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Altered social interactions in male juvenile cynomolgus monkeys prenatally exposed to bisphenol A

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ABSTRACT

Bisphenol A (BPA) is a widespread environmental contaminant, and humans are routinely exposed to BPA. We investigated whether prenatal exposure to BPA influences behavioral development in juvenile cynomolgus monkeys (*Macaca fascicularis*). Pregnant cynomolgus monkeys were implanted with subcutaneous pumps and exposed to 10 μg/kg/day BPA or vehicle (control) from gestational day 20 to 132. Both BPA-exposed and control juvenile monkeys (aged 1–2 years) were assessed using the peer-encounter test that was conducted to evaluate behaviors in social interaction with a same-sex, same-treatment peer. In the encounter test, prenatal BPA exposure significantly reduced *environmental exploration* and *presenting*, a gesture related to sexual reproduction, and increased *visual exploration*, but only in males; furthermore, it significantly reduced the typical sexual dimorphism of the aforementioned behaviors normally observed between male and female juvenile cynomolgus monkeys. This study demonstrates that prenatal BPA exposure affects behavioral development during adolescence and results in the demasculinization of key sexually dimorphic behaviors in male juvenile monkeys.

1. Introduction

Bisphenol A (BPA) is used to make polycarbonate and epoxy resins, resulting in widespread environmental contamination and human exposure. Furthermore, BPA has been detected in the majority of the human population (Braun et al., 2009; Calafat et al., 2008; Padmanabhan et al., 2008; Tsutsumi, 2005). Several studies have described adverse effects of BPA exposure on the reproductive system (Munoz-de-Toro et al., 2005; Timms et al., 2005; Vandenberg et al., 2007), progression of physical puberty (Howdeshell et al., 1999), adult behaviors (Diaz Weinstein et al., 2013; Luo et al., 2013), enhanced risks of obesity (Miyawaki et al., 2007; Somm et al., 2009), type 2 diabetes mellitus (Alonso-Magdalena et al., 2011), and tumor development (Betancourt et al., 2010). In addition, adverse effects of pre- and perinatal BPA exposure on brain development (Facciolo et al., 2005; Han et al., 2011; Kubo et al., 2001, 2003; McCaffrey et al., 2013; Rubin et al., 2006; Tando et al., 2007) and neurobehavioral development (Adriani et al., 2003; Aloisi et al., 2002; Fujimoto et al., 2006; Goncalves et al., 2010; Laviola et al., 2005; Negishi et al., 2003, 2004; Yu et al., 2011) are of growing concern, among which the disturbance of monoaminergic system development (Adriani et al., 2003; Kubo et al., 2001; Laviola et al., 2005; Negishi et al., 2004) and the lack of brain and/or behavioral sexual dimorphism (Fujimoto et al., 2006; Kubo et al., 2003; McCaffrey et al., 2013; Rubin et al., 2006) should be particularly noted. However, some studies argued that there are minimal or no adverse effects of BPA (Kobayashi et al., 2012; Kwon et al., 2000; Ryan et al., 2010), which leaves room for discussion regarding the risk of BPA exposure on human health and development.

Nonhuman primates such as macaque monkeys have a close evolutionary relationship with humans and have been successfully used in numerous biomedical fields, including research on prenatal exposure to environmental chemicals. The findings obtained with nonhuman primates are expected to fill the gaps in data between rodents and humans. The nonhuman primate model has been used to assess the effects of environmental contaminants, such as dioxin (Negishi et al., 2006), polychlorinated biphenyls (PCBs) (Levin et al., 1988; Nakagami et al., 2011; Rice and Hayward, 1999; Schantz et al., 1989), methylmercury (Burbacher et al., 2005; Gilbert et al., 1996; Rice, 1998a), and lead (Rice, 1998b), on behavioral developments. Moreover, a recent

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study reported that prenatal BPA exposure has adverse effects on the midbrain dopamine neurons and hippocampus spine synapses in rhesus monkeys (Elsworth et al., 2013).

We previously reported that male infant cynomolgus monkeys (aged 2–3 months) prenatally exposed to BPA exhibit female-like behaviors during mother–infant interactions (Nakagami et al., 2009), but it is uncertain whether these behavioral alterations are transient or long lasting. In the present study, animals from the prenatal exposure cohort were tested in the behavioral peer-encounter test that was conducted to evaluate behaviors in social interaction with a same-sex, same-treatment peer (Negishi et al., 2006) as juveniles (aged 1–2 years) to evaluate the long-term effects of developmental BPA exposure on social and nonsocial behaviors.

2. Materials and methods

2.1. Animals

In this study, the cynomolgus monkeys (*Macaca fascicularis*) were the offspring of 37 hematologically and serologically normal adult females (body weight: 2.5–4.0 kg; age: 5–13 years) (China National Scientific Instruments and Materials Import/Export Corporation, China) who were the same monkeys used in our previous study (Nakagami et al., 2009) and were exposed to BPA throughout pregnancy. Animal breeding, mating, and behavioral experiments were performed at the Shin Nippon Biomedical Laboratories (SNBL), Ltd., Japan. All animals were individually housed in stainless cages constructed according to the NIH guidelines (69 × 61 × 75 cm). The environment was maintained at 26 ± 2 °C with humidity of 50 ± 10% under a 12-h light–dark cycle. All animals received 108 g of food pellets (12 g × 9 pellets) (Teklad Global Certified 25% Protein Primate Diet, Harlan Sprague Dawley, Inc., IN, USA) once daily and water *ad libitum*. All animal cages used in this study were washed daily. Each female monkey with normal menstrual cycles (20–32 days) was caged with a healthy male monkey randomly selected from the breeding colony in SNBL for 3 days during the expected period for ovulation (11–15 days after menstruation) (one male and one female in a cage) and then housed individually. Consequently, no female monkey copulated with the same male monkey. A trained observer confirmed copulation or presence of intravaginal sperm. Gestational day 0 was defined as the median day of the 3-day mating period. The mothers and their infants [delivery day was designated as postnatal day (PND) 0] housed together until weaning at 7–8 months of age. After weaning, juvenile monkeys were housed individually with only visual access to other monkeys. At PND 0 and 28, body weight of offspring was measured after separation from its mother under anesthesia. At PND 196, 360, and 720, body weights of juvenile monkeys were measured under brief restraint in a small transfer cage. It should be noted that the juvenile monkeys used in this study were the same in our previous study (Nakagami et al., 2009) reporting behavioral alterations by prenatal BPA exposure in mother–infant interactions. Details regarding the breeding and outcomes are available in our previous publication (Nakagami et al., 2009).

2.2. Chemical treatment

The schedule of BPA administration is shown in Fig. 1A. In the present study, BPA exposure began on gestational day 20. Alzet® osmotic pumps (DURECT Corporation, CA, USA), which release a fixed volume of solution (6 μL/day) for 28 days, were surgically implanted into the dorsal subcutaneous tissue of the mother under light anesthesia (ketamine, 5 mg/kg, im). Eighteen pregnant monkeys received BPA (Tokyo Chemical Industry Corporation, Ltd., Japan) dissolved in a vehicle consisting of N,N-dimethylacetamide (Wako Pure Chemical Industries, Ltd., Japan) and polyethylene glycol (1:1) (Wako Pure Chemical Industries) through the osmotic pumps, and 19 received the vehicle. To administer 10 μg/kg/day BPA to pregnant monkeys, BPA concentration in the osmotic pumps was calculated before implantation using the following formula: body weight (kg) × 10 (μg/kg/day)/6 (μL). Pumps were replaced every 28 days [on gestational days 48, 76, 104, and 132 (extirpation)] under anesthesia with ketamine. The normal gestational period for cynomolgus monkeys is 160–170 days. Our previous study revealed route dependence [oral *vs.* subcutaneous (s.c.) administration] and remarkable interspecies differences (rats *vs.* cynomolgus monkeys) in the bioavailability of BPA [area under the curve for 24 h (AUC_{24h})] (Tominaga et al., 2006), in which the ratio of AUC_{24h} in cynomolgus monkeys exposed to BPA at 10 mg/kg by oral gavage to that in rat exposed to BPA in a similar manner was 5.9, and the ratio of AUC_{24h} in monkeys exposed to BPA by s.c. injection of dosage 10 mg/kg to that in monkeys exposed to the same dose of BPA orally was 443.6. In brief, this study indicated that the ratio of the bioavailability in monkeys exposed to BPA subcutaneously to that in rats exposed to BPA orally would be 2000 to 3000 [$AUC_{24\ h\ (monkey,\ s.c.,\ 10\ mg/kg)}/AUC_{24\ h\ (rat,\ p.o.,\ 10\ mg/kg)}$], which indicated that approximately a dosage of 10 μg/kg/day BPA administered by s.c. injection to monkeys was equivalent to an oral dosage of approximately 5 mg/kg/day in rats. To date, no-observed-adverse-effect-level (NOAEL) of BPA base on the results of studies using rats, which were conducted according to standardized toxicity test guidelines, was 5 mg BPA/kg/day by oral gavage (Tyl et al., 2002). This dose was used as a cutoff dose for low-dose effects in the National Toxicology Program's report of the endocrine disruptors low-dose peer review (Melnick et al., 2002). This was the reason why we selected 10 μg/kg/day BPA in the present study. All experiments were humanely performed in accordance with the guidelines for animal experimentation of SNBL and were approved by the Animal Care and Use Committee of the Graduate School of Agricultural and Life Sciences, University of Tokyo.

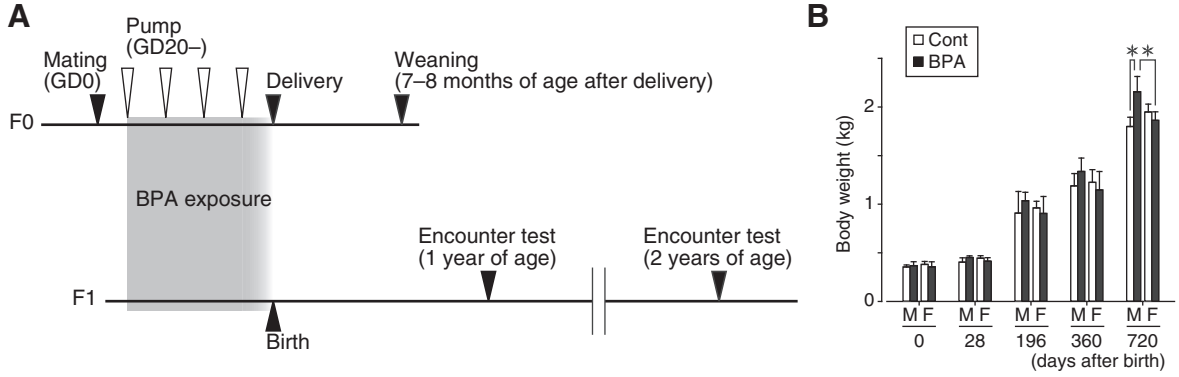

Fig. 1. Prenatal BPA exposure in cynomolgus monkeys. (A) The schedule of BPA administration and behavioral tests performed on the offspring. (B) Developmental increases in body weights of offspring (mean ± SD, n = 4/group).