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Effects of perinatal bisphenol A exposure during early development on radial arm maze behavior in adult male and female rats

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ABSTRACT

Previous work has shown that exposure to bisphenol A (BPA) can affect anxiety behavior. However, no studies have examined whether administration of this endocrine disruptor during the perinatal period has the potential to induce alterations in cognitive behavior in both adult males and females as assessed in an appetitive task. The goal of the current study was to determine whether exposure to different doses of BPA during early development alters performance on the 17-arm radial maze in adulthood in Long–Evans rats. Oral administration of corn oil (vehicle), 4 μg/kg, 40 μg/kg, or 400 μg/kg BPA to the dams occurred daily throughout pregnancy, and the pups received direct oral administration of BPA between postnatal days 1–9. Blood was collected from offspring at weaning age to determine levels of several hormones (thyroxine, thyroid stimulating hormone, follicle stimulating hormone, luteinizing hormone). One male and one female from each litter were evaluated on the 17-arm radial maze, a working/reference memory task, in adulthood. Results indicated that after exposure to BPA at both 4 and 400 μg/kg/day, rats of both sexes had decreased levels of FSH at weaning. There were no significant effects of BPA on performance on the radial arm maze in males or females. In conclusion, exposure to BPA during early development had modest effects on circulating hormones but did not affect performance on a spatial learning and memory task.

1. Introduction

Bisphenol A (BPA), an endocrine disruptor, is a chemical used in the production of many products including polycarbonate plastics, resins used to line cans, thermal or recycled paper, and certain dental sealants (Biedermann et al., 2010; Biles et al., 1997; Brotons et al., 1995; Krishnan et al., 1993; Olea et al., 1996; Ozaki et al., 2004). According to the recent National Health and Nutrition Examination Survey, approximately 93% of the U.S. general population shows detectable amounts of BPA metabolites in urine (Calafat et al., 2008). BPA has been classified as an endocrine disruptor due to its ability to bind to several hormone receptors, such as estrogen and thyroid receptors (Gould et al., 1998; Kuiper et al., 1997, 1998; Moriyama et al., 2002; Zoeller et al., 2005). The widespread use and endocrine-disrupting potential of BPA have given rise to the concern that perinatal exposure to BPA may exert long-lasting changes in cognitive behavior. The National Toxicology Program (NTP) (2008) reviewed published studies to conclude that there are some concerns for adverse neural and behavioral effects of developmental exposure of BPA on fetuses, infants, and children. However, the available investigations of long-lasting changes in cognitive behavior in both males and females are limited.

Several studies have employed the water maze to examine whether perinatal exposure to BPA alters spatial learning and memory in adult rodents, and mixed results have been reported (Goncalves et al., 2010; Nakamura et al., 2012; Xu et al., 2010). For example, oral exposure of 40 μg/kg/day BPA to males and females resulted in either enhancements or impairments relative to controls in terms of latency to find the platform and path length during the training days, depending on whether exposure occurred during gestation, during lactation, or during both developmental time periods (Goncalves et al., 2010). Another study examining performance in the water maze in males found impairments in the higher dose groups (50,000, 5000, and 500 μg/kg), but not in the lowest dose group (50 μg/kg) after oral administration to the dams during gestation and lactation (Xu et al., 2010). In contrast, two studies failed to find significant effects on water maze performance in males and females after gestational and lactational exposure with subcutaneous injections of 20 μg/kg/day BPA and gavage of 2.5 or 25 μg/kg/day BPA (Ferguson et al., 2012; Nakamura et al., 2012). The differences in results between these studies may be due to methodological differences, such as dose of BPA and route of administration. Two of the previously mentioned studies also reported impairments in the passive avoidance task in adult males and females (Goncalves et al., 2010; Xu et al., 2010). In

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addition, Kubo et al. (2001) found that 1500 μg/kg/day BPA abolished a sex difference in a passive avoidance task.

Although results from these studies suggest that BPA may alter water maze and passive avoidance performance, they may not generalize to other types of tasks. The water maze is based on rats finding the water aversive, and it has been shown that exposure to water at temperature ranges used in the previously discussed studies (around 25 °C) results in a two-fold increase in serum corticosterone concentrations (often used as an indicator of stress) compared to rats not exposed to the water (Sandi et al., 1997). Given the reports of changes in anxiety behavior after BPA (Adriani et al., 2003; Cox et al., 2010; Fujimoto et al., 2006; Gioiosa et al., 2007; Kubo et al., 2001, 2003; Patisaul and Bateman, 2008; Rubin et al., 2006; Ryan and Vandenbergh, 2006), water-induced changes in anxiety and stress may confound results found with the water maze. Similar concerns arise with passive avoidance behavior, which also uses an aversive stimulus (footshock) to induce motivation to perform the task (Goncalves et al., 2010; Kubo et al., 2001; Xu et al., 2010). Therefore, it is important to assess behavior in appetitive tasks to avoid this potential confound.

Only a few studies have used land-based maze tasks to assess effects of perinatal BPA on learning and memory. In Ryan and Vandenbergh (2006), female mice were exposed to 0, 2, or 200 μg/kg/day BPA by gavage during gestation and lactation. Mice were then tested on the Barnes maze around postnatal day (PND) 42 and the 8-arm radial arm maze about 1 week later. No treatment effects of BPA were seen in either task. Additionally, no differences in performance on the Barnes maze were seen at PNDs 47–50 when males and females were orally exposed to 2.5 or 25 μg/kg/day BPA during gestation and lactation (Ferguson et al., 2012). It should be noted that the ages of rodents tested correspond to adolescence/late adolescence, and therefore, it is unknown whether adult behavior was altered.

Given the available evidence, it is possible that BPA alters behavior or sex differences in behavior. However, more studies are needed to identify dose-dependent effects of perinatal exposure to BPA in both males and females, especially with respect to cognitive-related behaviors. A number of studies previously discussed did not use a range of doses of BPA that are relevant to human exposure and/or used only males or only females. The current study examines the long-term effects of early developmental exposure to BPA on acquisition of a 17-arm radial maze task in both male and female adult rats. There are several reasons for why this behavioral task was implemented. First, the 17-arm radial maze is a task that is considered challenging, making it more likely to be sensitive to subtle effects of chemical exposures. Second, previous results from our lab and others have demonstrated sex differences in performance on this radial arm maze task, with males committing fewer reference and working memory errors than females (Beatty, 1984; LaBuda et al., 2002; Mishima et al., 1986; Seymoure et al., 1996; Tees et al., 1981; Williams and Meck, 1991). The current experiment employed several doses of BPA to investigate whether perinatal exposure to BPA alters spatial learning in adult males and females.

2. Methods

2.1. Breeding and housing procedures

Adult (>80 days old) female and male Long–Evans rats were obtained from Harlan (Indianapolis, IN) and used as breeders. Due to the long length of time needed to behaviorally test the exposed offspring, two cohorts of animals, separated by one year, were used. Precautions were undertaken to minimize exposure to materials that might contain BPA (recycled paper, polycarbonate plastics, etc.). All rats in the current study were housed in clear, polysulfone cages (except during breeding) and given access to reverse osmosis water in glass or polysulfone bottles. The diet used (Harlan 2020X) contained low, but stable amounts of phytoestrogens. Prior to breeding, female breeders were pair- or triple-housed, while males were single-housed due to aggressive behavior towards cagemates. Breeding procedures did not begin until at least 2 weeks after arrival from Harlan. During the time between arrival and breeding, male and female breeders were handled at 3–5 times (at least once per week). After a bout of handling, the experimenter observed that each female breeder received 100 μl of tocopherol-stripped corn oil pipetted onto 1/2 of a cookie (Newman's Own Arrowroot flavor, Westport, CT) in their home cage. For breeding, one male and one female were placed in a metal breeding cage and were monitored daily for the presence of at least one sperm plug underneath the cage. Upon detection of a sperm plug, females were removed from breeding cages and were single-housed in polysulfone cages throughout the remainder of the experiment.

2.2. Dosing procedure

Dosing of pregnant females was initiated on the first day of detection of a sperm plug, which was designated as gestational day (GD) 0, and continued daily until parturition. BPA powder was suspended in tocopherol stripped corn oil to make different solutions (0, 0.01. 0.1, or 1 mg BPA/ml oil), and these solutions corresponded to daily doses of 0, 4, 40, or 400 μg BPA/kg of body weight/day throughout the entire pregnancy. Pregnant females were assigned to treatment groups according to body weight at GD 0 so that no differences in average body weight were evident between the groups at the start of dosing. Fresh solutions were prepared prior to each of the two cohorts of rats. During each day of dosing, females were weighed, and solutions were mixed with a stir bar to ensure homogeneity of the suspension. 0.4 μl of solution/g of body weight was pipetted on 1/2 of a cookie, and the cookie was allowed to dry for several minutes before administration to each dam in their home cages. Dams consumed the 1/2 of a cookie within 5 min.

The day of parturition was designated as postnatal day (PND) 0. Litter size and sex ratio were assessed on PND 1. At PND 2, litters were culled to a maximum of 10 pups while trying to maintain a balanced sex ratio in each litter as much as possible. The minimum litter size was 7. Similar to dosing of the pregnant females, solutions (0, 0.002, 0.02, and 0.2 mg BPA/ml of oil) were mixed daily and prepared prior to the start of each cohort. Direct oral administration of the same doses of BPA solutions that were given to dams (0, 4, 40, and 400 μg BPA/kg of body weight/day) were given to pups beginning the day after birth (PND 1) and continued until PND 9. This dosing regimen was chosen to model human gestational exposure as it is estimated that development processes that occur from birth to approximately PND 10 in rats correspond to the third trimester in humans (Clancy et al., 2007; Dobbing and Sands, 1979). Although many previous studies relied on lactational transfer of BPA from dams to pups as a route of exposure, recent evidence suggests that this method results in extremely low exposures to the pups (Doerge et al., 2010). In order to dose a litter, the dam was placed in a separate cage. Separation of the dam from the pups lasted around 10–15 min. Each pup was weighed and sexed, a pipette was placed in the mouth, and the solution was dispensed. Pups were given 2 separate bouts of oral dosing (with the second bout occurring immediately after the first) for a total volume of 2 μl solution/g body weight. The weights of each sex were averaged for the litter for later analysis.

2.3. Growth and development analyses

At PND 23, all offspring were weaned. 2 females and 2 males from each litter were weighed, ear-punched, and retained for future behavioral endpoints. Dams and remaining littermates were sacrificed at this time, and various measurements were recorded including body weight, liver weight, brain weight, and the number of implantation sites in the dams. Analyses of offspring body weight at weaning represent an average of all of the females and all of the males from each litter. All rats retained for later measures were double-housed with same sex littermates and handled once a week for the remainder of the experiment.