Does preconception paternal exposure to a physiologically relevant level of bisphenol A alter spatial memory in an adult rat?

Ying Fan a,e, Shibin Ding a, Xiaolei Ye b, Anne Manyande c, Dongliang He a, Nana Zhao b, Huiqin Yang a, Xin Jin a, Jian Liu a, Chong Tian a, Shunqing Xu b, Chenjiang Ying a,⁎

a Department of Nutrition and Food Hygiene, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
b School of Environmental Science and Public Health, Wenzhou Medical College, Wenzhou 325000, China
c School of Psychology, Social Work and Human Sciences, University of West London, London, UK
d Ministry of Education Key Laboratory of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
e Wuhan Mental Health Center, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

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ABSTRACT
Bisphenol A (BPA) is a ubiquitous environmental endocrine disrupting compound (EDC); public health concerns have been fueled by findings that maternal BPA exposure can change sex differences in the brain and in some behaviors. We investigated whether a physiologically relevant dose of BPA ingested by male rats before conception would affect spatial memory and hippocampal acetylcholinesterase (AchE) in their adult offspring. Twenty-two 60-day-old male rats (F0) received either a BPA diet (50 μg/kg/day) or vehicle alone for 10 weeks before being mated with non-exposed females. The paternal rats and their forty adult offspring’s (F1) behaviors were then examined in the Morris Water Maze (MWM) and their AchE activities in the hippocampus were evaluated. BPA exposure led to spatial memory deficits along with decreased AchE activities in the hippocampus (p = 0.01) in adult F0 rats. This paternal exposure also induced impairment in spatial memory acquisition in both sexes while retention only in females in F1 rats, as well as abolished sex differences in the hippocampus AchE. Overall, these data provide new evidence that paternal BPA exposure, at a “safe” dose, may induce transgenerational alterations in spatial memory in a sex-specific manner.

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Introduction
Among endocrine disruptors (EDCs), the xenoestrogen bisphenol A (BPA) deserves particular attention due to its widespread exposure to humans. Indeed, detectable levels of BPA have been found in samples of urine, blood, breast milk and saliva of both humans and nonhuman subjects (Patiasaul and Bateman, 2008). Recent findings suggest that chronic exposure to BPA in rodents during child development may result in extensive physical and mental alterations (Hajszan and Leranth, 2010; Wolstenholme et al., 2011). It has also been reported that BPA can interfere with the physiology and morphology of an adult brain. For example, acute or chronic BPA administration can impair both visual and spatial memories and synaptic plasticity in gonadal intact male rats (Eilam-Stock et al., 2012; Leranth et al., 2008) and block estrogen-induced memory enhancement in adult ovariectomized rats (Inagaki et al., 2012). However, in most of these behavioral studies, BPA was given during development and few have investigated the effects of adult BPA exposure in male subjects. In addition, most of the male subjects were exposed to BPA at high concentrations (Leranth et al., 2008) or administration was by subcutaneous injection (Eilam-Stock et al., 2012) which may not be relevant to the human condition as current human BPA exposure occurs with low dosage and mainly by oral ingestion. For these reasons, we chose to investigate the effects of chronic dietary exposure to BPA at a physiologically relevant level on spatial memory using an adult male rat model.

Another avenue for BPA effects on child development is through an action on infant–maternal interactions which may have an epigenetic impact on the behavior of the offspring (Wolstenholme et al., 2011). It has been observed that in cynomolgus monkeys, dams that ingested BPA during the gestation period cared less for their pups than did control females which resulted in increased resistance and exploration in their male offspring (Nakagami et al., 2009). The effects of this chemical on juvenile mice were more dramatic when the pups were reared by foster dams, as both diet and dam affected their social behavior and anxiety (Cox et al., 2010). Another cross-fostering study also revealed that the increase in anxiety-like behaviors and corticosterone response in F2 offspring of lipopolysaccharide-exposed mothers were reversed by the cross-fostering treatment and this phenotype was due to the reduction of maternal care experienced by the F2 animals (Walker 2012).
et al., 2012). This evidence therefore, confirms that maternal-mediated transmission may be dependent on maternal care rather than epigenetic mechanisms and that exposure to a gestational female does not necessarily constitute a transgenerational phenotype but rather multigenerational exposure. However, unless the germ cell is directly affected, then a transgenerational phenomenon is possible (Manikkam et al., 2012; Skinner et al., 2011).

Given the above-mentioned factors, a model based on paternal exposure seems plausible, because males do not have direct physiological or behavioral interaction with their offspring, since they only contribute sperm for the next generation. This, therefore, avoids confounding effects such as maternal behavior. This overall evidence suggests that there is a need for additional research to examine paternally mediated effects of BPA particularly, as BPA is a ubiquitous pollutant.

It is also important to note that a plethora of studies have examined developmental BPA exposure in laboratory animals but they have often generated inconsistent results. As a result, explicit guidelines for BPA research have been described. The suggested recommendations include the control of exogenous estrogens (Birnbaum et al., 2012), statistical control for litter effects, internal measures of BPA (Patisaul and Bateman, 2008) and the use of oral administration for the most relevant extrapolation to humans (Betancourt et al., 2012). However, the last recommendation could be problematic for laboratory animal research because the oral administration (typical method) of orogastric gavage can be stressful for rodents. Gestational stress can in addition, result in later alterations of offspring behavior; therefore, controlling or evaluating this potential confound would be beneficial. The proposed guidelines mentioned above were incorporated into the current study.

![Fig. 1. Effects of adult BPA exposure on spatial memory in male F0 rats. The MWM tests were performed in 144-day-old male rats after 10 week treatment with BPA (50 μg/kg/day). Data from the training test are averages of 4 trials per day for 5 consecutive days. (A) Distance moved: F0 BPA rats swam longer distance before reaching the platform than F0 controls. (B) Mean velocity: F0 BPA rats swam faster than F0 controls. (C) Escape latency: F0 BPA rats needed more time to find the platform than F0 controls. On the sixth day, the escape platform was removed and animals were reintroduced into the pool to perform the probe test. F0 BPA rats made fewer platform contact accuracies and spent less time in the platform quadrant than F0 controls during the entire 60 s trial (D and E, respectively). Mean ± S.E.M., n = 11, *p < 0.05 and **p < 0.01 vs. control.](image-url)
design. We include control of exogenous environmental estrogens, statistical control for litter effects and assessment of serum BPA levels. A new method for chronic diet BPA exposure which can mimic naturalistic exposure without any stress as well as allowing increasing the dose with high accuracy was implemented.

Acetylcholinesterase (AchE) activity in the hippocampus was also considered of interest in our study. AchE is a specific cholinergic marker for monitoring the functional state of acetylcholine neurons. Moreover, the cholinergic deafferentation of the hippocampus has been largely shown to induce memory impairments in different behavioral tasks. Similarly, some food and drugs traditionally used for anti-amaesia have been found to attenuate Morris water task deficits in rats by modulating the cholinergic system (Baitharu et al., 2013; Tsuruoka et al., 2012). For example, it has been demonstrated that some antioxidant protections such as the procyanidins significantly improve learning and memory impairments in aged memory-impaired rats in the MWM test by increasing acetylcholine contents and AchE activity in hippocampus (Xu et al., 2009).

Therefore, as a result of the recommendations about the methodology of BPA treatment, the projected use of adult rats in neurobehavioral toxicology and the availability of BPA-exposed paternal rats, an exploratory study involving two generations was conducted. Here, we exposed male rats to BPA (50 μg/kg/day) or vehicle alone for 10 weeks and then used those rats as sires. We then examined the spatial memory functions in the F1 offspring to assess the consequences of paternal BPA exposure on spatial memory. Finally, in order to gain further insight into what molecular changes might underlie the behavioral phenotype; we analyzed the hippocampal AchE, a cholinergic marker, chosen because of the significant role it plays in the processing of learning and memory.

**General methods**

**Animals**

Twenty-two 60-day-old Wistar male rats (Hubei Research Center of Laboratory Animal, China) were housed individually in special pathogen-free conditions, with ad libitum access to food and water in an environmentally controlled room (temperature, 21 ± 1 °C; relative humidity, 60 ± 10%) maintained on a 12-h light/dark cycle (lights off from 18:00 to 06:00). Glass water bottles and polypropylene cages were used in this study. Animals were maintained in accordance with the Guidelines for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academies Press, Washington (DC), 1996) and ethical approval was granted by the Ethics Committee of Tongji Medical College (Huazhong University of Science and Technology, Wuhan, China).

The twenty-two male rats (F0) were randomly assigned to receive either a BPA or a control diet every day until the end of the study (see below). After 10 week treatment, all males were mated to unexposed naive females. One male and two female rats were housed together at 4:00 pm and the male was removed to his own cage the next morning at 6:00 am. This breeding procedure was repeated 3 times and daily treatment of the sire continued during this time. Successful mating was determined by the presence of a vaginal plug. Dams were left undisturbed until the expected parturition. The day of delivery was considered postnatal day (PND) 0. The litter size was standardized to 8 pups (four/sex/litter, if possible) for each dam on PND 5. Pups were weaned on PND 25 and housed in pairs according to sex and litter. All offspring (F1) were left undisturbed from weaning until behavioral testing on PND 56 except for weekly weighing and observation. Paternal BPA did not alter litter size or sex ratios (data not shown). Body weights of the F0 generation showed no loss during the treatment with BPA (data not shown).

**Diet and BPA treatment**

A semi-purified, phytoestrogen-free powdered formula (AIN-93G, Test Diet, Beijing HFK Bio-Technology Co., Ltd., China) was used as a basal diet for all the animals. BPA (Sigma-Aldrich; purity 99%, CAS no. 80-05-7) dose selection in this experiment was based on the current USEPA’s reference for safe daily limit (50 μg/kg bw/day, USEPA, 1993, http://www.epa.gov/iris/subst/0356.htm) which is the value that shows no adverse effects of BPA on reproductive toxicity. Based on experience during our pretest, BPA was dissolved in corn oil and diluted with several stock solutions to the desired concentrations (20 μg/ml, 40 μg/ml, 60 μg/ml) and was stored at −20 °C. An automated algorithm calculated the necessary volume of stock solution based on the daily body weight of each rat. This solution was diluted in corn oil to form a total volume of 0.5 ml, which was then directly added into a 5 g basal powder diet and mixed. Finally, the mixture was placed into a robust ceramic jar in the home cage of each BPA rat. Each control rat received 5 g basal diet containing an equal volume of corn oil (0.5 ml) at the same time. Administration occurred at a defined time (8:00 am). Based on our experience, 5 g of food was consumed within 8 h; therefore the test diet was checked at 4 h intervals and was supplemented by sufficient basal diet if it has been completely consumed.

**Morris Water Maze**

Following a minimum of 2 week recovery since last mating, F0 males were subjected to the MWM test. The F1 generation underwent the same testing procedure on PND 56. In order to obtain unbiased results, only one male and one female per litter that were born on the same evening were observed in the present study. In the end, ten dams were used per group. All testing occurred between 9:00 and 10:30 to minimize circadian effects and to be consistent with the majority of published water maze research, which has used daytime testing (Beiko et al., 2004). Rats were handled for weighing daily (for F0 rats) or weekly (for F1 rats) prior to testing. To acclimatize the water maze pool, all subjects received a 2 day modified pre-training test according to Morris’s procedure (Morris, 1989). The MWM consisted of a circular dark tank (150 cm diameter and 70 cm depth) filled with cloudy water and divided into four quadrants: Northwest, Northeast, Southeast, and Southwest. A hidden platform was fixed in one of the four virtual quadrants during all training sessions. The water was kept at 23 ± 1 °C. Visual cues were placed in the water maze room. The training test lasted for 5 days and in each training session, 4 trials were performed at 30-min intervals. For each trial, the animals started from a different position in the water maze. In the training sessions, the learning progress was evaluated as the delay in reaching the hidden platform (escape); the length of the path (distance) and velocity were also recorded. On the sixth day the probe test was executed: the platform was removed and the animal

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**Fig. 2.** Effects of adult BPA exposure on the hippocampal AchE in male F0 rats. The hippocampal AchE concentrations were examined by the spectrophotometric method in 144-day-old male rats after 10 week treatment with BPA (50 μg/kg/day). F0 BPA rates exhibited reduced AchE activity than the controls. Mean ± S.E.M, n = 11, and *p < 0.05 vs. control.
swam freely for 60 s. The platform crossings and time spent on the platform quadrant were recorded. Data were obtained through a tracking video system (EthoVision®, NOLDUS).

**Analysis of hippocampal AchE activity**

About 30 min after behavioral assessment, animals were sacrificed under anesthesia and their hippocampi were quickly dissected out for biochemical estimation, washed with ice-cold 0.9% saline, weighed and stored at −80 °C until processing. Protein concentrations were determined by the method of BCA as described previously (Ruan et al., 2010). The activities of AchE were determined spectrophotometrically using the assay kit from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

**Serum analysis of BPA**

Unconjugated BPA was isolated and measured by the high performance liquid chromatography in F0 BPA group as described previously (Wolstenholme et al., 2011). An attempt was made to quantify levels in the rats consuming the BPA diet.

**Statistical analysis**

All statistical procedures were carried out using SPSS 18.0. After confirming that data were normally distributed using the Kolmogorov–Smirnov test, the statistical significance of daily performance in the MWM was analyzed using repeated measures analysis of variance (RM-ANOVA) with post-hoc LSD tests. Group comparisons of the probe test data plus hippocampal AchE data were analyzed using Student’s t-test for F0 rats while two-way ANOVA with post-hoc LSD tests for F1 rats. Differences were considered to be significant if \( p < 0.05 \).

**Results**

**Adult BPA exposure induces MWM deficits in F0 rats**

As expected, after 10-week treatment with BPA diet, the BPA group performed worse than the control group in the MWM (\( n = 11 \), each). The RM-ANOVA revealed that although F0 BPA rats swam faster [Fig. 1B; \( F(1, 20) = 6.25, p = 0.02 \)], they swam longer distance [Fig. 1A; \( F(1, 20) = 7.39, p = 0.01 \)] and spent more time [Fig. 1C; \( F(1, 20) = 5.88, p = 0.03 \)] to find a hidden platform than did the controls over the successive 5 days of training. During the final exposure to swimming in the pool when the escape platform was removed, F0 BPA rats also made fewer platform contact accuracies [Fig. 1D; \( t(20) = 4.57, p < 0.01 \)] and the duration was shorter in the platform quadrant [Fig. 1E; \( t(20) = 4.41, p < 0.01 \)]. These data inferentially suggest that both acquisition and retention of spatial memory were significantly impaired by BPA exposure.

**Adult BPA exposure decreases the hippocampal AchE activities in F0 rats**

To determine if BPA might affect the cholinergic system, we examined the brains from F0 rats after the final MWM test (\( n = 11 \), each). As shown in Fig. 2, F0 BPA group exhibited decreased AchE activity in the hippocampus relative to the controls \( (t(20) = 2.71, p = 0.01) \). We further analyzed the relationships between animals’ behavioral performance and the hippocampal AchE. A simple linear regression analysis indicates that decreased hippocampal AchE has a mild but statistically significant correlation with cognitive decline in the water maze training test (for Distance: \( r = 0.64, p = 0.04 \); for Latency: \( r = 0.75, p < 0.01 \); for Velocity: \( r = 0.62, p = 0.04 \)). This correlation was not observed in the probe test.

The present animal models mostly mimic a naturalistic BPA exposure in humans

The dietary level of BPA resulted in serum concentration of 12.712 ± 2.353 ng/ml in F0 BPA rats (\( n = 11 \)), which are within the normal range of human maternal blood, 0.3–18.9 ng/ml (Schönfelder et al., 2002), suggesting that this exposure route is most appropriate for modeling exposures with translational values to humans.

**Paternal BPA exposure induces MWM deficits in F1 rats in a sex-specific manner**

Forty unexposed offspring were subjected to the MWM tests at PND 56 (\( n = 10 \), each). The RM-ANOVA showed that both sexes of F1 offspring from paternal BPA swam greater distance [Fig. 3A; \( F(3, 36) = 17.30, p < 0.01 \)] and needed more time [Fig. 3C; \( F(3, 36) = 9.00, p < 0.01 \)] in finding a hidden platform than did the control offspring during the training test. However, F1 BPA females escaped significantly faster than the controls [Fig. 3B; \( F(3, 36) = 4.54, p < 0.01 \)]. During the probe test, F1 BPA males performed as well as the same-sex controls, whereas F1 BPA females failed to match this performance and consequently, a sex difference emerged in the form of a small but statistically significant male advantage among F1 BPA offspring in the measures of platform crossing [Fig. 3D; Group: \( F(1, 36) = 5.24, p = 0.03 \); Sex: \( F(1, 36) = 4.29, p = 0.04 \); Interaction: \( F(1, 36) = 5.24, p = 0.03 \); BPA females vs. BPA males, LSD < 0.01] and duration in the target quadrant [Fig. 3E; Group: \( F(1, 36) = 3.05, p = 0.09 \); Sex: \( F(1, 36) = 3.16, p = 0.08 \); Interaction: \( F(1, 36) = 5.58, p = 0.02 \); BPA females vs. BPA males, LSD < 0.01]. These data indicate that paternal exposure to a “USEPA-dose” of BPA can alter the normal gender-dependent pattern of retention which causes persistent spatial memory deficits in females but impairs the acquisition of spatial information without significantly affecting retention in males.

**Paternal BPA exposure diminishes sex differences in the hippocampal AchE**

The brains from F1 offspring were also examined after the MWM tests (\( n = 10 \), each). Unexpectedly, no significant difference was observed between F1 BPA and control offspring [Group: \( F(1, 36) = 2.83, p = 0.10 \); Group × Sex: \( F(1, 36) = 0.94, p = 0.34 \)]. As shown in Fig. 4, although not quite significant, F1 BPA females tended to produce lessened AchE activity than the same-sex controls (LSD = 0.07), and as a result, a typical sex difference was confirmed in control but not in BPA offspring [Sex: \( F(1, 36) = 6.95, p = 0.01 \); control female > control male, LSD = 0.02].

**Discussion**

Exposure to a physiologically relevant level of BPA in adult male rats: (a) disrupted spatial memory abilities, (b) reduced hippocampal AchE activity, (c) transmitted spatial memory deficits to their offspring and (d) diminished typical sex differences in hippocampal AchE activities in their offspring. The observation that an adult exposed to BPA produces impaired spatial memory is not novel. Of primary interest here are the previously unobserved transgenerational effects via the paternal lineage. As far as we know, this is the first study to describe learning and memory deficits due to preconception paternal exposure to BPA using multiple tests in a single study.

**Negative effects of BPA on spatial memory in F0 rats**

In the current study, the impairment in spatial memory in F0 BPA males was expected based on results that F0 BPA rats swam longer distance than controls when locating the hidden platform during acquisition training (Fig. 1A) and spent significantly less time in the target quadrant during probe trials (Fig. 1E). Increased escape latency during
the training session was also found in F0 BPA rats (Fig. 1C). The impairment in F0 BPA rats was substantial, with about 36% of decrease in the hippocampal AchE activity (Fig. 2). Similarly, previous studies have demonstrated that BPA induces memory impairment associated with the reduction in acetylcholine production and cholinergic fiber (Miyagawa et al., 2007; Tian et al., 2010). Undoubtedly, the acetylcholine system plays an important role in orchestrating major hippocampal functions. However, there is evidence that BPA, an estrogen agonist, can affect this region through both genomic (nuclear-receptor) and non-genomic (membrane receptors) pathways (Eilam-Stock et al., 2012; Hajszan and Leranth, 2010). Moreover, it is now clear that some xenoestrogens such as BPA can bind to membrane receptors and rapidly activate signaling pathways, even at low concentrations (Luine and Frankfurt, 2012). For example perinatal exposure to low-dose BPA significantly impairs spatial memory and expressions of N-methyl-D-aspartate (NMDA) receptor subunits and estrogen receptors β in the hippocampus (Xu et al., 2010).

On the other hand, F0 BPA rats exhibited signs of increased swimming speed throughout the test (Fig. 1B). An upsurge in swimming velocity is suggestive of enhanced motor behavior and/or a strong response to stress which is inversely related to the level of spatial memory (Zhou et al., 2011). Indeed, several studies have found that prenatal and neonatal exposures to low doses of BPA (2–20 μg/kg/day) caused hyper-locomotion in male rat offspring (Adriani et al., 2003; Zhou et al., 2011) and relatively high doses of BPA (100 or 500 μg/kg/day) could lead to cognitive deficits and increased movement in the elevated plus maze tested in mice (Tian et al., 2010). These effects were interpreted as due to alterations in the dopaminergic and NMDA systems. In addition, BPA has been shown to induce stress hyperactive behaviors and
increase corticosterone levels through other behavioral tests such as light–dark and Y-maze paradigms (Poinenova et al., 2010). Therefore, in the present study, it is also possible that FO BPA rats had changes in the neurochemical systems involved in the locomotor response to novelty-induced stress and/or in the locomotor habituation to novelty (Adriani et al., 2003; Masuo and Ishido, 2011) which can be interpreted as leading to the poor performance observed in the MWM.

Overall, the present data provide additional support to the view that BPA possibly affects more than one pathway of memory modulation in both developing and mature brains (Eilam-Stock et al., 2012; Zhou et al., 2011).

Sex-specific effects of paternal BPA on spatial memory in F1 rats

Interestingly, our present data shows that the deficits in MWM acquisition induced by BPA were transmitted to the second generation in both sexes, as evidenced by increased swimming distance (Fig. 3A) and delayed escape latency periods (Fig. 3C) but only female BPA offspring exhibited persistent deficits during the next probe test, showing fewer platform crossings (Fig. 3D) and shorter duration in the target quadrant (Fig. 3E). Furthermore, F1 BPA females maintained increased elevated velocity throughout the training sessions whereas the males remained normal (Fig. 3B). This result suggests that paternal BPA may increase the susceptibility to stress response in females but not in males. Similar to our results, previous reports have acknowledged that BPA exposure during pregnancy can cause distinct changes in the expression of sexually dimorphic placental gene transcripts between male and female conceptus (Rosenfeld, 2012) and decrease or abrogate sex differences in rearing behavior and emotional response (Gioiosa et al., 2007; Jones and Watson, 2012). Although the underlying mechanisms promoting the paternal transmission effects differ from that of maternal lineage, similar sex-specific effects may have occurred as early as the zygotic stage after paternal exposure to an undesired environment (Dunn and Bale, 2011; Pocar et al., 2012; Polanska et al., 2006). It is also known that BPA can upregulate clusterin in prostate and single-strand DNA breaks in spermatozoa in adult male rodents (De Flora et al., 2007). As a result, DNA-damaged spermatozoa can fertilize oocytes which transmit heritable genetic diseases to their offspring and consequently lead to pre- and post-implantation loss, malformations and deficits in learning behavior (Wolstenholme et al., 2011).

Nonetheless, it is surprising that while the behavioral phenotype was transmitted to the F1 generation, the AChE perturbations experienced following paternal BPA exposure were not, which showed no significant difference between BPA and control offspring. These results speak to the complexities of transgenerational transmission. It is possible that although the epigenetic marks responsible for water maze behavior appear to be stable in F1, epigenetic regulation of genes involved in the acetylcholine system may be more plastic, responding to the present environment in the F0. The brain may transgenerationally compensate for an altered neural circuitry in earlier generations to preserve homeostatic conditions, resulting in adaptive changes in intact acetylcholine system, which inevitably is not impaired by the functioning of whole neural circuitry for spatial navigation.

Moreover, we found that although not quite significant, female BPA offspring tended to produce decreased AChE activity than the control females (p = 0.07), and as a result, a typical sex-difference was abolished in BPA offspring (Fig. 4). It has been demonstrated that acetylcholine levels in the hippocampus will increase very fast following memory task conditions, which is essential for setting appropriate dynamics for encoding of new information within the hippocampal formation (Deiana et al., 2011). In this sense, it may be hypothesized that paternal BPA blocked the increase of acetylcholine production in females, which may translate into poor performance.

Finally, it should be noted that this study did not monitor the circulating gonadal hormones of pubertal rats at sacrifice. Therefore, it is also possible that the influence of these hormones brought additional complexity to the present model, because BPA can mimic or block gonadal hormone function, depending on the presence or absence of circulating gonadal hormones (Luni and Frankfurt, 2012).

Conclusions

The present study is certainly not without limitations. However, since our initial assumption was that males do not rear their offspring, paternal exposure could avoid the confounding contributions of behavioral factors, such as maternal care. It is now conceivable from our results and those of others that BPA can induce changes and affect behaviors in exposed males, including the loss of males’ attractiveness to females, increased restless and feminized behaviors (Jašarević et al., 2011; Jones and Watson, 2012). Alternatively, the effects on behavior seen in this study could have occurred because females were forced to mate with an impaired male. Thus, such aberrant mating behaviors of a male may have resulted in stress in a female, which subsequently affected offspring’s behaviors (Meek et al., 2007). Therefore, in order to differentiate between the effects of paternal BPA use and maternal stress effects on behavior in offspring, a detailed quantification of the possible differences in mating behavior between BPA-ingesting and control males would need to be carried out (Skinner and Anway, 2005). Nonetheless, these results are intriguing as they confirm that a “safe” BPA dose is sufficient to stably affect spatial memory through the male germ line, which may be dependent on epigenetic and sex-specific mechanisms. Therefore, protection from BPA exposure may not only be essential during gestation but also before conception, as shown here for the male. Again, these data suggest the need for further research into paternally mediated mechanisms in experimental animals as well as in humans.

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