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Transgenerational effects of prenatal bisphenol A on social recognition

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ABSTRACT

Bisphenol A (BPA) is a man-made endocrine disrupting compound used to manufacture polycarbonate plastics. It is found in plastic bottles, canned food linings, thermal receipts and other commonly used items. Over 93% of people have detectable BPA levels in their urine. Epidemiological studies report correlations between BPA levels during pregnancy and activity, anxiety, and depression in children. We fed female mice control or BPA-containing diets that produced plasma BPA concentrations similar to concentrations in humans. Females were mated and at birth, pups were fostered to control dams to limit BPA exposure to gestation in the first generation. Sibling pairs were bred to the third generation with no further BPA exposure. First (F1) and third (F3) generation juveniles were tested for social recognition and in the open field. Adult F3 mice were tested for olfactory discrimination. In both generations, BPA exposed juvenile mice displayed higher levels of investigation than controls in a social recognition task. In F3 BPA exposed mice, dishabituation to a novel female was impaired. In the open field, no differences were noted in F1 mice, while in F3, BPA lineage mice were more active than controls. No impairments were detected in F3 mice, all were able to discriminate different male urine pools and urine from water. No sex differences were found in any task. These results demonstrate that BPA exposure during gestation has long lasting, transgenerational effects on social recognition and activity in mice. These findings show that BPA exposure has transgenerational actions on behavior and have implications for human neurodevelopmental behavioral disorders.

Introduction

Humans encounter a variety of endocrine disrupting compounds (EDCs) on a daily basis (Bergman et al., 2013). Studies with rodents, primates, and other species demonstrate that exposure during development can increase disease risk as adults (Diamanti-Kandarakis et al., 2009; Flint et al., 2012; Kundakovic and Champagne, 2011; Skinner et al., 2011; Wolstenholme et al., 2011a). Moreover, some EDCs have long lasting actions. Generations after a prenatal exposure, disease rates continue to be higher in EDC-exposed lineages than controls (Anway and Skinner, 2006; Doyle et al., 2013; Jirtle and Skinner, 2007; Manikkam et al., 2013; Salian et al., 2009). The World Health Organization (WHO) recently stated that wildlife, laboratory animal, and human studies show a strong likelihood that early life EDC exposure increases risk for reproductive dysfunction, cancers, obesity, diabetes, and behavioral disorders (Bergman et al., 2013). The present study was conducted to determine the long lasting and perhaps permanent effects of one such EDC, Bisphenol A, on juvenile behaviors using a mouse model and exposures within the range typically found in humans.

Bisphenol A (BPA) is a man-made EDC commonly used as a plasticizer. This compound causes heritable transgenerational reductions in sperm quality and changes in behavior and gene expression (Manikkam et al., 2013; Wolstenholme et al., 2012). High prenatal and/or childhood BPA concentrations in urine are correlated with various social and emotional behaviors in children such as increased activity, impulsiveness, aggression, and poor emotional control (Braun et al., 2009, 2011; Harley et al., in press; Miodovnik et al., 2011). An earlier WHO report on safety of BPA also noted this potential problem (FAO/WHO, 2010). In animals, early life exposure to low doses of BPA increases social behavior and social interactions, anxiety and aggression, and causes some cognitive impairments (Cox et al., 2010; Kawai et al., 2003; Kundakovic et al., 2013; Patisaul and Bateman, 2008; Porrini et al., 2005; Wolstenholme et al., 2011b, 2012). BPA also affects maternal behavior (Cox et al., 2010; Della Seta et al., 2005; Kundakovic et al., 2013; Palanza et al., 2002), which can impact offspring behaviors in adulthood (Daxinger and Whitelaw, 2012; Skinner et al., 2011). We recently reported that human-relevant BPA exposure during gestation changed behavior in pairs of interacting juvenile mice, and decreased vasopressin (*Avp*) and oxytocin (*Oxt*) transcripts in embryonic brain (Wolstenholme et al., 2012). Both neuropeptides are part of the neural circuitry that controls social recognition (Bielsky and Young, 2004; Bielsky et al., 2005; Choleris et al., 2003; Lim and Young, 2006; Shepard et al., 2009). Moreover, these effects were transgenerational, present in both first (F1) and fourth (F4) generation mice.

In the present study, we exposed mice to the same human-relevant dose of BPA (Wolstenholme et al., 2012) throughout gestation. We tested the F1 generation offspring as juveniles, which were directly exposed to BPA during gestation. To determine if the effects we observed were

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transgenerational, juvenile mice in the F3 generation, the first generation not directly exposed to BPA, were tested. Changes found in this generation are transmitted via the germline and are likely to be permanent (Anway and Skinner, 2006; Jirtle and Skinner, 2007). We tested all mice for social recognition, a task regulated by vasopressin and oxytocin. In this task, repeated exposure to the same animal typically produces habituation, a decline in social investigation. When normal mice are exposed to a novel individual; investigation is elevated; this is dishabituation. Changes in social recognition may have many underlying mechanisms. Here, we used the open field to assess the possible contributions of anxiety and locomotor activity in juvenile BPA and control mice. In addition, we examined olfactory discrimination abilities in F3 adults to determine if BPA could have long-term effects on olfactory function.

Methods and materials

Animals

All procedures were conducted in compliance with the University of Virginia Animal Use and Care Committee. Mice were housed in a 12:12 light cycle (lights off at 1200 EST). All mice used in these studies were C57BL/6 originally purchased from the Jackson Laboratory. Adult female C57BL/6J mice from Jackson Laboratories (Bar Harbor, ME) were randomly assigned to either a phytoestrogen-free chow (n = 74, Harlan Teklad TD95092) or the same chow supplemented with 5 mg BPA per kg diet (n = 22, Harlan Teklad TD09386). Mice were switched to these diets 7–10 days before pairing with a male for two weeks. All females consumed their assigned diets and water *ad libitum*. At this BPA dose, we calculated BPA intake to be 20 μg per day (Wolstenholme et al., 2012). Free BPA levels in the plasma of pregnant dams consuming this dose of BPA averages 3.9 ng/ml (Wolstenholme et al., 2012), within the range (0.3–4.0 ng/ml) reported in pregnant women (Vandenberg et al., 2007).

Within 12 h after birth, pups were placed with foster dams (on control diet) that had given birth within the past 24 h. We did this to limit the offspring's BPA exposure to gestation, and because BPA may cause differences in maternal behavior which could effect offspring behavior (Cox et al., 2010; Kundakovic et al., 2013). Foster dams (n = 48) retained two biological pups, not included in the study, and received 4 fostered pups from the same litter (control litters n = 26, BPA litters n = 22). To distinguish the biological from foster pups, we clipped tail tips of the biological pups. All pups (control: n = 23 males, n = 19 females; BPA: n = 22 males, n = 20 females) were weaned at postnatal day 21 (PN21) when they were placed on standard chow (Harlan Teklad diet #7912) containing phytoestrogens, group housed (3–5 per cage) by litter and sex, and tested for behaviors. F1 and F2 brother–sister pairs (F1, BPA n = 9, control n = 9, F2, BPA n = 15 control n = 15) were used to produce the transgenerational offspring. As adults, males were introduced into the cages of adult females for mating (~2–3 weeks) and removed prior to birth of the pups. All subsequent generations consumed standard mouse chow containing phytoestrogens (Harlan Teklad diet #7912). As adults, the F3 mice used for the olfaction test were briefly placed on control diet during breeding to create F4 offspring for another study. Adults consumed standard chow for at least two weeks prior to testing.

Behavior tests

Each mouse was tested in only one behavior task. Only one mouse of each sex was tested in each litter to reduce potential litter effects. Social and odor recognition tasks were scored live between 1000 and 1200 h. Open field activity was conducted in the dark between 1200 and 1800 h and recorded under red light. Test boxes were cleaned with 10% ethanol and wiped dry between tests. All tests were scored by observers blind to the treatment conditions of the mice.

Juvenile social recognition

On PN21, juvenile mice were singly housed for 20 min in a standard mouse cage with bedding but no food or water. A small metal cylinder with a round top (10.16 cm diam. × 13.97 cm) and vertical bars (spaced 1 cm apart) was placed in the cage for 10 min. During the habituation phase, the same ovariectomized (OVX) C57BL/6J adult was repeatedly placed under the cylinder for eight 1-minute trials each separated by a 9-minute inter-trial interval. On the ninth trial, a novel OVX female was placed under the cylinder for 1 min (Imwalle et al., 2002; Tejada and Rissman, 2012). The time each juvenile (F1 control: n = 15 males, 10 females; F1 BPA: n = 14 males, 12 females, F3 control: n = 9 males, 8 females; F3 BPA n = 7 males, 8 females) spent investigating the cylinder and/or the stimulus female was recorded. Investigation is defined as contact with the head or body of the stimulus mouse at a distance less than 1 cm or directly touching the bars of the cylinder. Ovariectomized females were used as the stimulus mice to reduce possible variability caused by the estrus cycle.

Juvenile open field activity

Juvenile (PN23–24) mice were habituated to the behavioral testing room for one hour. The open field apparatus is a large white Plexiglas box (60 cm × 60 cm × 45 cm) divided evenly into a 25 (5 × 5) square grid. F1 and F3 mice from BPA (F1: control n = 8 males, 8 females, F3: n = 6 males, 7 females) and control (F1: n = 8 males, 9 females, F3: n = 6 males, 7 females) lineages were placed in one corner of the apparatus. Locomotor activity was recorded for 5 min by video and scored using Noldus Observer (Leesburg, VA). The box was divided into three zones, as described (Imwalle et al., 2002): corner, wall and center. Locomotor activity was scored as the number of grid lines crossed in each zone. The center zone consisted of the inner nine squares, the wall zone consisted of the outer twelve squares, and the corner zone consisted of the remaining four corner squares. Increased time spent in the center zone is interpreted as a low anxiety phenotype, while more time in the corners or next to the wall indicates higher anxiety.

Adult odor discrimination test

To assess olfactory abilities in mice from the BPA and control lineages, adult F3 males and females were tested in an odor habituation–dishabituation task (Yang and Crawley, 2009). Urine was collected from two sets of adult male mice (n = 4/group), pooled into two groups (pool A and B), aliquots were immediately frozen, and stored at −80 °C. On the test day, F3 adult mice from the control (n = 5 males and 6 females) and BPA lineages (n = 6 males and 6 females) were habituated to a clean cage for 30 min then repeatedly exposed to volatile odors from water or urine. Ten microliters of water or urine was dropped onto a piece of filter paper taped to a small plastic weigh boat and inverted onto an empty wire cage lid. Mice had three two-minute sessions with water with one-minute intervals between each presentation. This was followed by three presentations of one urine pool. In the third trial, the other urine pool was used. The order of presentation of the two urine pools was counter-balanced between groups; water was always presented first.

Statistical analysis

All data were analyzed using NCSS (Kaysville, Utah 2007). For social recognition and odor discrimination, we used two-way repeated measures ANOVA with diet and sex as main factors and trials as the repeated measure. For the social recognition test, data from trials 1–8 and trials 8–9 were subjected to separate two-way repeated measures ANOVAs with diet and sex as factors and trial as the repeated measure. To analyze open field behavior we used, two-way ANOVA with sex and diet as factors. Significant results were assessed by Fisher Exact post-hoc