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SHORT COMMUNICATION

Prolonged exposure to a low-dose of bisphenol A increases spontaneous motor activity in adult male rats

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Abstract We investigated the effects of bisphenol A (BPA), an environmental endocrine-disrupting chemical, on spontaneous motor activity in adult male rats. The rats were implanted intraperitoneally with mini-osmotic pumps containing either BPA (50 μg/kg body weight per day) in sesame oil (BPA-treated group) or sesame oil only (vehicle-treated group). Spontaneous motor activity during a 24-h period was measured over 5 days from day 9 to day 13 after implantation using an animal movement analysis system. Spontaneous motor activity during the last 2 h of the dark phase and during the first 1-h of the light phase was increased in the BPA-treated group. Total spontaneous motor activity during the 12-h light phase, but not the 12-h dark phase, was higher in the BPA-treated group than in the vehicle-treated group. These findings suggest that BPA may induce hyperactivity in adult male rats during the 12-h light phase, especially during the 2 h immediately preceding sleep-onset and 1 h immediately following sleep-onset.

Keywords Bisphenol A · Low dose · Spontaneous motor activity · Light phase · Adult male rats

Introduction

Bisphenol A (BPA) is an environmental endocrine-disrupting chemical that is ubiquitously present in the environment. BPA leaches from products containing polycarbonate plastics, such as baby bottles [1], food containers [2], and beverage containers [3], and sealants and dental composite resins [4]. Microgram amounts of BPA have been found in both extracted foods and water from autoclaved cans (4–23 μg/can) [2] and in saliva (9–931 μg) during the first 1 h after the application of a dental sealant (50 mg) [4]. The maximum amount (931 μg) recorded in saliva has been reported to represent 13.3 μg/kg body weight for a person weighing 70 kg and 37.2 μg/kg body weight for a child weighing 25 kg [5]. BPA has also been found in serum, breast milk, amniotic fluid, and placental tissue at birth in humans [6–8]. The concentration of BPA in various human biological fluids has been reported to be 2.0 ± 0.8 ng/ml in non-pregnant woman serum [7], 2.2 ± 1.8 ng/ml in fetal serum [7], 8.3 ± 8.9 ng/ml in amniotic fluid obtained at 15–18 weeks gestation [7], and 0.61 ± 0.20 ng/ml in breast milk [8]. These findings indicate that humans are persistently exposed to BPA and routinely ingest BPA.

In rodents, BPA induces abnormalities in nonreproductive behaviors, such as spontaneous motor activity, locomotor activity, and aggressive behavior [9–15]. In male rats aged 4–5 weeks, postnatal exposure to BPA increases spontaneous motor activity [9–12], while perinatal and postnatal exposure to BPA increases locomotor activity in female mice at 30 days of age and decreases it in male mice at the same age compared with the sex- and age-matched controls [13]. Pubertal exposure to BPA decreases locomotor activity in female mice at 60–70 days of age [14], and perinatal exposure to BPA temporarily activates aggressive behavior in male mice at 8 weeks of age [15]. Thus, exposure to BPA during the perinatal and/or postnatal periods leads to behavioral alterations in adulthood. However, there has also been a report of the oral administration of BPA to pregnant rats from gestational day 3

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until postnatal day 20 not affecting total spontaneous motor activity and the immobile time during the dark phase in male offspring at 12 weeks of age [16].

The question of whether the exposure of adult rodents to BPA induces behavioral alterations has not yet been answered. In the study reported here, we investigated whether the prolonged exposure of 8-week-old male rats to BPA would cause alterations in spontaneous motor activity using an animal movement analysis system.

Materials and methods

Animals and materials

Male Sprague Dawley (SD) rats (8 weeks of age) were obtained from Japan SLC (Shizuoka, Japan). ALZET mini-osmotic pumps (model 2004; pumping rate 0.25 μl/h; duration 28 days) were obtained from DURECT Co. (Cupertino, CA). BPA was obtained from Tokyo Kasei Co. (Tokyo, Japan), and sesame oil was obtained from Sigma-Aldrich (St. Louis, MO).

Treatment of animals

Male SD rats were maintained in a temperature- and light-controlled room (12/12-h light/dark cycle; lights on at 1900 hours and off at 0700 hours) and were allowed free access to a standard laboratory chow (CE-7 diet; Clea Japan Inc., Tokyo, Japan) and tap water in glass bottles. The rats were weighed and implanted intraperitoneally with ALZET mini-osmotic pumps under nembutal anesthesia. BPA was dissolved in sesame oil at a concentration of 1.83 ± 0.01 μg/μl ($n = 7$). The pumps were designed to deliver 50 μg of BPA/kg body weight per day based on initial body weight (BPA-treated group). The vehicle-treated group was implanted with the pump containing sesame oil only. The initial body weight of the rats was 219 ± 3 g in the vehicle-treated group ($n = 7$) and 220 ± 1 g in the BPA-treated group ($n = 7$). There were no differences in final body weight on day 28 after implantation between the two groups [vehicle-treated group ($n = 7$) 346 ± 11 g; BPA-treated group ($n = 7$) 350 ± 9 g].

All animals were treated humanely, and care was taken to alleviate suffering. The experimental protocols were reviewed and approved by the local Animal Ethics Committees at the Ehime Prefectural University of Health Sciences (Approval No. 14), Ehime, Japan.

Behavioral experiments

Spontaneous motor activity was measured using an animal movement analysis system (Scanet SV-10; MATYS, Toyama, Japan). The system consisted of a rectangular enclosure (480 × 300 mm) with side walls (height 60 mm). The side walls were equipped with 144 pairs of photosensors located 30 mm from the bottom edge and placed at 5-mm intervals. Each pair of photosensors scanned every 0.1 s to detect animal movements. Each rat was individually placed in a standard rat cage (445 × 276 × 204 mm) which was fixed at the center of the apparatus. The data on spontaneous motor activity were recorded separately for 48 consecutive periods of 30 min each. Spontaneous motor activity during a 24-h period was monitored over 5 days, from day 9 until day 13 after implantation.

Statistical analyses

Significant differences between the two independent groups were analyzed with the Mann–Whitney U test using StatView ver. 5.0 software (SAS Institute, Cary, NC). Significant differences among multiple groups in Fig. 2 were evaluated with analysis of variance (ANOVA). For all statistical analyses, the criterion for significance was $p < 0.05$. All values are expressed as the mean ± standard error of the mean (SEM).

Results

The mini-osmotic pumps containing either vehicle or BPA (50 μg/kg body weight per day) were implanted intraperitoneally into 8-week-old male rats, and spontaneous motor activity during a 24-h period was measured over 5 days from day 9 until day 13 after implantation using an animal movement analysis system. Figure 1 shows the mean spontaneous motor activity during the 5-day experimental period. Spontaneous motor activities at 1900, 2200 and 0200 hours were 1.41-, 1.73- and 1.63-fold, respectively, higher in the BPA-treated group [5,812 ± 529 counts/h ($n = 35$) at 1900 hours, $p < 0.05$; 1,406 ± 206 counts/h ($n = 35$) at 2200 hours, $p < 0.05$; 1,095 ± 178 counts/h ($n = 35$) at 0200 hours, $p < 0.05$] than in the vehicle-treated group [4,109 ± 418 counts/h ($n = 35$) at 1900 hours; 813 ± 145 counts/h ($n = 35$) at 2200 hours; 672 ± 119 counts/h ($n = 35$) at 0200 hours]. Spontaneous motor activity from 1700 to 1800 hours was 1.17-fold higher in the BPA-treated group [15,655 ± 634 counts/2 h ($n = 35$), $p < 0.005$] than in the vehicle-treated group [13,371 ± 520 counts/2 h ($n = 35$)]. All rats in both the vehicle-treated and BPA-treated groups were much more active during the dark phase than in the light phase. BPA did not alter the rhythm of spontaneous motor activity (Fig. 1).

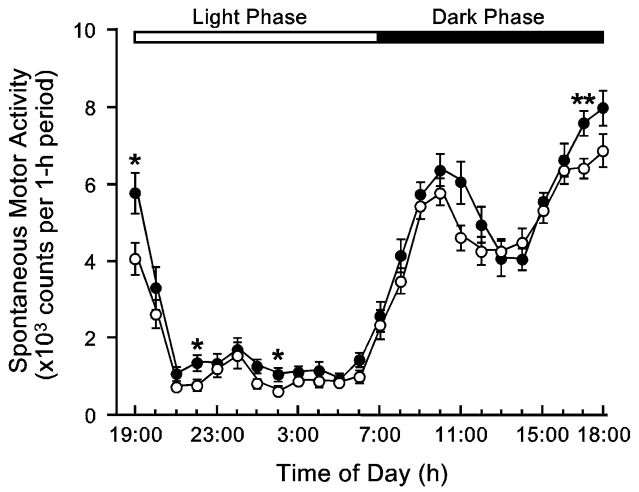

Fig. 1 Effects of bisphenol A (BPA) on spontaneous motor activity. Spontaneous motor activity was measured over 5 days from day 9 until day 13 after implantation. The mean spontaneous motor activity during the 5-day experimental period is as the mean ± standard error of the mean (SEM) for seven rats. $*p < 0.05$, $**p < 0.005$ (compared with the values of the vehicle-treated group at the corresponding time by the Mann–Whitney *U* test). *Open circle* Vehicle-treated group, *filled circle* BPA-treated group

Figure 2 shows the total spontaneous motor activity during the whole day, the 12-h light phase, and the 12-h dark phase for each day. In the whole day, BPA increased total spontaneous motor activity by 15 % on day 9, 18 % on day 10, 15 % on day 11, 16 % on day 12, and 13 % on day 13 compared with the vehicle-treated group on the corresponding day (Fig. 2a), but these differences were not significant [$F(9,60) = 1.896$, $p = 0.0698$ by ANOVA]. The sum of total spontaneous motor activity in the whole day during the 5-day experimental period was 1.15-fold higher in the BPA-treated group [442,127 ± 23,592 counts/5 days ($n = 7$), $p < 0.05$] than in the vehicle-treated group [383,317 ± 18,274 counts/5 days ($n = 7$)] (Fig. 3a). In the light phase, BPA increased total spontaneous motor activity by 27 % on day 9, 36 % on day 10, 34 % on day 11 ($p < 0.05$), 48 % on day 12 ($p < 0.05$), and 29 % on day 13 compared with the vehicle-treated group on the corresponding day [$F(9,60) = 3.136$, $p = 0.0037$] (Fig. 2b). The sum of total spontaneous motor activity in the light phase during the 5-day experimental period was 1.34-fold higher in the BPA-treated group [111,175 ± 7,266 counts/5 days ($n = 7$), $p < 0.01$] than in

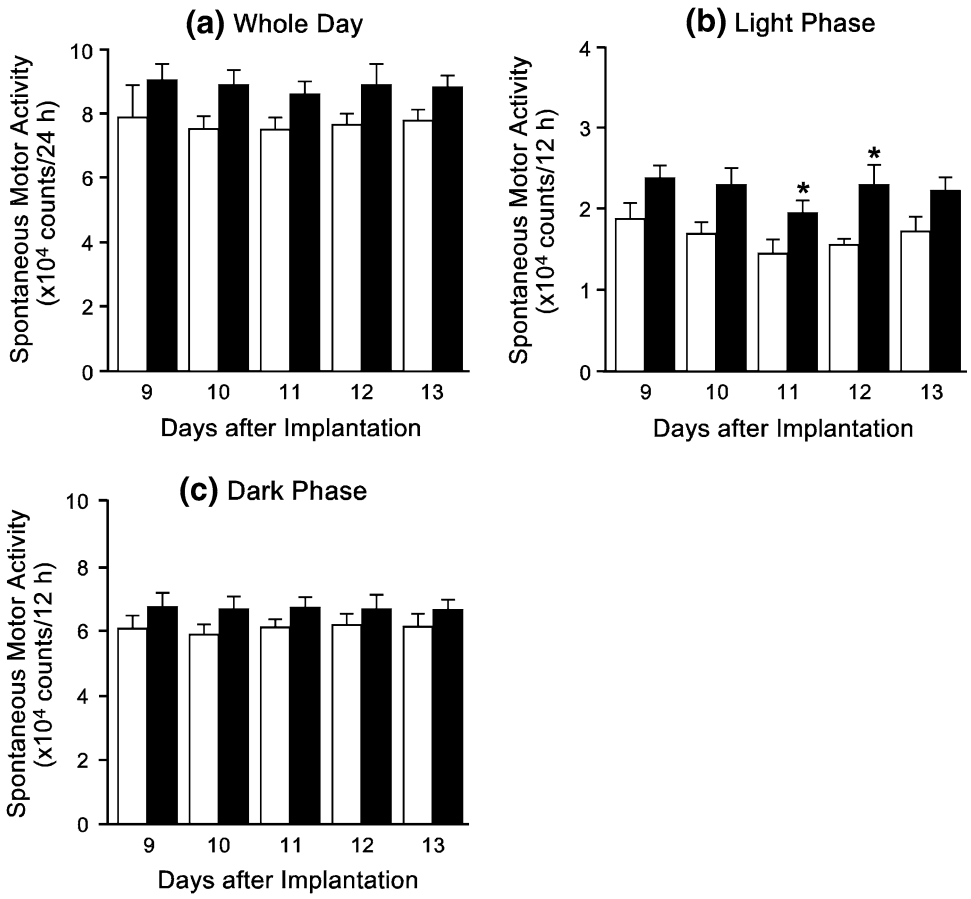

Fig. 2 Effects of BPA on total spontaneous motor activity in the whole day (**a**), the light phase (**b**), and the dark phase (**c**) on each day. Values are the mean ± SEM for seven rats. $*p < 0.05$ (compared with the values of the vehicle-treated group on the corresponding day by the Mann–Whitney *U* test)

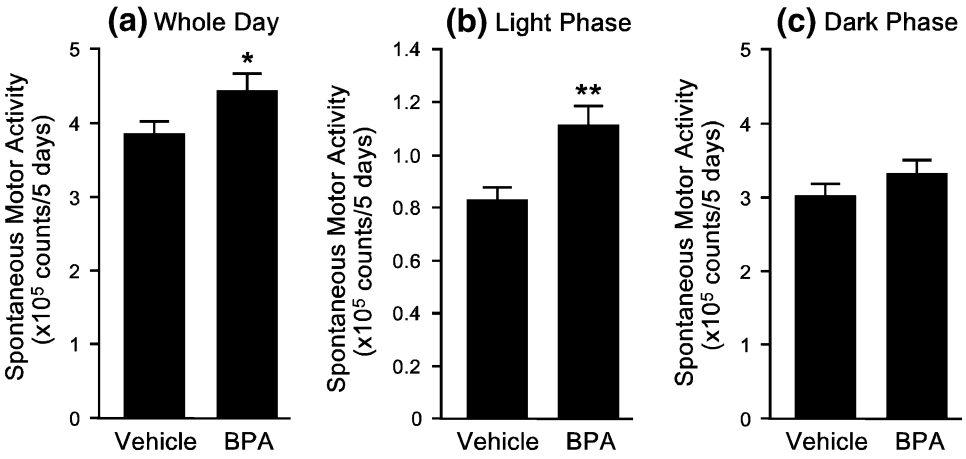

Fig. 3 Effects of BPA on the sum of total spontaneous motor activity in the whole day (**a**), the light phase (**b**), and the dark phase (**c**) during the 5-day experimental period. Values are the mean ± SEM for seven rats. $*p < 0.05$, $**p < 0.01$ (compared with the values of the vehicle-treated group by the Mann–Whitney *U* test)

the vehicle-treated group [82,774 ± 5,063 counts/5 days ($n = 7$)] (Fig. 3b). In the dark phase, BPA did not increase total spontaneous motor activity on each day compared with the vehicle-treated group on the corresponding day [$F(9,60) = 0.714$, $p = 0.6942$] (Fig. 2c). No difference in the sum of total spontaneous motor activity in the dark phase during the 5-day experimental period was observed between the BPA-treated group [330,952 ± 18,602 counts/5 days ($n = 7$)] and the vehicle-treated group [300,542 ± 17,138 counts/5 days ($n = 7$)] (Fig. 3c).

Discussion

The aim of this study was to investigate whether BPA induces behavioral alterations in adult rats. To this end, we administered BPA intraperitoneally to 8-week-old male rats using a mini-osmotic pump and measured spontaneous motor activity over 5 days from day 9 to day 13 after pump implantation. The results of our spontaneous motor activity measurements showed that prolonged exposure of adult rats to BPA increased total spontaneous motor activity in the light phase during the 5-day experimental period compared with the vehicle-treated group. In the dark phase, total spontaneous motor activity of the BPA-treated group did not differ from that of the vehicle-treated groups. These findings suggest that BPA induced hyperactivity in the light phase, but not the dark phase, in adult rats. Moreover, we found that BPA increased spontaneous motor activity during the last 2 h of the dark phase and during the first 1 h of the light phase, thus suggesting that BPA induced hyperactivity before and after sleep-onset.

In our study, we consistently administered BPA intraperitoneally at 50 μg/kg body weight per day to male rats from 8 to 11 weeks of age and found the induction of hyperactivity in the light phase over the 5 days from day 9 to day 13 after BPA administration. Masuo et al. [9] and Ishido et al. [10] administered BPA intracisternally at 0.2 μg/10 g body weight into 5-day-old male rats and found the induction of hyperactivity in the dark phase at 4–5 weeks of age. These doses of BPA are below or equivalent to the "Tolerable Daily Intake" as established by the European Food Safety Authority [EFSA Press Release: EFSA update advice on bisphenol A (30 September 2010); available at http://www.efsa.europa.eu/en/press/news/cef100930.htm]. In addition, these authors reported that the intracisternal administration of BPA at higher doses (2 and 20 μg/pup), which are below the "no observed adverse effect level" of BPA (50 mg/kg body weight per day) [17], also induced hyperactivity in the dark phase [9, 10]. Moreover, Ishido et al. [12] reported that oral administration of BPA at 600 μg/pup per day to male rats daily from 5 days to 3 weeks of age induced hyperactivity in the dark phase, but not the light phase, at 4–5 weeks of age. Taken together, the differences between our findings and those of previous studies may be due to the different timing of BPA exposure, but not to different doses and routes of BPA administration.

To date, the majority of studies looking at the impact of BPA on behavior have focused on exposure to BPA during the perinatal and/or postnatal periods [9–16] because the nervous system during these periods seems to be vulnerable to this chemical [18]. For example, Ishido et al. [10] found that a single intracisternal injection of BPA to 5-day-old male rats decreased gene expression levels of the dopamine D4 receptor in the striatum at 4 weeks of age and the dopamine transporter in the midbrain at 8 weeks of age. In our study, we administered BPA intraperitoneally into 8-week-old male rats because the nervous system has been reported to continue to remodel and change not just early in development but throughout the entire period of development and even during adulthood [18]. Whether BPA reaches the brain and affects the nervous system in adult rats remains unclear from our results. Funabashi et al. [19] reported that a single subcutaneous injection of BPA into 8-week-old ovariectomized rats increased the expression of

progesterone receptor mRNA in the frontal cortex of the brain and decreased it in the temporal cortex. Moreover, oral administration of BPA to adult female rats from the day of mating until weaning of the young has been reported to decrease the number of estrogen receptor-immunoreactive cells in the arcuate nucleus of hypothalamus of lactating rats [20]. These findings indicate that BPA passes through the blood−brain barrier and affects the brain in adult rats. The mechanism by which BPA administered intraperitoneally to adult rats induced this hyperactivity in the light phase is now the subject of a continuing investigation.

In conclusion, prolonged exposure of adult male rats to a low-dose of BPA induced hyperactivity during the 12-h light phase. BPA also induced hyperactivity during the 2 h immediately preceding sleep-onset and during the 1 h immediately following sleep-onset, suggesting that it may induce delayed sleep-onset.

Conflict of interest None.

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