3 days per week. At 15 weeks of age, behavioral tests (open-field test and PPI test) were performed. Care and treatment of rats were in accordance with the guidelines established by the Animal Experimentation and Ethics Committee of Kitasato University School of Medicine and were approved by the committee.

The Open-Field Test

Each rat was placed in the center of a square white box (width, 1.0 m; height, 0.4 m) for the open-field test as described in previous studies (Inada et al. 2003; Kobayashi et al. 2004). The locomotor behavior of each rat was recorded with a videocamera for 30 min. Videos were subsequently analyzed using an Image Open Field 2.15r (Obara Medical, Tokyo) and the total locomotor distance and locomotor distance were calculated every 5 min. During 30-min observations, the following behaviors were recorded as indexes: time of the first grooming for more than 3 s and instances of wall rearing (WR), center rearing (CR), face washing (FW), body washing (BW), defecation (fecal boil), and urination (urine deposited).

The PPI Test

The morning after the open-field test, the PPI test was performed using a method described in previous studies (Inada et al. 2003; Kobayashi et al. 2004). A Startle Response System SR-LAB ABS system (San Diego Instruments, San Diego, CA, USA) was used, which was composed of a startle chamber equipped with an electric sensor and a speaker mounted 24 cm above the floor to create an acoustic noise burst. Each rat was placed in a cylindrical holder in the chamber and allowed to acclimate for 5 min before the test session. In the PPI test session, four types of acoustic stimulation—a startle pulse of a burst of 120 dB (P alone) and combined trials of prepulse (PP; a burst of 70, 75, or 80 dB) followed by a pulse of 120 dB (PP70&P, PP75&P, or PP80&P)—were given to each rat in pseudorandom order. The numbers of acoustic stimulations in a test session were 11 for P alone, PP70&P, and PP75&P and 10 each for PP80&P and for no acoustic stimulation. The startle response was measured by an electric sensor. The mean value of the responses for respective stimulations in a session was calculated.

The percentage PPI of a startle response was calculated by the following formulas.

$$\%\text{PPI at PP70} = [1 - (\text{PP70\&P/P alone})] \times 100$$

$$\%\text{PPI at PP75} = [1 - (\text{PP75\&P/P alone})] \times 100$$

$$\%\text{PPI at PP80} = [1 - (\text{PP80\&P/P alone})] \times 100$$

Statistical Analyses

For dams, the mean values of body weight and daily intake of food and water and the number of F_1 rats, the number of implantations, and the number of implantations/number of F_1 rats were calculated. For F_1 rats, the mean values of body weight, the daily intake of food and water per body weight, the organ weight, and the indexes of the behavioral tests were calculated. Data were analyzed by Student's t test or Mann-Whitney U test for dams, and one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) test for F_1 rats, using Statview J-5.0 software (SAS Institute, Cary, NC, USA). The level of significance was $p < 0.05$.

Results

For dams, the mean value ± standard error of initial body weight in the control and 125-ppm TBT-treated groups was 240.8 ± 7.2 and 245.0 ± 8.7 g, respectively. The mean ± SE body weight at delivery was 418.8 ± 12.6 g for the control and 382.5 ± 10.5 g for the 125-ppm-treated groups. There were no significant differences between the groups for initial body weight or body weight at delivery. The mean value ± SE of the daily intake of food by the time of delivery was 80.0 ± 2.5 g/kg body weight for the control group and 65.1 ± 2.5 g/kg body weight for the treated group. The mean dose of TBT chloride exposure via their food in the 125-ppm groups estimated for each daily body weight and food intake by the time of delivery was 8.13 ± 0.13 mg/kg body weight.

The mean number of implantations was 15.0 ± 0.9 for the control and 13.3 ± 1.0 for the 125 ppm-treated groups. The mean number of live F_1 rats at delivery was 13.4 ± 0.9 for the control and 10.2 ± 1.5 for the 125 ppm-treated groups. The mean percentage of live F_1 rats among the number of implantations was 91.7% ± 2.9% for the control and 64.9% ± 8.3% for the 125 ppm-treated groups. These were significantly different by Mann-Whitney U test.

Figure 2 illustrates the mean body weight of female F_1 rats exposed to TBT chloride via the placenta, their dams' milk, and/or their food over the observation periods, which were from 9 weeks of age (observation day 0) to 15 weeks of age (observation day 43). On day 0, the mean body weight ± SE was 226.9 ± 4.8 g for the CC group, 192.5 ± 3.2 g for the TC group, 227.6 ± 4.7 g for the CT group, and 196.2 ± 6.6 g for the TT group. The mean values in the TC and TT groups were significantly lower than those in the CC and CT groups. Over the treatment period, the mean values of body weight in the TC and TT

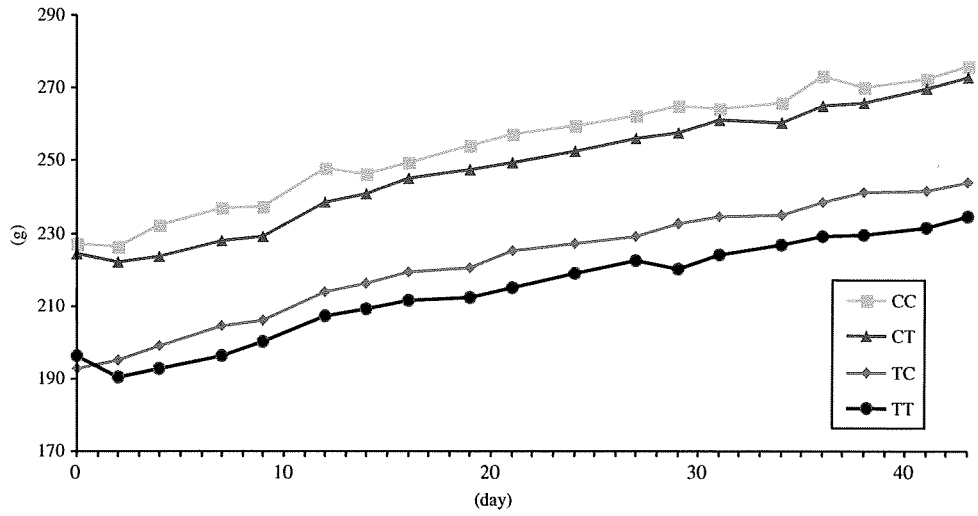

Fig. 2 Daily change in mean body weight of female F_1 rats exposed to TBT chloride via the placenta and their dams' milk and/or food. Day 0 = 9 weeks of age. *CC* control-control (no exposure), *TC* TBT-control (exposure to TBT via the placenta and their dams' milk), *CT* control-TBT (exposure to TBT via food at 9–15 weeks of age), *TT* TBT-TBT (exposure to TBT via the placenta, their dams' milk, and their food at 9–15 weeks of age). Mean values are indicated

groups were significantly lower than those in the CC and CT groups. On day 43, the mean body weight ± SE was 276.1 ± 4.3 g for the CC group, 243.9 ± 5.4 g for the TC group, 275.8 ± 6.1 g for the CT group, and 234.3 ± 6.4 g for the TT group.

Figure 3 illustrates the mean food consumption/body weight of the female F_1 rats over the observation period. The mean value in the CT group was significantly lower than that in the CC group on days 1–17, 19–33, and 38–43. The mean value in the TT group was significantly lower than that in the CC group on days 1–11, 14–30, and 34–43. In the comparison to the TC group, the mean value in the CT group was significantly lower on days 9–11, 14–18, and 31–40, and that in the TT group was significantly lower on days 1–33 and 38–40. The mean value in the TT group was also significantly lower than the mean value in the CT group on days 14–18, 20–21, 31–33, and 41–43. The mean food consumption/body weight ± SE over the observation period was 65.3 ± 0.6 g/kg for the CC group, 66.6 ± 1.0 g/kg for the TC group, 52.5 ± 1.2 g/kg for the CT group, and 55.8 ± 2.2 g/kg for the TT group. The mean dose of TBT chloride exposure via food estimated at each daily body weight and food intake was 6.56 ± 0.15 mg/kg body weight in the CT group and 6.97 ± 0.27 mg/kg body weight in the TT group.

Figure 4 illustrates the mean water consumption/body weight of female F_1 rats over the observation period. The mean value in the CT group was significantly lower than that in the CC group on days 10–14 and days 20–21. The mean value in the TT group was significantly lower than that in the CC group on days 3–7, 10–14, and 20–21. The mean value in the TT group was also significantly lower than that in the CT group on days 1–4 and that in the TC group on days 3–7 and 20–21. The mean water consumption/body weight ± SE over the observation period was 139.3 ± 11.4 g/kg for the CC group, 128.5 ± 6.8 g/kg for the TC group, 120.4 ± 7.2 g/kg for the CT group, and 115.1 ± 10.5 g/kg for the TT group.

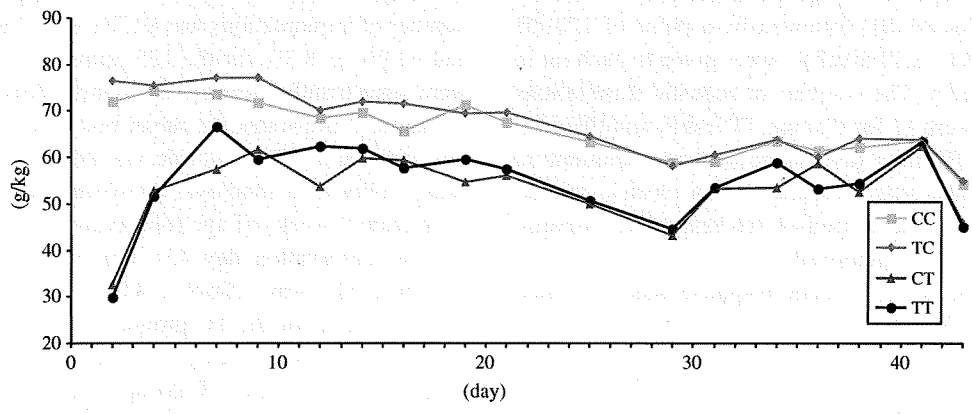

Fig. 3 Daily changes in mean food intake/body weight of female F_1 rats exposed to TBT chloride via the placenta and their dams' milk and/or food. Day 0 = 9 weeks of age. *CC* control-control (no exposure), *TC* TBT-control (exposure to TBT via the placenta and their dams' milk), *CT* control-TBT (exposure to TBT via food at 9–15 weeks of age), *TT* TBT-TBT (exposure to TBT via the placenta, their dams' milk, and their food at 9–15 weeks of age). Mean values are indicated

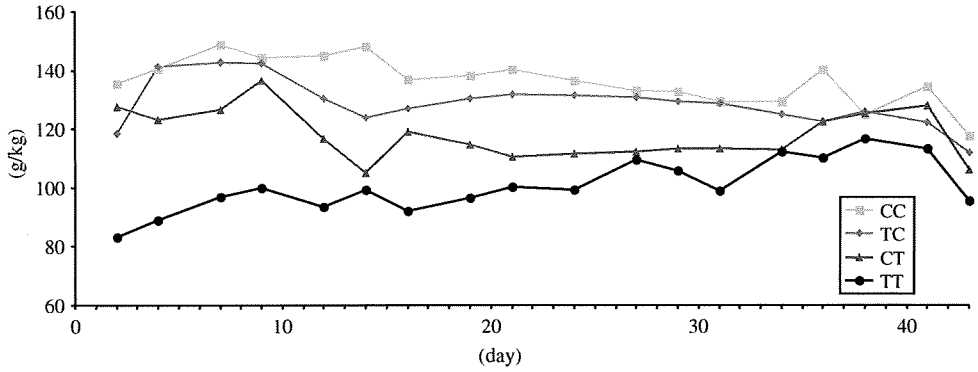

Fig. 4 Daily changes in mean water intake of female F_1 rats exposed to TBT chloride via the placenta and their dams' milk and/or food. Day 0 = 9 weeks of age. *CC* control-control (no exposure), *TC* TBT-control (exposure to TBT via the placenta and their dams' milk), *CT* control-TBT (exposure to TBT via food at 9–15 weeks of age), *TT* TBT-TBT (exposure to TBT via the placenta, their dams' milk, and their food at 9–15 weeks of age). Mean values are indicated

The locomotor distance every 5 min for 30 min in F_1 rats in the open-field test is illustrated in Fig. 5. For the locomotor distance every 5 min compared to the CC group, the mean values in the TC group were significantly lower during 5–10 and 15–25 min. The mean value in the CT group during 15–20 min was significantly lower than that in the CC group. The mean value in the TT group was significantly lower than that in the CC group during every 5 min after 5 min. That in the TT group was also significantly lower than that in the CT group during 20–25 and 25–30 min. For the total locomotor distance, the mean value (cm) ± SE in each group was as follows: 9334.6 ± 1428.9 in the CC group, 7371.5 ± 516.1 in the CT group, 6375.4 ± 896.7 in the TC group, and 4565.0 ± 393.1 in the TT group. The mean value in the TC and TT groups was significantly lower than that in the CC group ($p = 0.028$ for the TC group and $p = 0.001$ for the TT group).

The mean instances of typical behaviors in the groups are illustrated in Fig. 6. The mean instances of WR in the CT, TC, and TT groups was significantly lower than in the CC group. The mean instances of CR in the CT or TT groups was significantly lower than that in the CC group. The mean instances of FW or BW in the TT group was significantly lower than the respective mean value in the CT group. There were no significant differences among the groups for the time of the first grooming (data not shown).

PPI test results are reported in Table 1. There were no significant differences among groups in %PPI at any prepulse, 70, 75, or 80 dB.

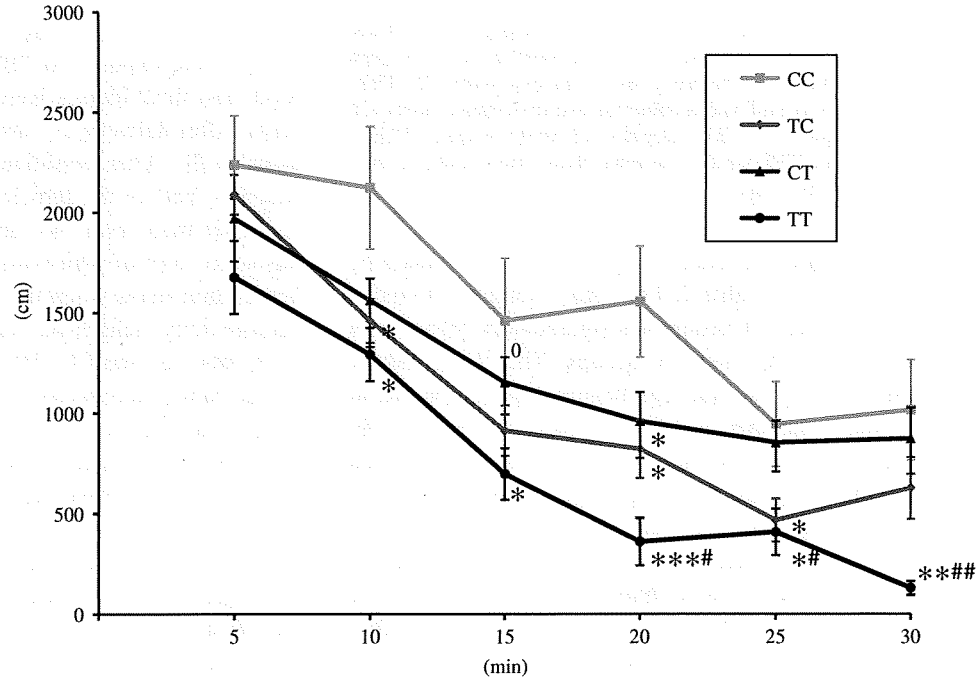

Fig. 5 Locomotor activity of F_1 rats exposed to TBT chloride via the placenta and their dams' milk and/or food. *CC* control-control (no exposure), *TC* TBT-control (exposure to TBT via the placenta and their dams' milk), *CT* control-TBT (exposure to TBT via food at 9–15 weeks of age), *TT* TBT-TBT (exposure to TBT via the placenta, their dams' milk, and their food at 9–15 weeks of age). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared to the CC group. # $p < 0.05$ and ## $p < 0.01$ compared with the CT group

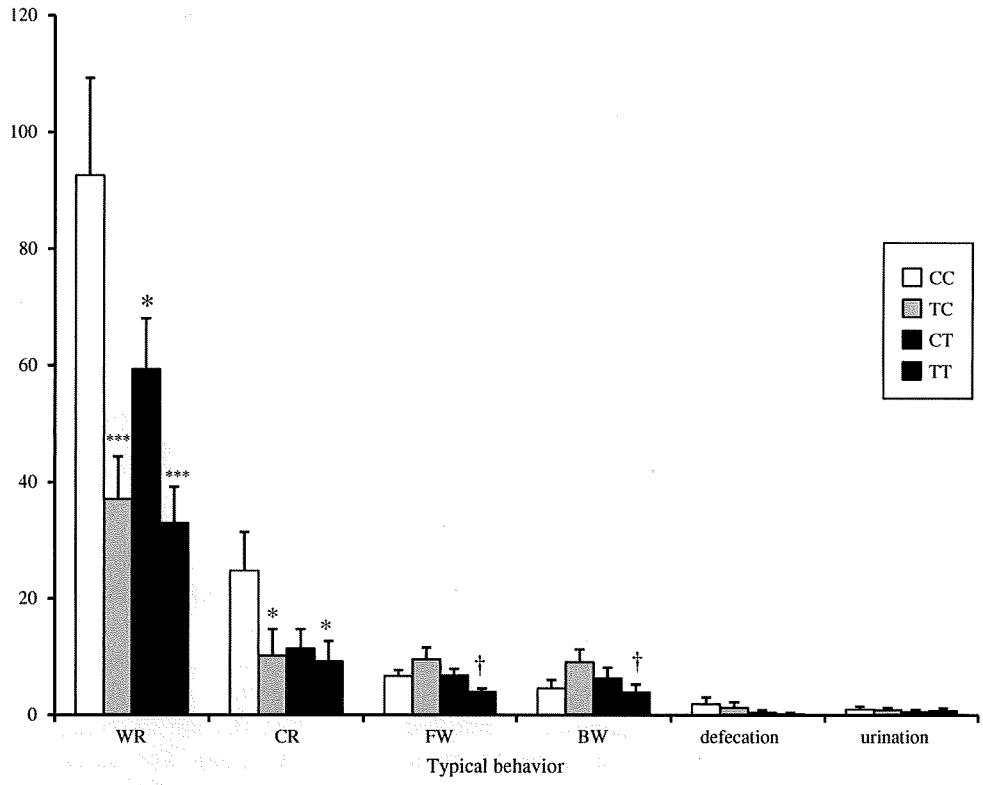

Fig. 6 Instances of typical behaviors of female F_1 rats exposed to TBT chloride via the placenta and their dams' milk and/or food in the open-field test. *CC* control-control (no exposure), *TC* TBT-control (exposure to TBT via the placenta and their dams' milk), *CT* control-TBT (exposure to TBT via food at 9–15 weeks of age), *TT* TBT-TBT (exposure to TBT via the placenta, their dams' milk, and their food at 9–15 weeks of age). *WR* wall rearing, *CR* center rearing, *FW* face washing, *BW* body washing. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with the CC group. † $p < 0.05$ compared with the TC group

Table 1 The prepulse inhibition (PPI) test in female F_1 rats exposed to TBT chloride via the placenta, their dams' milk, and/or their food

Group	%PPI at P70	%PPI at P75	%PPI at P80
CC	64.4 ± 8.4	53.9 ± 6.4	39.9 ± 4.8
TC	55.2 ± 6.8	43.1 ± 4.3	34.6 ± 4.9
CT	71.6 ± 14.8	46.7 ± 5.9	31.0 ± 6.8
TT	57.8 ± 7.7	47.1 ± 5.8	35.2 ± 3.9

Note: Mean ± SE. *%PPI at 70* %PPI when prepulse was 70 dB, *%PPI at 75* %PPI when prepulse was 75 dB, *%PPI at 80* %PPI when prepulse was 80 dB. *CC* control-control (no exposure), *TC* TBT-control (exposure to TBT via the placenta and their dams' milk), *CT* control-TBT (exposure to TBT orally at 9–15 weeks of age), *TT* TBT-TBT (exposure to TBT via the placenta, their dams' milk, or their food at 9–15 weeks of age)

Organ weights and relative organ weights of female F_1 rats are reported in Table 2. For organ weights, the mean liver weight in the CT group was significantly higher than that in the CC, TC, and TT groups. The mean kidney weight in the CT group was significantly higher than that in the TC group, and that in the TT group was significantly lower than that in the CC and TC groups. The mean spleen weight in the TC group was significantly lower than that in the CC and TT groups. The mean thymus weight in the CT and TT groups was significantly lower than that in the CC group. For relative organ weights, the mean relative liver weight in the CT and TT groups was significantly higher than that in the CC group. The mean relative kidney weight in the TC group was significantly higher than that in the CC and TT groups. The mean relative thymus weight in the CT and TT groups was significantly lower than that in the CC group.

Discussion

In the present study, the pregnant rats in the treatment groups were exposed to TBT chloride at 125 ppm in their food, and their fetuses were exposed to TBT via the placenta. After delivery, F_1 rats were exposed to TBT via their dams' milk. After cessation of the exposure to TBT after weaning, half of the female F_1 rats were exposed to TBT again via their food. We decided to use female F_1 rats in this study, half of which were exposed to TBT at the same age as their dams when they were pregnant. We compared neurotoxicity using behavioral tests among the four groups of F_1 rats, i.e., the CC, TC, CT, and TT groups.

For dams, there were no significant differences in body weight at delivery between control and TBT-exposed dams. In the previous study of adult mice exposed to TBT (Tsunoda et al. 2004), the only temporary decrease was in the body weight of adult mice exposed to TBT via their food at 125 ppm, but their final mean body weight was not significantly different from that of the controls. For the effects on the fetuses, a significantly lower ratio of the number of F_1 rats/the number of implantations in

Table 2 Organ weights (g) and relative organ weights of F_1 rats exposed to TBT chloride via the placenta, their dams' milk, and/or their food

Group	Liver	Kidney	Spleen	Thymus
Weight (g)				
CC	8.729 ± 0.261	1.896 ± 0.051	0.719 ± 0.035	0.414 ± 0.049
TC	8.118 ± 0.267	1.839 ± 0.036	0.614 ± 0.019*	0.345 ± 0.019
CT	10.585 ± 0.293***,†††	2.030 ± 0.064††	0.750 ± 0.043†	0.308 ± 0.025*
TT	8.626 ± 0.436###	1.686 ± 0.049*,††,###	0.662 ± 0.045	0.273 ± 0.025**
Relative weight (mg/g)				
CC	31.40 ± 0.62	6.82 ± 0.10	2.58 ± 0.11	1.51 ± 0.19
TC	34.56 ± 1.29	7.84 ± 0.24***	2.62 ± 0.11	1.48 ± 0.10
CT	38.19 ± 1.26***	7.33 ± 0.23	2.69 ± 0.11	1.12 ± 0.10*
TT	36.06 ± 1.90**	7.03 ± 0.17†††	2.76 ± 0.17	1.13 ± 0.08*

Note: Mean ± SD. *CC* control-control (no exposure), *TC* TBT-control (exposure to TBT via the placenta and their dams' milk), *CT* control-TBT (exposure to TBT orally at 9–15 weeks of age), *TT* TBT-TBT (exposure to TBT via the placenta, their dams' milk, or their food at 9–15 weeks of age)

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared to the CC group. ### $p < 0.001$ compared to the CT group. † $p < 0.05$, †† $p < 0.01$, and ††† $p < 0.001$ compared to the TC group

TBT-treated dams compared with control dams was observed, which suggests that TBT does not inhibit implantations in the uteri but inhibits the development of fetuses and induces fetal death.

After the development of F_1 rats, a significant decrease in body weight was observed in the TC and TT groups compared to the CC group from 9 to 15 weeks of age. Rats in both groups were exposed to TBT via the placenta and their dams' milk. In contrast, no significant decreases in body weight were induced via TBT-laced food after 9 weeks of age. There was no significant difference in body weight between the TC and the TT, or between the CC and the CT, groups. These results suggest that the effects of TBT on body weight occur by exposure via the placenta and their dams' milk and not via TBT-laced food after development. In the previous studies concerning the development of F_1 animals exposed to TBT, Konno et al. (2005) demonstrated that the body weight of mice exposed to TBT chloride at 50 ppm via drinking water was significantly lower than that in the control at 3 weeks of age, which is the time of weaning. For the development of rats, Carthew et al. (1992) reported a 16% reduction compared to the control for the body weight of female F_{344} rats exposed to TBT oxide in the diet from weaning to 6 weeks of age. Although there were differences in animal species, route of administration, and chemical forms, these values can be used as references for deciding the no-observed-adverse-effect level (NOAEL) of TBT for development. It should be noted that the inhibition of development continued after the cessation of TBT exposure in the TC group at the time of weaning. The inhibition of development of the F_1 generation caused by TBT exposure occurs from the fetal stage and continues during development regardless of cessation of the exposure to TBT.

A significant decrease in food and water consumption per body weight compared to the CC group was observed in rats in the CT and TT groups that were exposed to TBT via their food after 9 weeks of age. Appetite loss occurred in the groups exposed to TBT via food. The decrease in food and water intake was also observed in the previous study for adult mice exposed to TBT at 125 ppm in their food (Tsunoda et al. 2004). The decrease in food and water intake did not affect the body weight in the present study or in previous studies. This suggests that the extent of the decrease in the daily intake of food and water regarding effects on development may not be serious.

The relative thymus weight was decreased in the CT and TT groups. Carthew et al. (1992) also reported a 22.5% reduction compared with the control for relative thymus weight of female F_{344} rats exposed to 150 ppm TBT oxide in their food from weaning to 6 weeks of age. The decrease in thymus weight in rats exposed to TBT via their food in the present study was in accordance with the toxic effects of TBT on the thymus in previous studies summarized by Arakawa (2000). Regarding the spleen, which is also an important immune organ, its weight in the TC group was significantly lower than in the CC and CT groups, and there were no significant differences among the groups in relative spleen weight. In the previous study, the mean relative spleen weight of mice exposed to TBT chloride at 125 ppm in their food was low, but not significantly different, compared to that in the control (Tsunoda et al. 2004). It is suggested that the toxic effects of TBT on the immune system occur mainly in the thymus rather than the spleen.

The mean liver weight in the CT group was significantly higher than that in the other groups including the CC group. The relative liver weight in the CT group was also significantly higher than that in the CC group. The

increased metabolism of TBT in the liver (Wada 1985) may have induced the increase in liver weight in the CT group. The significant increase in relative liver weight in the TT group compared with the CC group was affected by the decrease in body weight in the TT group. In the previous study of adult mice exposed to TBT at 125 ppm in their food, there was no significant difference in relative kidney weight compared to the control (Tsunoda et al. 2004). For the kidney, the mean weight in the TT group was significantly lower than in the other groups. This decrease was affected by the decrease in body weight. The high mean kidney weight in the CT group and the high relative kidney weight in the TC group were also affected by the alterations in body weight in the respective groups.

The total locomotor distance in the open-field test was significantly decreased in the TC and TT groups compared with the CC group. The lowest mean total locomotor distance was observed in rats in the TT group exposed to TBT both via the placenta and their dams' milk and via their food. The mean value in the TT group was also significantly lower than that in the CT group. For locomotor activity every 5 min, the TT group showed a significantly lower value than the CC group after 5 min. For the TC group, a significantly lower locomotor distance for 5 min was observed in the three observation intervals, while a significantly lower locomotor distance was observed in only one interval for the CT group. TBT inhibits locomotor activity of F_1 rats, and the inhibitory effects via the placenta and their dams' milk was greater than that via their food. Although significant differences were not seen between the control and the TBT-treated groups in Aou and coworkers' (2000) study of the effects of TBT on the behavior of F_1 rats exposed via the placenta, their dams' milk, and their food, the total locomotor distance of the F_1 rats at 6 weeks of age was lower. The extent of the difference was different, but the results of the present study were in accordance with those of the previous study.

For adaptive responses in the open-field test, the mean instances of WR were significantly lower in the CT, TC, and TT groups compared with the CC group. The instances of CR were significantly lower in the TC and TT groups compared with the CC group. In the study by Aou et al. (2000) the total instances of rearing were also lower in the TBT-treated group. The rats' exploratory behaviors as adaptive responses were impaired by TBT exposure more strongly via the placenta and their dams' milk compared to via their food after 9 weeks of age. It should be noted that additive adverse effects were observed in the TT group that had been exposed to TBT via the placenta, their dams' milk, and their food.

In contrast to locomotor activity and adaptive responses, there were no significant differences in any %PPI in the PPI tests. The learning and cognitive function was not affected by TBT exposure under the protocol in the present study. The reasons why TBT inhibits locomotor activity and adaptive responses but does not inhibit learning or cognitive activity are unclear. In the previous study by Konno et al. (2005), specific ligand binding to *N*-methyl-D-aspartate (NMDA) receptors, which are involved in memory and learning, was inhibited in the cerebrum of F_1 mice exposed to TBT chloride via placenta and dams' milk. Arakawa (2000) showed alterations in trace elements in the hippocampus of rats that received a single intraperitoneal injection of 2.0 mg/g TBT chloride. Those studies suggested impairment of memory and learning by TBT but do not coincide with the results in the PPI test in the present study. There are two possible explanations. The PPI test may not be sensitive enough to detect the impairment of memory and learning by TBT. Or the alterations induced by exposure to TBT in brain tissues may not be sufficient to cause impairment of memory and learning.

To elucidate the neurotoxic mechanism of TBT, it may be useful to examine alterations in gene expressions. Recently, oligonucleotide microarrays have been developed and applied to the study of neurotoxic effects of environmental toxicants on the developing rat (Takahashi et al. 2009). It is of interest whether or not alterations in gene expression detected by the microarrays are observed under the protocol in this study. Also, in this study, the concentration of TBT in the food was set at 125 ppm, which was higher than those of reported TBT contaminations of fish and shellfish. To determine the NOAEL, lower concentrations of TBT should be tested, warranting further studies.

In conclusion, TBT induces inhibition of rat development by exposure via the placenta and their dams' milk but not via their food. The inhibition of development continues without exposure after weaning. For neurotoxicity effects, TBT impaired locomotor activity and, also, inhibited exploratory behaviors. The neurotoxic effects of TBT were greater with rats' exposure via the placenta and their dams' milk than via their food, and additive adverse effects were observed for both modes of exposure.

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Alterations in male infant behaviors towards its mother by prenatal exposure to bisphenol A in cynomolgus monkeys (*Macaca fascicularis*) during early suckling period

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Summary Bisphenol A (BPA) is an environmental chemical with physiological potencies that cause adverse effects, even at environmentally relevant exposures, on the basis of a number of studies in experimental rodents. Thus, there is an increasing concern about environmental exposure of humans to BPA. In the present study, we used experimentally controlled cynomolgus monkeys (*Macaca fascicularis*) to assess the influence of prenatal exposure to BPA (10 μg/(kg day)) via subcutaneously implanted pumps and examined social behaviors between infants and their mothers during the suckling period. Mother–infant interactions in cynomolgus monkeys had behavioral sexual dimorphism associated with sex of infant from early suckling period. Prenatal exposure to BPA altered the behaviors of male infants significantly; BPA-exposed male infants behaved as female infants. And it also affected some of female infant behaviors. Consequently, gestational BPA exposure altered some behaviors of their mothers,

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mainly in male-nursing mothers. These results suggest that BPA exposure affects behavioral sexual differentiation in male monkeys, which promotes the understanding of risk of BPA exposure in human.

1. Introduction

Bisphenol A (4,4′-isopropylidene-2-diphenol, BPA) is a high production volume chemical used in the manufacture of polycarbonate plastics, epoxy resins and polyester resins. Worldwide, humans are exposed to environmentally released BPA via foods, water, and some medical instruments. BPA is actually detected in human urine (Calafat et al., 2008), amniotic fluid (Engel et al., 2006; Tsutsumi, 2005), and maternal blood at delivery (Padmanabhan et al., 2008).

Toxicities of BPA have been widely studied in the past decade because of its physiological multi-potencies. BPA was first perceived as having a weak estrogenic potency (1/1000–1/100,000 of 17β-estradiol, the most potent endogenous estrogen) in *in vitro* and *in vivo* studies (Krishnan et al., 1993; Milligan et al., 1998; Steinmetz et al., 1997). Many studies focused on the abnormal development of reproductive organs in mice (Munoz-de-Toro et al., 2005; Rubin et al., 2006; Timms et al., 2005; Vandenberg et al., 2007) and physical puberty in mice (Howdeshell et al., 1999) and reproductive functions in sheep (Savabieasfahani et al., 2006) associated with exposure to BPA, because these biological processes depend on endogenous estrogens. However, subsequent studies using reporter-gene assays revealed anti-androgenic (Lee et al., 2003) and anti-thyroid hormone (Moriyama et al., 2002; Zoeller et al., 2005) activities of BPA. More recent studies have provided evidence of non-genomic effects of BPA, which have distinct mechanisms from classic genomic pathways through nuclear receptors in pancreatic a cells (Alonso-Magdalena et al., 2005), GH3/B6 pituitary tumor cells (Watson et al., 2005) and PC12 cells (Yoneda et al., 2003). The non-genomic potencies of BPA were equivalent to those of estradiol. Two *in vivo* studies showed anti-estrogenic potency of BPA in the rat cerebellum (Zsarnovszky et al., 2005) and rat hippocampus (MacLusky et al., 2005).

As a result of investigations of the toxicity of BPA, there is an increasing concern that BPA exposure alters behaviors substantially controlled by the central nervous system (CNS), especially transient *in utero* and/or neonatal BPA exposure. It has been reported that pre- or perinatal BPA exposure affects spontaneous novelty seeking and impulsive behavior (Adriani et al., 2003), response to fear-provoking stimuli (Negishi et al., 2004a), pain behaviors (Aloisi et al., 2002) and responses to dopaminergic drugs (Adriani et al., 2003; Laviola et al., 2005; Negishi et al., 2004a) in rats and mice. Furthermore, recent studies have shown that environmentally relevant exposure of experimental rodents to BPA caused disruption of sexual dimorphism in brain development (Fujimoto et al., 2006) and in specific brain regions such as the rostral periventricular preoptic area (Rubin et al., 2006), locus ceruleus (Kubo et al., 2003) and bed nucleus of the stria terminals (Funabashi et al., 2004). Thus, behavioral development and sexual dimorphism in the CNS is believed to be a sensitive end point in the assessment of toxicity to pre- or perinatal exposure to BPA, at least in rats and mice (Palanza et al., 2008).

One of the goals of many studies reporting adverse effects of BPA exposure in experimental animals is the extrapolation of the results to humans. However, previous studies in experimental rodents have often faced difficulties in extrapolating the results to assess risk in humans because of physiological differences between the two species. Experimental monkeys may prove useful in this regard because they are physiologically similar to humans. In the toxicological field, some studies have used monkeys to evaluate the adverse effects of exposure to environmental chemicals such as dioxin (Negishi et al., 2006; Tokuda et al., 2006; Yasuda et al., 2005) and polychlorinated biphenyl (Levin et al., 1988; Schantz et al., 1989).

In the present study, we evaluated infant and maternal behaviors in cynomolgus monkeys (*Macaca fascicularis*) to assess influence of prenatal exposure to BPA on the behavioral development of monkeys. We analyzed first each behavior observed in this study using univariate analysis, and then applied multivariate analysis to demonstration of behavioral alterations by BPA exposure. This is the first report that evaluates the influences of BPA exposure on the behavioral development of non-human primate.

2. Materials and methods

2.1. Animals and chemicals

2.1.1. Breeding

Thirty-seven hematologically (the numbers of red blood cells, white blood cells and platelets, hematocrit, and the concentration of hemoglobin) and serologically (the concentrations of total protein, bilirubin, creatinine, and minerals) normal female cynomolgus monkeys (*M. fascicularis*) (body weight: 2.5–4.0 kg; age: 5–13 years) imported from China National Scientific Instruments and Materials Import/Export Corporation in China were used in the present study, which was conducted at Shin Nippon Biomedical Laboratories (SNBL), Ltd., Japan (Table 1). Animal breeding, mating and behavioral experiments were performed in SNBL. All animals were kept individually at a temperature of 26 ± 2 °C, humidity of 50 ± 10%, 12 h/12 h light–dark cycle and 15 times ventilation/h in stainless steel cages as per the NIH guidelines (69 cm × 61 cm × 75 cm). All animals received 108 g of food pellets (Harlan Sprague Dawley, Inc., IN) once daily and had free access to drinking water. Female monkeys with normal menstrual cycles (20–32 days) were caged with a healthy male monkey during the time of expected ovulation (11–15 days after menstruation) and were again kept individually. Confirmation of copulation or intravaginal sperm by an observer defined the middle day of the 3-day mating as gestational day 0.

2.1.2. Chemical treatment

At gestational day 20, pregnancies were confirmed by ultrasonic diagnosis. Just after confirmation of normal

Table 1 Effect of BPA exposure on pregnancy and delivery in cynomolgus monkeys.

	Control		BPA	
	Male[a]	Female[a]	Male[a]	Female[a]
Number of pregnant animals	19		18	
Number of animals that delivered normally	6	10	4	9
Abortion (sex of Fl was unknown)	3		1	
Stillbirth	0	0	2	0
Neonatal death (prematured birth)	0	0	2	0
Death after weaning	1	0	0	0
Gestational length (day)[b]	161.7 ± 5.0	160.9 ± 6.4	159.3 ± 5.0	159.7 ± 7.0
Fl body weight at birth (g)[b]	369 ± 34	372 ± 33	368 ± 40	350 ± 51
At 168 days afterbirth (g)[b]	895 ± 152	937 ± 85	979 ± 75	898 ± 140
Number of animals observed	5	10	4	6

[a] Sex of infant.
[b] Normally delivered mothers and infants were used.

pregnancies, Alzet® osmotic pumps (DURECT Corporation, CA) which provide a fixed release of solution (6 μL/day) for 28 days were surgically implanted in dorsal subcutaneous tissues. This surgery was performed under brief anesthesia. A small incision (2–3 cm) was made in ventral skin after shaving hair. Subsequently, a pump was subcutaneously inserted from the incision, which was sutured quickly. All of surgical procedures were performed under sterile condition. Eighteen pregnant monkeys received bisphenol A (Tokyo Chemical Industry Corporation, Ltd., Japan) dissolved in vehicle consisting of N,N-dimethylacetamide (Wako Pure Chemical Industries, Ltd., Japan) and polyethylene glycol (1:1) (Wako Pure Chemical Industries, Ltd.) through osmotic pumps (Table 1). In order to administer BPA to pregnant monkeys at a dosage of 10 μg/(kg day), the concentration of BPA in the osmotic pumps was calculated before implantation as follows: body weight (kg) × 10 μg/(kg day)/6 μL. We exchanged pumps every 28 days (gestational days 48, 76, 104, and 132). The dose of BPA in the present study using monkeys aimed at the circulating level of BPA accomplished by 5 mg/(kg day) oral exposure in rats, because there was a concern about a "low-dose effect" of BPA, i.e., any adverse effect that results at a dose lower than the dose that induces general toxicities in rats (vom Saal et al., 2007). This trial calculation was introduced based on our previous studies (Negishi et al., 2004b; Tominaga et al., 2006), in which we examined the toxicokinetics of orally or subcutaneously administered BPA (10 mg/kg) in cynomolgus monkeys and compared the effects with those in rats to assess route dependencies and species differences in BPA concentrations. Nineteen pregnant control pregnant monkeys received a vehicle solution (no BPA) in the same manner as the pregnant monkeys received BPA (Table 1). Mother monkeys and their infants [delivery day was postnatal day (PND) 0] lived together in the same cage until weaning (6–10 months after birth (MAB)). All experiments were performed humanely according to the guidelines for animal experiments at the SNBL and were approved by The Animal Care and Use Committee of the Graduate School of Agricultural and Life Sciences, The University of Tokyo.

2.2. Observation of mother–infant interaction

2.2.1. Recording

Mother–infant interactions of 5 male and 10 female infants and their respective mothers in the control group and of 4 male and 6 female infants and respective mothers in BPA-exposed group were observed (Table 1). One control male infant, three BPA-exposed female infants, and their respective mothers were excluded from subjects for analyses of mother–infant interaction because qualities of some of their video images were not sufficient to analyze fine movements of infants. Just before the daily feeding (14:30), mother–infant interactions were recorded using a digital video camera for 12 min twice per week at 2 and 3 MAB (PND 31–60 and 61–90, respectively) in cages in which the stainless steel bars on the front of the cage were exchanged with a transparent acrylic board.

2.2.2. Video image analysis

The first 2 min of the video recording, considered as the acclimatization period, was disregarded; the following 10 min of the recording was used for the behavioral analysis Videotapes were scored using the Observer 5.0 (Noldus Information Technology, The Netherlands). Fourteen typical behaviors of infants (*approach to mother, autogrooming, clinging, environmental exploration, lip smacking, locomotion, nipple contact, orientation, outward looking, proximity, rejection, self-directed behavior, social exploration,* and *ventral contact*) and fourteen typical behaviors of mothers (*approach to infant, attack, autogrooming, environmental exploration, lip smacking, locomotion, orientation, outward looking, rejection, self-directed behavior, social exploration, social grooming, stereotypy,* and *threat*) were defined and evaluated in this study (see Supplementary Table 1). We applied one-zero sampling methods to the mother–infant interactions under blinded conditions, in which only the presence (1) or absence (0) of each behavior for 5 s was recorded (Altmann, 1974). A 10 min analysis produced 120 samples (1 or 0) and a frequency (the number of appearance among one hundred and twenty 5-s samples) for each behavior was determined. For each mother–infant pair, the mean frequencies per

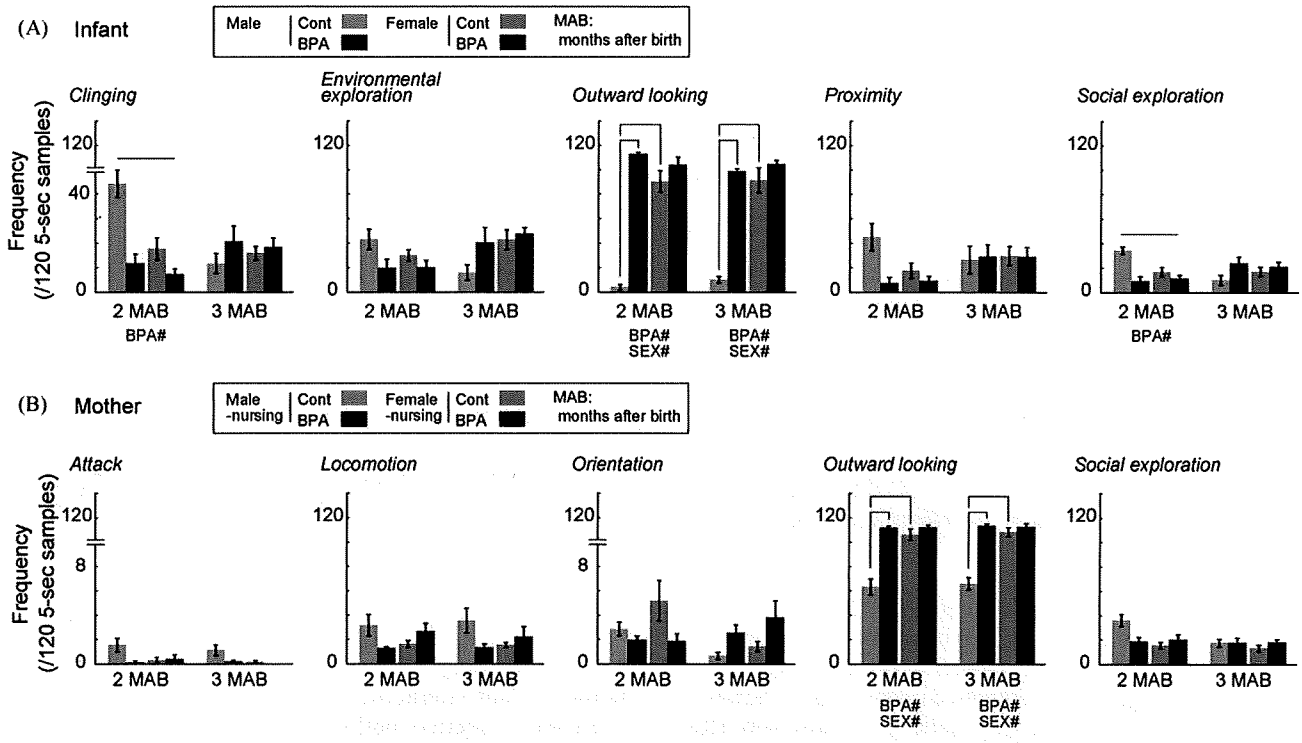

Figure 1 Frequencies (number of 5-s samples in which each behavioral category was observed) of infant (A) and maternal (B) behaviors affected by prenatal or gestational BPA exposure, respectively. Factors [infant: prenatal exposure to BPA (BPA), sex of infant (SEX) and age of infant (AGE); mother: gestational exposure to BPA (BPA), sex of its infant (SEX) and age of its infant (AGE)] whose effects were significant in three-way repeated-measures ANOVA are presented in each panel. When a two-way significant interaction (BPA × SEX or BPA × AGE) is observed, simple main effect at each level is tested and significant effect is shown as BPA# or SEX#. Data are presented as mean ± standard error of the mean (S.E.M.).

month (8 or 9 challenges per individual at 2 and 3 MAB) were used as individual frequencies.

2.3. Statistical analysis

2.3.1. Univariate analysis

The main effects of BPA exposure (BPA) and sex of the infant (SEX) in gestational length were investigated by two-way analysis of variance (ANOVA). The main effect of BPA, SEX, and days after birth (DAB; 0 and 168 days after birth) were investigated by three-way (BPA and SEX as between-subjects factors and DAB as a within-subjects factor) repeated-measures ANOVA, where alpha-value was set at 0.05. The frequencies of the appearance of each behavior expressed in the mother–infant interaction in 5 s, via one-zero sampling methods were first analyzed by three-way (BPA and SEX as between-subjects factors and age of infant (AGE) as a within-subjects factor) repeated-measures analysis of variance, where P-values were set at $0.0034(1-(1-0.05)^{1/14})$ each for infant and maternal behaviors to ensure that the alpha level of each was set at 0.05 (Supplementary Table 2). When a significant interaction between factors was observed in the ANOVA, an appropriate simple main effect test was done for further analysis at each level (Supplementary Tables 2 and 3).

2.3.2. Multivariate analysis

In the present study, we applied discriminant analysis to reveal the effect of BPA exposure on mother–infant interaction at each age of infant, which has for its main purpose to find linear combinations of independent variables that maximize the differences among the groups. Before the discriminant analysis, principal component analysis (PCA) was used to synthesize variables independent each other, because behaviors affected by BPA exposure (Fig. 1) had significant correlation each other (Supplementary Table 4), which were inappropriate for discriminant analysis. The frequencies of the appearance of each behavior significantly ($P < 0.0037$) affected by BPA exposure and marginally ($P < 0.05$) affected (five behaviors each in infants and mothers, Fig. 1 and Supplementary Table 2) at each age of infant were used for PCA (Supplementary Table 5). Variance–covariance matrix was used for PCA. Until the cumulative percentage of proportion rate of PC exceeded 80% (Supplementary Table 4), PCs were accepted.

At each age, we then performed discriminant analyses to discriminate four groups of infants (control male, control female, BPA-exposed male, and BPA-exposed female infants) and mothers (control male-nursing, control female-nursing, BPA-exposed male-nursing, and BPA-exposed female-nursing mothers) using PC scores. Discriminations were based on the *Mahalanobis* distance to centers of balance. This statistical analysis determines the most likely group of a test data set as a discriminated group. Correct discriminant rates (%) at each age were evaluated (Supplementary Table 6). In addition, in this analysis, the discriminant function is defined as the form:

$$D = \sum dZ$$

where D = discriminant score, d = weighing coefficient, and Z = PC score which was already standardized by PCA (Supplementary Table 7). Discriminant scores were analyzed by two-way (BPA and SEX as between-subject factors) ANOVA, where P-value was set at $0.0253(1-(1-0.05)^{1/2})$. When a significant interaction between factors was observed in the ANOVA at each age, an appropriate simple main effect test was done for further analysis at each level (Supplementary Table 8). All statistical analyses were performed using SPSS (SPSS Japan Inc., Japan) and StatView® (HULINKS Inc., Japan) software.

3. Results

3.1. General observations

Throughout this study, neither mothers nor infants showed gross physical abnormality regarded as an adverse effect of BPA exposure. Sixteen of 19 mothers in the control group and 13 of 18 mothers in the BPA-exposed group delivered normal infants (Table 1). The control mothers yielded 6 male and 10 female infants, while the BPA-exposed mothers yielded 4 male and 9 female infants. Three abortions and one death after weaning occurred in the control group (Table 1); the behavioral analysis in the present study included the data for the monkey that died. One abortion, two stillbirths (both males) and two neonatal death (both males) occurred in the BPA-exposed group (Table 1). Two-way ANOVA for gestational length and two-way repeated-measures ANOVA for infant body weight indicated that neither BPA exposure nor sex of infant affected gestational length and infant body weight at birth and 168 days after birth ($P > 0.2$) (Table 1). All weaned animals except one, who died 300 days after birth, in the control group survived healthy for 3 years until they were humanely sacrificed.

3.2. Univariate analysis

The frequencies of behaviors of infants and mothers in the mother–infant interaction were analyzed by three-way repeated-measures (BPA exposure (BPA) and sex of infant (SEX) as between-subjects factors and age of infant (AGE) as a within-subjects factor) ANOVA, which indicated significant effects and/or significant interactions ($P < 0.0037$ in infants and mothers) related to BPA on three infant behaviors (*clinging*, *outward looking*, and *social exploration*) and one maternal behaviors (*outward looking*) (Supplementary Table 2 and 3). In addition, ANOVA implicated marginal effects and/or marginal interactions ($P < 0.05$) related to BPA on two infant behaviors (*environmental exploration* and *proximity*) and four maternal behaviors (*attack*, *locomotion*, *orientation*, and *social exploration*). The frequencies of these behaviors of infants and mothers were shown in Fig. 1A and B. Other behaviors are presented in Supplementary Fig. 1A and B. As shown in Fig. 1A, *clinging* and *social exploration* were significantly reduced by prenatal BPA exposure at 2 months after birth (MAB), which were apparent in male infants. *Outward looking* of infant was significantly increased by BPA exposure in male infants both at 2 and 3 MAB. Only in control infants, significant sexual difference in the frequencies of infant *outward looking* was observed. Although ANOVAs failed to indicate significant effects of prenatal exposure on other two infant behaviors, *environmental exploration* and *proximity* seemed to be affected especially in male infants. Some of maternal behaviors also seemed to be affected by gestational BPA exposure (Fig. 1B). Among these behaviors, *outward looking* of mother was significantly increased by BPA exposure in male-nursing mothers both at 2 and 3 MAB. Only in control mothers, outward looking was observed more frequently in female-nursing mothers than in male-nursing mothers, as observed in infant *outward looking*. About other behaviors where ANOVAs indicated marginal effects and/or marginal interactions ($P < 0.05$), on the whole, it seemed that BPA-exposed male-nursing mothers show altered behaviors after gestational BPA exposure compared with control male-nursing mothers.

3.3. Multivariate analysis

As observed in univariate analyses, some behaviors of infants and mothers seemed to be affected intricately by BPA exposure. To understand BPA-induced behavioral alterations using multivariate, we tried to perform discriminant analysis using behaviors potentially affected by BPA exposure (*clinging*, *environmental exploration*, *outward looking*, *proximity*, and *social exploration* in infants; *attack*, *locomotion*, *orientation*, *outward looking*, and *social exploration* in mothers). There were, however, significant correlations between these behaviors (Supplementary Table 4), which meant that the frequencies of these behaviors were inappropriate as variables for discriminant analysis. Thus, we performed principal components analysis to obtain principal components, synthetic variables statistically independent each other, from the frequencies of behaviors enumerated above at each age prior to discriminant analysis. And then, principal component scores defined by the sum of frequency multiplied by corresponding factor loading of each behavior (Supplementary Table 5) were thrown into subsequent discriminant analysis. Discriminant analysis discriminated control male infants or control male-nursing mothers most correctly in the discrimination of infants or mothers, respectively, and frequently failed to discriminate other group subjects correctly (Supplementary Table 6). To visualize these discriminations, we calculated discriminant scores from principal component scores, the sum of principal component score multiplied by weighing coefficient, at each age (Supplementary Table 7) and analyzed by two-way (BPA and AGE as between-subjects factors) ANOVA (Supplementary Table 8). Prenatal BPA exposure significantly ($P < 0.0253$) affected discriminant scores of male infants but not those of female infants (Fig. 2A). Discriminant scores of BPA-exposed male infants both at 2 and 3 MAB were closer to those of control female infants than those of control male infants and significant sexual differentiation observed in control infants disappeared completely in BPA-exposed infants. Gestational exposure to BPA also affected discriminant scores of male-nursing, but not female-nursing, mothers significantly ($P < 0.0253$) (Fig. 2B) and the differentiation dependent on the sex of its infant, which was observed only in control mothers, disappeared in BPA-exposed mothers. As observed in infant behaviors, discriminant scores of BPA-exposed male-nursing mothers were closer to those of control female-nursing mothers than those of control male-nursing mothers.

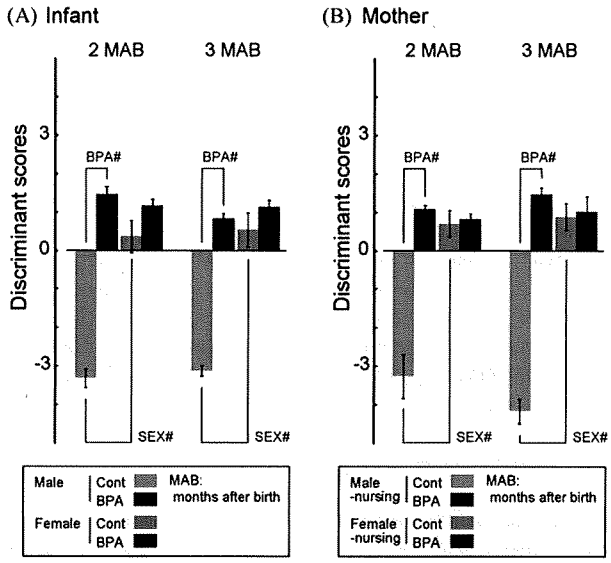

Figure 2 Scores of discriminant function of infant (A) and mother (B) at each age. Discriminant scores were the sum of principal component scores, which were separately generated from five behaviors shown in Fig. 1 at each age, multiplied by corresponding weighing coefficient. Factors [infant: prenatal exposure to BPA (BPA) and sex of infant (SEX); mother: gestational exposure to BPA (BPA) and sex of its infant (SEX)] whose effects were significant in two-way ANOVA are presented in each panel. When a two-way significant interaction (BPA × SEX) is observed, simple main effect at each level is tested and significant simple main effect is shown as BPA# or SEX#. Data are presented as mean ± S.E.M.

4. Discussion

Adverse effects of BPA were intensively examined over a decade in various experimental animals. One goal of these studies was risk assessment in humans. In the present study, we used cynomolgus monkeys because of their phylogenic similarities to humans.

In the present study, BPA was infused (10 μg/(kg day)) via subcutaneously implanted pumps to maintain a continuous and fixed exposure. Our previous studies assessing route dependencies (per oral vs. subcutaneous) and species differences (rats vs. cynomolgus monkeys) in serum BPA concentrations (Negishi et al., 2004b; Tominaga et al., 2006) suggested that the dose used in the present study in monkeys seemed to correspond to an oral dose of 5 mg/(kg day) or less in rats. There was a concern about a "low-dose effect" of BPA, i.e., any adverse effect that results at a dose lower than the dose that induces general toxicities in rodents (<5 mg/(kg day) per oral) (vom Saal et al., 2007), and we also aimed to assess low-dose effect of BPA exposure in monkeys. While the human exposure level of BPA was estimated to be less than 1 μg/(kg day) orally (Kang et al., 2006), the exposure level in the present study was thought to be still high relative to environmentally relevant exposure levels in humans, because BPA was directly (subcutaneously) injected. However, when we tried to measure the circulating BPA level in pregnant monkeys used in the present study, a commercial enzyme-linked immunosorbent assay kit (Wako Pure Chemical Industries, Ltd.) revealed that the concentration of BPA in plasma from BPA-exposed female monkeys at gestational day 50 (30 days after pump implantation) was less than 12.5 ng/mL which was the detection limit of this assay. On the other hand, circulating level of BPA in human mothers at delivery ranged between 0.5 and 22.3 ng/mL (Padmanabhan et al., 2008).

This study was the first step in investigating the effects of prenatal exposure to a "relatively low dose" of BPA on the development of experimentally controlled non-human primates, especially on the behavioral development. In the present study, infant monkeys were reared only by their mothers, without direct interaction with any other monkeys. Although only two ages were analyzed in this study, it seemed that, as male infants of the control group grew, their social interaction with their mothers decreased (increase of *locomotion* and decrease of *clinging*, *nipple contact*, and *ventral contact*) (Fig. 1 and Supplementary Fig. 1). Univariate analyses and discriminant analyses suggested that, even during the early suckling period, infants showed behavioral dimorphism based on their genetic sex. Sex of the infant also affected maternal behaviors. Previous studies reported, in rhesus monkey (*Macaca mulatta*), behavioral sexual differentiations of mounting (male infants showed more frequent mounting behaviors females did) (Goy and Deputte, 1996) and vocalization (males used more frequent geckers and noisy screams than females did) (Tomaszycki et al., 2001) in mother–infant interactions and infant–yearling infant interactions (females showed higher interests in other infants than males did) (Herman et al., 2003), in which several mothers and infants were housed together. In contrast, we used individually housed cynomolgus monkeys to obtain uniformed subjects having less individual difference, which delivered its offspring. Consequently, the offspring could interact only with its mother. Thus, experimental monkeys used in this study experienced very limited interactions with others. The species difference in itself and differences in feeding condition would affect behaviors, which make it difficult to compare their behaviors with those observed in these previous studies. In general, male infant and male-nursing mother are seemed to be more active than female infant and female-nursing mother in rhesus monkeys at early suckling period. It was apparent that non-human primates form sexual dimorphism even in early neonatal period as like human (Benenson et al., 1999; Connellan et al., 2000).

It was obvious that the infant *outward looking* and maternal *outward looking* were influenced most remarkably by BPA exposure among 14 infant and 14 maternal behaviors, respectively, mainly in male infant and male-nursing mothers, while some other behaviors also seemed to be affected by BPA exposure. Non-exposed male infants and male-nursing mothers were less interested in environmental visual stimulation than non-exposed females and female-nursing mothers, respectively, during early suckling period, while BPA-exposed males and male-nursing mothers frequently showed *outward looking*. It might be that *outward looking* of one tempted that of the other. Discriminant analyses clearly indicated that male infants and male-nursing mothers were more vulnerable to BPA exposure than female infants and female-nursing mothers, and that BPA-exposed male infant and male-nursing mothers showed female-like or female-nursing-like behaviors, respectively, which were also implied by other behaviors even if there was no statistical significance in univariate analyses.

As mentioned above, maternal behaviors as well as infant behaviors also seem to be altered by gestational exposure to BPA. There is a possibility that BPA directly alters maternal behaviors, and these alterations affect the behaviors of infants, as reported in rats (Della Seta et al., 2005). However, with regard to maternal behaviors, influences by BPA exposure were observed mainly in male-nursing mother monkeys. If gestational exposure to BPA directly affects maternal behaviors, more common behavioral alterations would be expected between male- and female-nursing mothers. Thus, it is relevant that *in utero* BPA exposure of male fetuses disturbed their behavioral sexual differentiation and that these feminized behaviors in BPA-exposed male infant caused female-nursing-like behaviors in the BPA-exposed male-nursing mothers. Additional studies, e.g. a fostering study, may shed further light on this important issue.

It should be noted that discriminant scores presented in this study are statistically calculated from principal components, synthetic variables consisting of selected behaviors, which made it difficult to give them any appropriate behavioral explanation using psychological terms. Thus, we here only give these behavioral alterations the disturbance of behavioral sexual differentiation in male infants by prenatal BPA exposure.

It is well known that male-specific prenatal transient androgen masculinize the male brain; testosterone itself and/or estradiol aromatized from testosterone are believed to cause cellular responses in neurons and to induce sexual dimorphism of the brain. Prenatal sex hormones, especially testosterone, also in rhesus monkeys play important role in development of behavioral sexual dimorphism as well as reproductive function. Prenatal testosterone masculinized juvenile and adult copulatory behavior and defeminized female-typical sexual initiation in female rhesus monkeys (Wallen, 2005) and increased yawning behavior in female rhesus monkeys to male-like level (Graves and Wallen, 2006). On the other hand, testosterone blockade by flutamide accelerated pubertal development in male rhesus monkeys (Herman et al., 2006). In addition, this blockade also strengthened click-evoked otoacoustic emissions (CEOAEs) (McFadden et al., 2006) and improved spatial memory ability (Herman and Wallen, 2007) in male rhesus monkeys (CEOAE strength and spatial memory ability were substantially greater in females than males). Given this scenario, the observed feminization of male infants in this study can be explained, at least in part, by the anti-estrogenic or anti-androgenic action of BPA. A recent study revealed that prenatal BPA exposure in rats inhibited serum testosterone surge of male neonates (Tanaka et al., 2006). This might be the reason, at least partially, for feminized behaviors of male monkey infants. Some studies reported disturbances in sexual differentiation in response to pre- or perinatal BPA exposure in rats (Fujimoto et al., 2006; Kubo et al., 2001, 2003) and mice (Rubin et al., 2006). It is necessary to consider physiological activities of BPA other than its weak estrogenicity. In fact, two studies have reported on the anti-estrogenic activity of BPA *in vivo* in brain (MacLusky et al., 2005; Zsarnovszky et al., 2005). Several reports have suggested that BPA has anti-androgenic (Lee et al., 2003) and anti-thyroid hormone like activities (Moriyama et al., 2002; Zoeller et al., 2005) in addition to non-genomic activities at very low doses (Zsarnovszky et al., 2005). More recent study reported epigenetic disruption, DNA hypomethylation, by prenatal BPA exposure in mice (Dolinoy et al., 2007). Although the mechanism by which BPA causes disturbance in brain sexual differentiation is still unclear, the above-mentioned potential activities of BPA should be considered.

We assessed mother–infant interactions behaviorally by observing behaviors twice per week, which might be infrequent compared with usual behavioral studies. Such infrequent observations made variations large especially in infrequently observed behaviors such as attack and orientation, which were unfavorable for statistical analyses. Individual data conducted from more frequent observations would contribute to more reliable statistical analysis and might indicate more subtle but important behavioral alterations by BPA exposure.

This study demonstrated in cynomolgus monkeys that prenatal BPA exposure might affect sexual differentiation in the interactive behaviors between infant and mother. The observed female-like behaviors in male monkeys in response to prenatal exposure to BPA indicates the need for a novel paradigm for assessing the risk of humans to BPA.

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Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.psyneuen.2009.03.005.

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