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Review

Effects of developmental exposure to bisphenol A on brain and behavior in mice ☆, ☆☆

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ABSTRACT

Bisphenol A (BPA) is a widespread estrogenic chemical used in the production of polycarbonate, and epoxy resins lining food and beverage cans and in dental sealants. During fetal life the intrauterine environment is critical for the normal development, and even small changes in the levels of hormones, such as estradiol or estrogen-mimicking chemicals, can lead to changes in brain function and consequently in behavior. We review here a series of ethological studies on the effects of maternal oral exposure during the last part of gestation (prenatal exposure) or from gestation day 11 to postnatal day 7 (perinatal exposure) to a low, environmentally relevant dose of BPA (10 μg/kg bw/day) on behavioral responses of CD-1 mouse offspring. We examined both male and female offspring and found that maternal exposure to BPA affected: (1) behavioral responses to novelty before puberty and, as adults; (2) exploration and activity in a free-exploratory open field; (3) exploration in the elevated plus maze and (4) sensitivity to amphetamine-induced reward in the conditioned place preference test. A consistent effect of the maternal exposure to BPA is that in all these different experimental settings, while a significant sex difference was observed in the control group, exposure to BPA decreased or eliminated the sex difference in behavior. In addition, exposure of female mice to BPA in both adulthood or during fetal life altered subsequent maternal behavior. These findings, together with those from other laboratories, are evidence of long-term consequences of maternal exposure to low-dose BPA at the level of neurobehavioral development.

1. Introduction

A number of environmental compounds referred to as estrogenic endocrine disrupters (EEDs) are able to bind to estrogen receptors (ERs) and interfere with the normal cellular development in target tissues. For example, studies have demonstrated estrogenic activity *in vitro* and *in vivo* for the naturally occurring phytoestrogens (e.g., soybeans), some pesticides (e.g., *o,p′*-DDT, methoxychlor and lindane), products associated with plastics (bisphenol A (BPA) and nonylphenol), pharmaceuticals (ethinylestradiol, diethylstilbestrol), or industrial chemicals (polychlorinated biphenyls, PCBs) (Sonnenschein and Soto, 1998). Many studies on these EEDs in animal models reported effects on the reproductive system (Richter et al., 2007). However, more subtle and insidious effects of EEDs can emerge as interference with brain developmental trajectories that result in behavioral alterations.

BPA is a widespread estrogenic chemical that is produced in excess of 6 billion pounds per year for use as the monomer that is polymerized to manufacture polycarbonate plastic food and beverage containers, the resin lining of metal cans, dental sealants, and as an additive in a wide array of other products (Vandenberg et al., 2007). Although assessments of all of the sources that account for current levels found in human tissues are limited, several studies have now confirmed detectable levels of BPA in human populations in developed countries (Calafat et al., 2007; Vandenberg et al., 2007). Most relevant to the studies described in this review, BPA has been measured in amniotic fluid, maternal and fetal plasma, and placental tissue at birth (Ikezuki et al., 2002; Schonfelder et al., 2002), and in breast milk of lactating mothers (Kuruto-Niwa et al., 2007; Sun et al., 2004). BPA was reported by Dodds and Lawson in 1936 to be a full estrogen agonist, before chemical engineers determined in the 1950s that

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this hormonally active drug could be polymerized to produce polycarbonate plastic. BPA is an estrogen agonist that can bind to both classical nuclear ERs, ER$_\alpha$ and ERβ (Welshons et al., 2006). In addition, recent studies have revealed that low levels of BPA can induce rapid, membrane-initiated estrogenic effects (Wozniak et al., 2005; Zsarnovszky et al., 2005), suggesting that low levels of BPA exposure might interfere with normal estrogenic signaling (Wetherill et al., 2007). The developing brain is exquisitely sensitive to estrogen and therefore might be particularly vulnerable to xenoestrogen exposure (Montano et al., 1995).

During mammalian brain development, estrogen participates in the organization of neural circuits that control a broad spectrum of neuroendocrine, behavioral, and cognitive functions. Alterations in the estrogenic milieu of the developing central nervous system (CNS) influence critical aspects of cellular differentiation, including neurite extension and branching, synapse formation, myelination, the expression of neurotransmitters and neuropeptides, and cell death and survival (Arnold and Gorski, 1984). Through its neurotrophic and differentiation promoting effects, estrogen also appears to be crucial for the sexual differentiation of CNS structures and functions. During a critical period of brain development that in mice and rats, extends from late prenatal life until the first 1–2 weeks after birth, estrogen irreversibly organizes male-type specific circuitries (Arnold and Gorski, 1984; Welshons et al., 2006). Estrogen is actively synthesized in the brain from testosterone by neurons expressing the enzyme aromatase (McEwen and Alves, 1999). While it was initially assumed that the brain of the female fetus was completely protected from defeminization or mascolinization by the plasma estrogen-binding glycoprotein alpha-fetoprotein (AFP), which binds circulating maternal/fetal estradiol, this was shown to be incorrect (Montano et al., 1995). Instead, it appears that it is the combination of maternal/fetal estradiol in blood and intracellular produced estradiol (due to aromatization of testosterone) that results in defeminization and masculinization of the male but not the female brain in rats and mice. Some isoforms of AFP bind estradiol in rodents with high affinity (Mizejewski and Jacobson, 1987), while non-steroidal estrogens, such as BPA and the drug diethylstilbestrol (DES), that exhibit a lower affinity for plasma estrogen-binding glycoproteins, bypass this cell uptake regulatory mechanism, thus increasing the fraction of the total measurable amount of the compound in blood that is able to pass from blood into cells and bind to nuclear ERs (Nagel et al., 1997, 1999).

Estrogenic chemicals can thus interfere with the male- and female-typical development of brain areas that control the occurrence and pattern of a wide range of behaviors required for reproduction, such as sexual behaviors, as well as social and non-social behaviors in adult life. Disruption of the normal processes of masculinization of males and feminization of females may undermine the survival and reproductive success (i.e., fitness) of exposed individuals (Parmigiani et al., 1998). Many studies indicate that the developing fetus is more sensitive to estrogenic chemicals relative to the adult (Bern, 1992; vom Saal et al., 2007). Mothers can pass EEDs to their offspring transplacentally, and after birth by breastfeeding newborns (Vandenberg et al., 2007).

The laboratory mouse is a good experimental model to investigate the effects of developmental exposure to EEDs on certain types of behavioral systems that are differently expressed in adult males and female, such as the occurrence and pattern of social behavior (e.g., aggression, parental behavior) and non-social behaviors (e.g., exploration, emotionality, activity patterns, learning, memory). In mice and rats, as in other mammals, non-reproductive behaviors also show sex differences in quantity of performance expressed rather than being present in one sex and absent in the other (Goy and McEwen, 1980). Although some of these sex differences reflect activational (transient) effects of estradiol and testosterone in the blood of adult males and females (Palanza et al., 2001b; Zimmerberg and Farley, 1993), differential actions of gonadal steroids during the perinatal period plays a crucial role in permanently organizing sexual dimorphisms in behavior and its underlying neural substrates (Arnold and Gorski, 1984).

We discuss here a series of experiments examining the effects of maternal exposure to BPA, at doses within the range of human exposure, on behavioral responses of male and female CD-1 house mice (Mus domesticus). We fed pregnant and lactating mice 10 μg/kg/day BPA. This dosage is far below the United States Environmental Protection Agency (US-EPA) lowest observed adverse effect level (LOAEL; 50 mg/kg/day) that was used to calculate a reference dose (estimated to be safe for daily human exposure) of 50 μg/kg/day (IRIS, 1988). Our previous research on behavioral effects of developmental exposure to estrogenic endocrine disruptors (Palanza et al., 1999a, b, 2001a, b) has suggested that male and female mice show differing sensitivities to EEDS in relation to different behavioral systems. Prenatal exposure to different EEDS mainly affected emotional, explorative and maternal behavior in females, whereas the development of sexual and aggressive behavior was only marginally affected in males. Based on this background, the present analysis focused on the effects of BPA on maternal behavior and on the behavioral systems involved in the response to novelty and reward.

1.1. Effects of BPA on maternal behavior

When studying the impact of hormonal perturbations during early development on behavioral responses as an index of altered brain function, it should be considered that behavior is influenced by a large variety of environmental factors affecting both the mother and the offspring, and their interactions, in early and later phases of development. For instance, perturbations of the stimuli received by the pups from the mother may account for alterations of the neuroendocrine system and behavior as expressed in adulthood (Benus and Roendigs, 1997; Francis et al., 1999). In studies where fetuses or newborns are directly treated or exposed through treatment of the mother to a chemical, not only in toxicological but also in pharmacological and endocrinological studies as well, the analysis are generally only addressed to the developing organism, while possible alterations of the mother–offspring interactions are not taken into account and thus not monitored.

Maternal behavior in mammalian species is the result of the complex interaction between the developing offspring and the lactating dam (reviewed in: Rosemblatt et al., 1979; Fleming et al., 1999). During pregnancy and the prepartum period, numerous hormones, such as progesterone, prolactin and estradiol, organize and activate the neuroendocrine substrates responsible for the expression of maternal behavior. After parturition, the hormonal control wanes and the female depends only upon stimulation by her suckling offspring to maintain her maternal responsiveness (Svare, 1989). Slight perturbations in any of the components of the mother–infant interaction may result in alterations of the behavior of the mother and/or of the offspring during development (Fleming et al., 1999). Therefore, an EDC-induced alteration in the female hormonal milieu and/or an EDC effect on early development of her offspring might be reflected by an alteration of the behavior shown by the dam during lactation. Ethological observations of maternal–infant interactions may thus be a sensitive index of perturbations due to exposure to very low doses of estrogenic EDCs, which may act directly on the

neuroendocrine system of the dam and on the development of her offspring.

We have examined the effects of BPA treatment on maternal behavior following exposure of mice to BPA during prenatal development and/or adulthood (Palanza et al., 2002a). The CD-1 mice used in this study were maintained as an outbred colony at the laboratory of the Division of Biological Sciences, University of Missouri-Columbia. Mice were time-mated (gestation day, GD, 0 = day of vaginal plug) and on GD 14–18, 14 pregnant mice were fed tocopherol stripped corn oil vehicle and nine pregnant mice were fed 10 μg/kg bw/day BPA using an electronic micropipette (as opposed to gavage, which is more stressful). The day of birth was considered postnatal day (PND) 1, and offspring were weaned on PND 20. At 2.5 months of age, F_1 female offspring from vehicle- and BPA-treated dams were mated and fed vehicle or 10 μg/kg bw/day BPA on GD 14–18. There were four groups of F_1 females that were exposed during gestation–adulthood to vehicle–vehicle ($N = 20$), vehicle–BPA ($N = 15$), BPA–vehicle ($N = 15$), and BPA–BPA ($N = 15$). F_1 dams were observed in their home cages during the dark phase of the light cycle, when mice are most active. Maternal behavior was observed every 4 min during a 120 min period on PND 2–15. On PND 1, F_2 pups were counted, weighed, and sex was determined. Litters were culled to 10 pups, with equal numbers of male and female pups when possible. Pups were weighed during the lactation period and cliff-drop aversion and righting reflex were evaluated in all pups of a subset of 8 l/group on PND 3, 5, 7 and 9. The dosing and the analysis design are summarized in Table 1. For statistical analyses, all pup data were adjusted for litter by including litter as a main effect variable and dividing the *F*-value for treatment by the *F*-value for litter. Data were analyzed by ANOVA, Holms *t*-test, and/or Fisher protected least squared difference test.

BPA treatment did not affect gestational body weight gain in F_0 or F_1 dams. Statistically significant effects for F_1 maternal behavior collapsed across 14 observation days are presented in Table 2. Exposure to BPA either during gestation or in adulthood resulted in a decrease in the percent of time dams spent nursing and in the nest but increased the percent of time the dams resting alone, grooming, out of the nest or nest building. Increased activity was also observed in the group exposed to BPA in adulthood. The only significant effect observed in mice exposed to BPA during gestation and then again during pregnancy in adulthood was increased time resting. There were no significant differences between the 4 groups in the number of live F_2 pups/litter, sex ratio, or body weight at birth, or in weight gain during the lactation period. No significant effects were observed for cliff aversion or righting reflexes development. The finding that BPA exposure did not affect the weaning weights of the pups suggests an adequate level of maternal care across the groups, albeit the reported differences in the amount of nursing behavior. Naturally occurring variations in maternal cares—with no effects on offspring weights—have been reported in rats as related to estrogen-modulated differences in oxytocin receptors levels in specific brain areas (Champagne et al., 2001) and to long-term effects on offspring endocrine and behavioral profile in adulthood (Francis et al., 1999).

We hypothesized that the changes seen in maternal behavior may be the result of a direct effect of BPA on the neuroendocrine substrates underlying the initiation of maternal behavior. The possible mechanisms underlying the observed alterations in maternal behavior of adult females just exposed during late pregnancy to BPA (vehicle–BPA group) can be due to a direct effect of BPA on the neuroendocrine system of the dam. In this study, females were fed BPA during the last 5 days of pregnancy; and it is possible that the subsequent effects on maternal behavior were due, in part, to biologically active BPA remaining in treated females after parturition during the time that they were lactating, since it has been reported that the metabolism of BPA to its inactive conjugated (glucuronidated) metabolite is reduced during pregnancy (Matsumoto et al., 2002). It is well recognized that estrogen levels have to drop at parturition for normal lactation to occur. Thus, while estrogen is important in mice for the initiation of maternal behavior prior to parturition (Fleming et al., 1999), our findings here show that exposure to manmade estrogenic chemicals during late pregnancy has the effect of reducing subsequent nursing behavior. These findings are consistent with a previous study examining the effects on maternal behavior of exposure to a low dose of the estrogenic pesticide methoxychlor during pregnancy in CD-1 mice (Palanza et al., 2002a). Furthermore, a recent work showed that exposure of rats during pregnancy and lactation to 40 μg/kg/day BPA reduced their maternal behavior (Della Seta et al., 2005). Interestingly, prolonged administration of BPA to lactating female rats induced a decrease in estrogen receptor-α cells in the maternal arcuate nucleus, a hypothalamic area involved in activating

Table 2
Effects on maternal behavior in F_1 female mice fed bisphenol A (BPA; 10 μg/kg/day) during gestation and/or adulthood

Percent time	Bisphenol A exposure during gestation and/or adulthood		
	BPA–vehicle	Vehicle–BPA	BPA–BPA
Nursing	↓15%	↓14%	↔
Nest building	↑73%	↑146%	↔
Resting alone	↑67%	↑29%	↑46%
Grooming	↑25%	↑18%	↔
Active	↔	↑18%	↔
In nest	↓12%	↓10%	↔
Out of nest	↑17%	↑12%	↔

↓,↑ Statistically significant increases/decreases compared to vehicle–vehicle group, ↔ no statistically significant effect. From Palanza et al. (2002a).

Table 1
Transgenerational exposure of mice with corn oil control (vehicle) or 10 μg/kg/day bisphenol A (BPA)

F_0	F_1		F_2
	Dosing	Resulting treatment	
Exposure			
Vehicle	Vehicle	Vehicle–vehicle (20)	Only maternal dosing
	BPA	Vehicle–BPA (15)	
BPA	Vehicle	BPA–vehicle (15)	Only maternal dosing
	BPA	BPA–BPA (15)	
Measures			
		Maternal behavior PND 1–15	Litter size, sex ratio growth rate, reflex development

(*N*) refers to the number of dams per resulting treatment group.

reproductive behavior, including maternal behavior (Aloisi et al., 2001).

Females that were exposed to BPA only during fetal development (BPA–vehicle group) showed similar changes in their maternal behavior as those described above for female mice exposed to BPA for the first time during late pregnancy (vehicle–BPA group). The BPA–vehicle females exhibited lower nursing and nest-related behavior, and increased out-of-nest behaviors (particularly, resting alone and self-grooming) relative to control (vehicle–vehicle) dams. The decrease in maternal behavior as a result of *in utero* BPA exposure might be due to an interference in the organization of the neuroendocrine substrates underlying the expression of maternal behavior later in life. This would suggest that the interactions of an adult postpartum female with her pups may be a sensitive measure of hormonal perturbation during the lactating female's prior fetal life.

An intriguing finding here is the absence of an effect on maternal behavior in females first exposed to BPA during fetal life and then again in adulthood during late pregnancy. One hypothesis is that fetal exposure to BPA results in permanent changes in systems that maintain homeostasis. This shift in homeostatic mechanisms may alter the subsequent response to chemical exposure at a later time in life relative to the response that would occur with no prior exposure to the chemical. There has been speculation that short-term exposure to chemicals, such as BPA, could lead to different outcomes relative to long-term exposure, such as in a multigenerational study (NTP, 2001). However, more comprehensive studies involving administration of endocrine disrupting chemicals at different times in life, which include physiological measures as well as behavior, will be required to answer this question.

1.2. Effects of BPA on the offspring's brain and behavioral development

We have also conducted studies examining the effects of perinatal exposure, via maternal treatment during pregnancy and lactation, to BPA on non-social behaviors sensitive to the action of perinatal gonadal hormones. The interplay among developmental stage at the time of exposure, age of testing, and response endpoint can be of considerable value in the study of the possible mechanisms of action of estrogenic chemicals on the development of brain and behavior.

In these experiments we used a procedure that allowed oral administration of the chemical to the pregnant and lactating female without handling at all the female and her offspring. This is a critical issue, since handling procedures can be stressful to animals, and stressful events during pregnancy can change the hormonal milieu of the mother and affect neuroendocrine development of the offspring (Montano et al., 1991; vom Saal et al., 1990). Before and after time-mating, female mice were trained to spontaneously drink a small volume (50 μl) of corn oil from a modified syringe (without the needle and with a larger hole) introduced through the cage top every day. All females easily learned to drink the oil as soon as the syringe was introduced; this procedure allows accurate administration of chemicals without the stress associated with gavage or injection. Pregnant female mice spontaneously drank daily doses of corn oil with or without the BPA 10 μg/kg/day) from GD 11 to PND 8 (perinatal exposure—"exploration and anxiety" experiments) or only from GD 11 to 18 (prenatal exposure—"brain dopaminergic function" experiment). Within 12 h after parturition (which occurred on GD 19), the sex and weight of live newborn pups was recorded, and each litter was culled to 10 pups (3–6 males and 3–6 females) in order to reduce litter size and sex-ratio variability that could impact the growth and development of pups during the early postnatal period. When 25–26 days old, offspring were weaned, and mice were group-housed with same-sex littermates (3–6 mice/group). For each test, only one male and one female from each litter was examined ($N = 12–14$/sex/treatment/test). We examined control and BPA-exposed offspring for exploratory behaviors before and after puberty and for conditioned place preference (CPP) induced by amphetamine.

2. Exploration and anxiety

We assessed explorative and emotional behaviors of the maternally exposed offspring at different ages and in different experimental settings (Gioiosa et al., 2007). Mice underwent a (1) novelty test before puberty (PND 28–30); (2) a free-exploratory open field test as adults; (3) the elevated plus maze as adults. All of these behavioral paradigms show sex differences in rodents, including mice (Laviola et al., 2005; Leret et al., 1994; Palanza et al., 2001b, 2002b), and can be sensitive to alterations in the endocrine milieu (Leret et al., 1994). Our hypothesis was that if such differences were decreased, reversed or enhanced by perinatal BPA exposure, then a reasonable inference is that developmental exposure to BPA interfered with sexual differentiation of the brain (Weiss, 2002).

2.1. Novelty test before puberty

A transparent Plexiglas cage ($40 \times 25 \times 15$ cm) was divided into two compartments (A—familiar and B—novel; 20×25 cm each) by a partition of white opaque polypropylene with a small opening in the middle that could be opened and closed by the experimenter. Male or female sibling groups were housed in compartment A of the apparatus. After 24 h, all mice but one were randomly removed from the cage, so that only one mouse was tested and the door dividing the two compartments was opened thus allowing the mouse to enter the novel area (compartment B). This test can measure the propensity of exploration (curiosity) and preference for novelty, amount of locomotion, and also serves as an index of anxiety. The results showed that in the control group, female mice entered the novel compartment more quickly, spent more time in it, and were less active (lower locomotion levels) when compared to males. Pre-puberal unexposed females thus showed a behavioral profile suggestive of lower anxiety and higher propensity to explore a novel environment (which suggests that females showed lower risk assessment levels than males), but, at the same time, they seemed to be less active as compared to control males. Relative to controls, pre-puberal BPA-exposed females entered the novel compartment less quickly, spent less time in it, spent more time exhibiting grooming behaviors, and were more active than controls, suggesting that BPA resulted in an increase in risk assessment behaviors in females.

2.2. Free-exploratory open field

This test was conducted in an apparatus with a home-cage area and an unfamiliar arena (an open-field—OF) of $73 \times 110\,cm^2$, bordered by a 50-cm high wall and in which a bright and a dark zone were created. One male and female per litter were individually housed in the home-cage section, and after 24 h the barrier between the compartments was removed allowing entrance into the OF. A cut-off of 10 min was used for those animals that did not enter the OF. Starting from the first entrance in the OF, behavioral observation lasted 5 min. The results showed that adult control females displayed higher exploration than

males when challenged to explore a novel environment; control females spent more time exploring the arena and showed more returns between OF and home-cage, and when in the OF arena, they spent more time in the bright zone and in the central area of OF relative to males. Compared to same-sex controls, BPA-exposed females showed decreased frequencies of returns while BPA-exposed males displayed increased returns between home-cage and OF (BPA-exposed females were more similar to control males while BPA-exposed males were more similar to control females). Thus, a consistent finding was that BPA-exposed animals showed no behavioral sex differences, while control animals showed significant behavioral sex differences.

2.3. Elevated plus maze

The elevated-plus-maze paradigm is one of the most widely used animal tests for the study of anxiety (Rodgers, 1997). It consists of two open arms and two closed arms that extended from a common central platform. A mouse was placed in the center, and tests lasted 5 min. In the conventional form of the test, anxiety is routinely assessed by measures of open-arm avoidance, while locomotor activity is most reliably measured by the frequency of closed arm entries. A series of additional ethological measures, which include stretched attend postures, head-dipping and grooming, have been linked through factor analysis to risk assessment, directed exploration and displacement activity, respectively (Rodgers and Johnson, 1995).

Our data indicate that controls' behavioral profile differed between males and females, with females being more explorative on the elevated plus maze. Females entered more frequently the open arms, spent more time in the center and were more active than males. BPA-exposed females displayed a lower frequency of open-arm entries and spent less time in the center, showing a behavioral profile more similar to that of control males. Consequently, BPA-exposed males and females did not differ in their behavioral response in the elevated plus maze, an effect mainly due to changes in BPA-exposed females' behavior relative to female controls. Interestingly, traditional indexes of anxiety (time spent in open arms) did not differ in relation to sex, and this was not affected by BPA exposure. Farabollini et al. (1999) previously reported decreased anxiety in the elevated plus maze in male rats perinatally exposed to BPA, as indicated by the increased time spent in the open arms and decreased time spent in risk assessment by the exposed rats.

In summary, as a general result, we found that while control mice showed sex differences on a number of behavioral responses at both ages and in all the test paradigms, mice perinatally exposed to BPA showed decreased or no sex differences (Table 3). Unexposed female mice, when allowed to explore a novel environment, were more reactive and explorative as compared to unexposed males, either prior to puberty or in adulthood. Developmental exposure to the estrogenic pollutants BPA resulted in behavioral alterations mainly in females, similar to findings with methoxychlor (Palanza et al., 2002b). Females showed levels of exploratory behavior more similar to the typical behaviors observed in control males than to those recorded in the control females. Altogether these findings may well be seen as indexes of reduced reactivity of BPA-exposed females to novel stimuli and are consistent with an estrogenic action of BPA, and possible "defeminization" or "masculinization" effects of developmental exposure to these compounds.

However, we also found that BPA-exposed males showed female-type behavior on a few measures. The overall result was a reduction or a reversal of sexual differences in exposed mice, relative to those displayed by controls. The fact that sexual behavioral differences occur before puberty (i.e., before the increase of sexual hormones production is activated by gonads), and that exposure to estrogenic compounds decreased differences in response to a challenge (novelty), suggest an interference of BPA in the processes of development and organization of the CNS and possibly of hormone and/or neurotransmitter receptor systems of both sexes (reviewed in Richter et al., 2007).

3. Brain dopaminergic function

The specificity of the developmental changes affecting a central neurochemical system can be evaluated by assessing the effects of a psychoactive agent targeting that system upon the behavioral responses known to be modulated by that system. For this reason we assessed in adult animals the possibility that prenatal exposure to BPA may influence the development of brain

Table 3
Sex differences in behavioral test responses of pre-puberal and adult mice pre- or peri-natally exposed to vehicle or to bisphenol A (BPA 10 μg/kg bw/day).

Behavioral test	Behavioral response	Control	BPA
Novelty test (pre-puberty)[a]	Latency to novelty	$F<M$	$\downarrow F=M$
	Exploration of novelty	$F>M$	$\uparrow F>M$
	Risk assessment	$F<M$	$F>M$
	Locomotion	$F<M$	$F=M$
	Self-grooming	$F<M$	$\uparrow F>M$
Free-exploratory open field[a]	Exploration	$F>M$	$\downarrow F=M$
	Locomotion	$F>M$	$\downarrow F<M\uparrow$
	Time center	$F>M$	$F=M$
	Time bright area	$F>M$	$F=M$
	Returns home	$F>M$	$F<M\uparrow$
Elevated plus maze[a]	Entrance open arms	$F>M$	$F=M$
	Time center	$F>M$	$F<M\uparrow$
	Time closed arms	$F<M$	$F=M$
Conditioned place preference (amphetamine induced)[b]	Preference for drug-paired compartment (reward memory)	$F>M$	$\downarrow F=M$

$F=M$, level of behavior do not significantly differ in males and female; $F>M$, levels of behavior significantly higher in females than males; $F<M$ levels of behavior significantly lower in females than males. $\downarrow,\uparrow$ statistically significant decrease or increase of behavior following BPA exposure, respectively.

[a] Exposure to BPA from gestation day 11 to postnatal day 7 (from Gioiosa et al., 2007).

[b] Exposure to BPA from gestation day 11 to 18 (from Laviola et al., 2005).

dopaminergic systems by investigating potential changes in the reinforcing effects of amphetamine, using a widely validated paradigm, the CPP (Laviola et al., 2005). This paradigm provides a measure of incentive memory of rewarding drug effects, which impinge on drug action within mesolimbic dopamine systems. The CPP paradigm consists of a three compartment opaque rectangular box; two cues, one visual (white or black walls) and one tactile (wide or narrow mesh floor), were associated with each of the two end-compartments. The white compartment of the apparatus was repeatedly paired with the administration of amphetamine (1 or 2 mg/kg i.p.), whereas the black one was paired with an injection of saline. Using a split-litter design, one male and one female from each litter were randomly assigned to be conditioned with saline or one of the two amphetamine doses. On the test day, mice were allowed free-access to both compartments, and a preference for the drug-paired side is taken as an index of the drug's positive reinforcing properties.

As a general result, amphetamine treatment produced increased locomotor activity in mice regardless of prenatal exposure to BPA or vehicle. With respect to amphetamine-induced place conditioning, females as a whole were more responsive than males, thus confirming previous results (Laviola et al., 1994). When compared to unexposed female mice, BPA-exposed females failed to show amphetamine-induced conditioning. Males showed no changes due to the prenatal treatment. Thus, exposure during fetal development to BPA was apparently responsible in female mice for impairment of brain reward pathways targeted by the drug.

Reduced novelty seeking and increased neophobia were also found in female rats perinatally exposed to BPA (Adriani et al., 2003). This behavioral profile could be related to gender-specific alterations in the function of brain neurochemical systems involved in the response to amphetamine. As release of dopamine within the dorsal and ventral striatum is known to be involved in the behavioral effects that follow amphetamine administration (Kelly et al., 1975; Staton and Solomon, 1984), it is reasonable to assume that a potential alteration in the behavioral effects of amphetamine administration could be an index of BPA-induced long-term effects on the dopaminergic function of the brain.

In rats, motor activity and motivation to explore were depressed at adulthood following perinatal exposure to BPA (Adriani et al., 2003; Farabollini et al., 1999) and perinatal exposure to BPA abolished sex differences in behavioral patterns in an open field (Kubo et al., 2003). Locus coeruleus and dopaminergic system are known to be involved in the regulation of animal reactivity to a novel environment and in the CNS, the dopaminergic system have been reported to be affected by early developmental exposure to estrogenic endocrine disruptors. Sexual dimorphism in the locus coeruleus has been detected in rats, with females showing larger volume, higher number of neurons and greater dopamine-β-hydroxylase-immunoreactive cells than males (Pinos et al., 2001). In addition, perinatal exposure to low doses of BPA in rats reversed the sexual difference in volume and number of cells of the locus coeruleus (Kubo et al., 2003). The mesolimbic and nigrostriatal dopaminergic systems represent major structures of the CNS underlying locomotor activity, novelty induced behavior, reward learning, attention deficit (Andersen and Teicher, 2000; Berridge and Robinson, 1998; Braak and Braak, 2000). *In utero* and lactational exposure to PCB-77 resulted in elevations in concentrations of dopamine in the frontal cortex, and of dopamine and its metabolites in the *substantia nigra* in periadolescent and in adult rats (Seegal et al., 1997).

In our study, developmental exposure to BPA resulted in alterations in the psychopharmacological profile of female mice, suggesting an impairment of brain reward pathways targeted by amphetamine, possibly involving brain monoaminergic (particularly dopaminergic) circuits (Laviola et al., 2005). On this basis, it may be supposed that BPA might interact with some steps in the development and organization of the monoaminergic system during the perinatal period. A convincing body of evidence indicates that estrogen can modulate basal and amphetamine-stimulated levels of dopamine release in rodent striatum as measured by *in vivo* microdialysis (Becker, 1999) and intrauterine exposure to estradiol has been reported to have a significant effect on the organization of monoamine systems within the fetal hypothalamus (Kaylor et al., 1984). Developmental exposure to BPA has been shown to alter D_1 receptor expression and density in male mice (Suzuki et al., 2003).

Rubin et al. (2006) have reported that mice perinatally exposed to low doses of BPA showed alterations in sexually dimorphic population of tyrosine hydroxylase containing neurons in the anteroventral periventricular preoptic area (AVPV), and in the open field behavior, an effect consistent with an estrogenic activity of BPA on the developing brain. This is consistent with our preliminary data on the effect of prenatal exposure to BPA or the synthetic estrogen DES on the number of neurons producing tyrosine hydroxylase in the locus coeruleus of pre-puberal mice. We found that, while control animals showed sex difference in the number of TH-stained neurons in the LC, the exposure to BPA eliminates this difference, as did DES (Ponzi et al., 2006).

However, none of these studies described here can presently delineate all of the mechanisms involved in BPA's actions on the developing brain, and further studies are needed to clarify possible mechanisms underlying BPA's actions on brain development and effects on behavior. At present, it must be recognized that in addition to its well documented estrogenicity, BPA may exert other effects on the developing brain. A number of studies have suggested that some of the neurobehavioral effects of xenoestrogens, such as BPA, cannot be explained by an estrogenic action of this compound, related just to its binding to nuclear ERs alpha and beta (Wetherill et al., 2007). Furthermore, MacLusky et al. (2005) have recently reported anti-estrogenic effects of BPA on hyppocampal synaptogenesis. BPA has also been shown to exert estrogenic or anti-estrogenic effects in the rat cerebellum, according to the background concentration of estradiol (Zsarnovszky et al., 2005).

4. Conclusions

The literature on long-term brain and behavioral effects of developmental exposure to BPA, that we have briefly reviewed here, indicates that altered behavior is a conspicuous endpoint produced by low-dose exposure to BPA. Behavioral alteration has the advantage of revealing both direct and indirect effects of contamination exposure. Indeed, our research on long-term effects of developmental exposure to BPA in mice, as well as evidence from other laboratories (Rubin et al., 2006), clearly indicates that behavioral endpoints can be more sensitive than other endpoints as biomarkers of exposure, either in terms of chemical concentration, response time or both. We have shown that sexually dimorphic behaviors are particularly sensitive to developmental interference produced by chemicals with endocrine disrupting properties, by examining both reproductive and non-reproductive behaviors.

A common issue emerging from developmental studies in mice and rats is that exposure to BPA, as well as to other endocrine disrupting chemicals, can diminish, eliminate, reverse or widen sex differences in behaviors, thus interfering with normal sexual differentiation of the brain. These behavioral changes are associated with changes in monoamines (Ishido et al., 2004, 2007; Patisaul et al., 2006; Rubin et al., 2006). However, as Weiss

(2002) points out, explicit recognition of sex differences in performance is not a prominent feature of toxicological studies, except for reproductive capacity studies. Neurotoxicity testing does not typically recognize sex differences in behavioral responses as an experimental outcome.

Animal models may contribute to elucidating the impact of endocrine disrupters on brain development and behavior, but when designing laboratory experiments, the species used, gender, age, experience, environmental features, and their interactions are all aspects to evaluate in order to recreate the context and hence the function of the particular behavioral patterns under examination (Parmigiani et al., 1998). For example, developmental effects of toxicants are sometimes misinterpreted to refer exclusively to direct and specific damage to the developing nervous system, while they may depend, at least partially, on alterations to delicate reciprocal mother/pup relationships (Palanza et al., 2001a, b, 2002b; Della Seta et al., 2005). An analysis of maternal behavior is therefore important when assessing the effects of chemicals administered via maternal treatment. It is well known that both variations in maternal care and the response of pups to maternal cues can be responsible for variations in the rate of maturation, such as differences in growth rate or neurobehavioral responses. The effects on offspring of administration of chemicals to pregnant/lactating females can thus be amplified due to an interaction of effects on the mother as well as effects on the offspring (Fleming et al., 1999). As the results of these ethological studies indicate, exposure through a non-stressful administration procedure (i.e., allowing females to drink corn oil in which the compound is dissolved) of pregnant female mice to a low, environmentally relevant dose of an endocrine disrupter produced subtle alterations in subsequent maternal behavior and in their offspring's behavioral development; environmentally relevant levels refer to doses that could be encountered by wildlife and humans in the daily environment.

The low dose of BPA that caused elimination of the sexual difference in exploration observed in unexposed animals are far below the acceptable daily intake considered "safe" for humans by the FDA (US Food and Drug Administration). This should be a cause of concern for public health, confirming that low-dose exposure to weak environmental estrogens during brain sexual differentiation can influence subsequent sexually differentiated adult behaviors. In the conceptual frame of evolutionary theory, sex differences in behavior are thought to reflect adaptive differences of behavioral strategies in coping as resulting from sexual selection. Longitudinal studies on effects of endocrine disrupting chemicals should be carried out in order to evaluate in which contexts, and with what intensity, eliminating or reversing sex differences could have relevance to population dynamics, and whether behavioral alterations occur in systems influencing reproductive success and thus individual fitness.

References

Adriani, W., Della Seta, D., Dessi-Fulgheri, F., Farabollini, F., Laviola, G., 2003. Altered profiles of spontaneous novelty seeking, impulsive behavior, and response to D-amphetamine in rats perinatally exposed to bisphenol A. Environ. Health Perspect. 111, 395–401.

Aloisi, A.M., Della Seta, D., Ceccarelli, I., Farabollini, F., 2001. Bisphenol-A differently affects estrogen receptors-α in estrous-cycling and lactating female rats. Neurosci. Lett. 310, 49–52.

Andersen, S.L., Teicher, M.H., 2000. Sex differences in dopamine receptors and their relevance to ADHD. Neurosci. Biobehav. Rev. 24, 137–141.

Arnold, A.P., Gorski, R.A., 1984. Gonadal steroid induction of structural sex differences in the nervous system. Annu. Rev. Neurosci. 7, 413–442.

Becker, J.B., 1999. Gender differences in dopaminergic function in striatum and nucleus accumbens. Pharmacol. Biochem. Behav. 64, 803–812.

Benus, R.F., Roendigs, M., 1997. The influence of the postnatal maternal environment in accounting for differences in aggression and behavioural strategies in *Mus domesticus*. Behaviour 134, 623–641.

Bern, H.A., 1992. The fragile fetus. In: Colborn, T., Clement, C. (Eds.), Chemically Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Princeton Scientific Publishing, Princeton, pp. 9–16.

Berridge, K.C., Robinson, T.E., 1998. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res. Rev. 28, 309–369.

Braak, H., Braak, E., 2000. Pathoanatomy of Parkinson's disease. J. Neurol. 247 (Suppl. 2) (II/3-10).

Calafat, A.M., Ye, X., Wong, L.-Y., Reidy, J.A., Needham, L.L., 2007. Exposure of the US population to bisphenol A and 4-tertiary-octylphenol: 2003–2004. Environ. Health Perspect. (Online 24 October 2007).

Champagne, F., Diorio, J., Sharma, S., Meaney, M.J., 2001. Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. Proc Natl Acad Sci USA 98 (22), 12736–12741.

Dodds, E.C., Lawson, W., 1936. Synthetic oestrogenic agents without the phenanthrene nucleus. Nature 137, 96.

Della Seta, D., Minder, I., Dessí-Fulgheri, F., Farabollini, F., 2005. Bisphenol-A exposure during pregnancy and lactation affects maternal behavior in rats. Brain Res. Bull. 65 (3), 255–260.

Farabollini, F., Porrini, S., Dessi-Fulgheri, F., 1999. Perinatal exposure to the estrogenic pollutant bisphenol A affects behavior in male and female rats. Pharmacol. Biochem. Behav. 64, 687–694.

Fleming, A.S., O'Day, D.H., Kraemer, G.W., 1999. Neurobiology of mother–infant interactions: experience and central nervous system plasticity across development and generations. Neurosci. Biobehav. Rev. 23, 673–685.

Francis, D., Diorio, J., Liu, D., Meaney, M.J., 1999. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. Science 286, 1155–1158 (New York, NY).

Gioiosa, L., Fissore, E., Ghirardelli, G., Parmigiani, S., Palanza, P., 2007. Developmental exposure to low doses of environmental estrogens alters sex differences in exploration and emotional behavior in mice. Horm. Behav. 52 (3), 307–316.

Goy, R., McEwen, B., 1980. Sexual Differentiation of the Brain. MIT Press, Cambridge, MA.

Ikezuki, Y., Tsutsumi, O., Takai, Y., Kamei, Y., Taketani, Y., 2002. Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. Human Reprod. 17, 2839–2841.

IRIS, 1988. Bisphenol A. (CASRN 80-05-7). US-EPA Integrated Risk Information System Substance File, vol. 2002.

Ishido, M., Masuo, Y., Kunimoto, M., Oka, S., Morita, M., 2004. Bisphenol A causes hyperactivity in the rat concomitantly with impairment of tyrosine hydroxylase immunoreactivity. J. Neurosci. Res. 76, 423–433.

Ishido, M., Yonemoto, J., Morita, M., 2007. Mesencephalic neurodegeneration in the orally administered bisphenol A-caused hyperactive rats. Toxicol. Lett. 173, 66–72.

Kaylor Jr., W.M., Song, C.H., Copeland, S.J., Zuspan, F.P., Kim, M.H., 1984. The effect of estrogen on monoamine systems in the fetal rat brain. J. Reprod. Med. 29, 489–492.

Kelly, P.H., Seviour, P.W., Iversen, S.D., 1975. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res. 94, 507–522.

Kubo, K., Arai, O., Omura, M., Watanabe, R., Ogata, R., Aou, S., 2003. Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats. Neurosci. Res. 45, 345–356.

Kuruto-Niwa, R., Tateoka, Y., Usuki, Y., Nozawa, R., 2007. Measurement of bisphenol A concentrations in human colostrum. Chemosphere 66, 1160–1164.

Laviola, G., Dell'Omo, G., Chiarotti, F., Bignami, G., 1994. d-amphetamine conditioned place preference in developing mice: relations with changes in activity and stereotypies. Behav. Neurosci. 108, 514–524.

Laviola, G., Gioiosa, L., Adriania, W., Palanza, P., 2005. d-Amphetamine-related reinforcing effects are reduced in mice exposed prenatally to estrogenic endocrine disruptors. Brain Res. Bull. 65, 235–240.

Leret, M.L., Molina-Holgado, F., Gonzalez, M.I., 1994. The effect of perinatal exposure to estrogens on the sexually dimorphic response to novelty. Physiol. Behav. 55, 371–373.

MacLusky, N.J., Hajszan, T., Leranth, C., 2005. The environmental estrogen bisphenol A inhibits estrogen-induced hippocampal synaptogenesis. Environ. Health Perspect. 113, 675–679.

Matsumoto, J., Yokota, H., Yuasa, A., 2002. Developmental increases in rat hepatic microsomal UDP-glucuronosyltransferase activities toward xenoestrogens and decreases during pregnancy. Environ. Health Perspect. 110, 193–196.

McEwen, B.S., Alves, S.E., 1999. Estrogen actions in the central nervous system. Endocr. Rev. 20, 279–307.

Mizejewski, G.J., Jacobson, H.I., 1987. Biological Activities of Alpha-Fetoprotein. CRC Press, Boca Raton.

Montano, M.M., Wang, M.H., Even, M.D., vom Saal, F.S., 1991. Serum corticosterone in fetal mice: sex differences, circadian changes, and effect of maternal stress. Physiol. Behav. 50, 323–329.

Montano, M.M., Welshons, W.V., vom Saal, F.S., 1995. Free estradiol in serum and brain uptake of estradiol during fetal and neonatal sexual differentiation in female rats. Biol. Reprod. 53, 1198–1207.

Nagel, S.C., vom Saal, F.S., Thayer, K.A., Dhar, M.G., Boechler, M., Welshons, W.V., 1997. Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol. Environ. Health Perspect. 105, 70–76.

Nagel, S.C., vom Saal, F.S., Welshons, W.V., 1999. Developmental effects of estrogenic chemicals are predicted by an in vitro assay incorporating modification of cell uptake by serum. J. Steroid Biochem. Mol. Biol. 69, 343–357.

NTP, N.T.P., Final Report of the Endocrine Disruptors Low Dose Peer Review Panel. Endocrine Disruptors Low Dose Peer Review. 〈http://ntp-server.niehs.nih.gov/htdocs/liason/LowDoseWebPage.html〉, Raleigh, NC, 2001, pp. Requests for hard copies of the NTP report and inquiries about the Endocrine Disruptors Low-Dose Peer Review can be made to the NTP Liaison and Scientific Review Office (NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709; t: 919-541-0530; f: 919-541-0295; liaison@starbase.niehs.nih.gov).

Palanza, P., Morley-Fletcher, S., Laviola, G., 2001a. Novelty seeking in periadolescent mice: sex differences and influence of intrauterine position. Physiol. Behav. 72, 255–262.

Palanza, P., Gioiosa, L., Parmigiani, S., 2001b. Social stress in mice: gender differences and effects of estrous cycle and social dominance. Physiol. Behav. 73, 411–420.

Palanza, P.L., Howdeshell, K.L., Parmigiani, S., vom Saal, F.S., 2002a. Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. Environ. Health Perspect. 110 (Suppl. 3), 415–422.

Palanza, P., Morellini, F., Parmigiani, S., vom Saal, F.S., 2002b. Ethological methods to study the effects of maternal exposure to estrogenic endocrine disrupters: a study with methoxychlor. Neurotoxicol. Teratol. 24, 55–69.

Parmigiani, S., Palanza, P., vom Saal, F.S., 1998. Ethotoxicology: an evolutionary approach to the study of environmental endocrine-disrupting chemicals. Toxicol. Ind. Health 14, 333–339.

Patisaul, H.B., Fortino, A.E., Polston, E.K., 2006. Neonatal genistein or bisphenol-A exposure alters sexual differentiation of the AVPV. Neurotoxicol. Teratol. 28, 111–118.

Pinos, H., Collado, P., Rodriguez-Zafra, M., Rodriguez, C., Segovia, S., Guillamon, A., 2001. The development of sex differences in the locus coeruleus of the rat. Brain Res. Bull. 56, 73–78.

Ponzi, D., Palanza, P., Maruniak, J.A., Parmigiani, S., vom Saal, F.S., 2006. Sexual dimorphism in the number of TH-immunostained neurons in the Locus Coeruleus of young mice is eliminated by prenatal exposure to Bisphenol A. In: Fifth Forum of European Neuroscience, Abstract, A008.21.

Richter, C.A., Birnbaum, L.S., Farabollini, F., Newbold, R.R., Rubin, B.S., Talsness, C.E., Vandenbergh, J.G., Walser-Kuntz, D.R., vom Saal, F.S., 2007. *In vivo* effects of bisphenol A in laboratory rodent studies. Reprod. Toxicol. 24, 199–224.

Rodgers, R.J., 1997. Animal models of 'anxiety': where next? Behav. Pharmacol. 8, 477–504.

Rodgers, R.J., Johnson, N.J., 1995. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. Pharmacol. Biochem. Behav. 52, 297–303.

Rosemblatt, S., Siegel, H.I., Mayer, A.D., 1979. Progress in the study of maternal behavior in the rat: hormonal, nonhormonal, sensory, and developmental aspects. In: Rosemblatt, J.S., Hinde, R.A., Beer, C., Busnel, M.C. (Eds.), Advances in the Study of Behavior. Academic Press, New York, pp. 225–311.

Rubin, B.S., Lenkowski, J.R., Schaeberle, C.M., Vandenberg, L.N., Ronsheim, P.M., Soto, A.M., 2006. Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. Endocrinology 147, 3681–3691.

Schonfelder, G., Wittfoht, W., Hopp, H., Talsness, C.E., Paul, M., Chahoud, I., 2002. Parent bisphenol A accumulation in human maternal-fetal-placental unit. Environ. Health Perspect. 110, A703–A707.

Seegal, R.F., Brosch, K.O., Okoniewski, R.J., 1997. Effects of in utero and lactational exposure of the laboratory rat to 2,4,2′,4′- and 3,4,3′,4′-tetrachlorobiphenyl on dopamine function. Toxicol. Appl. Pharmacol. 146, 95–103.

Sonnenschein, C., Soto, A.M., 1998. An updated review of environmental estrogen and androgen mimics and antagonists. J. Steroid Biochem. Mol. Biol. 65, 143–150.

Staton, D.M., Solomon, P.R., 1984. Microinjections of d-amphetamine into the nucleus accumbens and caudate-putamen differentially affects stereotipy and locomotion in the rat. Physiol. Psychol. 12, 159–162.

Sun, Y., Irie, M., Kishikawa, N., Wada, M., Kuroda, N., Nakashima, K., 2004. Determination of bisphenol A in human breast milk by HPLC with column-switching and fluorescence detection. Biomed. Chromatogr. 18, 501–507.

Suzuki, T., Mizuo, K., Nakazawa, H., Funae, Y., Fushiki, S., Fukushima, S., Shirai, T., Narita, M., 2003. Prenatal and neonatal exposure to bisphenol-A enhances the central dopamine D1 receptor-mediated action in mice: enhancement of the methamphetamine-induced abuse state. Neuroscience 117, 639–644.

Svare, B., 1989. Recent advances in the study of female aggressive behaviour in mice. In: Brain, P.F., et al. (Eds.), House Mouse Aggression. Harwood Academic Press, Chur, pp. 135–159.

Vandenberg, L.N., Hauser, R., Marcus, M., Olea, N., Welshons, W.V., 2007. Human exposure to bisphenol A (BPA). Reprod. Toxicol. 24, 139–177.

vom Saal, F.S., Quadagno, D.M., Even, M.D., Keisler, L.W., Keisler, D.H., Khan, S., 1990. Paradoxical effects of maternal stress on fetal steroids and postnatal reproductive traits in female mice from different intrauterine positions. Biol. Reprod. 43, 751–761.

vom Saal, F.S., Akingbemi, B.T., Belcher, S.M., Birnbaum, L.S., Crain, D.A., Eriksen, M., Farabollini, F., Guillette Jr., L.J., Hauser, R., Heindel, J.J., Ho, S.M., Hunt, P.A., Iguchi, T., Jobling, S., Kanno, J., Keri, R.A., Knudsen, K.E., Laufer, H., Leblanc, G.A., Marcus, M., McLachlan, J.A., Myers, J.P., Nadal, A., Newbold, R.R., Olea, N., Prins, G.S., Richter, C.A., Rubin, B.S., Sonnenschein, C., Soto, A.M., Talsness, C.E., Vandenbergh, J.G., Vandenberg, L.N., Walser-Kuntz, D.R., Watson, C.S., Welshons, W.V., Wetherill, Y., Zoeller, R.T., 2007. Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. Reprod. Toxicol. 24, 131–138.

Weiss, B., 2002. Sexually dimorphic nonreproductive behaviors as indicators of endocrine disruption. Environ. Health Perspect. 110 (Suppl. 3), 387–391.

Welshons, W.V., Nagel, S.C., vom Saal, F.S., 2006. Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. Endocrinology 147, S56–S69.

Wetherill, Y.B., Akingbemi, B., Kanno, J., McLachlan, J.A., Nadal, A., Sonnenschein, C., Watson, C.S., Zoeller, R.T., Belcheer, S.M., 2007. *In vitro* molecular mechanisms of bisphenol A action. Reprod. Toxicol. 24, 178–198.

Wozniak, A.L., Bulayeva, N.N., Watson, C.S., 2005. Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor-a mediated Ca++ fluxes and prolactin release in GH3/B6 pituitary tumor cells. Environ. Health Perspect. 113, 431–439.

Zimmerberg, B., Farley, M.J., 1993. Sex differences in anxiety behavior in rats: role of gonadal hormones. Physiol. Behav. 54, 1119–1124.

Zsarnovszky, A., Le, H.H., Wang, H.S., Belcher, S.M., 2005. Ontogeny of rapid estrogen-mediated extracellular signal-regulated kinase signaling in the rat cerebellar cortex: potent nongenomic agonist and endocrine disrupting activity of the xenoestrogen bisphenol A. Endocrinology 146, 5388–5396.