was removed, and the animal swam freely for 60 s. The time spent in the platform quadrant was recorded, as an indicator of memory retention. The path length and velocity were also recorded. Data were obtained through a tracking video system (EthoVision®, NOLDUS).

3. Statistical analysis

Data were expressed as mean ± SEM. When the assumptions of homogeneity and normality of variance were verified, one- or two-way ANOVA was carried out to determine the main effect of sex and treatment and possible sex × treatment interactions. One-way ANOVA was performed first between litters for each treatment and sex, to exclude litter effects. Once litter effect was excluded, statistical tests were performed. The ANOVA was followed by a Student–Newman–Keuls post hoc test. If the assumption of homogeneity of variance was violated, a Kruskal–Wallis nonparametric ANOVA followed by a Mann–Whitney test was performed. The significance level adopted was 5%.

4. Results

4.1. Step-down inhibitory avoidance task (IAT)

The training session step-down latency among groups did not differ significantly in a Kruskal–Wallis analysis of variance ($p > 0.05$).

Latency time in the test session showed no significant differences between sexes, in both STM ($p = 0.239$) and LTM ($p = 0.625$). The test results for STM (Fig. 1A) and LTM (Fig. 1B), expressed as mean and interquartile range (25/75), are shown for each sex and treatment. PRE-LAC-treated females and males showed impairment in conditioning memory as measured in the inhibitory avoidance task, both in the STM and LTM. LAC-treated males showed similar failure rates for both memory types.

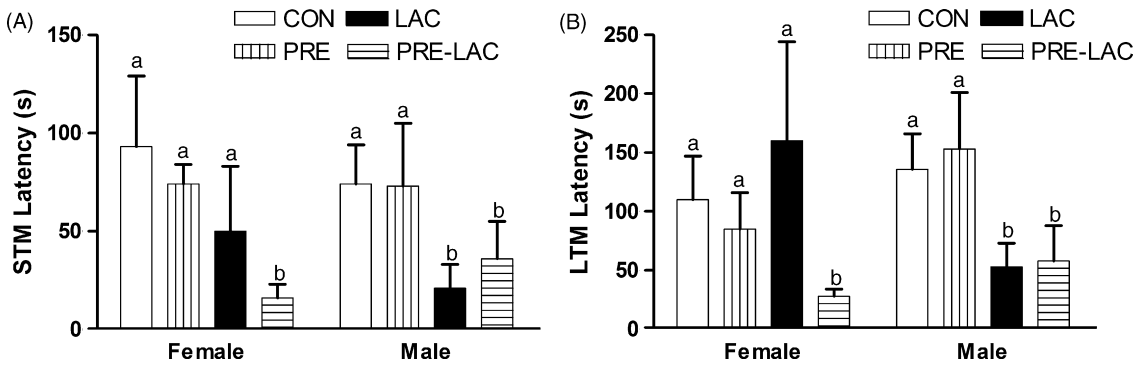

Fig. 1. The effect of BPA on latency of step-down in the step-down inhibitory avoidance task (in s) in 16-week-old female and male rat pups, on A) short-term memory (STM), and B) long-term memory (LTM). Mother rats were exposed to one of the following treatments: CON (vehicle); PRE (BPA 40 μg/kg/day during pregnancy); LAC (BPA 40 μg/kg/day during lactation); PRE-LAC (BPA 40 μg/kg/day during pregnancy and lactation). Differences between bars having the same letters are not significant at the 5% probability level. The results are expressed as the mean and interquartile range (25/75).

4.2. Open field test (OF)

Locomotor activity: the number of crossings differed between sexes ($p < 0.001$). A treatment effect was also observed ($p < 0.05$). Additionally, a sex × treatment interaction was observed [$F(3, 169) = 3.68$, $p < 0.05$] (Fig. 2). Females showed no impairment from the BPA treatment, but LAC and PRE-LAC males had poorer performances than CON males and all female groups.

Exploratory activity: females showed more rearings (38.58 ± 1.65) than did males (24.44 ± 1.22) [$F(1, 171) = 35.80$, $p < 0.001$]. The treatment also had a significant effect [$F(3, 169) = 5.28$, $p < 0.005$]. The PRE-LAC and LAC-treated rats showed fewer rearings than the CON and PRE groups (Table 1). An interaction of sex × treatment was not found ($p > 0.05$).

Emotional state: grooming and defecation events showed no differences in either sex or treatment ($p > 0.05$) (Table 1).

4.3. Object recognition (OR)

During the training session, no differences were observed ($p > 0.05$) between either sexes or treatments, in the exploration time between identical objects (data not shown). The object recognition performance differed between the sexes in both STM [$F(1, 171) = 4.14$, $p < 0.05$] and LTM [$F(1, 171) = 20.72$, $p < 0.001$]. STM and LTM were, for females, 0.69 ± 0.012 and 0.51 ± 0.013, and for males, 0.51 ± 0.013 and 0.58 ± 0.017, respectively. The treatment effects are shown in Fig. 3. STM performance was altered by the treatments [$F(3, 169) = 4.01$, $p < 0.005$]. The PRE-LAC group showed poorer performance than the CON group. The LTM group showed a significant difference for treatments [$F(3, 169) = 2.75$, $p < 0.05$]. The CON group performed better than the PRE-LAC group. No sex × treatment interaction was found ($p > 0.05$).

4.4. Morris water maze (MWM)

Path length: no sex difference ($p > 0.05$) was observed in the learning phase. However, the training session was affected by treatments [$F(15, 2366) = 5.07$, $p < 0.001$] (Fig. 4). On training day 1, the PRE-LAC group traveled a longer distance than the PRE and LAC groups. On day 2, the distance covered by the CON group was shorter than the LAC group, and the PRE-LAC path length was shorter compared to the PRE and LAC treatments. On day 4, the distance traveled by the CON group was shorter than the LAC and longer than the PRE and PRE-LAC-treated groups. On day 5, the distance covered by the CON group was shorter than the LAC and PRE-LAC treatments, and the PRE performance was poorer than the LAC and PRE-LAC-treated rats. In the test session, females (1238.02 ± 19.98 cm) traveled a greater distance than males (1158.42 ± 28.17 cm) [$F(1, 171) = 5.40$, $p < 0.05$]. On the test day, the distance covered showed a significant treatment effect [$F(3, 169) = 3.46$, $p < 0.05$] (Table 2).

Velocity: During the training session, there was no difference between the sexes [$F(5, 859) = 1.63$, $p > 0.1$]. Treatments had a significant effect [$F(15, 2366.2) = 4.43$, $p < 0.001$] on the training

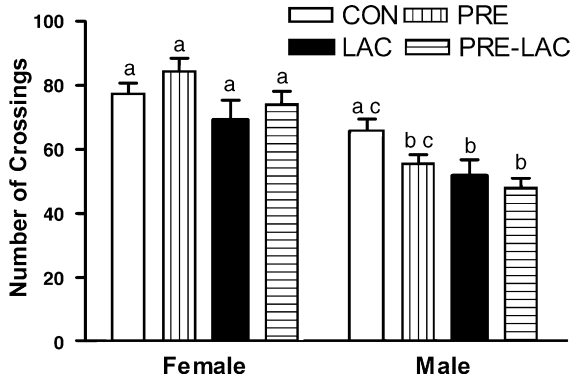

Fig. 2. The effect of BPA on the number of crossings in 16-week-old pups. Mother rats were exposed to one of the following treatments: CON (vehicle); PRE (BPA 40 μg/kg/day during pregnancy); LAC (BPA 40 μg/kg/day during lactation); PRE-LAC (BPA 40 μg/kg/day during pregnancy and lactation). Differences between bars having the same letters are not significant at the 5% probability level. The results are expressed as mean ± SEM.

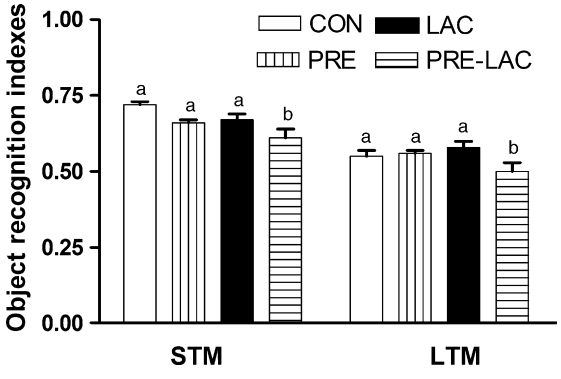

Fig. 3. The effect of BPA on object recognition indexes in 16-week-old pups for: short-term memory (STM) and long-term memory (LTM). Mother rats were exposed to one of the following treatments: CON (vehicle); PRE (BPA 40 μg/kg/day during pregnancy); LAC (BPA 40 μg/kg/day during lactation); PRE-LAC (BPA 40 μg/kg/day during pregnancy and lactation). Differences between bars having the same letters are not significant at the 5% probability level. The results are expressed as mean ± SEM.

Table 1
Effect of BPA on number of rearings, grooming, and defecations (during 5 min) in female and male rats.

	CON	PRE	LAC	PRE-LAC
Rearings	33.78 ± 2.19	35.74 ± 2.10	24.94 ± 2.32[a]	27.84 ± 2.0[a]
Groomings	0.69 ± 0.09	0.89 ± 0.11	0.5 ± 0.12	0.69 ± 0.14
Number of fecal boli	1.77 ± 0.28	0.84 ± 0.18	1.42 ± 0.31	0.80 ± 0.32

[a] LAC and PRE-LAC-treated rats showed fewer rearings ($p < 0.05$) than rats receiving the CON and PRE treatments. The results are expressed as mean ± SEM.

Table 2
Path length, velocity, and time spent in the platform quadrant during the water maze retention test: effect of BPA treatments.

	CON	PRE	LAC	PRE-LAC
Path length (cm)	1202.57 ± 30.90	1140.31 ± 27.41	1213.67 ± 37.38	1307.62 ± 48.87[a]
Velocity (cm/s)	20.61 ± 0.50	20.06 ± 0.48	21.48 ± 0.68	22.81 ± 0.82[b]
Time in PQ(s)	19.51 ± 0.74	17.66 ± 0.78	16.95 ± 0.85	15.8 ± 0.89[c]

PQ, platform quadrant.
[a] PRE-LAC rats traveled longer distance than other treatments.
[b] PRE-LAC rats showed greater velocity compared to rats receiving the CON and PRE treatments.
[c] PRE-LAC rats remained for a shorter time in the platform quadrant than did rats receiving the CON treatment. The results are expressed as mean ± SEM.

(Fig. 5). On day 1, the velocity of the CON group was higher than for the PRE and LAC treatments. On days 2 and 3, the PRE-LAC treatment group showed the highest velocity of all the treatment groups. On day 4, the LAC velocity was less compared to the CON and PRE-LAC treatments. On day 5, the LAC velocity was slower than the PRE-LAC. In the test session, there was a difference between the sexes [$F(1, 171) = 3.88$, $p < 0.05$]. The velocities of females and males were 21.50 ± 0.34 and 20.33 ± 0.49 cm/s, respectively. On the test day, the velocity was altered by treatment [$F(3, 169) = 3.43$, $p < 0.05$]. The CON and PRE groups showed slower velocities compared to the PRE-LAC group (Table 2).

Latency: The training session showed no difference by sex [$F(5, 859) = 1.88$, $p > 0.05$]. The latency showed a difference by treatments [$F(15, 2366.2) = 4.804$, $p < 0.05$] (Fig. 6). On day 2, the CON group showed a lower latency time than all the other groups. On days 3 and 5, the LAC group showed the highest latency time compared to all other treatments. On day 4, the CON group showed a shorter latency time than the LAC group. A significant decrease in latency time between the first and the last day of training was observed, demonstrating that all groups had learned the task. In the retention test, no difference was found between the sexes [$F(1, 171) = 0.85$, $p > 0.1$], but a difference was found for treatment [$F(3, 169) = 3.17$, $p < 0.05$]. The CON group remained longer on the platform than did the PRE-LAC-treated rats (Table 2).

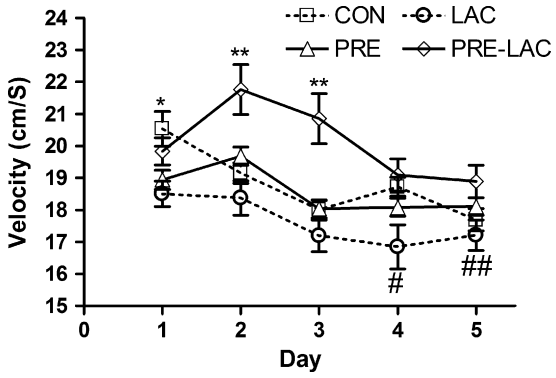

Fig. 5. Morris water maze training. Effect of BPA treatment on velocity (cm/s) in 16-week-old pups. Mother rats were exposed to one of the following treatments: CON (vehicle); PRE (BPA 40 μg/kg/day during pregnancy); LAC (BPA 40 μg/kg/day during lactation); PRE-LAC (BPA 40 μg/kg/day during pregnancy and lactation). *CON was higher ($p < 0.05$) than the PRE and LAC treatments. **PRE-LAC treatment had the highest velocity ($p < 0.05$) compared to all other treatments. #LAC velocity was lower ($p < 0.05$) than CON and PRE-LAC treatments. ##LAC was lower ($p < 0.05$) than PRE-LAC. The results are expressed as mean ± SEM.

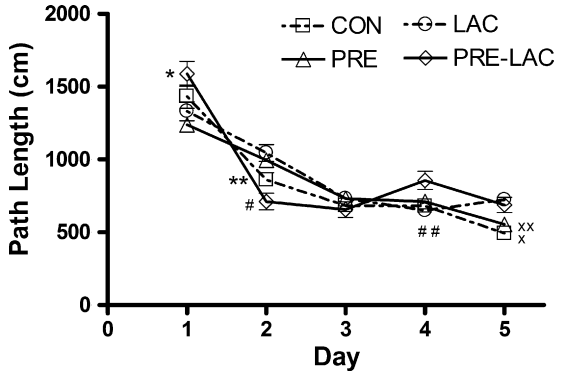

Fig. 4. Morris water maze training. Effect of BPA treatment on path length (cm) in 16-week-old pups. Mother rats were exposed to one of the following treatments: CON (vehicle); PRE (BPA 40 μg/kg/day during pregnancy); LAC (BPA 40 μg/kg/day during lactation); PRE-LAC (BPA 40 μg/kg/day during pregnancy and lactation). *PRE-LAC was higher ($p < 0.05$) than PRE and LAC. **CON was lower ($p < 0.05$) than LAC. #PRE-LAC was inferior ($p < 0.05$) compared to PRE and LAC. ##CON was lower ($p < 0.05$) than LAC and higher ($p < 0.05$) compared with PRE and PRE-LAC. xCON was lower ($p < 0.05$) than LAC and PRE-LAC. xxPRE performance was poorer ($p < 0.05$) than LAC and PRE-LAC. The results are expressed as mean ± SEM.

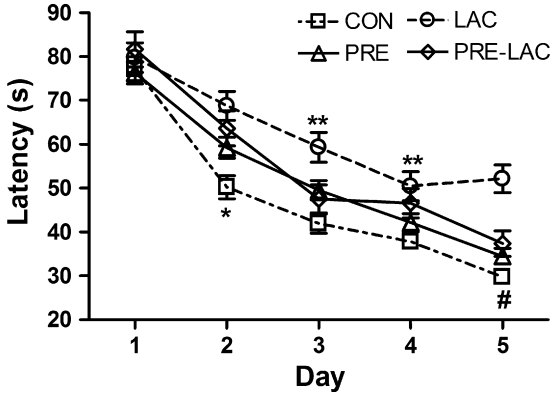

Fig. 6. Morris water maze training. Effect of BPA treatment on escape latency time (s) in 16-week-old pups. Mother rats were exposed to one of the following treatments: CON (vehicle); PRE (BPA 40 μg/kg/day during pregnancy); LAC (BPA 40 μg/kg/day during lactation); PRE-LAC (BPA 40 μg/kg/day during pregnancy and lactation). *CON showed a shorter latency time than all other treatments. **LAC showed a higher latency time compared to all other treatments. #CON showed a shorter latency time than the LAC treatment. The results are expressed as mean ± SEM.

5. Discussion

Memory formation is a process that involves many biochemical and molecular events in specific brain regions (Izquierdo and Medina, 1997). Soon after learning, memory is very susceptible to external and internal interferences (Gioiosa et al., 2007). We used the IAT test to evaluate the possible interference of BPA exposure during pregnancy and/or lactation with STM and LTM, in a paradigm of aversive learning. The aversive learning model is normally adaptive, and it contributes to remembering situations of fear and anxiety, such as post-traumatic stress (Maren, 2003). In IAT we found no significant difference between males and females in the latency time to step-down from the platform, suggesting a lack of difference in the consolidation of memory between the sexes. However, when the treatments were compared within each sex, the groups had different performances, suggesting an effect of BPA exposure with sex differences on STM and LTM memory consolidation. Females and males of the PRE-LAC treatment group showed impairment in STM and LTM. Besides the LAC treatment, only males also showed disturbances in STM and LTM, suggesting a greater tendency to susceptibility of males to impairment caused by BPA. The mechanisms involved in STM and LTM are essentially different, but some common pathways are known in the CA1 and the entorhinal cortex (Cammarota et al., 2007). BPA possibly affected more than one pathway of memory modulation. In our study, the females and males in the PRE-LAC group that showed memory disruption, were exposed to BPA for approximately 41 days of intrauterine development and postnatal growth, a period that coincides with major organogenesis and cellular differentiation (Newbold et al., 2007). In our laboratory, female rats of 5 months of age exposed to BPA during pregnancy and lactation showed higher T_4 levels. This impairment of TH homeostasis has some similarity to thyroid syndrome resistance and coincided with the females and males of the PRE-LAC group, which showed memory impairment. TH is already known for its role in brain development, learning, and memory processes (Köhrle, 2008). On the other hand, males from the LAC group also showed STM and LTM impairment, and this period (21 days) overlaps with the period of cellular differentiation. Males were more susceptible to impairment by BPA exposure for this memory type than were females, showing a sex-specific BPA effect.

Locomotor activity, exploratory habits, and emotional behavior of female and male rats were investigated in the OF test. In agreement with Fujimoto et al. (2007) and Gioiosa et al. (2007), locomotor activity was higher in females than males; but this difference was not observed between the control treatments. Similarly to the IAT test, males suffered a negative effect of BPA treatment on locomotion performance (PRE-LAC and LAC) (Fig. 2). In addition, Kubo et al. (2001) demonstrated, only in females, a negative effect of BPA on locomotion: females exposed to BPA scarcely moved. Perhaps the differences between these findings are related to the differences in the doses administered. In our study, the impairment effects of BPA were attained using a low dose.

Continuing in the OF task, we evaluated the exploration behavior by means of the number of rearings of each animal. We found a less intense exploratory instinct in males compared to females. Kubo et al. (2001) also reported that females show a greater tendency to explore new environments. In the treated animals, regardless of sex, the PRE-LAC and LAC groups showed a deficit in exploratory behavior, suggesting a negative effect of BPA on the normal capacity of the animal to explore environments (Table 1). As described by Fujimoto et al. (2006), the negative effect of BPA on the number of rearings suggested a loss in sexual dimorphism. Finally, to investigate emotional behavior, we evaluated the frequencies of fecal boli and grooming events. Differently from anxiety, the response to fear is more complex; according to Küçük et al. (2008), the number of fecal boli is a function of the animal's response to fear in situations considered dangerous, and therefore describes the emotional state of the animal. However, in our study, the frequencies of fecal boli and grooming did not differ between males and females, nor between the treatments. No consistent defecation rate results were found by Küçük et al. (2008) in the evaluation of emotional behavior. The OR paradigm of learning involves the free exploratory behavior associated with the ability of an animal to explore a novel object more than a familiar one. In the OR task, females showed different results from males in STM and LTM. For STM, we found better performance in females compared to males. On the other hand, males were better in the LTM compared to females. Gender differences in brain function and learning are determined by many factors. These factors include the organizational and activational effects of sex hormones, mainly by exposure during early brain development (Dalla and Shors, 2009).

Although STM and LTM have different pathways of modulation (Izquierdo et al., 1998), the BPA treatment during pregnancy and lactation (PRE-LAC) resulted in impairment in the performance of the STM and LTM tests (Fig. 3). The role of estrogens in memory is already known. Fernandez et al. (2008) reported that the administration of 17β-estradiol (E_2), infused via the hippocampus, facilitated the consolidation of memory for OR. E_2 was responsible for activation of an extracellular signal regulated by kinases (ERK), which is considered a limiting factor for various forms of memory consolidation and learning. In our study, BPA impaired memory consolidation as evaluated by the IAT and OR tests, suggesting its antagonist action to E_2. There are different concepts of the importance of spatial skills. For mammals, one theory supports the idea that spatial ability is better developed in males because of their different roles, including hunting and territory expansion (Jacobs et al., 1990). This increased spatial ability in males reflects a larger hypothalamus than females in polygamous species, which have greater intraspecific competition. Another theory suggests a greater spatial ability of females based on their capability to recognize objects and territories (Eals and Silverman, 1997). In addition to these concepts, an animal's spatial ability is an extremely important aspect of its biology, to allow it to perform daily tasks. In our study we evaluated spatial ability by the MWM, which recorded the distance and speed attained by the animals, in addition to the time of latency to find the hidden platform in the tank. These parameters evaluated the animals' ability to form spatial memory. The impaired performance in the MWM is attributed to possible damage in brain regions that are essential for coordinating spatial memory, such as the hippocampus, the striatum, the cerebellum, and several areas of the cortex (D'Hooge and Deyn, 2001).

In our study, the total distance traveled by the animals in the MWM task was longer in females than in males, as evaluated by us in the OF task, supporting the idea of locomotion superiority in females. This contrasts with the finding by Healy et al. (1999), of no differences between the sexes. Considering the treatments in both sexes during the training, in general the PRE-LAC group traveled the longest distance. The PRE-LAC group also performed better on the test day, compared to the PRE group. Differently from the OF task, the locomotion activity evaluated in the MWM was not decreased by BPA exposure. These results may be due to differences in tasks; the MWM task requires a forced movement (swimming), and not a spontaneous natural movement such as walking in OF.

During the days of training, there was no difference in velocity between the sexes; but on the test day, the females reached a higher speed compared to males. When the individual treatments during training were evaluated, again the PRE-LAC group showed the highest speed. On the test day, the PRE-LAC group continued to be faster compared to the CON and PRE groups.

For the latency time to find the platform, there was no difference between the sexes, in agreement with the observations of Healy et

al. (1999). During the training there was a progressive decrease in the latency time to find the platform, confirming that the animals had learned during that period. In the different treatments, regardless of sex, the CON group showed the greatest ability to find the platform, while the LAC group was the least able on training days 3 and 5. On the test day, the CON group remained longer in the platform quadrant, showing better spatial ability compared to the PRE-LAC group. Macbeth et al. (2008) assessed the influence of estrogens on spatial memory. They observed an improvement in spatial memory in pregnant rats, a period which coincides with high levels of circulating estrogens. In our study, the exposure to BPA in pups during the gestation and lactation periods (PRE-LAC) impaired the spatial ability of the animals, suggesting an antagonistic effect of BPA on the action of estrogen.

The tasks that we imposed did not involve social or reproductive parameters. However, different memory types, locomotor, and exploratory activities were impaired by transplacental and lactational exposure to BPA. We observed changes in memory and behavior induced by a low dose of BPA, which may be related to sexual differentiation of the brain. Our study showed the abolishment of behavioral sex differences, and additionally suggested that estrogen and thyroid disruption may be involved in memory impairment, as seen in the OR and MWM tasks.

In summary, nervous and endocrine systems interact to maintain organism homeostasis. The alteration of any signal pathway can affect these functions. Since rapid changes occur during development in prenatal and postnatal ages, the organisms as targets for endocrine disruptors also change. In this sense, it is important to understand the general aspects of development and, more specifically, nervous system development. This knowledge can help interpret toxic effects when considered as the endpoint of behavior and memory. Traditionally, developmental neurotoxicology shows alterations induced by toxins, in specific sensitive "windows" of development. However, when the chemical insult is subtle, gross alterations are not evident and appear only through long-term behavioral deviance. During prenatal and postnatal life, critical phases of neurodevelopment are evidenced. Gliogenesis and apoptosis initiate in the early embryo and continue in postnatal life. Similarly, differentiation and synaptogenesis begin in the late embryo and progress during postnatal life. Therefore, functional organization includes fetal and postnatal life. Myelination starts at the end of fetal life and continues during postnatal life. Proliferation and migration processes are extended from embryo until the 15th day of postnatal life. Only organogenesis and neurulation occur during the embryonic phase only.

In this study, the longest period of BPA exposure (gestation and lactation – PRE-LAC group) generally displayed the worst performance in all tests. Thus, it was verified that over prenatal and early postnatal life the nervous system is sensitive to suffer functional disturbances induced by BPA.

Additionally, BPA exposure during breastfeeding (LAC group), a period when differentiation, functional organization, myelination and other neurodevelopmental processes occur, impaired male and female performance in some tests. Natural behavioral differences between females and males disappear. These results suggested a loss of sexual dimorphism, with behavioral defemenization and demasculinization in females and males, respectively.

BPA exposure during gestation (PRE group) resulted in no apparent change in memory and behavioral tasks. Nevertheless, BPA exposure in the PRE-LAC group caused important detrimental effects on both sexes. These results permitted inferring that organogenesis and other developmental processes that occur during the gestation period were covertly affected by BPA, in as much as additional BPA exposure in the lactation period significantly increased the negative effects when compared with BPA exposure only in the lactation period. Memory and behavioral tasks emerge as a sensitive end point to evaluate endocrine disruption. In fact, we observed sex-specific alterations induced by a low dose of BPA and loss of behavioral sexual dimorphism. These findings cause concern, since they indicate the risk associated with BPA exposure.

Conflicts of interest

All authors declare they have no potential competing interest.

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References

Aguiar, R.B., Dickel, O.E., Cunha, R.W., Monserrat, J.M., Barros, D.M., Martinez, P.E., 2006. Estradiol valerate and tibolone: effects on memory. Pharmacol. Biochem. Behav. 85, 689–696.

Barros, D.M., Izquierdo, L.A., Mello e Souza, T., Ardenghi, P., Pereira, P., Medina, J.H., Izquierdo, I., 2000. Molecular signaling pathways in the cerebral cortex are required for retrieval of one-trial inhibitory avoidance learning in rats. Behav. Brain Res. 114, 183–192.

Barros, D.M., Mello e Souza, T., de David, T., Choi, H., Agguzoli, A., Madche, C., Ardenghi, P., Medina, J.H., Izquierdo, I., 2001. Simultaneous modulation of retrieval by dopaminergic D1, β noradrenergic, serotonergic-1A and cholinergic muscarinic receptors in cortical structures of the rat. Behav. Brain Res. 124, 1–7.

Calafat, A.M., Kuklenyik, Z., Reidy, J.A., Caudill, S.P., Ekong, J., Needham, L.L., 2005. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. Environ. Health Perspect. 113 (4), 391–395.

Cammarota, M., Bevilaqua, L.R.M., Medina, J.H., Izquierdo, I., 2007. Studies on short-term avoidance memory. In: Bermudez-Rattoni, F. (Ed.), Neural plasticity and memory: from genes to brain imaging. CRC Press, Boca Raton, pp. 193–208.

Dalla, C., Shors, T.J., 2009. Sex differences in learning processes of classical and operant conditioning. Physiol. Behav. 97, 229–238.

Dekant, W., Völkel, W., 2008. Human exposure to bisphenol A by biomonitoring: methods, results and assessment of environmental exposures. Toxicol. Appl. Pharmacol. 228, 114–134.

D'Hooge, R., Deyn, P.P., 2001. Applications of the Morris water maze in the study of learning and memory. Brain Res. Rev. 36, 60–90.

Eals, M., Silverman, I., 1997. The hunter–gatherer theory of spatial sex differences: proximate factors mediating the female advantage in recall of object arrays. Ethol. Sociobiol. 15, 95–105.

Fernandez, S.M., Lewis, M.C., Pechenino, A.S., Harburger, L.L., Orr, P.T., Gresack, J.E., Schafe, G.E., Frick, K.M., 2008. Estradiol-induced enhancement of object memory consolidation involves hippocampal extracellular signal-regulated kinase activation and membrane-bound estrogen receptors. J. Neurosci. 28 (35), 8660–8667.

Friedman, H.R., Goldman-Rakic, P.S., 1988. Activation of the hippocampus and dentate gyrus by working-memory: a 2-deoxyglucose study of behaving Rhesus monkeys. J. Neurosci. 8, 4693–4706.

Fujimoto, T., Kubo, K., Aou, S., 2006. Prenatal exposure to bisphenol A impairs sexual differentiation of exploratory behavior and increases depression-like behavior in rats. Brain Res. 1068, 49–55.

Fujimoto, T., Kubo, K., Aou, S., 2007. Environmental impacts on brain functions: low dose effects of bisphenol A during perinatal critical period. Int. Congr. Ser. 1301, 226–229.

Gioiosa, L., Fissore, E., Ghirardelli, G., Parmigiani, S., Palanza, P., 2007. Developmental exposure to low-dose estrogenic endocrine disruptors alters sex differences in exploration and emotional responses in mice. Horm. Behav. 52, 307–316.

Hanet, N., Lancon, A., Delmas, D., Jannin, B., Chagnon, M.-C., Cherkaoui-Malki, M., Latruffe, N., Artur, Y., Heydel, J.-M., 2008. Effects of endocrine disruptors on genes associated with17β-estradiol metabolism and excretion. Steroids 73, 1242–1251.

Healy, S.D., Braham, S.R., Braithwaite, V.A., 1999. Spatial working memory in rats: no differences between the sexes. Proc. R. Soc. Lond. 266, 2303–2308.

IRIS, 1998. Bisphenol A (CASRN 80-05-7). US. Environmental Protection Agency, Washington, DC, Available: http://www.epa.gov/NCEA/iris/subst/0356.htm (accessed on 12.03.09).

Izquierdo, I., Medina, J.H., 1997. Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. Neurobiol. Learn. Mem. 68, 285–316.

Izquierdo, I., Medina, J.H., Izquierdo, L.A., Barros, D.M., Souza, M.M., Souza, T.M., 1998. Short- and long-term memory are differentially regulated by monoaminergic systems in the rat brain. Neurobiol. Learn. Mem. 69, 219–224.
Jacobs, L.F., Gaulin, S.J.C., Sherry, D.F., Hoffman, G.E., 1990. Evolution of spatial cognition: sex-specific patterns of spatial behavior predict hippocampal size. Proc. Natl. Acad. Sci. Evol. 87, 6349–6352.
Köhrle, J., 2008. Environment and endocrinology: the case of thyroidology. Ann. Endocrinol. 69, 116–122.
Kubo, K., Arai, O., Ogata, R., Omura, M., Hori, T., Aou, S., 2001. Exposure to bisphenol A during the fetal and suckling periods disrupts sexual differentiation of the locus coeruleus and of behavior in the rat. Neurosci. Lett. 304, 73–76.
Kubo, K., Arai, O., Omura, M., Watanabe, R., Ogata, R., Aou, S., 2003. Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats. Neurosci. Res. 45, 345–356.
Küçük, A., Gölgeli, A., Saraymen, R., Koç, N., 2008. Effects of age and anxiety on learning and memory. Behav. Brain Res. 195, 147–152.
Macbeth, A.H., Gautreaux, C., Luine, V.N., 2008. Pregnant rats show enhanced spatial memory, decreased anxiety, and altered levels of monoaminergic neurotransmitters. Brain Res. 1241, 136–147.
Maren, S., 2003. What the amygdala does and doesn't do in aversive learning. Learn. Mem. 10, 306–308.
Medina, J.H., Izquierdo, I., 1995. Retrograde messengers, long-term potentiation and memory. Brain Res. Rev. 21, 185–194.
Mlynarcıkova, A., Kolena, J., Fickova, M., Scsukova, S., 2005. Alterations in steroid hormone production by porcine ovarian granulose cells caused by bisphenol A and bisphenol A dimethacrylate. Mol. Cell. Endocrinol. 244, 57–62.
Morris, R., 1984. Developments of a water–maze procedure for studying spatial learning in the rat. J. Neurosci. Met 11 (1), 47–60.
Newbold, R.R., Padilla-Banks, E., Snyder, R.J., Phillips, T.M., Jefferson, W.N., 2007. Developmental exposure to endocrine disruptors and the obesity epidemic. Reprod. Toxicol. 23, 290–296.
Patisaul, H.B., Polston, E.K., 2008. Influence of endocrine active compounds on the developing rodent brain. Brain Res. Rev. 57 (2), 352–362.
Prange-Kiel, J., Rune, G.M., 2006. Direct and indirect effects of estrogen on rat hippocampus. Neuroscience 138, 765–772.
Schönfelder, G., Wittfoht, W., Hopp, H., Talsness, C.E., Paul, M., Chahoud, I., 2002. Parent bisphenol A accumulation in the human maternal–fetal–placental unit. Environ. Health Perspect. 110 (11), 703–707.
Vandenberg, L.N., Maffini, M.V., Sonnenschein, C., Rubin, B.S., Soto, A.M., 2009. Bisphenol-A and great divide: A review of controversies in the field of endocrine disruption. Endocr. Rev. 30, 75–95.
Vom Saal, F.S., Hughes, C., 2005. An extensive new literature concerning low-dose effects of Bisphenol A shows the need for a new risk assessment. Environ. Health Perspect. 113 (8), 926–933.
Zoeller, R.T., Bansal, R., Parris, C., 2005. Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist *in vitro* increases serum thyroxine and alters RC3/Neurogranin expression in the developing rat brain. Endocrinology 146 (2), 607–612.