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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 a spatial learning and memory task.

Keywords

BPA; T4; thyroxine; cognition; spatial learning

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.,

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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 has 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 $\mu\text{g/kg/day}$ 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, 5,000, and 500 $\mu\text{g/kg}$), but not in the lowest dose group (50 $\mu\text{g/kg}$) 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 $\mu\text{g/kg/day}$ BPA and gavage of 2.5 or 25 $\mu\text{g/kg/day}$ 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 addition, Kubo et al. (2001) found that 1500 $\mu\text{g/kg/day}$ 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 Vandenberg, 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 Vandenberg (2006), female mice were exposed to 0, 2, or 200 $\mu\text{g/kg/day}$ 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 PND 47-50 when males and females were orally exposed to 2.5 or 25 $\mu\text{g/kg/day}$ 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 ½ 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 ½ 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 ½ of a cookie within 5 minutes.

The day of parturition was designated as postnatal day (PND) 0. Litter size and sex ratio was 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 minutes. 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.

Beginning at PND 25, females were checked daily for vaginal opening, and males were checked daily for preputial separation beginning on PND 30 to determine onset of puberty. Puberty onset was averaged from the 2 female and 2 male offspring from each litter. Prior to food restriction for behavioral testing in adulthood, weights for all rats were recorded around PND 85, and these weights were used for analysis of adult body weights. Ad libitum food was returned immediately upon completion of behavioral testing. Although body weights were also measured prior to sacrifice (around PND 140), these were not analyzed due the variability in time (2-5 weeks) between the end of food deprivation and sacrifice. At the time of sacrifice liver and brain weights of the offspring were also recorded. These measures taken in adulthood from the offspring (adult body, brain, and liver weights) were calculated as an average of the two offspring from each sex that were available from each litter.

2.4 Hormone Serum Analyses

At weaning, trunk blood was collected from littermates that were not used for further behavioral endpoints. Blood samples were spun in a centrifuge at 3500 rpm for 10 minutes, and then serum was harvested and stored at -20°C until thawed for assays. Serum thyroxine (T4) concentration was measured by radioimmunoassay based on previously reported methods (Schneider et al., 2006; Taylor et al., 2008) from a maximum of one male and one female from each litter. 5 µl of serum was diluted into 200 µl of glycine acetate with 0.25% bovine serum albumin (GAB) per tube and was assayed in duplicate. Standards were

prepared by addition of known amounts of L-T4 to 200 μ l of GAB including the equivalent of 5 μ l of charcoal-extracted rat serum. 50 μ l of polyclonal rabbit anti-T4 antibody (Cat#20-TR40, Fitzgerald Industries International, Concord, MA) diluted 1:20,000 in GAB/0.05 M EDTA was then added to each tube and incubated for 3 days when approximately 0.006 μ Ci of [¹²⁵I]-T4 was added. On the fifth day, 50 μ l of a 200 μ g/ml (10 μ g) solution of rabbit immunoglobulin was added, followed by 100 μ l of a goat anti-rabbit (GAR) secondary antibody solution (Cat#R0881, Sigma, St. Louis, MO) prepared at 60% of the manufacturer's recommended volume for a final dilution of approximately 1:8. Tubes were incubated at room temperature for 30 minutes before addition of 1 ml of a 25% wt/vol solution of PBS/PEG. Tubes were then centrifuged, aspirated, and counted in a gamma counter. The sensitivity of the assay was determined by 3 standard deviations from the zero standard and 3 standard deviations from the nonspecific binding level. The serum T4 assay had lower and upper limits of detection of 0.05 μ g/dl and 10.5 μ g/dl, respectively, for a 5 μ l sample. All samples were run in a single assay. The intra-assay coefficient of variation was 8.1%. TSH (thyroid stimulating hormone), LH (luteinizing hormone), and FSH (follicle stimulating hormone) were analyzed using a MILLIPLEX MAP Rat Pituitary Magnetic Bead Panel (Millipore, Billerica, MA). The commercial kit provided quality control samples measured within expected parameters. The mean intra-assay variations for TSH, LH and FSH assays, respectively, were 4.3, 0.8 and 11.8%.

2.5 Radial Arm Maze

Starting in adulthood (PND 85), two female and two male rats from each litter were weighed and placed on food restriction. During this time, rats were daily given 3 pellets of the food reward (Purified Rodent Tablets; 45 mg; TestDiet, St. Louis, MO) that was used during behavioral training. The target weight was 85-90% ad libitum, and due to sex-specific increases in body weight during this age, the target weight was increased by 5 grams weekly for males only. All rats were weighed daily prior to behavioral evaluation, and if one of the pair-housed rats weighed significantly less in relation to the goal weight, these rats were briefly separated for the purposes of feeding. Only 1 female and 1 male from each litter were tested in the 17-arm radial arm maze. The maze was made of wood, painted gray, and located in a room (380 x 442 cm) with a moderate amount of both 2- and 3-dimensional cues placed on the walls and floors. It consisted of a central round platform (diameter = 87 cm) from which 17 arms (69 cm long x 11 cm wide) radiated out. Each arm had a taller wall on one side (11 cm) and a shorter wall on the other side (2.5 cm). The height of the central platform and the arms was 66 cm from the floor.

Habituation began 5 days after the start of food deprivation and consisted of placing each rat in the center platform. One pellet of food reward was placed in each arm in a random location and 2 pellets were placed in the center platform. Each rat was allowed to run freely for 12 minutes for 4 consecutive days to acclimate to the apparatus.

Starting on the day after completion of habituation, rats were run once a day for 6 days/week for 4 weeks. Every day, one food reward pellet was placed at the end of 11 out of the 17 arms. The remaining arms did not contain a food reward. The arms that were baited and unbaited were consistent in their spatial location throughout the entire behavioral evaluation period, so that rats had to remember not only which arms they had already visited within a day (i.e., working memory), but also the arms that never contained food reward (i.e., reference memory). An entry into an arm was recorded when all four limbs were inside an arm. Reentry into a previously baited arm was categorized as a working memory error, while entry into an arm that never contained food rewards was categorized a reference memory error. Once a day, rats were placed in the center of the maze and allowed to explore freely until all food rewards were eaten or for a maximum of 15 minutes. Rats were then placed back into their home cage with their untrained littermate, and the maze was cleaned

with a 10% ethanol solution prior to running the next rat. The arms were rotated 2 positions every 4 days to reduce the possibility that remaining odors could be used as cues this task. The experimenter who tracked the radial arm maze behavior was blind to the treatment group of the rats.

2.6 Statistical Analyses

Measures from the dams and from the growth and development in the exposed offspring (weaning and adult body weights, puberty onset, and liver weight/body weight) were analyzed with ANOVAs (SPSS; version 19; SPSS Inc., Chicago, IL) with treatment as the independent factor and cohort as a covariate. In addition, early postnatal body weight was analyzed with a repeated measure (postnatal age) ANOVA with treatment as the independent factor and cohort as a covariate. Due to the large, well-known sex differences in these measures from the offspring, males and females were analyzed separately.

Hormone concentrations in the offspring were analyzed with a repeated measure (sex) ANOVA, the between-subject variable was treatment, and cohort was used as a covariate. Post hoc tests for significant treatment effects were run between the control and each treatment group because these were the only comparisons of interest. ANOVAs were used as posthoc tests in order to include cohort as a covariate. In cases where data was available from more than one littermate per sex, averages were calculated for each sex of each litter.

ANOVAs of reference and working memory errors were performed using treatment as a between-subject factor, cohort as a covariate, and both block and sex as repeated measures. Blocks were defined as the average number of errors for 6 days (each week) of running for each animal.

3. Results

3.1 Growth and Development Measures

Adult female rats that were exposed to BPA during their pregnancy did not exhibit any significant differences in gestational weight gain or other measures taken at weaning including brain weight, relative liver weight (liver weight/body weight), or number of implantation sites (Table 1). Litter size and sex ratio of the offspring were not significantly affected by BPA treatment (Table 1). Sample sizes for each treatment group were 0 µg/kg (9), 4 µg/kg (10), 40 µg/kg (13), 400 µg/kg (12). The remaining results are those obtained from the offspring of these dams.

Results of BPA exposure during early development on body weight in early postnatal development, at weaning, and in adulthood (PND 85) are shown in Figure 1. BPA treatment did not affect body weight in males or females at any age or at any of the doses examined. Additionally, no significant treatment effects in males or females were found in puberty onset, adult brain weights, or adult relative liver weights (Table 2). Cohort was significant for puberty onset in both females ($F_{(1,39)} = 53.6$; $p < .001$) and males ($F_{(1,39)} = 10.0$; $p < .005$) and in females only for adult brain weight ($F_{(1,39)} = 4.8$; $p < .05$). Sample sizes for each treatment group were 0 µg/kg (9), 4 µg/kg (10), 40 µg/kg (13), 400 µg/kg (12).

There was a significant effect of treatment ($F_{(3,29)} = 3.3$; $p < .05$) and cohort ($F_{(1,29)} = 6.3$; $p < .05$) in serum T4 concentrations at weaning. However, there are no differences between control and treated BPA groups (Figure 2A). BPA treatment did not significantly alter thyroid stimulating hormone (TSH) (Figure 2B). There was a significant sex difference ($F_{(1,29)} = 23.7$; $p < .001$), with females having higher TSH concentrations than males, and a significant sex x cohort interaction ($F_{(1,29)} = 7.5$; $p < .05$). The ratio of T4/TSH in males and females at weaning was not altered by developmental BPA exposure (data not shown).

Follicle stimulating hormone (FSH) was altered by BPA exposure, as seen by a significant treatment effect ($F_{(3,29)} = 3.5$; $p < .05$; Figure 2C). FSH was decreased significantly in the 4 $\mu\text{g/kg}$ ($F_{(1,11)} = 15.1$; $p < .005$) and in the 400 $\mu\text{g/kg}$ groups ($F_{(1,13)} = 5.7$; $p < .05$), compared to the control group. Additionally, there was a overall significant effect of sex ($F_{(1,29)} = 10.7$; $p < .005$), with females having a higher concentration than males, and a sex x cohort interaction ($F_{(1,29)} = 8.4$; $p < .01$). LH concentrations also were significantly different between the sexes ($F_{(1,29)} = 12.8$; $p < .005$) such that females had higher concentrations than males. Sample sizes for all of the analyses of hormones were 0 $\mu\text{g/kg}$ (6), 4 $\mu\text{g/kg}$ (8), 40 $\mu\text{g/kg}$ (10), 400 $\mu\text{g/kg}$ (10).

3.2 Reference Memory Errors

All behavioral graphs depict averages for each block (6 days) of evaluation. Reference memory errors are displayed in Figure 3. There was no significant treatment effect or interaction of treatment x block. However, there was a significant effect of block ($F_{(3,117)} = 9.1$; $p < .001$) with errors decreasing across blocks of sessions, indicating that both sexes learned to avoid the arms that never contained food.

3.3 Working Memory Errors

Working memory errors are shown in Figure 4. No significant treatment, treatment x block, or block effects were observed. There was a significant sex difference ($F_{(1,39)} = 4.5$; $p < .05$) in this measure with males committing fewer errors than females.

4. Discussion

The current study is the first to measure whether perinatal exposure, at a time that is meant to mimic human gestational exposure, to BPA affects both reference and working memory in adult rodents, as assessed through the 17-arm radial maze. No significant effects of BPA could be detected on radial arm maze performance in males or females. This supports previous findings in which female mice did not exhibit changes in performance on an 8-arm radial maze task after developmental exposure to BPA at doses similar to the current study (Ryan and Vandenberg, 2006). On the other hand, perinatal BPA at two different doses did affect FSH levels at weaning age in both males and females.

This cognitive task was chosen because 17 arms (compared to the 8-arm radial maze) make this task difficult and more likely to detect small exposure-related differences than easier tasks. There was a significant effect of block in both males and females in reference memory errors with performance improving across the weeks of training. Therefore, it would be possible to see both enhancements and impairments in this measure. Unlike reference memory errors, no significant effects of block were seen in working memory errors. Working memory was taxed in this task and did not detectably improve across weeks of evaluation in any group, but all groups were able to perform at a similar level.

BPA at doses of 4 and 400 $\mu\text{g/kg}$ decreased the level of FSH at weaning age in both male and female rats. Previous studies have examined changes in FSH plasma levels in response to early developmental exposure to BPA in males, while none have examined females. Similar to results found in the current study, Cardoso et al., (2010) found decreased levels of FSH in male Wistar rats at PND 35 after oral exposure to 2500 $\mu\text{g/kg/day}$ BPA during gestation and lactation. In contrast, elevations in FSH levels at PND 18 and 25 were seen in male Wistar rats after subcutaneous injections of very large doses of BPA from PND 2-12 (Atanassova et al., 2000). In this study, 500 μg of BPA was given to pups without scaling to their body weight. This would result in a dose of 50,000 $\mu\text{g/kg}$ body weight in a pup that

weighed 10 g. Thus, doses closer to that used in the current study result in similar results, a decrease in FSH, to what was found here.

Importantly, the changes in FSH levels seen here were apparent two weeks after the discontinuation of treatment. Given that BPA has a short half-life (Völkel et al., 2002; 2005), it is highly unlikely that BPA was still present in the blood of the offspring at the time of sacrifice, suggesting that BPA exposure induces at least short-term changes in hormone concentrations. Females are closer to puberty by weaning age, and this accounts for higher levels of FSH and LH in females compared to males. The implications of the changes in FSH due to BPA at weaning are unknown, especially given that puberty onset was not altered. The idea that early exposure to BPA may induce changes in hormone measurements that outlast the dosing period is supported by reports of effects of developmental BPA exposure on later levels of testosterone, estrogen, luteinizing hormone, and progesterone and in some of their respective receptors (Bai et al., 2011; Fernandez et al., 2010; Kendig et al., 2012; Rubin et al., 2001). More studies are needed to determine the immediate effects of BPA during dosing, and whether the changes in FSH reported here persist into adulthood.

No changes from BPA treatment were detected in T4 or TSH. This is consistent with studies in which BPA at doses from 2.5 to 40000 µg/kg administered throughout gestation and lactation failed to alter levels of T4 in males or females at weaning age (Ferguson et al., 2011; Kobayashi et al., 2005). These results contrast with other studies in which the thyroid hormone system is altered by BPA. For example, BPA antagonizes thyroid hormone receptors and increases expression of thyroid responsive genes (Moriyama et al., 2002; Zoeller et al., 2005). Also, there are reports of alterations to thyroid hormone concentrations in the offspring following maternal exposure (Xu et al., 2007; Zoeller et al., 2005). It is unknown whether BPA exposure in the present study led to thyroid hormone disruption during the dosing period itself.

The current study found no significant effects of BPA on all of the maternal endpoints, such as litter size and gestational weight gain, which are consistent with past studies that used comparable doses of BPA (Ema et al., 2001; Ferguson et al., 2011; Negishi et al., 2003; Somm et al., 2009; Tinwell et al., 2002; Tyl et al., 2002, 2008). Also, many of the growth and development measures in male or female offspring, such as body weight and puberty onset, were not altered by BPA exposure. There are many studies in rodents which support the finding that BPA does not affect either vaginal opening, preputial separation, or body weight (Ema et al., 2001; Kato et al., 2006; Kobayashi et al., 2002; Nakamura et al., 2012; Newbold et al., 2007; Ryan et al., 2010; Stump et al., 2010; Tinwell et al., 2002; Tyl et al., 2002, 2008). However, the negative findings reported here are in contrast to reports that earlier vaginal opening is seen after subcutaneous injections of 50 µg/kg to rats from PND 0-3 or 20 µg/kg to mice from GD 11-17 (Adewale et al., 2009; Honma et al., 2002). Other studies that have used a longer duration of treatment (that includes both gestational and lactational exposure) and an oral route of administration do not find significant effects of BPA on vaginal opening at doses below 50 mg/kg/day (Ema et al., 2001; Howdeshell, et al., 1999; Ryan et al., 2010; Tinwell et al., 2002; Tyl et al., 2002). Therefore, duration of dosing and route of administration may play a role in whether puberty onset, measured by vaginal opening, is unaffected or accelerated by early exposure to BPA. Additionally, there are numerous reports of increased body weight, mostly in females, after exposure to developmental BPA (Honma et al., 2002; Howdeshell et al., 1999; Miyawaki et al., 2007; Nikaido et al., 2004; Patisaul and Bateman, 2008; Rubin et al., 2001; Somm et al., 2009). It is unclear as to why some studies see significant changes in body weight after perinatal exposure to BPA while others do not, but one possibility is that the type of diet may interact

with BPA exposure to produce different effects on body weight. We will be investigating this question in future experiments.

In conclusion, perinatal exposure to BPA led to decreases in serum FSH in both sexes at weaning age, after termination of the dosing. These changes were not, however, reflected in the timing of puberty which was not altered by BPA treatment. In addition, no effects of perinatal BPA were found on body weight at any age, nor did it detectably affect an appetitive spatial learning and memory task in adult rats.

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References

- Adelewa HB, Jefferson WN, Newbold RR, Patisaul HB. Neonatal bisphenol-a exposure alters rat reproductive development and ovarian morphology without impairing activation of gonadotropin-releasing hormone neurons. *Biol Reprod.* 2009; 81:690–9. [PubMed: 19535786]
- Adriani W, Seta DD, Dessi-Fulgheri F, Farabollini F, Laviola G. Altered profiles of spontaneous novelty seeking, impulsive behavior, and response to D-amphetamine in rats perinatally exposed to bisphenol A. *Environ Health Perspect.* 2003; 111:395–401. [PubMed: 12676589]
- Atanassova N, McKinnell C, Turner KJ, Walker M, Fisher JS, Morley M, et al. Comparative effects of neonatal exposure of male rats to potent and weak (environmental) estrogens on spermatogenesis at puberty and the relationship to adult testis size and fertility: evidence for stimulatory effects of low estrogen levels. *Endocrinology.* 2000; 141:3898–907. [PubMed: 11014247]
- Bai Y, Chang F, Zhou R, Jin PP, Matsumoto H, Sokabe M, Chen L. Increase of anteroventral periventricular kisspeptin neurons and generation of E2-induced LH-surge system in male rats exposed perinatally to environmental dose of bisphenol-A. *Endocrinology.* 2011; 152:1562–1571. [PubMed: 21303948]
- Beatty WW. Hormonal organization of sex differences in play fighting and spatial behavior. *Prog Brain Res.* 1984; 61:315–30. [PubMed: 6241318]
- Biedermann S, Tschudin P, Grob K. Transfer of bisphenol A from thermal printer paper to the skin. *Anal Bioanal Chem.* 2010; 398:571–6. [PubMed: 20623271]
- Biles JE, McNeal TP, Begley TH, Hollifield HC. Determination of bisphenol-A in reusable polycarbonate food-contact plastics and migration to food-stimulating liquids. *J Agric Food Chem.* 1997; 45:3541–4.
- Brotons JA, Olea-Serrano MF, Villalobos M, Pedraza V, Olea N. Xenoestrogens released from lacquer coatings in food cans. *Environ Health Perspect.* 1995; 103:608–12. [PubMed: 7556016]
- Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003–2004. *Environ Health Perspect.* 2008; 116:39–44. [PubMed: 18197297]
- Cardoso N, Pandolfi M, Ponzo O, Carbone S, Szwarcfarb B, Scacchi P, et al. Evidence to suggest glutamic acid involvement in Bisphenol A effect at the hypothalamic level in prepubertal male rats. *Neuro Endocrinol Lett.* 2010; 31:512–6. [PubMed: 20802452]
- Clancy B, Finlay BL, Darlington RB, Anand KJ. Extrapolating brain development from experimental species to humans. *Neurotoxicology.* 2007; 28:931–7. [PubMed: 17368774]
- Cox KH, Gatewood JD, Howeth C, Rissman EF. Gestational exposure to bisphenol A and cross-fostering affect behaviors in juvenile mice. *Horm Behav.* 2010; 58:754–61. [PubMed: 20691692]
- Doerge DR, Vanlandingham M, Twaddle NC, Delclos KB. Lactational transfer of bisphenol A in Sprague-Dawley rats. *Toxicol Lett.* 2010; 199:372–6. [PubMed: 20933065]
- Dobbing J, Sands J. Comparative aspects of the brain growth spurt. *Early Hum Dev.* 1979; 3:79–83. [PubMed: 118862]

- Ema M, Fujii S, Furukawa M, Kiguchi M, Ikka T, Harazono A. Rat two-generation reproductive toxicity study of bisphenol A. *Reprod Toxicol*. 2001; 15:505–23. [PubMed: 11780958]
- Ferguson SA, Law CD Jr, Abshire JS. Developmental treatment with bisphenol a or ethinyl estradiol causes few alterations on early preweaning measures. *Toxicol Sci*. 2011; 124:149–60. [PubMed: 21813462]
- Ferguson SA, Law CD, Abshire JS. Developmental treatment with bisphenol A causes few alterations on measures of postweaning activity and learning. *Neurotoxicol Teratol*. 2012; 34:598–606. [PubMed: 23041373]
- Fernandez M, Bourguignon N, Lux-Lantos V, Libertun C. Neonatal exposure to bisphenol a and reproductive and endocrine alterations resembling the polycystic ovarian syndrome in adult rats. *Environ Health Perspect*. 2010; 118:1217–1222. [PubMed: 20413367]
- Fujimoto T, Kubo K, Aou S. Prenatal exposure to bisphenol A impairs sexual differentiation of exploratory behavior and increases depression-like behavior in rats. *Brain Res*. 2006; 1068:49–55. [PubMed: 16380096]
- Gioiosa L, Fissore E, Ghirardelli G, Parmigiani S, Palanza P. Developmental exposure to low-dose estrogenic endocrine disruptors alters sex differences in exploration and emotional responses in mice. *Horm Behav*. 2007; 52:307–16. [PubMed: 17568585]
- Goncalves CR, Cunha RW, Barros DM, Martinez PE. Effects of prenatal and postnatal exposure to a low dose of bisphenol A on behavior and memory in rats. *Environ Toxicol Pharmacol*. 2010; 30:195–201. [PubMed: 21787652]
- Gould JC, Leonard LS, Maness SC, Wagner BL, Conner K, Zacharewski T, et al. Bisphenol A interacts with the estrogen receptor alpha in a distinct manner from estradiol. *Mol Cell Endocrinol*. 1998; 142:203–14. [PubMed: 9783916]
- Honma S, Suzuki A, Buchanan DL, Katsu Y, Watanabe H, Iguchi T. Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. *Reprod Toxicol*. 2002; 16:117–22. [PubMed: 11955942]
- Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, vom Saal FS. Exposure to bisphenol A advances puberty. *Nature*. 1999; 401:763–4. [PubMed: 10548101]
- Kato H, Furuhashi T, Tanaka M, Katsu Y, Watanabe H, Ohta Y, et al. Effects of bisphenol A given neonatally on reproductive functions of male rats. *Reprod Toxicol*. 2006; 22:20–9. [PubMed: 16311018]
- Kendig EL, Buesing DR, Christie SM, Cookman CJ, Gear RB, Hugo ER, Kasper SN, Kendzierski JA, Ungi KR, Williams K, Belcher SM. Estrogen-like disruptive effects of dietary exposure to bisphenol A or 17alpha-ethinyl estradiol in CD1 mice. *Int J Toxicol*. 2012; 31:537–550. [PubMed: 23160314]
- Kobayashi K, Miyagawa M, Wang RS, Sekiguchi S, Suda M, Honma T. Effects of in utero and lactational exposure to bisphenol A on somatic growth and anogenital distance in F1 rat offspring. *Ind Health*. 2002; 40:375–81. [PubMed: 12502241]
- Kobayashi K, Miyagawa M, Wang RS, Suda M, Sekiguchi S, Honma T. Effects of in utero and lactational exposure to bisphenol A on thyroid status in F1 rat offspring. *Ind Health*. 2005; 43:685–690. [PubMed: 16294924]
- Krishnan AV, Stathis P, Permuth SF, Tokes L, Feldman D. Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology*. 1993; 132:2279–86. [PubMed: 8504731]
- Kubo K, Arai O, Ogata R, Omura M, Hori T, Aou S. Exposure to bisphenol A during the fetal and suckling periods disrupts sexual differentiation of the locus coeruleus and of behavior in the rat. *Neurosci Lett*. 2001; 304:73–6. [PubMed: 11335058]
- Kubo K, Arai O, Omura M, Watanabe R, Ogata R, Aou S. Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats. *Neurosci Res*. 2003; 45:345–56. [PubMed: 12631470]
- Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β . *Endocrinology*. 1997; 138:863–70. [PubMed: 9048584]

- Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology*. 1998; 139:4252–63. [PubMed: 9751507]
- LaBuda CJ, Mellgren RL, Hale RL. Sex differences in the acquisition of a radial maze task in the CD-1 mouse. *Physiol Behav*. 2002; 76:213–7. [PubMed: 12044593]
- Mishima N, Higashitani F, Teraoka K, Yoshioka R. Sex differences in appetitive learning of mice. *Physiol Behav*. 1986; 37:263–8. [PubMed: 3737737]
- Miyawaki J, Sakayama K, Kato H, Yamamoto H, Masuno H. Perinatal and postnatal exposure to bisphenol a increases adipose tissue mass and serum cholesterol level in mice. *J Atheroscler Thromb*. 2007; 14:245–52. [PubMed: 17938543]
- Moriyama K, Tagami T, Akamizu T, Usui T, Saijo M, Kanamoto N, et al. Thyroid hormone action is disrupted by bisphenol A as an antagonist. *J Clin Endocrinol Metab*. 2002; 87:5185–90. [PubMed: 12414890]
- Nakamura K, Itoh K, Dai H, Han L, Wang X, Kato S, et al. Prenatal and lactational exposure to low-doses of bisphenol A alters adult mice behavior. *Brain Dev*. 2012; 34:57–63. [PubMed: 21277127]
- Negishi T, Kawasaki K, Takatori A, Ishii Y, Kyuwa S, Kuroda Y, et al. Effects of perinatal exposure to bisphenol A on the behavior of offspring in F344 rats. *Environ Toxicol Pharmacol*. 2003; 14:99–108. [PubMed: 21782668]
- Newbold RR, Jefferson WN, Padilla-Banks E. Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive tract. *Reprod Toxicol*. 2007; 24:253–8. [PubMed: 17804194]
- Nikaido Y, Yoshizawa K, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N, et al. Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring. *Reprod Toxicol*. 2004; 18:803–11. [PubMed: 15279878]
- NTP (National Toxicology Program). NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A. 2008:08–5994.
- Olea N, Pulgar R, Perez P, Olea-Serrano F, Rivas A, Novillo-Fertrell A, et al. Estrogenicity of resin-based composites and sealants used in dentistry. *Environ Health Perspect*. 1996; 104:298–305. [PubMed: 8919768]
- Ozaki A, Yamaguchi Y, Fujita T, Kuroda K, Endo G. Chemical analysis and genotoxicological safety assessment of paper and paperboard used for food packaging. *Food Chem Toxicol*. 2004; 42:1323–37. [PubMed: 15207384]
- Patisaul HB, Bateman HL. Neonatal exposure to endocrine active compounds or an ERbeta agonist increases adult anxiety and aggression in gonadally intact male rats. *Horm Behav*. 2008; 53:580–8. [PubMed: 18308321]
- Rubin BS, Lenkowski JR, Schaeberle CM, Vandenberg LN, Ronsheim PM, Soto AM. Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. *Endocrinology*. 2006; 147:3681–91. [PubMed: 16675520]
- Rubin BS, Murray MK, Damassa DA, King JC, Soto AM. Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. *Environ Health Perspect*. 2001; 109:675–80. [PubMed: 11485865]
- Ryan BC, Hotchkiss AK, Crofton KM, Gray LE Jr. In utero and lactational exposure to bisphenol A, in contrast to ethinyl estradiol, does not alter sexually dimorphic behavior, puberty, fertility, and anatomy of female LE rats. *Toxicol Sci*. 2010; 114:133–48. [PubMed: 19864446]
- Ryan BC, Vandenberg JG. Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice. *Horm Behav*. 2006; 50:85–93. [PubMed: 16540110]
- Sandi C, Loscertales M, Guaza C. Experience-dependent facilitating effect of corticosterone on spatial memory formation in the water maze. *Eur J Neurosci*. 1997; 9:637–42. [PubMed: 9153570]
- Schneider MJ, Fiering SN, Thai B, Wu SY, St Germain E, Parlow AF, et al. Targeted disruption of the type 1 selenodeiodinase gene (Dio1) results in marked changes in thyroid hormone economy in mice. *Endocrinology*. 2006; 147:580–9. [PubMed: 16223863]
- Seymour P, Dou H, Juraska J. Sex differences in radial arm performance: Influence of rearing environment and room cues. *Psychobiology*. 1996; 24:33–7.

- Somm E, Schwitzgebel VM, Toulotte A, Cederroth CR, Combescure C, Nef S, et al. Perinatal exposure to bisphenol a alters early adipogenesis in the rat. *Environ Health Perspect.* 2009; 117:1549–55. [PubMed: 20019905]
- Stump DG, Beck MJ, Radovsky A, Garman RH, Freshwater LL, Sheets LP, et al. Developmental neurotoxicity study of dietary bisphenol A in Sprague-Dawley rats. *Toxicol Sci.* 2010; 115:167–82. [PubMed: 20164145]
- Taylor MA, Swant J, Wagner JJ, Fisher JW, Ferguson DC. Lower thyroid compensatory reserve of rat pups after maternal hypothyroidism: correlation of thyroid, hepatic, and cerebrocortical biomarkers with hippocampal neurophysiology. *Endocrinology.* 2008; 149:3521–30. [PubMed: 18372327]
- Tees RC, Midgley G, Nesbit JC. The effect of early visual experience on spatial maze learning in rats. *Dev Psychobiol.* 1981; 14:425–38. [PubMed: 7274582]
- Tinwell H, Haseman J, Lefevre PA, Wallis N, Ashby J. Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A. *Toxicol Sci.* 2002; 68:339–48. [PubMed: 12151630]
- Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, et al. Two-generation reproductive toxicity study of dietary bisphenol A in CD-1 (Swiss) mice. *Toxicol Sci.* 2008; 104:362–84. [PubMed: 18445619]
- Tyl RW, Myers CB, Marr MC, Thomas BF, Keimowitz AR, Brine DR, et al. Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. *Toxicol Sci.* 2002; 68:121–46. [PubMed: 12075117]
- Volkel W, Bittner N, Dekant W. Quantitation of bisphenol A and bisphenol A glucuronide in biological samples by high performance liquid chromatography-tandem mass spectrometry. *Drug Metab Dispos.* 2005; 33:1748–57. [PubMed: 16103135]
- Volkel W, Colnot T, Csanady GA, Filser JG, Dekant W. Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. *Chem Res Toxicol.* 2002; 15:1281–7. [PubMed: 12387626]
- Williams CL, Meck WH. The organizational effects of gonadal steroids on sexually dimorphic spatial ability. *Psychoneuroendocrinology.* 1991; 16:155–76. [PubMed: 1961837]
- Xu X, Liu Y, Sadamatsu M, Tsutsumi S, Akaike M, Ushijima H, et al. Perinatal bisphenol A affects the behavior and SRC-1 expression of male pups but does not influence on the thyroid hormone receptors and its responsive gene. *Neurosci Res.* 2007; 58:149–55. [PubMed: 17412439]
- Xu XH, Zhang J, Wang YM, Ye YP, Luo QQ. Perinatal exposure to bisphenol-A impairs learning-memory by concomitant down-regulation of N-methyl-D-aspartate receptors of hippocampus in male offspring mice. *Horm Behav.* 2010; 58:326–33. [PubMed: 20206181]
- Zoeller RT, Bansal R, Parris C. Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain. *Endocrinology.* 2005; 146:607–12. [PubMed: 15498886]

Highlights

- Pregnant females received 0, 4, 40, or 400 $\mu\text{g/kg/day}$ BPA throughout gestation.
- Male and female offspring were orally given the same dose from postnatal day 1-9.
- As adults, rats were trained on the 17-arm radial maze with baited and unbaited arms.
- BPA did not significantly alter adult performance in either males or females
- BPA doses of 4 and 400 $\mu\text{g/kg/day}$ decreased FSH levels in both sexes at weaning

Figure 1.

Body Weight in BPA-Exposed Offspring. BPA did not alter body weight in males or females during early postnatal development (PND 1-9; Figures A and B), weaning (PND 23; Figure C), or in adulthood (PND 85; Figure D). Sample sizes (n) for each sex in each group were 0 µg/kg(9), 4 µg/kg(10), 40 µg/kg(13), 400 µg/kg(12).

Figure 2.

Concentrations of Hormones in BPA-Exposed offspring. There were no differences due to treatment in T4 (A), TSH (B) and LH (D). (C) FSH was significantly decreased in rats that received 4 or 400 µg/kg BPA. Sample sizes for each treatment group were 0 µg/kg (6), 4 µg/kg (8), 40 µg/kg (10), 400 µg/kg (10). * $p < .05$ compared to controls of both sexes

Figure 3. Reference Memory Errors. BPA did not significantly alter reference memory errors in females or males. Block was significant ($p < .001$). Sample sizes (n) for each sex in each group were 0 µg/kg(9), 4 µg/kg(10), 40 µg/kg(13), 400 µg/kg(12).

Figure 4.

Working Memory Errors. BPA did not significantly alter working memory errors in females or males. Sex was significant ($p < .05$). Sample sizes (n) for each sex in each group were 0 µg/kg(9), 4 µg/kg(10), 40 µg/kg(13), 400 µg/kg(12).

Table 1

Maternal Measures

Dose (µg/kg)	Gestational Weight Gain (g)	Brain Weight (g)	Relative Liver Weight	No. of Implantation Sites	Litter Size	Sex Ratio (#males/ #females)
0	115.7±4.8	1.93±0.03	0.0594±0.0030	12.2±0.9	10.8±0.9	1.05±0.22
4	118.7±5.6	1.85±0.04	0.0587±0.0019	12.7±0.4	11.4±0.7	1.06±0.19
40	127.5±4.5	1.91±0.02	0.0582±0.0009	12.7±0.6	11.8±0.7	0.77±0.10
400	123.2±6.3	1.91±0.02	0.0617±0.0014	13.0±0.6	12.0±0.6	0.91±0.12

Table 2

Offspring Measures

Dose ($\mu\text{g/kg}$)	Sex	Puberty Onset (PND)	Relative Liver Weight (Liver Weight/BW)	Brain Weight (g)
0	Female	33.2 $\pm$ 1.0	0.0330 $\pm$ 0.0004	1.85 $\pm$ 0.02
4	Female	32.7 $\pm$ 1.0	0.0323 $\pm$ 0.0007	1.84 $\pm$ 0.01
40	Female	31.5 $\pm$ 0.6	0.0321 $\pm$ 0.0005	1.84 $\pm$ 0.01
400	Female	32.5 $\pm$ 0.8	0.0323 $\pm$ 0.0007	1.84 $\pm$ 0.03
0	Male	43.2 $\pm$ 0.6	0.0332 $\pm$ 0.0007	1.99 $\pm$ 0.03
4	Male	42.6 $\pm$ 0.4	0.0327 $\pm$ 0.0007	1.97 $\pm$ 0.02
40	Male	42.5 $\pm$ 0.3	0.0333 $\pm$ 0.0005	1.95 $\pm$ 0.02
400	Male	42.8 $\pm$ 0.4	0.0338 $\pm$ 0.0006	1.99 $\pm$ 0.02