Title
Differing Pattern of the Development of Mother–Infant Interactions in Cynomolgus Monkeys Due to Exposure of an Environmental Chemical, Bisphenol

Journal
International Journal of Comparative Psychology, 21(2)

ISSN
0889-3675

Authors
Nakagami, Akiko
Koyama, Takamasa
Kawasaki, Katsuyoshi
et al.

Publication Date
2008

DOI
10.46867/ijcp.2008.21.02.02

International Journal of Comparative Psychology, 2008, **21**, 70-83.

Differing Pattern of the Development of Mother–Infant Interactions in Cynomolgus Monkeys Due to Exposure of an Environmental Chemical, Bisphenol A

Akiko Nakagami and Takamasa Koyama
Japan Women's University, Japan

Katsuyoshi Kawasaki
Hoshi University, Japan

Takayuki Negishi
Aoyama Gakuin University, Japan

Toshio Ihara
Shin Nippon Biomedical Laboratories, Japan

Yoichiro Kuroda
Tokyo Metropolitan Institute for Neurosciences, Japan

Yasuhiro Yoshikawa
University of Tokyo, Japan

Recent studies have focused on the effects of low doses of Bisphenol A (BPA) on the central nervous system, which may prevent sexual dimorphism of the brain in rodents. To assess sensitivity to BPA, mother–infant behaviors in the cynomolgus monkey were studied longitudinally after treating the mothers with low-dose BPA during pregnancy. Mother–infant interaction was observed for 6 months after the birth of the infants. In conclusion, male offspring of BPA-treated females showed female-like behavior patterns. Prenatal BPA exposure altered infant behavior in the early stages of mother–infant interaction, and male infants were affected more seriously than females.

More than a decade has passed since the 1996 publication of *Our Stolen Future* by Colborn, Dumanoski, and Myers. They stated, "For all these systems, normal development depends on getting the right hormone messages in the right amount to the right place at the right time. As this elaborate chemical ballet rushes forward at a dizzying pace, everything hinges on timing and proper cues. If something disrupts the cues during a critical period of development, it can have serious lifelong consequences for the offspring" (p. 46). If traces of environmental endocrine disrupters (EEDs) persist and no decrease in their environmental concentrations have been observed, it is clear that the hormone exposure problem during the fetal period is related to the difference in the mouse's behavior. Reproductive capacity has addressed this issue (e.g. (Dhar, & vom Saal, 1992; Even, Walker, Keisler, Caldwell, Kier, & vom Saal, 1996; vom Saal & Dhar, 1992). Many studies (Calafat, Ye, Wong, Reidy, Needham, 2008; Cao & Corriveau, 2008; Kang, Kondo & Katayama , 2006; Le, Carlson, Chua, & Belcher, 2008; Mahalingaiah et al., 2008;

We are grateful to Dr. Takashi Yoshida for his helpful advice. This research was partially supported by KAKENHI (Grant-in-Aid for Scientific Research (S), 14104020) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. This study was also supported by the Core Research for Evolutional Science and Technology (CREST) program of the Japan Science and Technology Agency. Correspondence concerning this article should be addressed to Akiko Nakagami, Department of Psychology, Japan Women's University, 1-1-1 Nishi-ikuta, Tama-ku Kawasaki-shi, Kanagawa, 214-8565 Japan (anakagami@st.jwu.ac.jp).

Welshons, Nagel, & vom Saal, 2006; Tsai, 2006;) . Bisphenol A (BPA) is an EED that may be released from products such as polycarbonate plastics and epoxy resins which have been used to manufacture food containers. BPA mimics estrogen as well. Recent studies have focused on the effects of low-dose BPA on the central nervous system (CNS), particularly, its effect in preventing the development of sexual dimorphism in the brain of rodents. Kubo et al. (2003) showed the following result in rats: in the control group, females were more active in open-field behavior and had a larger locus coeruleus (LC) volume than males. BPA abolished and inverted the sex differences of both open-field behavior and the LC volume, respectively, without affecting the reproductive system; whereas the rats showed normal phenotype of sexual organs. The behavioral regulatory system in the brain is likely to be more sensitive to EEDs than the systems that regulate reproduction. Moreover, the brain is sensitive to BPA at concentrations lower than the tolerable daily intake (TDI) in humans. Kubo et al. (2003) suggested that the abnormalities in sexual differentiation produced by BPA differ from those caused by resveratrol (RVT) and diethylstilboestrol (DES). Their study showed that DES and RVT affected the LC volume and reproductive system, while BPA abolished and inverted the sex differences of open-field behavior and the LC volume, without affecting the reproductive system. Animal studies indicate that DES acts on parts of the developing fetus other than the reproductive tract, including the brain, the pituitary gland, the mammary glands in the breast, and the immune system, causing permanent changes (Colborn et al., 1996). According to Hines (1996), in animals, exposure to DES or higher levels of estrogen causes “dramatic and permanent changes in brain structure and behavior” (p. 64). The brain and behavior of rats were altered by BPA (Fujimoto, Kubo, & Aou, 2006); that is, BPA affected brain development and altered behavior such as open-field behavior or maternal behavior among rats and mice (Della Seta, Minder, Dessi-Fulgheri, & Farabollini, 2006; Palanza, Howdeshell, Parmigiani, & vom Saal, 2002; Rubin et al., 2006). Moreover, there is clear, although indirect, evidence of long-term alterations in the monoaminergic functions of the brain after perinatal BPA exposure (Adriani, Della Seta, Dessi-Fulgheri, Farabollini, & Laviola, 2003; Negishi et al., 2004). Kuroda (2003) suggested a “spatial–temporal complex pollution theory of toxic chemical substances” (p. 23), which emphasized the importance of the duration and site of exposure of the fetal and infant brain to toxic chemicals. These chemicals (EED, BPA, PCB, etc.) are known to affect neurogenesis during this crucial period as well as during the prenatal, perinatal, and infant periods. Various symptoms and abnormal behaviors have been observed because of slightly altered functions or neural impairment in specific cerebral regions. Studies during the prenatal period involving different species and different methods of administration showed that rats excreted BPA immediately after oral and subcutaneous administration; in contrast, monkeys absorbed BPA very rapidly, maintained a high concentration in the blood, and excreted the compound at a slower rate (Negishi et al., 2004; Tominaga et al., 2006). Chemicals in the blood easily pass into the brain because the blood–brain barrier is not fully functional during infancy. Therefore, the brain of the fetus and neonate is intrinsically sensitive to environmental changes. Environmental factors affecting the brain during this period may cause irreversible and permanent changes (Aou, 2004).

Monkeys exhibit sexual differences in behavior in early life. In normal primate mother-infant relations, it is generally recognized that male rhesus monkey infants are more active and more playful than their female counterparts (Hansen, 1966). Male infants in the wild have been reported to leave their mothers sooner than female infants (Itani, 1959). The female rhesus monkeys (*Macaca mulatta*) remain closely attached to the mothers; the males interact more frequently with other members at the periphery of the troop (Mitchell, 1968). Thus male infants are more active than female infants, while female infants are more frequently in contact with the mother. Rodents exposed to BPA showed behavioral changes and the LC volume of male rodents was equal to that of the females'. This indicates that the brain of the male rat was feminized. Is the sexual difference impaired in the early stage of development reversed later in primates? We consider that it is important for us to identify whether these alterations appear in primates and in which stage of development amelioration occurred.

We considered these neurobehavioral effects to be a result of BPA exposure. In the present study, we administered low-dose BPA during the prenatal period to cynomolgus monkeys (*Macaca fascicularis*), a phylogenetically close relative and a useful model for humans. We report that mother-infant interactions were observed longitudinally. We also observed social interactions between mother and infant immediately after birth and evaluated the behaviors in the offspring to assess sensitivity toward BPA.

Method

Subjects

Colony-bred adult female cynomolgus monkeys (*M. fascicularis*) (body weight 3–4kg, generally healthy) were maintained and bred at Shin Nippon Biomedical Laboratories Ltd. in Kagoshima, Japan. The animals were housed in stainless-steel cages, and received approximately 108g (12g × 9 pieces) of solid diet (Harlan Tekland, Harlan Sprague Dawley Inc., Indianapolis, IN, USA), on a daily basis, which was supplied once a day at 15 h; any food remaining by 9 h the next day was removed. The animals had free access to drinking water.

All mothers were primiparaous monkeys. They were housed individually during pregnancy in stainless steel cages (69cm × 61cm × 75cm) according to the National Institute of Health guidelines. In addition, they continued to care for their offspring until weaning. The duration of pregnancy and body weight of the offspring at birth were recorded (Table 1).

Table 1
Effect of BPA-exposure on gestation length and body weight at birth in cynomolgus monkeys.

	Control		BPA	
	Male	Female	Male	Female
Number of pregnant animals	19		18	
Number of animals normally delivered	6	10	4	9
Gestational length (days)[a]	161.7 ± 5.0	160.9 ± 6.4	159.3 ± 5.0	159.7 ± 7.0
F1 body weight at birth (g)[a]	369 ± 34	372 ± 40	368 ± 40	350 ± 51
Number of animal subjected to observations	6	9	4	6

[a]Normally delivered mothers and infants were used.
Values are mean ± SD.

Maternal BPA Exposure

Eighteen pregnant monkeys received BPA (10μg/kg/day) in a mixture of N, N-dimethylacetamide and polyethylene glycol (400) (1:1) through Alzet® osmotic pumps. These pumps were surgically implanted in the dorsal subcutaneous tissue and each pump released a fixed amount of the solution (6μl/day) from 20 days of pregnancy until delivery. The pumps were renewed every 28 days. Oral administration of 1mg/kg is an effective low dose among rats. Species differences of ADME (absorption, distribution, metabolism and excretion) of BPA between rodents and primates are 1:10, and blood concentration of BPA in subcutaneous injection (s.c.) is 10 times higher than per os (p.o.). (Tominaga et al., 2006). Thus, BPA concentrations were calculated as [body weight (kg) × 10 (μg/kg/day)/6 (μl)]. Control pregnant monkeys received only the vehicle solution using the same osmotic pumps.

Observation Procedure

Each mother and infant lived together in the same cage (69cm × 61cm × 75cm). We observed mother–infant behavior twice a week for a period of 1 week during the first 90 days after birth (the infant has greater developmental changes in the early stage of development) and once a week during the period from 90 to 180 days after birth. The observation started in the morning before food was given. Interactions between the mother and infant in their home cage were recorded for 20min using a digital video camera. The front mesh of the cage was replaced with an acrylic sheet for the observation period.

Table 2
Behavioral Categories of Infant in the Mother–Infant Interactions

Item (Abbreviation)	Explanation
Social behaviors	
Approach (Appr)	Approach mother within infant's reach.
Contact cling (Cntc)	Active body contact, excluding ventral contact.
Lip smacking (Lips)	Rhythmic movements of the lips, sometimes with an occasional click noise.
Nipple contact (Nipc)	Sucking mother's nipple.
Orient (Ornt)	Paying attention to animals and humans outside the cage. Reaction to mainly voice and noise.
Proximity (Prox)	Sitting or standing within mother's reach.
Reject/withdraw (Rjwz)	Rejection of mother's approach or withdrawing to the corners of cage, sometimes shaking its head.
Social exploration (Soce)	Nonaggressive interactions with mother such as licking and visual exploratory action toward her.
Ventral contact (Vntc)	Abdominal clinging to mother.
Nonsocial behaviors	
Auto grooming (Autg)	Grooming oneself.
Environmental exploration (Enve)	Exploratory actions toward surroundings with forelimbs or mouth (licked the glass).
Locomotion (Loco)	Moving on foot or brachiating by clinging to tall limbs and branches.
Outward interest (Outw)	Looking out of the cage from the gap between the acrylic front board and the wall, observing the wire on the floor.
Self-directed behavior (Slfd)	Stimulating oneself with its mouth, hand, or foot, and biting or licking for a long time.
Stereotypy (Styp)	Repetition of the same pattern behaviors in the monotonous rhythm.

We did not use video for five minutes at the beginning of the observation. We used the later 10 minutes for observation to prevent bias, because the part immediately after the beginning of the recording was not suitable for observation. We omitted the first and last 5 minutes of a 20

minute recording period to avoid human influence caused by the technician who switched on and off the video camera. Video recordings of the mother and infant were analyzed separately by the one–zero sampling method every 5s. Infants were observed exhibiting 15 behaviors (Table 2) that were defined beforehand. Each observer was blind to all information about the subjects (BPA group or control group, sex). We used The Observer 5.0 (Noldus Information Technology, Netherlands) to analyze the video observations. The total inter-observer agreement (Cohen's kappa) was 0.88.

We obtained approval for the study and conducted the experiments according to a guideline by IACUC Institutional Animal Care and Use Committee, Graduate School of Agricultural and Life Sciences, the University of Tokyo.

Statistical Analysis

For each behavioral item, we calculated the percentage of total observation time. Each behavioral change was expressed graphically as an average for each month. Infant behaviors were first analyzed by canonical discriminant analyses. Each behavioral category was then analyzed by a three-way analysis of variance (ANOVA). All statistical analyses were performed using SPSS (SPSS Inc.) and StatView (Hulinks Inc.). Significance was set at $p < .05$.

Results

General Observations

During experimental exposure to BPA, three fetal and two neonatal deaths occurred among 18 pregnant animals in the experimental group. Of the 19 animals in the control group, three fetal deaths and one neonatal death were observed. Gestation period and body weights at birth until weaning of the surviving animals are summarized in Table 2. BPA exposure had no significant effect on sex ratio, gestation length, or body weight at birth. In addition, no external abnormalities were observed in either group.

Effect of BPA Exposure on Four Classes of Infant Behaviors

Infant behaviors were analyzed for nine social behaviors and six nonsocial behaviors. The four groups (males and females of the BPA group and males and females of the control group) were discriminated by canonical discriminant analysis based on age-pooled data sets. Discriminant scores of all subjects in the four groups were plotted separately in two dimensions (Figure 1). The result showed that male infants of the control group were distinguishable from the remaining three groups. Control females, BPA females, and BPA males showed similar behavioral tendencies; that is, BPA males showed behavior similar to the female groups (BPA and control female infants). The cumulative contribution to the two functions in the canonical discriminant analysis was 97.1% (Function 1, 89.3%; Function 2, 7.8%). The behaviors contributing to Function 1 were outward interest, i.e. locomotion, orientation, ventral contact, and social exploration. Function 1 represents a measure of static or dynamic mother–infant relationships.

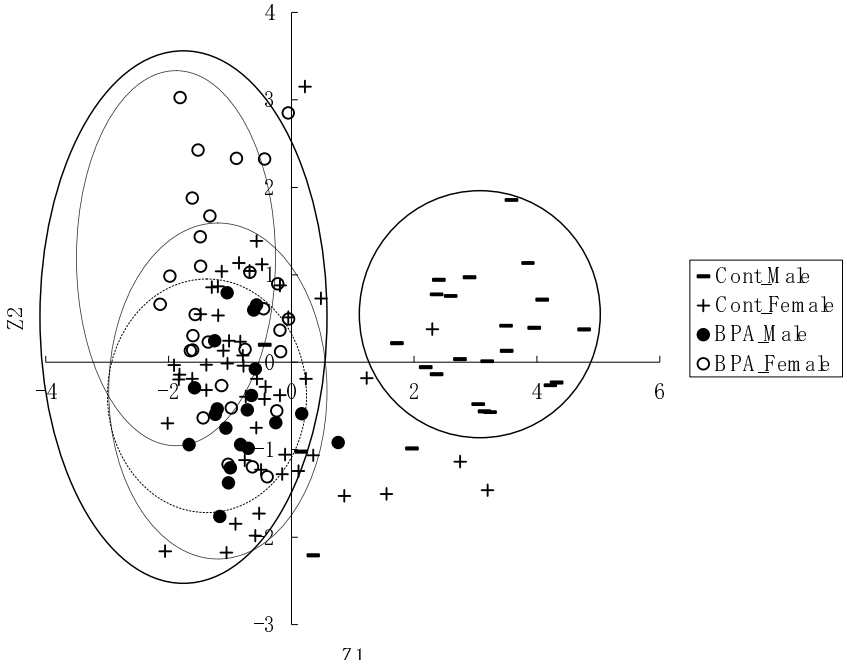


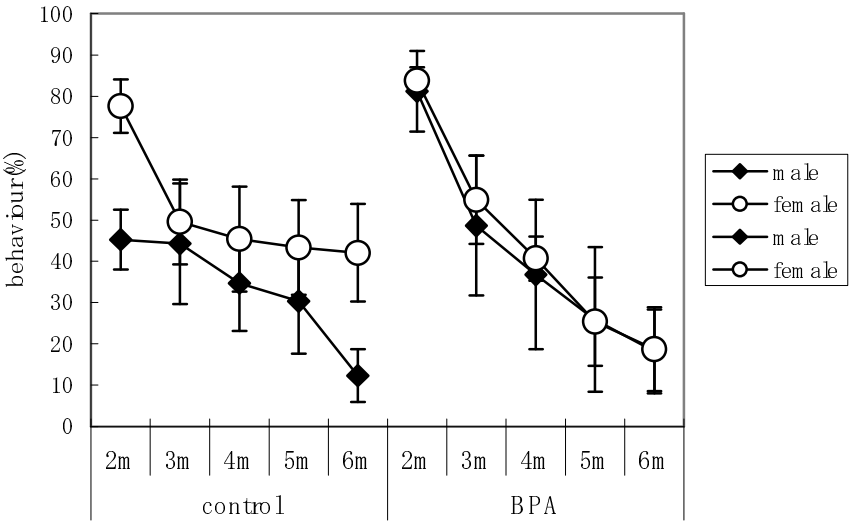
Figure 1. Canonical discriminant analysis (four groups). Subjects were classified into four groups (males & females of the BPA group and males & females of the control group, i.e., cont_male…) in consideration of the sexual differences in behaviors. Z1 represents the measure of static or dynamic mother–infant relationship.

Developmental Changes

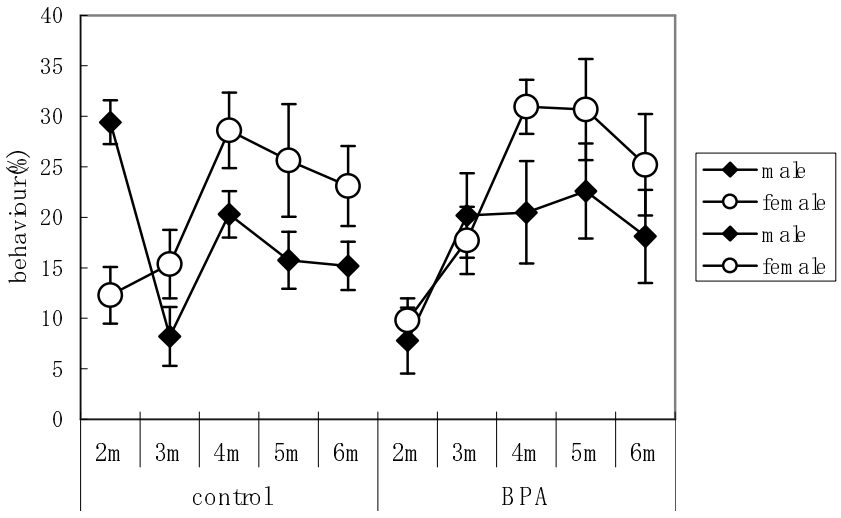
The developmental changes associated with behaviors that contributed to function 1 of the canonical discriminant analysis are indicated in Figure 2. As for these behaviors, analyses of variance (ANOVA) were performed in order to explain the developmental profile. A three-way ANOVA was performed across categories (treatment, sex, and age) to explain which factors caused significant changes. A significant interaction between age and treatment was shown in locomotion ($F_{4,84} = 2.69$, $p < 0.04$), ventral contact ($F_{4,84} = 2.66$, $p < .04$), social exploration ($F_{4,84} = 5.03$, $p < .001$), and outward interest ($F_{4,84} = 4.98$, $p < .001$) among the behaviors contributing to function 1 in the canonical discriminant analysis.

In addition, a two-way ANOVA (one factor about age) was performed to examine the developmental profile on the behavior categories related to the significant interaction of age. These analyses included: (1) two-way ANOVA between the BPA and control groups with two factors: sex × age, and (2) two-way ANOVA (treatment × age) factored by sex.

Ventral contact



Social exploration



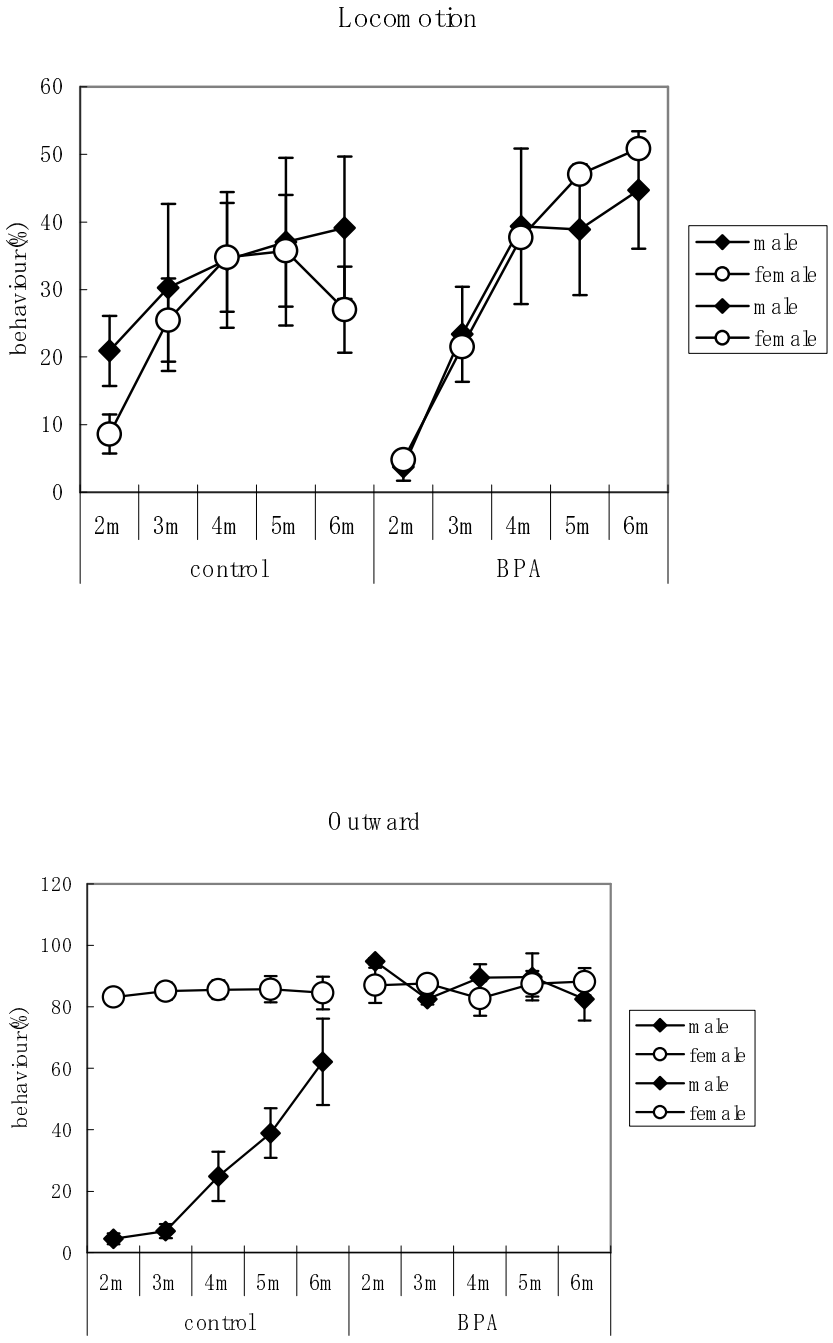


Figure 2. Developmental changes of infant behaviors.

Outward Interest. The three-way ANOVA showed a significant main effect of treatment ($F_{1, 21} = 31.3$, $p < 0.01$) as well as an age/treatment interaction. A significant main effect was also shown for sex ($F_{1, 21} = 16.63$, $p < .01$) and age ($F_{4, 84} = 3.70$, $p < 0.01$). The two-way ANOVA (sex × age) by treatment, showed a significant main effect of sex ($F_{1, 13} = 27.46$, $p < 0.01$) and age ($F_{4, 52} = 8.07$, $p < 0.02$) in the control group. There was also a significant interaction between sex and age ($F_{1, 13} = 5.76$, $p < 0.01$). This result reflects a difference in Outward Interest: males slowly increased, whereas females remained flat from the early stage of development. On the other hand, the main effect and the interaction were not significant in the BPA group. Males and females in the BPA group, as well as females of the control group remained constant in the frequency of Outward Interest from the early stage of development.

Locomotion. The two-way ANOVA (sex × age) showed a main effect of age to be significant in both the BPA group ($F_{4, 32} = 16.04$, $p < 0.01$) and control group ($F_{4, 52} = 3.62$, $p < 0.01$). Both groups showed increase of the behavior with age. Subsequently, another two-way ANOVA (treatment × age) showed a significant main effect ($F_{4, 56} = 15.0$, $p < 0.01$) in females and a significant interaction (treatment × age) ($F_{4, 28} = 2.69$, $p < 0.05$) in males. Locomotion of females increased in both the control group and the BPA group. However, males showed development in the BPA group but not in the control groups.

Ventral Contact. A two-way ANOVA (sex × age) showed that only the main effect of age was significant in both the BPA group and control group: ($F_{4, 32} = 18.22$, $p < 0.01$), ($F_{4, 52} = 5.11$, $p < 0.01$). This indicates that Ventral Contact decreased with development in both groups. Another two-way ANOVA (treatment × age) was performed with respect to both sexes and the results showed the main effect of age to be significant in males ($F_{4,28} = 6.22$, $p < 0.01$) and females ($F_{4,56} = 17.76$, $p < 0.01$). Both males and females exhibited a similar decrease of Ventral Contact as they developed.

Social Exploration. As a result of the two-way ANOVA (sex × age), the control group showed a significant main effect of age ($F_{4,52} = 3.85$, $p < 0.01$) and a significant interaction of age and sex ($F_{4,52} = 3.91$, $p < 0.01$), while a main effect of age was significant ($F_{4,32} = 7.83$, $p < 0.01$) in the BPA group. This is an indication that the developmental pattern of Social Exploration is different in males and females of the BPA group but similar in males and females of the control group. According to the two-way ANOVA (treatment × age), male infants showed a significant interaction ($F_{4, 28} = 7.34$, $p < 0.01$) and female infants showed a significant main effect of age ($F_{4, 28} = 9.6$, $p < 0.01$). This indicates that female infants in both the control group and the BPA group presented similar developmental changes, but in contrast male infants showed different developmental changes of Social Exploration in both groups.

Discussion

Previous rodent studies have shown that low-dose BPA exposure affected sexual behaviors and brain dimorphism in offspring (Della Seta et al., 2006; Fujimoto et al., 2006; Fujimoto, Gioiosa, Fissore, Ghirardelli, Parmigiani, & Palanza, 2007; Kawai et al., 2003; Kubo, & Aou, 2007; Kubo et al., 2003; Kubo et al., 2001; Porrini et al., 2005; Rubin et al., 2006; Tando et al.,

2007). Only two studies have reported the effect of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin exposure on nonhuman primates (Negishi et al., 2006; Schantz & Bowman, 1989), but no studies have addressed the effect of BPA exposure on primate offspring. Findings from rodent studies would be applicable to other species, including humans. Therefore, the present study is an important step in demonstrating the effects of BPA in primates.

The evidence that BPA exposure affects behavior of offspring has statistical significance. Overt behavior is considered the final output of CNS activities, and therefore, behavioral changes suggest the possibility of neurological and neuroendocrinological alteration in the brain.

The present study revealed that prenatal exposure to BPA prevented the sexual differentiation (dimorphism of the brain gender) of infant behaviors, such as Outward Interest and social exploration, and the development of ventral contact and locomotion behaviors in male infants. These observations led to two conspicuous features of prenatal exposure to BPA in cynomolgus monkeys. First, BPA exposure altered infant behaviors in the early developmental stages of the mother-infant interaction. Some of the behaviors in the BPA-exposed group were different from those in the control group, and male behaviors in the BPA-exposed group were feminized. A developmental difference was seen between the BPA-exposed and control groups with regard to locomotion and ventral contact. The control group showed sex differences at an early developmental stage, but the BPA-exposed group did not. Second, male infants were more susceptible to a low dose of BPA. Behaviors in the BPA-exposed males were similar to female behaviors. This shows that prenatal exposure to BPA influenced male infant behavior immediately after birth. Female infant rhesus monkeys received, as well as reciprocated, more positive physical contact than did male infant rhesus monkeys (Mitchell, 1968). Minami (1997) reported that sexual difference was generally apparent in social contacts. Ventral contact rapidly decreased as the infant developed; however, females often showed ventral contact for a longer period than males. Males exhibited locomotion behavior earlier than females. These behavioral differences in the mother-infant interactions were related not only to the experience of the mother but also to the sex of the infant (Mitchell & Brandt, 1970). This difference between sexes gradually increased and appeared among infants at an early stage of development; it influenced their growth and maturation through mother–infant interactions. Our results showed that the effect of BPA exposure on the sexual differentiation of the brain in monkeys was similarly estrogenic in nature, since it interfered with the sexual differentiation and social behavior from an early developmental stage. Normal brain development requires internal signals, such as environmental sensory stimuli, hormones, and growth factors at appropriate times and in appropriate amounts from the perinatal period to the lactation period. Sex hormones play an important role in the sexual differentiation of the brain. When cerebral neurons were exposed to testosterone during the third and fourth fetal months in humans, the brain showed masculine characteristics (Arai, 2004). Such differentiation was evident in a wide range of behaviors, from learning to emotional regulation, and in the autonomic nervous system. It also influenced higher brain functions such as homeostasis, stress responses, emotion, and memory (Aou, 2004). Tominaga et al. (2006) examined how BPA passed through the monkey's placenta on fetal days 50, 80, and 120. BPA was almost

completely excreted in urine within 24hr on fetal day 50, depending on maternal metabolism. However, on fetal day 80, increased blood concentration of BPA in the fetus is higher than that of mother, and was found to be circulating in the CNS of the growing brain. Yoshikawa (2005) considered that nearly the entire amount of BPA can pass through the placenta and affect the spatial-temporal development of the fetal brain.

In the present study, prenatal BPA exposure influenced the development of the brain gender in the CNS. Low-dose exposure to BPA resulted in feminization of masculine behavior. This low-dose exposure during the fetal period functioned as an estrogenic agent and prevented the sexual differentiation of male infants. It is believed that BPA can penetrate the CNS; thus, BPA passes through the placenta and possibly impairs the sexual differentiation of the behavior in the brain. It has been said that transgenderism is physiological in origin, probably because of neurological accidents during the prenatal period (Hood, 2005). Although our results cannot be applied directly to humans, they do support the concept of endocrine-disrupting chemicals destabilizing the sexual differentiation process in the brains of nonhuman primates.

As seen in this study, behavioral feminization of male infants during the early neonatal period diminished in the later stages suggesting that the effects of BPA exposure did not necessarily continue after birth. Further observations are needed to examine whether the effects last until the later developmental stages.

Our study focused on behaviors in the early period of development. Future research into what kinds of sexual behavior would be affected and at which reproductive age the effects are exhibited would be of value. Recently, it is suspected that BPA may be affecting the nervous systems of people in Japan. Therefore researchers should begin to reassess the effects of BPA, not only in rodents and non-human primates, but in humans as well.

References

Adriani, W., Della Seta, D, Dessi-Fulgheri, F., Farabollini, F., & Laviola, G. (2003). Altered profiles of spontaneous novelty seeking, impulsive behavior, and response to D-amphetamine in rats perinatally exposed to Bisphenol A. *Environmental Health Perspectives,* **111**(4), 395-401.

Aou, S. (2004). Bisphenol A and behavioural developmental disorder. *BRAIN MEDICAL,* **16**(4), 325–330. (In Japanese).

Arai, Y. (2004). Sex differentiation of central nervous system – brain of man and woman. *Nippon Rinsho,* **62**(2), 281-292.

Calafat, A. M., Ye, X., Wong, L-Y, Reidy, J. A., & Needham, L. L. (2008). Exposure of the U.S. population to Bisphenol A and 4-tertiary-Octylphenol: 2003–2004. *Environmental Health Perspectives,* **116**(1), 39-44.

Cao, X. L. & Corriveau, J. (2008) Migration of Bisphenol A from polycarbonate baby and water bottles into water under severe conditions. *Journal of Agricultural and Food Chemistry,* **56**(15), 6378-81.

Colborn, T., Dumanoski, D., & Myers, J. P. (1996). *Our stolen future: Are we threatening our fertility, intelligence, and survival?: A scientific detective story.* New York: Penguin.

Della Seta, D., Minder, I., Dessi-Fulgheri, F., & Farabollini, F. (2006). Bisphenol-A exposure during pregnancy and lactation affects maternal behavior in rats. *Brain Research Bulletin,* **65**(3), 255–260.

Even. M. D., Dhar, M. G., & vom Saal, F. S. (1992). Transport of steroids between fetuses via amniotic fluid in relation to the intrauterine position phenomenon in rats. *Journal of Reproduction and fertility,* **96**(2), 709-716.

Fujimoto, T., Kubo, K., & Aou, S. (2006). Prenatal exposure to Bisphenol A impairs sexual differentiation of exploratory behavior and increases depression-like behavior in rats. *Brain Research,* **1068**(1), 49–55.

Fujimoto, T., Kubo, K., & Aou, S. (2007). Environmental impacts on brain functions: Low dose effects of Bisphenol A during perinatal critical period. *International Congress Series,* **1301**, 226–229.

Gioiosa, L., Fissore, E., Ghirardelli, G., Parmigiani, S., & Palanza, P. (2007). Developmental exposure to low-dose estrogenic endocrine disruptors alters sex differences in exploration and emotional responses in mice. *Hormones and Behavior,* **52**(3), 307–316.

Hansen, E. W. (1966). The development of maternal and infant behavior in the rhesus monkey. *Behaviour,* **27**, 107-149.

Hines, M., & Sandberg E. C. (1996). Sexual differentiation of cognitive abilities in women exposed to diethylstilbestrol (DES) prenatally. *Hormones and Behavior* **30**(4), 354-63.

Hood, E. (2005). Are EDCs blurring issues of gender? *Environmental Health Perspectives,* **113**(10), A670–677.

Itani, J. (1959). Paternal care in the wild Japanese monkey, *Macaca fuscata fuscata*. *Primates,* **2**(1), 61-93.

Kang, J. H., Kondo, F., & Katayama Y. (2006). Human exposure to Bisphenol A. *Toxicology,* **226**(2-3), 79-89.

Kato, H., Furuhashi, T., Tanaka, M., Katsu, Y., Watanabe, H., Ohta, Y., et al. (2006). Effects of Bisphenol A given neonatally on reproductive functions of male rats. *Reproductive Toxicology,* **22**(1), 20–29.

Kawai, K., Nozaki, T., Nishikata, H., Aou, S., Takii, M., & Kubo, C. (2003). Aggressive behavior and serum testosterone concentration during the maturation process of male mice: The effects of fetal exposure to Bisphenol A. *Environmental Health Perspectives,* **111**(2), 175–178.

Kubo, K., Arai, O., Ogata, R., Omura, M., Hori, T., & Aou, S. (2001). Exposure to Bisphenol A during the fetal and suckling periods disrupts sexual differentiation of the locus coeruleus and of behavior in the rat. *Neuroscience Letters,* **304**(1–2), 73–76.

Kubo, K., Arai, O., Omura, M., Watanabe, R., Ogata, R., & Aou, S. (2003). Low dose effects of Bisphenol A on sexual differentiation of the brain and behavior in rats. *Neuroscience Research,* **45**(3), 345–356.

Kuroda, Y. (2003). Effects of endocrine disruptors on brain development — Development of novel assay systems for risk assessment — (ESS18). *Environmental Sciences,* **10**, 23-33.

Le, H. H., Carlson, E. M., Chua, J. P., & Belcher, S. M. (2008). Bisphenol A is released from polycarbonate drinking bottles and mimics the neurotoxic actions of estrogen in developing cerebellar neurons. *Toxicology Letters,* **176**(2), 149-56.

Mahalingaiah, S., Meeker, J. D., Pearson, K. R., Calafat, A. M., Ye, X., Petrozza, J., & Hauser, R. (2008). Temporal variability and predictors of urinary bisphenol a concentrations in men and women. *Environmental Health Perspectives,* **116**(2), 173-178.

Minami, T. (1997). Contact behavior in early mother-infant relations in macaques. *The Japanese Journal of Animal Psychology,* **47**(2), 129–136.

Mitchell, G. D. (1968). Attachment differences in male and female infant monkeys. *Child Development,* **39**(2), 611–620.

Mitchell, G. D. & Brandt, E. M. (1970). Behavioral differences related to experience of mother and sex of infant in the rhesus monkey. *Developmental Psychology,* **3**, 149.

Montano, M. M., Welshons, W. V., & vom Saal, F. S. (1995). Free Estradiol in serum and brain uptake of Estradiol during fetal and neonatal sexual differentiation in female rats. *Biology of Reproduction* **53**, 1198-1207.

Nage1, S. C., vom Saal, F. C., Thayer, K. A., Dhar, M. G., Boechler, M., & Welshons, W. V. (1997). Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens Bisphenol A and Octylphenol. *Environmental Health Perspectives,* **105**(1), 70-76.

Negishi, T., Kawasaki, K., Suzaki, S., Maeda, H., Ishii, Y., Kyuwa, S., et al. (2004). Behavioral alterations in response to fear-provoking stimuli and tranylcypromine induced by perinatal exposure to Bisphenol A and nonylphenol in male rats. *Environmental Health Perspectives,* **112**(11), 1159–1164.

Negishi, T., Shimomura, H., Koyama, T., Kawasaki, K., Ishii, Y., Kyuwa, S., et al. (2006). Gestational and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin affects social behaviors between developing rhesus monkeys (*Macaca mulatta*). *Toxicology Letters,* **160**(3), 233–244.

Negishi, T., Tominaga, T., Ishii, Y., Kyuwa, S., Hayasaka, I., Kuroda, Y., et al. (2004). Comparative study on toxicokinetics of Bisphenol A in F344 rats, monkeys (*Macaca fascicularis*), and chimpanzees (*Pan troglodytes*). *Experimental Animals,* **53**(4), 391–394.

Palanza, P. L., Howdeshell, K. L., Parmigiani, S., & vom Saal, F. S. (2002). Exposure to a low dose of Bisphenol A during fetal life or in adulthood alters maternal behavior in mice. *Environmental Health Perspectives,* **110**(3), 415–422.

Porrini, S., Belloni, V., Della Seta, D., Farabollini, F., Giannelli, G., & Dessi-Fulgheri, F. (2005). Early exposure to a low dose of Bisphenol A affects socio-sexual behavior of juvenile female rats. *Brain Research Bulletin,* **65**(3), 261–266.

Rubin, B. S., Lenkowski, J. R., Schaeberle, C. M., Vandenberg, L. N., Ronsheim, P. M., & Soto, A. M. (2006). Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of Bisphenol A. *Endocrinology,* **147**(8), 3681–3691.

Schantz, S. L. & Bowman, R. E. (1989). Learning in monkeys exposed perinatally to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Neurotoxicology and Teratology,* **11**(1), 13–19.

Schantz, S. L., Ferguson, S. A., & Bowman, R. E. (1992). Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on behavior of monkeys in peer groups. *Neurotoxicology and Teratology,* **14**(6), 433–446.

Tando, S., Itoh, K., Yaoi, T., Ikeda, J., Fujiwara, Y., & Fushiki, S. (2007). Effects of pre- and neonatal exposure to Bisphenol A on murine brain development. *Brain & Development,* **29**(6), 352–356.

Tominaga, T., Negishi, T., Hirooka, H., Miyachi, A., Inoue, A., Hayasaka, I., & Yoshikawa, Y. (2006). Toxicokinetics of Bisphenol A in rats, monkeys and chimpanzees by the LC-MS/MS method. *Toxicology,* **226**(2–3), 208–217.

Tsai, W. T. (2006). Human health risk on environmental exposure to Bisphenol-A: A review. *Journal of Environmental Science and Health. Part C, Environmental Carsinogenesis & Ecotoxicology Reviews.* **24**(2), 225-255.

vom Saal, F. S. & Dhar, M. G. (1992). Blood flow in the uterine loop artery and loop vein is bidirectional in the mouse: Implications for transport of steroids between fetuses. *Physiology & Behavior,* **52**(1), 163-171.

Walker, S. E., Keisler, L. W., Caldwell, C. W., Kier, A. B., & vom Saal, F. S. (1996). Effects of altered prenatal hormonal environment on expression of

autoimmune disease in NZB/NZW mice. *Environmental Health Perspectives,* **104**(4), 815-821.

Welshons, W. V., Nagel, S. C., & vom Saal, F. S. (2006) Large effects from small exposures. III. Endocrine mechanisms mediating effects of Bisphenol A at levels of human exposure. *Endocrinology,* **147**(6), 56–69.

Yoshikawa, Y. (2005). Experimental behavioral tests using monkey and rat offspring born from mothers exposed perinatally to EDCs. *Japanese Journal of Psychopharmacology,* **25**(3), 115-124.