Exposure to a Low Dose of Bisphenol A during Fetal Life or in Adulthood Alters Maternal Behavior in Mice

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Maternal behavior in mammals is the result of a complex interaction between the lactating dam and her developing offspring. Slight perturbations of any of the components of the mother–infant interaction may result in alterations of the behavior of the mother and/or of the offspring. We studied the effects of exposure of female CD-1 mice to the estrogenic chemical bisphenol A (BPA) during fetal life and/or in adulthood during the last part of pregnancy on subsequent maternal behavior. Pregnant females were fed daily doses of corn oil (controls) or 10 μg/kg body weight BPA during gestation days 14–18. As adults, the prenatally treated female offspring were time-mated and again fed either corn oil (controls) or the same doses of BPA on gestation days 14–18, resulting in four treatment groups: controls, prenatal BPA exposure, adult BPA exposure, and both prenatal and adult BPA exposure. Maternal behavior was then observed on postnatal days 2–15 and reflex responses were examined in the offspring. Dams exposed to BPA either as fetuses or in adulthood spent less time nursing their pups and more time out of the nest compared with the control group. Females exposed to BPA both as fetuses and in adulthood did not significantly differ from controls. No alterations in postnatal reflex development were observed in the offspring of the females exposed to BPA. 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. *Key words:* development, endocrine disruptors, house mice, maternal behavior, non-genomic transmission. *Environ Health Perspect* 110(suppl 3):415–422 (2002).
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Endocrine-disrupting chemicals (EDCs) are synthetic chemicals (i.e., pesticides) or naturally occurring substances (i.e., phytoestrogens) that are released into the environment and can interfere with the endocrine system of vertebrates (*1*). Certain EDCs can mimic or antagonize the endogenous sex hormones (estrogen and testosterone) and alter the normal hormone balance during development, which is crucial in regulating sexual differentiation of the neuroendocrine system of vertebrates. In traditional toxicology studies, man-made chemicals are tested for their capacity to induce gross abnormalities or lethality after the administration of a dose typically much higher than would be encountered in the environment. Conversely, the main focus of EDC research is on the functional changes that occur in endocrine-sensitive tissues due to exposure to low, environmentally relevant doses during critical periods in organ development. Functional changes, such as changes in behavioral responses or neural function, have not typically been examined in toxicologic studies, and, as stated by Colborn et al. (*2*), "Functional changes pose challenges in documenting the extent of a lesion, especially in the case of neuroendocrinological damage." A central nervous system (CNS) deficit may become evident only upon a specific kind of behavioral challenge, and the consequences of exposure to endocrine disruptors can be subtle. Examination of both learned and unlearned behaviors may reveal subtle deficits in CNS function, which may not be accompanied by demonstrable tissue pathology. Recent studies have shown that exposure to low doses of EDCs during early development are related to altered behavioral responses in rodents (*3–7*) and abnormal neurobehavioral development in humans, including a decrease in intelligence quotient (*8,9*).

The neuroendocrine-gonadal axis regulates the developmental organization and adult expression of behaviors critical for mammalian survival and reproduction, such as competitive aggression, exploration, and sexual and parental behaviors (*10*). The expression of these behaviors determines the fitness of an individual, and thus neurobehavioral alterations induced by EDCs may impact the survival and fitness of an individual in the environment. Thus, ethology, the evolutionary study of behavior, may provide a framework for integrating a functional perspective (i.e., evolutionary significance) to studies on proximate mechanisms that can account for behavioral alterations induced by developmental exposure to EDCs. Animal models aid in elucidating the impact of endocrine disruptors on brain development and behavior by taking into consideration the natural behavior of the animal (*11*).

In the present study, we hypothesized that ethological observations of maternal behavior may 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/or on the development of her offspring. Maternal behavior in mammals is regulated both by maternal hormones and stimulation by the nursing offspring [reviewed in Fleming et al. (*12*)]. During pregnancy and the prepartum period, progesterone, prolactin and, most important, 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 (*13*). 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 (*12*). For instance, it is well known that stress-induced perturbations in maternal behavior account for alterations in the neuroendocrine system and behavior of the offspring in adulthood (*14–16*). 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.

The purpose of our study was to examine the impact of low-dose exposure to the estrogenic chemical bisphenol A (BPA) on the maternal behavior of CD-1 mice exposed *in utero* (via their mothers) and/or during their own pregnancy as adults. BPA is the monomer that is used as a component in the interior lining of food and beverage cans, in some dental sealants, and in the production of polycarbonate plastic products, including baby bottles. Our prior research has demonstrated that feeding pregnant female mice doses of BPA within the range of human

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exposure significantly alters the development and function of the reproductive organs of the offspring in CF-1 mice (*17–19*).

We conducted a detailed ethological analysis of maternal behavior for the 2 weeks after parturition. Dams were observed in their home cages during the dark phase of the light cycle, when mice are most active. Because maternal behavior can affect, and be affected by, the physical and behavioral responses of pups, we also examined the offspring for changes in postnatal growth and the development of reflexes. The mouse is an altricial species; that is, the pups are born in a highly immature condition after a short pregnancy (18–20 days). Several reflexes and responses appear at successive postnatal stages in parallel with somatic changes. The time of appearance and subsequent complete maturation of various reflexes show considerable regularity, thus providing a tool to assess whether growth and neurobehavioral development are modified by exposure to hormone-mimicking chemicals and, in turn, whether changes in pup development are related to changes in maternal behavior (*11,20*).

Methods

Animals, Husbandry, and Mating Procedures

Animals were maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and all procedures were approved by the University of Missouri Animal Care and Use Committee. CD-1 mice (*Mus musculus domesticus*) were initially purchased from Charles River Laboratories (Wilmington, MA, USA) and were maintained as an outbred colony at the University of Missouri. The animals were housed in 18 × 29 × 13-cm polypropylene mouse cages on corn cob bedding. Pregnant and lactating females were fed Purina 5008 (soy-based) breeder chow, and after weaning, animals were fed Purina 5001 (soy-based) chow. Water was provided *ad libitum* in glass bottles and was purified by ion exchange followed by a series of carbon filters. Rooms were maintained at 25 ± 2°C under a 12:12-hr light:dark (L:D) cycle, with the lights on at 1100 hr.

Adult (3–4 month old) virgin female mice were time-mated by being placed into the cage of a stud male for 4 hr beginning at 0800 hr (at the end of the dark phase of the L:D cycle). Mating was verified by the presence of a vaginal plug (day 0 of gestation). After mating, pregnant females were housed three per cage.

Chemical Administration

Time-mated pregnant mice were fed 0 (vehicle control; n = 14) or 10 µg/kg/day BPA (Sigma Chemical, St. Louis, MO, USA; n = 9) dissolved in tocopherol-stripped corn oil (ICN, Aurora, OH, USA) on days 14–18 of gestation, during the fetal period of differentiation of the brain and urogenital system. The doses were delivered in a 30-µL volume with an electronic micropipetter into the mouth of the animals. Mice were picked up by the skin between the shoulders and held upright. The pipette tip was placed into the mouth with the pipette tip gently touching the roof of the mouth, and the oil was ejected from the pipetter. Mice readily consume corn oil, and this procedure is not stressful for the dams (*20*). On day 17 of pregnancy, females were individually housed in 18 × 29 × 13-cm clear polycarbonate mouse cages to allow observation of maternal behavior. Pregnant dams were weighed on gestation days 14, 16, and 18 to monitor weight gain during pregnancy, and the average weight between gestation days 14–18 was used to calculate the doses of BPA/kg body weight. The dams gave birth on day 19 of pregnancy, which is also postnatal day (PND) 1. The offspring were weaned on PND 20.

As adults (2–2.5 months of age), the prenatally exposed female offspring were time-mated and fed the same treatments of 0 (vehicle control; n = 51) or 10 µg/kg/day BPA (n = 31) on days 14–18 of gestation, following the same procedure as described above. As a result, a total of four treatment groups were established based upon the prenatal and adult exposure of the F_1 generation to vehicle control (OIL) or BPA (Table 1).

Maternal Behavior

Maternal behavior was assessed by observing lactating females in their home cages during an observation period of 120 min on PNDs 2–15. Previous experiments indicated that the mice were most active during the dark phase of the L:D cycle and that alteration due to exposure to hormonally active agents was detectable only during the active (dark) phase (*21*). Thus, in this experiment the observation period started at 0900 hr and was conducted entirely during the dark phase with the aid of 25-W red lights; mice do not see red light, and this does not shift their activity cycles.

Each dam was observed once every 4 min for a total of 30 observations. During each 4-min observation period, the experimenter recorded which behaviors the lactating female was displaying at the moment of observation. The maternal behaviors monitored were as follows:

a) In nest: The female was anywhere inside the nest, regardless of the behavior being exhibited at the moment of observation.

b) Nursing: The female was allowing the pups to suckle; this category did not necessarily imply that the whole litter was nursing, or that the female was adopting the nursing posture with her body arched over the pups.

c) Licking pups: The female was licking or grooming her pups.

d) Nest building: The female was engaged in some aspect of nest building, while she was either inside or outside the nest itself.

e) Eating/drinking: The female was nibbling at a food pellet or drinking from the water bottle.

f) Grooming: The female was grooming her own body.

g) Active: The female was moving about the cage.

h) Resting: The female was lying motionless outside the nest, not involved in any other form of behavior and with no pup attached to her nipples.

i) Forced nursing: The female was outside the nest and engaged in another behavior, but was reached and suckled by one or more pups, which she was trying to avoid.

Two additional categories were created by combining data: nest-related behavior and out-of-nest behavior. Nest-related behavior was a total of the observations per PND for nursing, nest-building and in nest activities. The out-of-nest category was a total of the observations per PND for active, eating/drinking, grooming, and resting when exhibited out of the nest and not in contact with any pup.

Measurements of the Offspring's Postnatal Development

Within 12 hr of delivery on PND 1, the following variables were measured: the number of pups per litter, ratio of male to total number of pups (sex ratio), and body weight of each pup. Litters were culled to 10 pups (5 males and 5 females whenever possible; litter size is typically 12), then returned to their mothers. All the pups were weighed on PNDs 3, 5, 7, 9, and 15 to monitor growth rate. For a subset of litters (n = 8 litters/treatment group), each pup within a litter was weighed and tested for cliff-drop aversion and righting reflexes on PNDs 3, 5, 7, and 9.

In more detail, the pup body weights were measured with a digital balance accurate to 0.01 g. Cliff-drop aversion is a measure of

Table 1. Transgenerational dosing design of mice with OIL or 10 µg/kg/day bisphenol A (BPA).[a]

Generation	Treatment			
F_0	OIL		BPA	
F_1	OIL	BPA	OIL	BPA
	Resulting treatment groups (n)			
	OIL–OIL (20)	OIL–BPA (15)	BPA–OIL (15)	BPA–BPA (15)

n, number of dams per treatment group. [a]The F_1 males from the same original litter were placed into either treatment group (OIL or BOA).

development of motor coordination and anxiety level; the higher the anxiety, the slower the pup is to complete the reflex. To measure the cliff-drop aversion reflex, each pup was placed on a table with the forepaws and face over the edge of the table. The experimenter measured the time it took for the pup to turn away from the cliff, until it was parallel to the edge of the table. Animals were given a maximum of 120 sec to complete the test. Animals that fell asleep or fell off of the cliff were assigned the maximum latency of 120 sec. The animals that fell landed in the experimenters' outstretched hands. The righting reflex provides information concerning motor coordination and vestibular maturation. The righting reflex involved placing a pup on its back and measuring the amount of time it took the pup to turn over with all four feet on the ground. Animals were tested for a maximum of 120 sec. Animals that did not turn over within 120 sec were assigned this score.

Statistical Analysis

All analyses were conducted using the Statistical Analyzing System, General Linear Model procedure (SAS Institute, Inc., Cary, NC, USA). Maternal body weights were analyzed by repeated-measures analysis of variance (ANOVA). The scores of the maternal behavior observations were converted to percentages of the maximum frequency possible (30) for each observational period. The maternal behavior data of the BPA-exposed females (*in utero* and/or during gestation) were log-transformed and analyzed by repeated-measures ANOVA, with two between-group factors (*in utero* exposure × gestational exposure) and one within-group factor (PNDs).

The number of pups per litter, sex ratio, and body weight of pups were analyzed by ANOVA. The body weights of the offspring were analyzed for individual PNDs because the pups were not individually identified within a litter. All pup data were adjusted for litter to control for maternal effects. The cliff-drop aversion and righting reflex data were analyzed by combining all pup measures for a particular reflex and computing an overall litter average; the overall litter averages were subsequently log-transformed and processed by repeated-measures ANOVA.

The post hoc comparisons of overall maternal behavior effects (collapsed across postnatal observation day) were made with the Holms *t*-test for multiple comparisons, a modified sequentially rejective Bonferroni *t*-test (*22*). The post hoc analyses of all the remaining data were made using Fisher's protected least-squared difference. The confidence level for rejecting the null hypothesis was $p < 0.05$.

Results

Maternal Body Weight during Gestation

For the F_0 generation, the average body weight during gestation was similar across the treatment groups (mean ± SE: OIL controls = 50.12 ± 1.64; BPA = 51.00 ± 1.84 g); these females were 3–4 months old. The F_1 female offspring of these mothers were timemated when they reached adulthood. The average gestational body weights of the BPA-exposed dams were not statistically different from the OIL control dams. Body weights (mean ± SE) for the four groups (fetal treatment via the mother–adult treatment during pregnancy) were OIL–OIL = 45.7 ± 1.3 g; OIL–BPA = 45.2 ± 1.3 g; BPA–OIL = 46.6 ± 1.2 g; BPA–BPA = 47.4 ± 1.3 g. The F_1 females were about 1 month younger and thus slightly lighter than the F_0 females.

Maternal Behavior

Figure 1 shows the data for maternal behavior collapsed across the 14 observation days. The findings are described below for each behavioral measure.

Nursing. The analysis of nursing behavior revealed a significant interaction between life stage at exposure (during fetal life or during pregnancy in adulthood) and treatment (BPA or OIL; $p < 0.001$). Specifically, dams exposed to BPA only as fetuses (BPA–OIL) or only during their own pregnancy (OIL–BPA) spent significantly ($p < 0.05$) less time nursing their pups compared with control (OIL–OIL) dams (Figure 1A). However, the nursing behavior of dams exposed to BPA first as fetuses and then again during their own pregnancy (BPA–BPA) did not differ from that of control dams (Figure 2C). The interaction of PND with treatment was not significant, and the BPA–OIL dams appeared to nurse less than controls throughout lactation (Figure 2A), whereas OIL–BPA dams appeared to nurse less than controls mainly in the first half of lactation (Figure 2B).

Nest building. The analysis of nest building propensity revealed a significant interaction between life stage at exposure and treatment ($p < 0.005$). Both BPA–OIL and OIL–BPA dams spent more time nest building than control (OIL–OIL) dams ($p < 0.05$; Figure 1B). There was also a significant interaction between PND and treatment ($p < 0.05$). The BPA–OIL mothers spent significantly more time nest building during

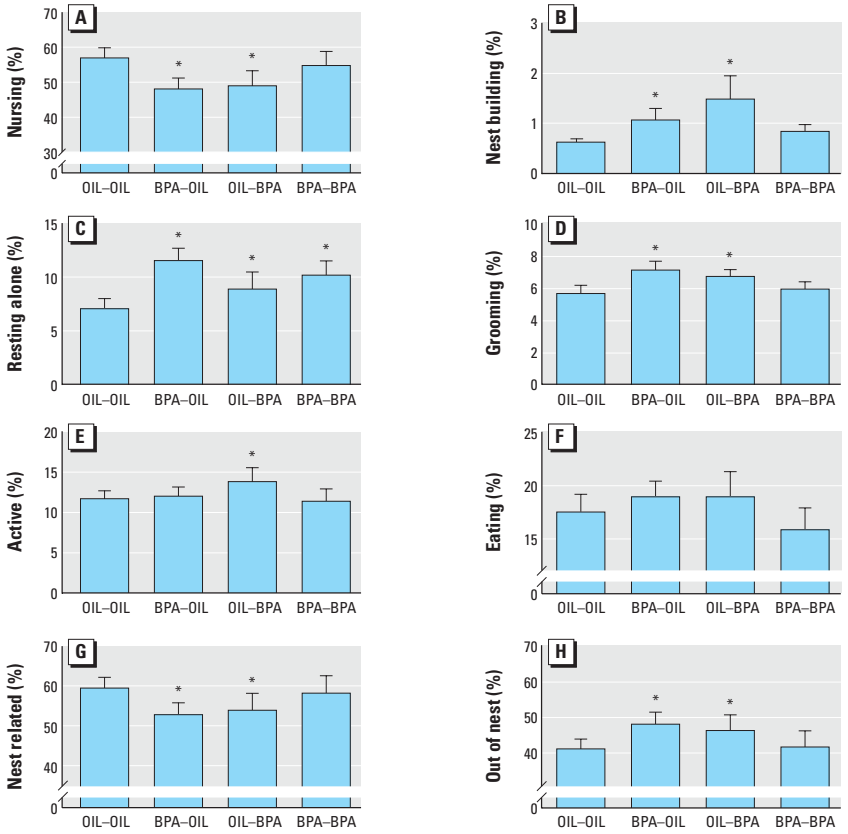

Figure 1. Average percent time (mean ± SE) spent on maternal behavior variables during PNDs 2–15 for dams exposed to 10 µg/kg/day BPA only *in utero* (BPA–OIL), only during gestation (OIL–BPA), or both *in utero* and during gestation (BPA–BPA). *Significantly different from control (OIL–OIL) (Holms *t*-test, $p < 0.05$).

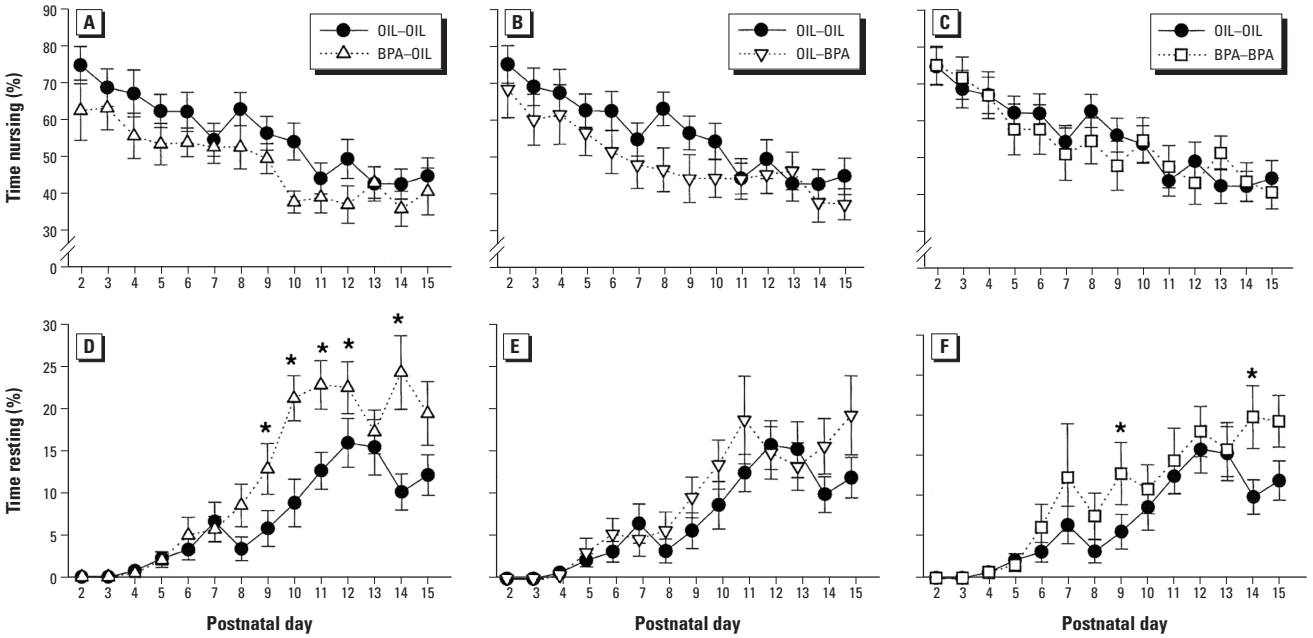

Figure 2. Percentage of time (mean ± SE) spent nursing (*A–C*) and resting alone (*D–F*) on PNDs 2–15 for dams exposed to 10 µg/kg/day BPA *in utero* (BPA–OIL), only during gestation (OIL–BPA), or both *in utero* and during gestation (BPA–BPA). *Significantly different from control (OIL–OIL) (Fisher's least-squared difference, $p < 0.005$–0.05).

early lactation (from PND 2 to PND 5) than control mothers (data not shown). The OIL–BPA dams also showed a similar trend with increased nest-building activity compared with control dams early in lactation (data not shown). The BPA-BPA mothers were not significantly different from controls in nest-building behavior.

Resting. There was a significant interaction between life stage at exposure and treatment for resting behavior ($p < 0.05$). Dams exposed to BPA (including BPA–OIL, BPA–BPA, and OIL–BPA groups) spent significantly more time resting away from the nest than OIL–OIL dams ($p < 0.05$; Figure 1C), consistent with the decrease in nursing behavior reported above. There was also a significant interaction between PND and treatment ($p < 0.05$), reflecting the fact that the differences between controls and the BPA-treated females were greatest during the middle period of lactation on PND 9–14 (Figure 2D–F).

Grooming. For grooming behavior, there was a significant interaction between life stage at exposure and treatment ($p < 0.001$). Both BPA–OIL and OIL–BPA dams spent significantly ($p < 0.05$) more time self-grooming relative to control (OIL–OIL) dams (Figure 1D). However, the rate of self-grooming was similar between BPA–BPA and control dams. The interaction between PND and treatment was not significant (Figure 3A–C).

Active. There was significant interaction between life stage at exposure and treatment for the variable active behavior ($p < 0.05$). OIL–BPA dams were significantly more active than OIL–OIL dams across the observation period ($p < 0.05$), whereas BPA–BPA and BPA–OIL dams were similar to controls (Figure 1E). There was no significant interaction between PND and treatment for active behavior (Figure 3D–F).

Eating/drinking. There was a significant interaction between life stage at exposure and treatment for eating and drinking ($p < 0.001$). Although the post hoc comparisons did not find significant differences between the treatment groups, the dams prenatally exposed to BPA (BPA–OIL) and dams gestationally exposed to BPA (OIL–BPA) tended to spend more time in eating and drinking behavior than controls (Figure 1F). There was no significant interaction between PND treatment for eating and drinking behavior.

Nest-related behavior. The variable of nest-related behavior was calculated by combining the observations for nursing, nest-building, and in-nest behaviors. There was a significant interaction between life stage at exposure and treatment for nest-related behavior ($p < 0.001$; Figure 1G). BPA–OIL and OIL–BPA dams spent less time in nest-related behavior than controls ($p < 0.05$). As expected, the frequency of nest-related behaviors decreased across PNDs (Figure 4A–C). The interaction of PND and treatment was not significant, and BPA–BPA dams did not differ significantly from controls.

Out-of-nest behaviors. The variable of out-of-nest behaviors was calculated by combining the observations for active, eating/drinking, grooming, and resting behaviors that occurred away from the pups. There was a significant interaction between life stage at exposure and treatment for out-of-nest behaviors ($p < 0.001$). BPA–OIL and OIL–BPA dams, but not BPA–BPA dams, spent significantly more time out of the nest, thus away from their pups, than controls ($p < 0.05$; Figure 1H). The interaction between PND and treatment was not significant (Figure 4D–F).

Remaining behaviors. BPA exposure did not significantly influence either in-nest, licking, or forced nursing behaviors.

Offspring Postnatal Development

Litter parameters at birth and growth rate. There were no significant differences in the number of pups per litter alive on the day of birth, the sex ratio (males/total pups per litter), or body weight at birth, based on treatment. Regardless of treatment, males weighed significantly more than females on the day of birth, as well as during PND 3–15 ($p < 0.001$). Treatment did not influence the body weight of offspring on PNDs 3–15.

Cliff-drop aversion reflex. PND was a significant factor in the analysis of cliff avoidance reflexes ($p < 0.001$), and the offspring completed the avoidance response more quickly as they aged (Figure 5A–C). However, there was no significant effect of treatment on cliff-drop aversion behavior.

Righting reflex. Pups exhibited the righting reflex more rapidly as they aged, and there tended to be an effect of treatment for this behavior ($p = 0.06$). Specifically, the off-

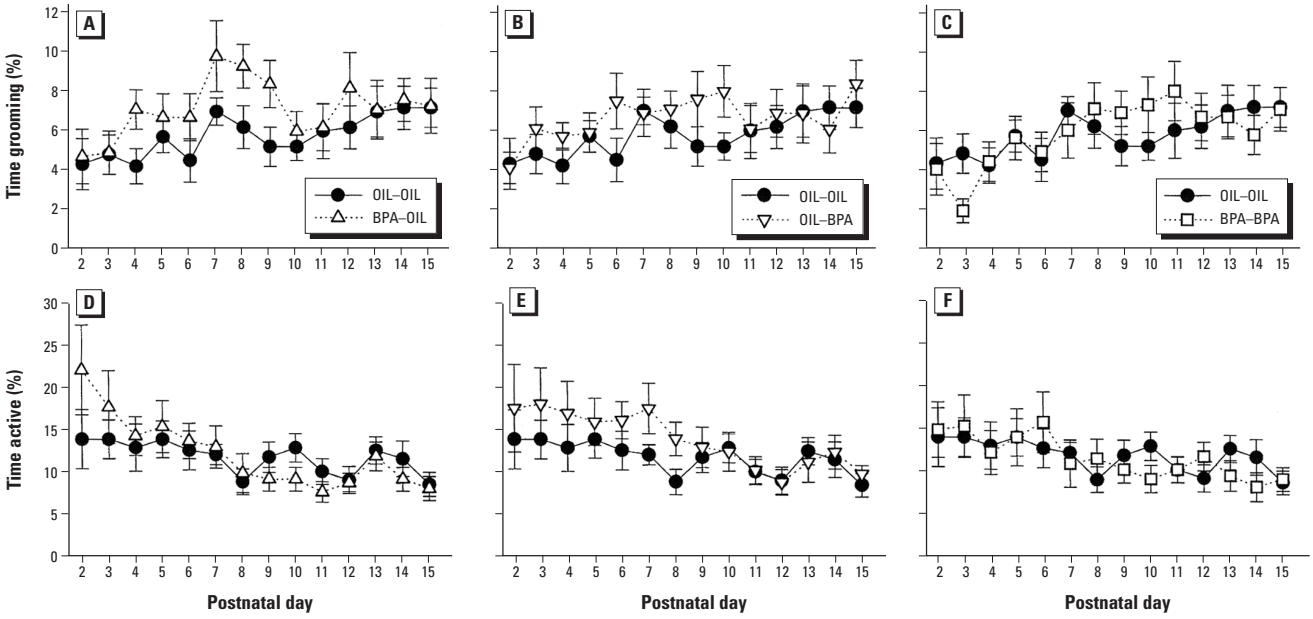

Figure 3. Percentage of time (mean ± SE) spent grooming (*A–C*) and active (*D–F*) on PNDs 2–15 for dams exposed to 10 µg/kg/day BPA only *in utero* (BPA–OIL), only during gestation (OIL–BPA), or both *in utero* and during gestation (BPA–BPA).

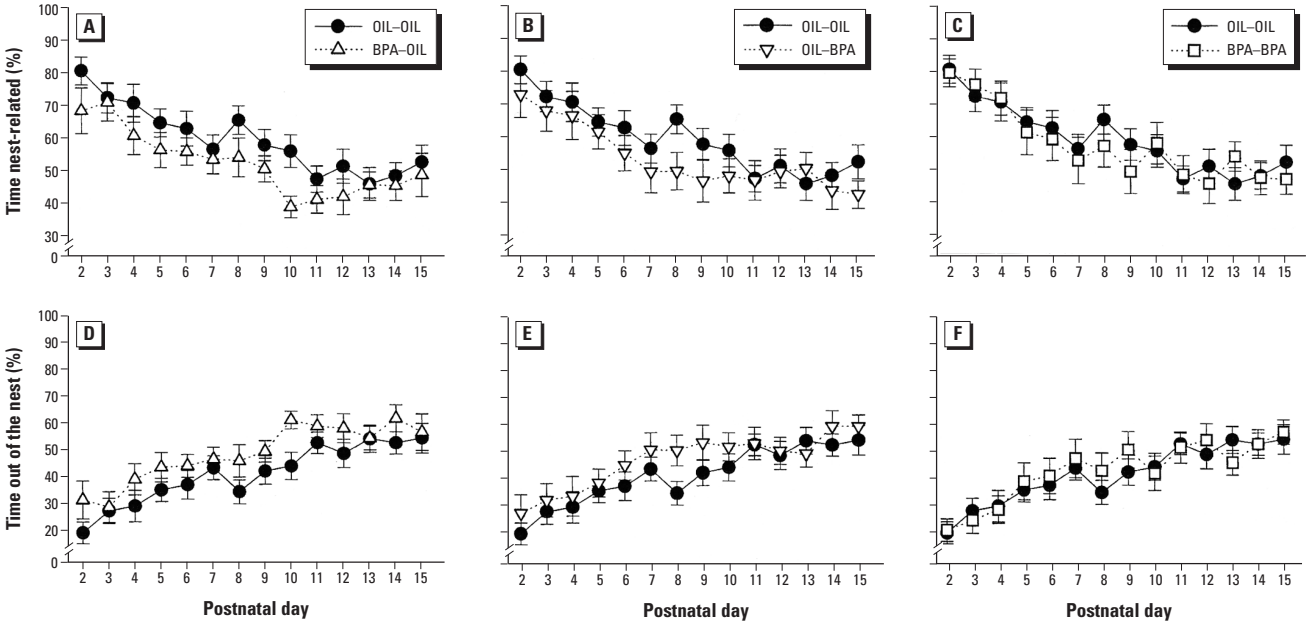

Figure 4. Percentage of time (mean ± SE) spent in nest-related (*A–C*) and out-of-nest behavior (*D–F*) behaviors on PNDs 2–15 for dams exposed to 10 µg/kg/day BPA only *in utero* (BPA–OIL), only during gestation (OIL–BPA), or both *in utero* and during gestation (BPA–BPA).

spring of BPA–OIL dams tended to take longer than offspring of control (OIL–OIL) dams to complete the righting reflex, with the difference occurring on PNDs 3 and 5 (Figure 5D–F).

Discussion

This detailed analysis of maternal behavior has shown a significant alteration in maternal behavior of female mice that were exposed to a low dose of BPA. Females that were exposed to BPA only as fetuses or only as adult dams in late pregnancy exhibited lower levels of nursing behavior toward their offspring and higher amounts of behaviors outside the nest (active, resting, and self-grooming) regardless of PND. An unexpected finding here is the absence of an effect on maternal behavior in females first exposed to BPA during fetal development 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 life stage relative to the response that would occur with no prior exposure to the chemical. There has been speculation that short-term exposure

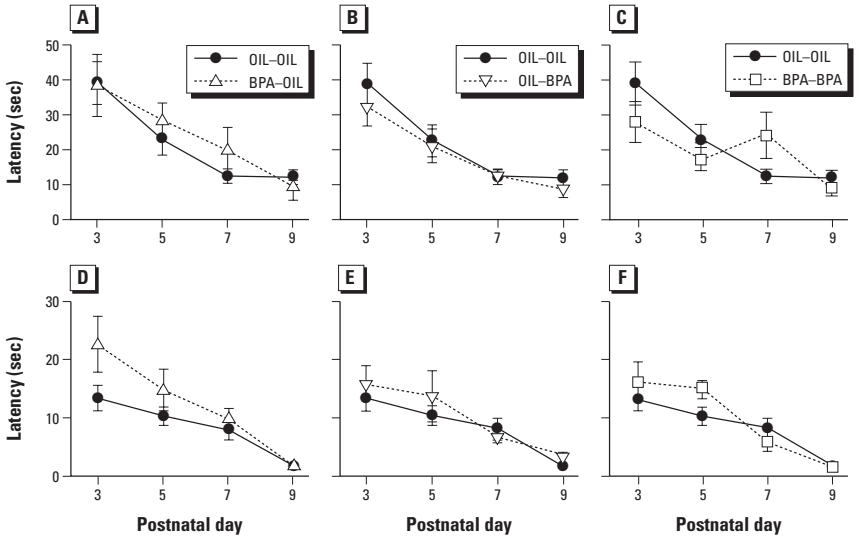

Figure 5. Latency in seconds (mean ± SE) to perform the cliff-drop aversion (*A–C*) and righting (*D–F*) reflexes for offspring of dams exposed to 10 µg/kg/day BPA only *in utero* (BPA–OIL), only during gestation (OIL–BPA), or both *in utero* and during gestation (BPA–BPA).

to chemicals, such as BPA, could lead to different outcomes relative to long-term exposure, such as in a multigenerational study (*23*). Our findings are thus intriguing, but a much broader study involving administration of EDCs at different times in life, which includes physiological measures as well as behavior, will be required to answer this question.

To evaluate the significance of the perturbation in maternal behavior in females exposed to BPA only as fetuses or during pregnancy, we can consider them in relation to the natural changes in maternal behavior that occur during development of the pups. As the pups age, lactating females spend progressively less time in the nest and increase the amount of time self-grooming and resting alone out of the nest. Thus, there is a natural decline in nursing and other maternal behaviors, which leads to weaning in mice as in other mammals (*24*). The time spent by lactating females in the nest and nursing is considered a reliable index of the "motivation" of the dam to nurse her pups (*25*).

The changes throughout the lactational period that we observed in control females are consistent with those in previous studies in mice (*25,26*). Relative to the control profile, females exposed to BPA only as adults during the last 5 days of their pregnancy (OIL–BPA group) showed a lower propensity to nurse and stay in the nest. These OIL–BPA females were also more active than control females and spent more time grooming and resting alone outside the nest. Interestingly, 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 (*11,21*). Dams exposed during late pregnancy to a very low dose of methoxychlor (20 µg/kg/day) spent significantly less time during the dark period of the light cycle in the nest and nursing the pups, and more time out of the nest compared with the control group.

The critical question regarding the decrease in maternal behavior for females in the OIL–BPA group is how BPA and other estrogenic chemicals can alter neuroendocrine systems that mediate the expression of parental care. Two main hypotheses could explain the possible mechanisms underlying the observed alterations in maternal behavior: *a*) a direct effect of BPA on the physiology and the neuroendocrine system of the dam, or *b*) an alteration of pup development and behavior resulting in a different initial stimulation and subsequent maintenance of maternal behavior during lactation. In this study, we monitored the body weight and development of neuromuscular reflexes of the offspring on different PNDs. We assessed two reflexive responses, the righting reflex and cliff-drop aversion, which can provide information concerning physical and motor development as well as sensory function and/or processing. Neither the pups' growth rates nor the two reflexive responses differed in relation to maternal treatment. Thus, it appears that these reflexive behaviors are not altered by developmental exposure to BPA via the mother. However, it is still possible that other changes in pups caused by developmental exposure to BPA (discussed below) could be contributing to the decrease in maternal behavior seen in females treated with BPA only as adults during pregnancy.

An alternative hypothesis concerning the basis of disruption of maternal behavior by BPA could be via a nonspecific toxic effect on the dam's metabolism and milk production. Although this hypothesis may fit with the increased time spent grooming by BPA-treated females, it does not explain their decreased propensity to nurse, because lower milk quality and lower production have been suggested to be compensated by an increase in time spent nursing (*27–29*).

In two different studies of women, Rogan et al. (*30*) and Gladen and Rogan (*31*) reported that a significantly shorter duration of lactation was related to increasing breast milk concentration of DDT. Mothers with higher levels of DDT breast-fed their children for a markedly shorter time, and it is well known that estrogen interferes with lactation. In this regard, it has been reported that pregnant mice accumulate BPA with repeated exposures during late pregnancy (*32*). In the present study, females were fed BPA during the last 5 days of pregnancy; 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.

Although estrogen is important in mice for the initiation of maternal behavior (*12,13*), our findings here, and those reported by Morellini et al. (*21*) and Palanza et al. (*11*) for methoxychlor-exposed mice, show that exposure to man-made estrogenic chemicals during late pregnancy has the effect of reducing subsequent maternal nursing behavior. In more detail, the initiation of maternal behavior after pregnancy is influenced by circulating hormones (estrogens, progesterone, and prolactin) (*13,33*). It has been reported in rats that individual differences in maternal behavior are also related to differences in oxytocin receptor levels in the specific brain regions that regulate maternal responses: the medial preoptic area, the lateral septum, the central nucleus of the amygdala, the paraventricular nucleus of the hypothalamuss and the bed nucleus of the stria terminalis (*34*). Oxytocin receptor levels are, in turn, modulated by differences in estrogen sensitivity in these brain regions (*34*).

Females that were exposed to BPA only during fetal development (BPA–OIL group) showed changes in their maternal behavior similar to those described above for female mice exposed to BPA for the first time during late pregnancy (OIL–BPA group). The BPA–OIL females exhibited lower nursing and nest-related behavior and increased out-of-nest behaviors (particularly, resting alone and self-grooming) relative to control 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 mother–offspring interactions may be a sensitive measure of hormonal perturbation during prior fetal life.

Our findings reported here add to a growing list of adverse effects due to fetal exposure to doses of BPA far below those previously predicted to cause no effect. The current lowest observed adverse effect level for BPA is 50 mg/kg/day, and the acceptable daily intake is set at 50 μg/kg/day. Other low-dose effects of BPA that have been reported in rodents include an accelerated rate of embryonic development (*35,36*), accelerated growth and early puberty in females (*19*), and changes in the mammary gland (*37*), vagina (*38*), prostate (*17,39–42*), sperm production (*18*) [see also Sakaue et al. (*43*)], epididymis (*40*), and pituitary response to estradiol (*44*). In two reports that failed to find any low-dose effects of BPA, there was also a failure to find any effects of the positive control chemical, diethylstilbestrol, which was also examined (*45,46*).

Phenotypic alteration induced by the consumption by pregnant females of food contaminated with an EDC (at doses within the range of human exposure) may be "inherited" by the offspring. We refer here to epigenetic inheritance, which may be related to modifications in gene activity rather than changes in the sequence of bases (*47–49*). Permanent changes in the activity of genes regulating the functions described above, including maternal behavior, that are disrupted by developmental exposure to BPA could thus be related to differential methylation of these genes during critical periods in tissue differentiation. The similar maternal behavior alterations induced by both gestational (OIL–BPA) and *in utero* (BPA–OIL) exposure to BPA might be related to the nongenomic transmission of these maternal behavior patterns across generations. Cross-fostering studies in rats have shown that the offspring inherit the behavior (i.e., higher vs. lower level of maternal nursing and licking/grooming of the pups) from the nursing mother and not the biological mother (*14,50*). In the present study, the *in utero* exposed dams are indeed females whose mothers had been exposed during the last 5 days of pregnancy to the same BPA dose as the gestationally exposed females.

A critical issue concerns the potential consequences of an alteration in maternal behavior for the development of behavioral and neuroendocrine responses of offspring subjected to a different quality of maternal care relative to controls. Although the lack of differences in the growth rates and neurobehavioral development of the pups suggests an adequate level of maternal care across the groups, long-lasting influences of maternal factors in shaping brain development and function in offspring have been demonstrated (*12,49,51*). For example, the behavior of a mother toward her offspring can program behavioral and neuroendocrine responses to stress in adulthood by altering the expression of genes related to these responses (*50*). Early maternal stimulation in the nest produces a dampening of the offspring's emotional reactivity to novelty and stress when they become adults (*16,52,53*); in particular, touch and licking stimulation are correlated with nursing behavior. Furthermore, recent studies indicate that the quality of the infants' nest experiences may well affect their subsequent behavior with their own offspring (*14*).

In the case of females exposed as fetuses to BPA, even though there was not a disruption in their offsprings' growth or in the two reflexes that were studied, the alteration by BPA in maternal behavior could result in more subtle changes in their offspring, even though the offspring were not exposed themselves to an EDC. This type of transgenerational effect can have profound implications for the impact of endocrine disruptors on evolutionary processes. Even slight perturbations, due to exposure to environmental contaminants, may alter the adaptive capability of an organism, and on a larger scale, slight shifts of the population mean for some behavioral responses may potentially have significant effects on population dynamics (*54,55*).

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