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Dose-dependent behavioral disturbances after a single neonatal Bisphenol A dose

Henrik Viberg*, Anders Fredriksson, Sonja Buratovic, Per Eriksson

Department of Environmental Toxicology, Uppsala University, Norbyvägen 18A, SE-75236 Uppsala, Sweden

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ABSTRACT

Bisphenol A is widely used in polymer products for food and beverage packaging, baby bottles, dental sealants, and fillings, adhesives, protective coatings, flame retardants, water supply pipes, and compact discs, and is found in the environment and in placental tissue, fetuses and breast milk. We have recently reported that neonatal exposure to other environmental pollutants can induce persistent aberrations in spontaneous behavior and also affect learning and memory functions in the adult animal. Furthermore, recent reports indicate that pre- and perinatal exposure to Bisphenol A can induce neurotoxic effects. The present study indicates that a single exposure to Bisphenol A on postnatal day 10 can alter adult spontaneous behavior and cognitive function in mice, effects that are both dose–response related and long-lasting/irreversible. Earlier studies on neonatal exposure to persistent organic pollutants (POPs) have shown the cholinergic system to be a target of neurotoxicity, but here only minor effects on the nicotine-induced behavior was seen. Furthermore, Morris swim-maze and the elevated plus-maze did not reveal any effects on spatial learning and anxiety-like behaviors. The present findings show similarities with effects earlier reported after pre- and perinatal exposure to Bisphenol A, and also with effects seen after a single postnatal exposure to other POPs, such as PBDEs, PCBs and PFCs.

1. Introduction

Bisphenol A (BPA; 2,2-bis(4-hydroxy-phenyl)propane), CAS number (80-05-7) is a monomer widely used in polymer products, such as epoxy, polyester–styrene, and polycarbonate resins used in food and beverage packaging, baby bottles, dental sealants, and fillings, adhesives, protective coatings, flame retardants, water supply pipes, and compact discs. BPA leaches from the products leading to widespread environmental contamination, thereby ending up in humans, e.g., BPA has been detected in both the saliva and in the serum of pregnant women as well as fetuses, placental tissue, amniotic fluid, follicular fluid, and breast milk (Ikezuki et al., 2002; Schonfelder et al., 2002; Ye et al., 2006). Studies have also shown that BPA can work as an endocrine active compound and as an agent altering DNA methylation (Wolstenholme et al., 2011).

During the fetal and neonatal period the developing brain is undergoing numerous essential developmental processes leading to the maturation of the brain into its highly developed adult functions. This period, the brain growth spurt (BGS) (Davison and Dobbing, 1968; Dobbing and Sands, 1979), is characterized by a series of rapid fundamental developmental changes, for example, maturation of dendritic and axonal outgrowth, the establishment of neural connections, and synaptogenesis and proliferation of glia cells with accompanying myelinization (Davison and Dobbing, 1968; Kolb and Whishaw, 1989). This is also the period when animals acquire many new motor and sensory abilities (Bolles and Woods, 1964) and when spontaneous motor behavior peaks (Campbell et al., 1969). In mammals, the period of BGS in terms of onset and duration varies from species to species. In the human, it begins during the third trimester of pregnancy and continues throughout the first 2 years of life, whereas in rodents the BGS is neonatal, spanning the first 3–4 weeks of life and reaching its peak around postnatal day 10.

In recent studies we have shown that a single oral neonatal exposure to certain environmental contaminants, such as DDT, polybrominated diphenyl ethers (PBDEs), perfluorinated compounds (PFCs) and polychlorinated biphenyls (PCBs), during the period of rapid brain development can cause similar persistent disturbances in spontaneous motor behavior in novel home environment and dysfunctions in learning and memory in adult animals (Eriksson, 1997, 1998; Johansson et al., 2008a; Viberg et al., 2003a, 2004).

Several studies have reported that prenatal, perinatal or repeated postnatal exposure to BPA can induce neurotoxic effects in mice. For example, prenatal exposure (gestational day 0–16) to BPA has been seen to induce accelerating cortical neuronal differentiation/migration leading to abnormal architecture postnatally (Nakamura et al., 2007, 2006) and these results are supported by a study showing that BPA can induces changes in both dendritic and synaptic development in cultured fetal cells (Yokosuka

* Corresponding author. Tel.: +46 18 4717695; fax: +46 18 4716425.
E-mail address: Henrik.Viberg@ebc.uu.se (H. Viberg).

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et al., 2008). Furthermore, studies have demonstrated that perinatal BPA-exposure (both during gestation and lactation) changed motivation to explore, anxiety behaviors, and spatial learning/memory (Farabollini et al., 1999; Porrini et al., 2005; Ryan and Vandenbergh, 2006). These effects are similar to effects earlier seen in our neonatal animal model after a single oral, neonatal exposure to environmental contaminants such as PBDEs, PFCs and PCBs. In a recent study Nakamura and coworkers studied effects in behavior in the open-field test, elevated plus maze (EPA) and Morris water maze tests, in adolescent and young adult mice after prenatal and lactational exposure (gestational day 0 to postnatal day 21) to BPA. The study showed altered behavior in both male and female mice exposed to BPA, where mice moved a significantly shorter distance in the open field test as well as in the EPM. Other variables in the open field, EPM and Morris water maze tests did not show any differences between the control animals and the animals exposed to BPA during gestation and throughout lactation (Nakamura et al., 2011).

The extensive use of BPA and the fact that humans are exposed to BPA during the fetal and newborn period, together with indications of developmental neurotoxicity from BPA, call for further investigations of possible developmental neurotoxic effects from exposure to BPA during the defined critical period of brain development occurring during the neonatal/newborn period. Therefore the aim of the present study was to investigate if a single oral dose of BPA, during the peak of the BGS, on postnatal day 10, can induce adult functional behavioral effects in mice.

2. Materials and methods

2.1. Chemicals and animals

Pregnant NMRI mice were purchased from Scanbur, Sollentuna, Sweden and were housed individually in plastic cages in a room with an ambient temperature of 22 °C and a 12/12 h cycle of light and dark. The animals were supplied with standardized pellet food (Lactamin, Stockholm, Sweden) and tap water *ad libitum*. The size of the litters was adjusted to 10–14 mice, within the first 48 h after birth, by the killing of excess pups. The litters contained pups of both sexes. At the age of 4–5 weeks the litters were separated into male and female animals. The males and females were kept in their litters (in treatment groups) with their siblings, and were placed and raised in groups of 4–7, in a room for male or female mice only, under the same conditions as detailed above. Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), after approval from the local ethical committees (Uppsala University and Agricultural Research Council) and by the Swedish Committee for Ethical Experiments on Laboratory Animals, approval number C185/9.

Bisphenol A (purity >99%, CAS number 80-05-7 and linear formula $(CH_3)_2C(C_6H_4OH)_2$) was purchased from Sigma–Aldrich, Stockholm, Sweden. Bisphenol A was dissolved in a mixture of egg lecithin (Merck, Darmstadt, Germany) and peanut oil (*Oleum arachidis*) (1:10) and then sonicated with water to yield a 20% (w:w) fat emulsion vehicle containing 0.032, 0.32 or 0.48 mg Bisphenol A/ml (0.14, 1.4 or 2.1 μmol/ml, respectively). The use of a 20% fat emulsion vehicle was to give a more physiologically appropriate absorption and hence distribution (Keller and Yeary, 1980; Palin et al., 1982), since the fat content of mouse milk is around 14%.

At the age of 10 days, both male and female mice, were given 0.32, 3.2 or 4.8 mg Bisphenol A/kg body weight (1.4, 14 or 21 μmol Bisphenol A/kg body weight), as a single oral dose via a metal gastric tube. These doses are the same on a molar basis as in our earlier studies of environmental pollutants inducing adult behavioral disturbances (Eriksson, 1997, 1998; Johansson et al., 2008a; Nakamura et al., 2011; Viberg et al., 2003a, 2004). These doses may be higher than what is used in several other studies, but it should be considered that the present study uses one single oral dose compared to the repeated or continuous dosing through gestation and lactation period common in most other studies. Control mice, male and female, received 10 ml of the 20% fat emulsion vehicle per kg body weight. The control group and the three different Bisphenol A groups each consisted of at least 15 animals from 3 different litters and only male mice were used in the behavioral studies.

The animals were weighed four times during the course of the experiment, first on the day of administration of Bisphenol A (day 10), then at time of separation of males and females (28–30 days of age), then at 10 weeks of age and finally at the time of sacrifice at 6 months of age. Also during the course of the experiment, the animals were checked once a day for clinical signs of toxic effects by visual examination.

2.2. Spontaneous behavior

At an adult age of 2 and 5 months, the male mice were subjected to spontaneous behavior testing in a novel home environment. 12 animals were randomly taken from 3 to 4 different litters in each treatment group at 2 months of age and 14 animals were tested at 5 months of age. The animals were tested between 08:00 and 12:00 h under the same ambient light and temperature conditions as in their cages. Twelve mice were randomly picked from 3 different litters in the different treatment groups. Motor activity was measured for a 60 min period, divided into 3 × 20 min periods, in an automated device consisting of 12 cages (40 cm × 25 cm × 15 cm) placed within two series of infrared beams (low and high level) (Rat-O-Matic, ADEA Elektronik AB, Uppsala, Sweden) (Fredriksson, 1994; Eriksson et al., 2001b). Three variables were measured:

Locomotion: Counting took place when the mouse moved horizontally through the low-level grid of infrared beams.

Rearing: Movement in the vertical plane was registered at a rate of 4 counts/s, when a single high level beam was interrupted, i.e., the number of counts obtained was proportional to time spent rearing.

Total activity: All types of vibration within the cage, i.e., those caused by mouse movements, shaking (tremors), and grooming, were registered by a pick-up (mounted on a lever with a counterweight), connected to the test cage.

2.3. Nicotine-induced behavior

Directly after the second spontaneous behavior test at 5 months of age, nicotine-induced behavior was studied. The 14 male mice tested in the spontaneous behavior test were picked up from the test cage and were directly given a single subcutaneous injection 80 μg nicotine base/kg body weight (nicotine-bi-(+)-tartrate, Sigma, USA) or 10 ml 0.9% NaCl/kg body weight subcutaneously. This amount of nicotine is known to cause an increased activity in normal adult NMRI mice (Eriksson et al., 2000; Viberg et al., 2002). Directly after the nicotine injection, the mice were replaced in the test chamber. The nicotine-induced behavior was measured during another 60 min period (60–120 min) (in the same way as the spontaneous behavior was measured) were motor activity was recorded with regard to the three variables locomotion, rearing, and total activity. The 60 min period was divided into 3 × 20 min periods.

2.4. Elevated plus maze

At the age of 3 months 12 male mice from each of the 4 treatment groups were tested in the elevated plus maze. The test procedure, adopted from Lister, measures the number of entries that the mice made into the open arms, as described below, and the time that they spent there (Lister, 1987). In the plus-maze apparatus, made of plywood, two diametrically placed open arms (white floor with no wall, 30 cm × 6 cm) face two closed arms (black floor with walls, 30 cm × 6 cm × 30 cm) mounted 50 cm above the floor. Testing took place from 09:00 to 14:00 h. The mice were transferred to the test laboratory in their regular "home" cages at least 60 min before they were submitted to the EPM. The mouse was placed on the central platform (white floor, 6 cm × 6 cm) of the apparatus, facing "north" toward the closed arms. A video camera was used to monitor the behavior of the animal. Twelve mice were picked randomly from three different litters in each treatment group and were tested only once in this test. The number of entries into the open and closed arms and the time spent in each of the arms were measured for 5 min. Arm entry was defined as all four paws in the arm. The maze apparatus was cleaned after each trial with a towel soaked in hot water.

2.5. Morris water maze

At the age of 4 months 15 male mice from each of the 4 treatment groups were tested in the Morris swim maze. The Morris type swim maze (Morris, 1981) was a circular grey tub, 102 cm in diameter, filled with water at 23 °C to a depth of 15 cm from the brim. In the centre of the "northeast" quadrant of the tub, a platform was submerged 1 cm beneath the water's surface. The platform consisted of a metal mesh, 12 cm in diameter. The relative positions of the pool and the observer were the same every day the tests were performed. The ability of each mouse to locate the submerged platform was observed for five consecutive days. The mouse was given five trials each day, and its latency to locate the platform was recorded. Testing was conducted between 09:00 and 14:00 h. Before the first trial each day, the mouse was placed on the submerged platform for 30 s. It was then released in the "south" position, facing the wall of the tub, and was allowed 30 s to locate the platform. If the mouse failed the task within 30 s it was gently placed on the platform again for 30 s. After each trial, the mouse remained on the platform for 30 s. All the mice in the study were subjected to five trials each day, and the tests were performed on four consecutive days. In other words, trials 1 through 20 were performed on days 1 through 4. On day 5, the platform was moved to the centre of the tub's "northwest" quadrant, for reversal trials; otherwise the day 5 procedure was identical to that of the previous four days. Latencies to reach the platform were recorded by the observer. The first 20 trials (days 1–4) measured spatial learning ability of the mouse and the last five trials (day 5) its relearning ability.

Table 1
Body weight at different ages after exposure to a single oral dose of Bisphenol A on postnatal day 10.

Treatment	Time of body weight			
	PND 10	4 weeks	10 weeks	6 months
Control	5.2 ± 0.6	17.5 ± 1.8	37.7 ± 2.5	42.0 ± 5.6
Low (0.32 mg/kg bw)	5.4 ± 0.8	15.1 ± 2.5**	36.3 ± 2.3	42.4 ± 2.5
Middle (3.2 mg/kg bw)	5.4 ± 0.4	16.0 ± 1.1*	36.1 ± 2.1	40.7 ± 2.6
High (4.8 mg/kg bw)	5.1 ± 0.5	12.8 ± 1.3***,+++	36.3 ± 1.6	41.6 ± 2.5

Statistical analysis between the different treatment groups at each time point were made with one-way ANOVA with Newman–Keul's post hoc test. Statistical differences between BPA exposed groups and the control group are indicated by: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Statistical differences between the high BPA exposed group (4.8 mg/kg bw) and the middle and low (3.2 and 0.32 mg/kg bw, respectively) BPA exposed group are indicated by: +++$P < 0.001$.

2.6. Statistical analysis

The statistical data concerning body weight were evaluated by one-way ANOVA (analysis of variance) with Newman–Keul's post hoc test.

The statistical data obtained from the spontaneous and nicotine-induced behavior tests were evaluated by ANOVA using a split-plot design, with pairwise testing between treated and control groups, using a Tukey's HSD test (honestly significant differences) test (Kirk, 1968).

The statistical data obtained from the elevated plus maze test were evaluated by one-way ANOVA with Tukey's HSD post hoc test.

The statistical data obtained from day 1 to day 4 in the Morris water maze test were evaluated by general linear model with Tukey's HSD post hoc test. Statistical analysis for the behavioral data of day 5 was submitted to general linear model test with pairwise testing between the three different BPA exposed and vehicle- treated groups, using Tukey's HSD post hoc test.

3. Results

3.1. Body weight

There were no clinical signs of toxic symptoms in the mice at any given time during the experimental period. The mean body weights of the 10 days old animals did not differ significantly ($P > 0.1$) between the control animals and the three different BPA exposed groups (Table 1). At the time of separation the mean body weight of all three BPA exposed groups was significantly lower than the body weight for the control group (Table 1) and the animals exposed to the highest dose of BPA (4.8 mg/kg body weight) on PND 10 showed significantly lower body weights than animals exposed to the lowest (0.32 mg/kg body weight) and middle (3.2 mg/kg body weight) dose of BPA. At 10 weeks of age and at 6 months of age (time of sacrifice) there were no significant differences in the body weights of any of the three groups exposed to BPA on PND 10 compared to the control group.

3.2. Spontaneous behavior in 2 and 5 months old mice

Results from the spontaneous behavioral variables locomotion, rearing, and total activity in 2 months old and 5 months old, NMRI mice, after exposure to a single oral dose of 0.32 mg, 3.2 mg and 4.8 mg, BPA/kg body weight at an age of 10 days, are shown in Figs. 1 and 2, respectively.

Neonatal exposure to BPA showed that there were significant group × period interactions after two months [$F_{6, 88} = 130.03$; $F_{6, 88} = 105.86$; $F_{6, 88} = 138.12$] and after five months [$F_{6, 104} = 127.30$; $F_{6, 104} = 113.77$; $F_{6, 104} = 127.38$], for the locomotion, rearing, and total activity variables, respectively (Figs. 1 and 2). Pairwise testing between BPA exposed and control groups showed a significant dose-related change in all three test variables. In control mice, there was a distinct decrease in activity in all three behavioral variables over the 60-min period, which is normal for control animals and shows the ability to habituate. Mice exposed neonatally to the lowest dose of BPA (0.32 mg/kg body weight) did not significantly differ in activity in any of the three behavioral variables during any of the three 20-min periods. Male mice receiving 3.2 or 4.8 mg BPA/kg body weight showed a dose–response dependent difference in activity for locomotion, rearing and total activity, where these animals showed a significantly decreased activity during the first 20-min (0–20-min) period and a significantly increased activity during the last 20-min period (40–60 min), compared to the control animals.

3.3. Nicotine-induced behavior in 5 months old mice

Results from the nicotine-induced behavior test in 5 months old mice neonatally exposed to 0.32, 3.2 or 4.8 mg BPA/kg body weight, or the 20% fat emulsion vehicle are shown in Fig. 3. The mice were given a single subcutaneous injection of 80 μg nicotine base/kg body weight or 10 ml 0.9% NaCl/kg body weight and observed for another 60 min period (60–120 min from base-line). There were significant treatment × period interactions [$F_{14, 96} = 25.92$, $F_{14, 96} = 9.38$, $F_{14, 96} = 32.51$] for the variables locomotion, rearing, and total activity, respectively. Pairwise testing between the nicotine-injected and saline-injected mice showed a significant increase in response to nicotine in animals neonatally exposed to the vehicle as well as the 3 different doses of BPA, during the first 20-min period (60–80 min) for locomotion and total activity. This increased activity after nicotine injection is normal and shows that both control animals and animals exposed neonatally to BPA react the same way. In the rearing variable the control group and the low dose BPA group responded with a significantly increased activity during the first 20-min period (60–80 min), but the animals exposed on postnatal day 10 to 3.2 or 4.8 mg BPA/kg body weight did not react with an increased activity. It is also worth noticing that, during the third 20-min period (100–120 min), mice exposed neonatally to 3.2 or 4.8 mg BPA/kg body weight and injected with saline or nicotine, were significantly more active than the control group (receiving saline) and the lowest BPA treatment (0.32 mg/kg body weight, receiving saline) and these mice again displayed a hyperactive condition in the locomotion, rearing and total activity variables, as observed during period 40–60 min period in the spontaneous behavior test.

3.4. Elevated plus-maze in 3 months old mice

Results from the elevated plus-maze test in 3 months old mice neonatally exposed to 0.32, 3.2 or 4.8 mg BPA/kg body weight, or the 20% fat emulsion vehicle are shown in Fig. 4. There were no significant differences in the number of entries into open arms or time spent in the open arms [$F_{3, 44} = 0.48$, $F_{3, 44} = 0.87$; pairwise testing $P \geq 0.05$] between control animals and BPA exposed animals.

3.5. Morris swim-maze in 6 months old mice

Results from the Morris swim-maze test in 4 months old mice neonatally exposed to 0.32, 3.2 or 4.8 mg BPA/kg body weight, or the 20% fat emulsion vehicle are shown in Fig. 5. During the acquisition period of spatial learning ability, measured from day 1 to day 4, all mice, regardless of treatment, improved their ability to locate

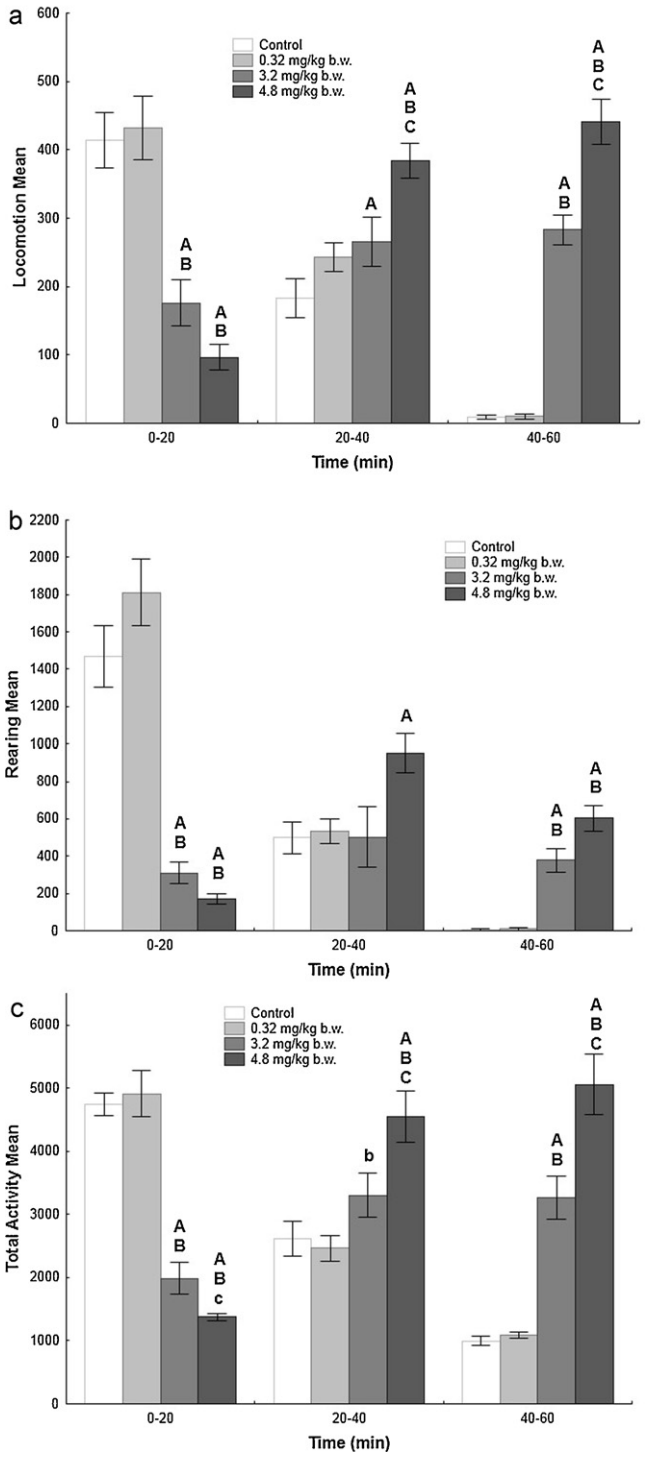

Fig. 1. Spontaneous behavior in 2 months old NMRI male mice exposed to a single oral dose of either 20% fat emulsion vehicle or 0.32, 3.2 or 4.8 mg Bisphenol A/kg body weight (1.4, 14 or 21 μmol Bisphenol A/kg body weight) on postnatal day 10. The data were subjected to an ANOVA with split-plot design and there were significant group × period interactions [$F_{6,88} = 130.03$; $F_{6,88} = 105.86$; $F_{6,88} = 138.12$] for the variables locomotion (a), rearing (b), and total activity (c), respectively. Pairwise testing between BPA exposed and control animals was performed using Tukey's HSD tests. The statistical differences are indicated as: (A) significantly different vs. controls, $P \leq 0.01$; (B) significantly different vs. low dose BPA (0.32 mg/kg body weight), $P \leq 0.01$; (b) significantly different vs. low dose BPA (0.32 mg/kg body weight), $P \leq 0.05$; (C) significantly different vs. middle dose BPA (3.2 mg/kg body weight), $P \leq 0.01$ and (c) significantly different vs. middle dose BPA (3.2 mg/kg body weight), $P \leq 0.05$. The height of the bars represents the mean value ± SD.

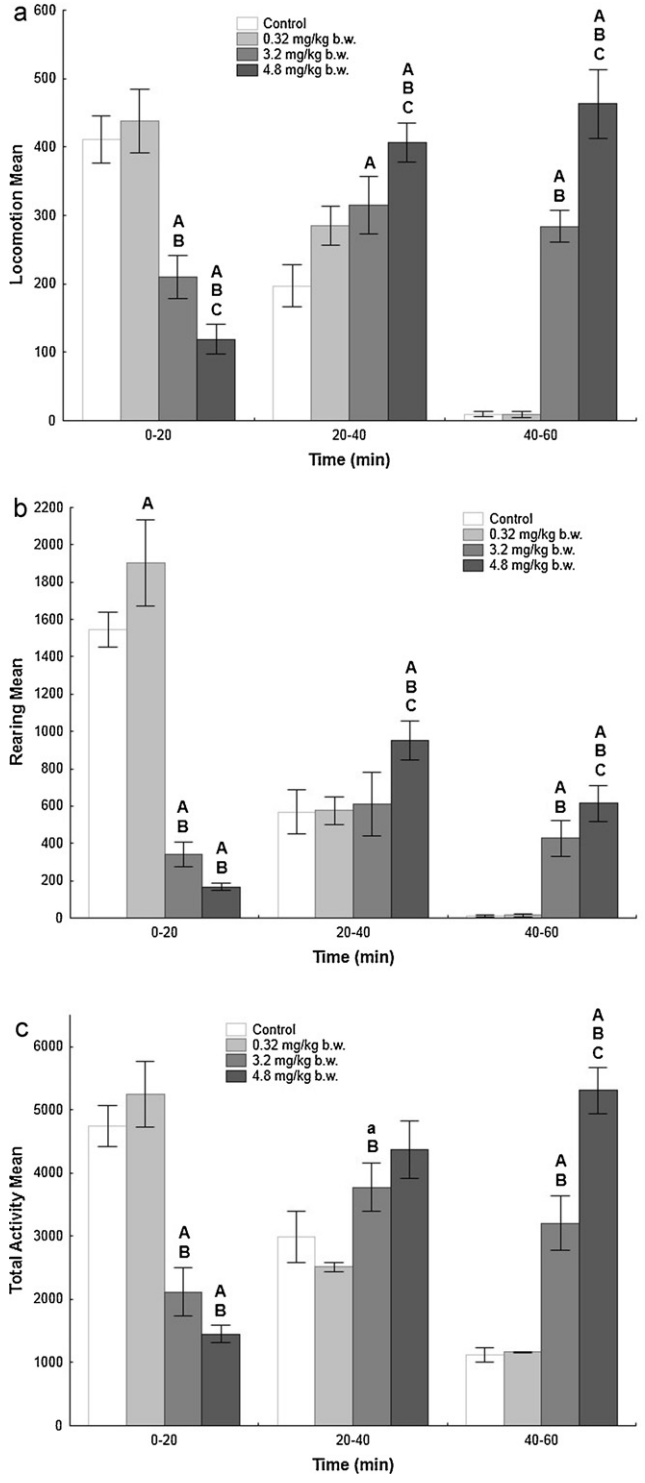

Fig. 2. Spontaneous behavior in 5 months old NMRI male mice exposed to a single oral dose of either 20% fat emulsion vehicle or 0.32, 3.2 or 4.8 mg Bisphenol A/kg body weight (1.4, 14 or 21 μmol Bisphenol A/kg body weight) on postnatal day 10. The data were subjected to an ANOVA with split-plot design and there were significant group × period interactions [$F_{6,104} = 127.30$; $F_{6,104} = 113.77$; $F_{6,104} = 127.38$] for the variables locomotion (a), rearing (b), and total activity (c), respectively. Pairwise testing between BPA exposed and control animals was performed using Tukey's HSD tests. The statistical differences are indicated as: (A) significantly different vs. controls, $P \leq 0.01$; (a) significantly different vs. controls, $P \leq 0.05$; (B) significantly different vs. low dose BPA (0.32 mg/kg body weight), $P \leq 0.01$ and (C) significantly different vs. middle dose BPA (3.2 mg/kg body weight), $P \leq 0.01$. The height of the bars represents the mean value ± SD.

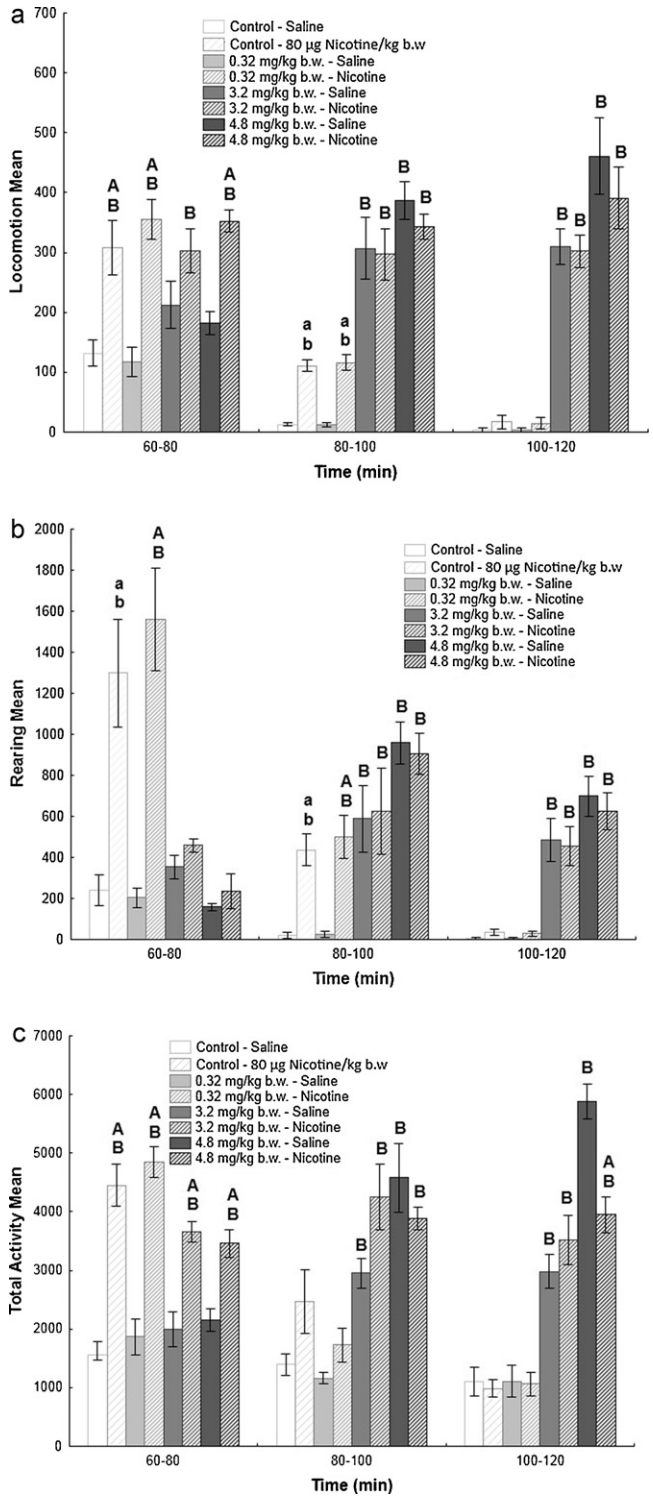

Fig. 3. Nicotine-induced behavior of 5 months old NMRI male mice exposed to a single oral dose of either 20% fat emulsion vehicle or 0.32, 3.2 or 4.8 mg Bisphenol A/kg body weight (1.4, 14 or 21 μmol Bisphenol A/kg body weight) on postnatal day 10. The nicotine-induced behavior was studied by using 80 μg nicotine base per kg body weight subcutaneously and 10 ml 0.9% NaCl/kg body weight subcutaneously. The data were subjected to an ANOVA with split-plot design and there were significant group × period interactions [$F_{14, 96} = 25.92$, $F_{14, 96} = 9.38$, $F_{14, 96} = 32.51$] for the variables locomotion (a), rearing (b), and total activity (c), respectively. Pairwise testing between nicotine-injected animals and saline-injected was performed using Tukey's HSD tests. The statistical differences are indicated as: (A) significantly different vs. internal control group, i.e., saline-injected, $P \leq 0.01$; (a) significantly different vs. internal control group, i.e., saline-injected, $P \leq 0.05$; (B) significantly different vs. saline–saline, $P \leq 0.01$ and (b) significantly different vs. saline–saline, $P \leq 0.05$. The height of the bars represents the mean value ± SD.

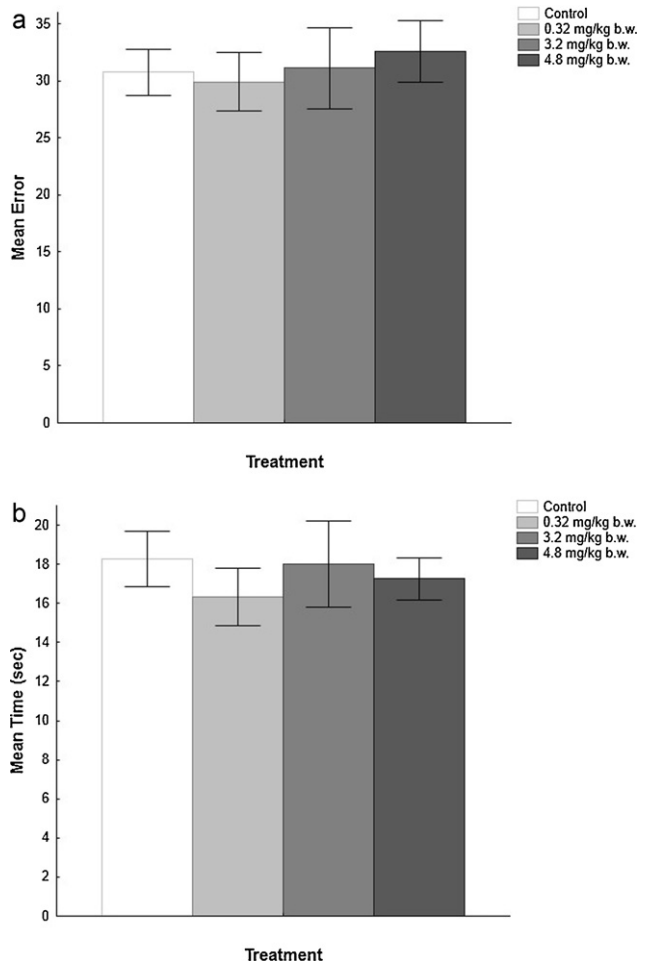

Fig. 4. Elevated plus-maze in 3 months old NMRI male mice exposed to a single oral dose of either 20% fat emulsion vehicle or 0.32, 3.2 or 4.8 mg Bisphenol A/kg body weight (1.4, 14 or 21 μmol Bisphenol A/kg body weight) on postnatal day 10. The data, number of entries into the open arms (a) and the time spent in the open arms (b) were subjected to an ANOVA with pairwise testing between BPA exposed and control animals using Tukey's HSD tests. The height of the bars represents the mean value ± SD.

the platform [$F_{3, 165} = 27.03$]. General linear model test revealed no significant treatment × time interactions among the three different BPA groups and controls [$F_{9, 165} = 1.43$]. On day 5 the platform was relocated for relearning by reversal trials. In the first trial on day 5, control mice displayed longer latency than in the last trial on day 4. This is normal behavior during relearning because, initially, the mouse searches near the previous platform location. The control animals as well as the BPA groups significantly improved their ability to find the new location on day 5 [$F_{4, 220} = 12.89$], and there were no significant treatment × time interactions among the three different BPA groups and controls [$F_{12, 220} = 0.74$] indicating normal relearning in control and BPA exposed animals.

4. Discussion

Recent research from our group has shown that neonatal exposure to different environmental pollutants during a defined critical developmental phase of the nervous system can induce persistent neurotoxic effects in mice. There are several reports showing that fetuses and newborns can be and are exposed to Bisphenol A. The present study shows that a single neonatal exposure to BPA can cause irreversible neurotoxic effects in mice, manifested as altered adult spontaneous behavior connected to reduced cognitive function.

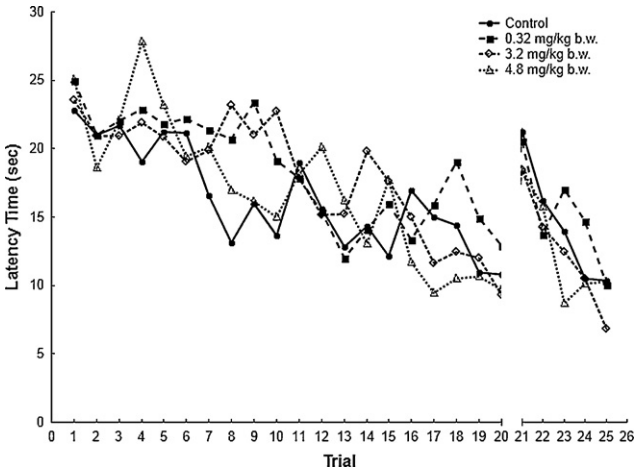

Fig. 5. Swim maze performance in 4 month old NMRI male mice exposed to a single oral dose of either 20% fat emulsion vehicle or 0.32, 3.2 or 4.8 mg Bisphenol A/kg body weight (1.4, 14 or 21 μmol Bisphenol A/kg body weight) on postnatal day 10. Latencies in locating the platform were measured during acquisition period (days 1–4) and during the relearning period (day 5). The behavioral data from day 1 to 4 were submitted to General Linear Model using a split-plot design and pairwise testing using Tukey's HSD test. The behavioral data from day 5 were submitted to General Linear Model using a split-plot design and pairwise testing using Tukey's HSD test.

The BPA exposed mice did not show any clinical signs of toxic symptoms during the experimental period and there was no difference in body weight on the day of BPA exposure (PND 10) between the BPA exposed animals and the control animals. On the day of separation (age of 4 weeks) the body weights of the BPA exposed animals were significantly lower compared to the control animals. Furthermore, when comparing the body weights between the different BPA exposures it was seen that the animals exposed to the high dose of BPA (4.8 mg/kg body weight) had significantly lower body weights than the low and middle dose groups. When analyzing the body weights again at 10 weeks and 6 months of age there were no significant differences between the BPA exposed animals and the controls. These results are supported by results from a recent study by Nakamura et al. (2011), where exposure to BPA from gestational day 0 to postnatal day 21, in mice, gave rise to lower weight gain at PND 20–21, but at 10 weeks of age this difference in body weight had disappeared, just as in our present study.

In 2 months old mice exposed to BPA a clear neurotoxic effect was seen, manifested as altered adult spontaneous behavior in novel home environment and reduced habituation. Habituation, here defined as a decrease in the three behavioral variables locomotion, rearing and total activity in response to the diminished novelty of the test chamber over the 60-min period, was observed in the control animals and in the mice neonatally exposed to the low dose of BPA (0.32 mg/kg body weight). In the animals neonatally exposed to the middle or high dose of BPA (3.2 or 4.8 mg/kg body weight, respectively) a significantly decreased activity during the first 20-min period (0–20 min) was seen, but during the last 20-min period (40–60 min) a significantly increased activity was evident, compared to the control animals and the lowest dose of BPA. In a recent study by Nakamura et al. (2011) it was seen that prolonged prenatal and lactational exposure to BPA induced a decreased activity in the open-field test at 10 weeks of age. This test was performed for 10 min and the results support our findings, since we also observed a decreased activity during the first 20 min of the spontaneous behavior test, in 2 months old animals. This type of alteration in spontaneous behavior has earlier been seen after neonatal exposure to other environmental pollutants, for example brominated flame retardants and perfluorinated compounds (Johansson et al., 2008a; Viberg et al., 2003a,b).

When comparing the animals exposed to the different doses of BPA a clear dose–response relation is seen in both 2 and 5 months old mice. As mentioned the low dose did not differ from the control animals, but both the middle and the high dose animals significantly differed from the control and low dose animals. Furthermore, the animals exposed on postnatal day 10 to the high dose of BPA (4.8 mg/kg body weight) were significantly more hypoactive during the first 20-min period (0–20 min) and significantly more hyperactive during the last 20-min period (40–60 min) compared to the animals exposed to the middle dose of BPA (3.2 mg/kg body weight).

The neurotoxic effects in adult spontaneous behavior were again tested at 5 months of age. The same pattern of neurotoxic effects was seen again, with the low dose not differing from the control animals, but the middle and high dose significantly differing from both the control and low dose. The dose–response relation was still evident. These results indicate that the developmental neurotoxic effects from neonatal BPA exposure are long-lasting and probably irreversible, as earlier been seen with other environmental pollutants (Eriksson, 1998; Johansson et al., 2008a; Viberg et al., 2003a,b). In addition, it is interesting to note that when looking at the nicotine-induced behavior test it is clear that the hyperactivity seen during the last 20-min period of the spontaneous behavior test (40–60 min) is not transient over the next 60-min period either, since animals exposed neonatally to the middle or high dose of BPA, receiving saline in this test, are still hyperactive active compared to the controls during the last 20-min period of the nicotine-induced behavior test (100–120 min). This further strengthens the hyperactive condition in neonatally exposed mice, resulting in an inability of appropriate integration of new sensory information in a novel home environment.

The results from the elevated plus-maze test did not reveal any differences between the control animals and the BPA exposed animals. These results are supported by the recent study by Nakamura et al. (2011) where no significant effects were seen in elevated plus-maze in number of entries into open arms and total time spent in open arms. However, that study showed a weak effect in total distance moved, which may be attributed to the fact that the BPA exposed animals actually moved significantly less than the control animals during the 10 min period measured in the open-field test. On the contrary, there are studies, with prolonged gestational BPA exposure, that display differences in the elevated plus-maze (Cox et al., 2010; Patisaul and Polston, 2008). Therefore, it is hard to draw any hard conclusions about the anxiety-like behaviors measured in this type of behavior test, since the route and timing of exposure as well as the dose seem to be of importance for the behavioral outcome.

The spatial learning ability was evaluated in Morris swim-maze and did not reveal any differences between the control animals and the neonatally BPA exposed animals. These results are also supported by the above mentioned study by Nakamura et al. (2011) where no significant effects were seen in the Morris swim-maze test, which has also been reported for juvenile male rats after repeated oral exposure to BPA from postnatal day 0–14 (Carr et al., 2003). Barnes maze test is another type of test to evaluate spatial learning and in mice exposed pre- and postnatally to BPA no effects were seen (Ryan and Vandenbergh, 2006), indicating that BPA does not likely affect spatial learning.

In several of our previous studies on developmental neurotoxicity of environmental pollutants, for example brominated flame retardants, chlorinated biphenyls and perfluorinated compounds, the altered spontaneous behavior has been accompanied by marked changes in the response to adult nicotine exposure (Eriksson, 1998; Eriksson et al., 2000; Johansson et al., 2008b;

Viberg et al., 2002). The normal reaction to the given nicotine dose is an instant increase in the level of activity, but animals neonatally exposed to the mentioned environmental pollutants react totally opposite, with an instant significant decrease in the level of activity. These changes are indications of that the cholinergic transmitter system is affected. In several cases we have also seen that the compounds changing the reaction to the nicotine also induce changes in the density of cholinergic nicotinic and muscarinic receptors in the brain (Ahlbom et al., 1994; Eriksson, 1998; Viberg et al., 2003a, 2004, 2005, 2007). Since the neonatal BPA exposure in the present study did not alter the adult reaction to nicotine in the same magnitude as earlier seen for other environmental pollutants it is possible that BPA induces the neurotoxic effects through a different mechanism than for example brominated flame retardants, chlorinated biphenyls and perfluorinated compounds, but at the same time the involvement of the cholinergic system cannot be excluded. On the other hand there are indications that developmental BPA exposure can affect the cholinergic system. In a study by Miyagawa et al. (2007) prenatal and neonatal exposures to BPA failed to induce anxiogenic effects and learning impairment, but the prenatal and neonatal exposures to BPA showed a dramatic reduction in choline acetyltransferase-like immunoreactivity in the hippocampus of mice, which is a marker of acetylcholine (ACh) production. Further studies are needed in order to establish the possible effects exerted by BPA on the developing cholinergic system.

In conclusion, the present study shows that neonatal exposure to BPA can cause developmental neurotoxic effects, manifested as altered adult spontaneous behavior in a novel home environment. These effects are dose–response related and long-lasting or irreversible. Since this study did not indicate any major effects on the cholinergic system in the induction of the neurotoxic effects further studies are needed to elucidate the mechanistic background to the behavioral effects. The similarities between these effects and effects seen after a single neonatal exposure to other persistent organic pollutants motivate studies on interactive effects of BPA exposure in combination with other known neurotoxic environmental pollutants. It is also of great interest to compare the present study with a similar study in female mice, since most of the published studies so far focus on the estrogenic and anti-estrogen action of BPA and the sexually dimorphic effects on behavior.

Conflict of interest

No competing interest.

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