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Perinatal Exposure to the Estrogenic Pollutant Bisphenol A Affects Behavior in Male and Female Rats

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FARABOLLINI F., S. PORRINI, AND F. DESSÌ-FULGHERI. *Perinatal exposure to the estrogenic pollutant Bisphenol A affects behavior in male and female rats.* PHARMACOL BIOCHEM BEHAV **64**(4) 687–694, 1999.—Bisphenol A (BPA) is an environmental estrogen with potentially aversive effects on public health. In rats, we studied the effects of perinatal exposure to BPA on nonsocial behaviors partly influenced by gonadal hormones. BPA was administered orally to one group of mother rats at a concentration within the range of human exposure from 10 days before mating until the weaning of the pups. In a second group, BPA was given at a higher dosage during a critical period for brain organization, i.e., from day 14 of gestation until day 6 after birth. The offspring of the treated mothers were tested in the holeboard and the elevated plus-maze at 85 days of age. Various aspects of nonsocial behavior were affected by BPA, differently in males and females, confirming that exposure to a weak environmental estrogen in the period of sexual differentiation of the brain can influence adult behavior. However, contrary to our expectation, a clear masculinization of females was not observed. In general, the factor analysis indicated that in treated males both the motivation to explore and anxiety are reduced, while in females, motor activity and motivation to explore are depressed. Because there were no substantial differences between the two modalities of BPA administration, we suggest that the prolonged treatment with the low dosage compensates for the higher dosage given during a shorter steroid-sensitive period. This may be a cause of concern for public health, given the greater incidence of prolonged exposure of humans to low concentrations released into the environment. © 1999 Elsevier Science Inc.

Bisphenol A Estrogens Environmental estrogens Nonsocial behavior
Brain organization Holeboard Elevated plus-maze Sex differences

THERE is increasing concern about the potential negative impact on public health of a class of environmental chemicals with estrogenic activity: the potential effects of these environmental estrogens include breast cancer (7,37), falling sperms counts (3), and a variety of reproductive abnormalities (5,13,14). Nevertheless, reliable direct evidence of the harmful effects of these substances at the concentrations found in the environment is lacking or controversial. As it was recently stressed (8), much debate is generated by the difficulty of extrapolating in vitro effects to an in vivo situation and by the use of the breast and uterus as models to assess the estrogenic activity of the substances.

Given the well-known ability of estrogens to affect sexual differentiation of the brain during a critical period, i.e., perinatal life (16), it is likely that behavioral systems organized under the influence of gonadal hormones can be affected by precocious exposure to environmental estrogens. Behavior, as the final point of a cascade of events, may reveal subtle effects not easily detectable at each single step of the process.

In addition to reproductive behavior, a variety of behavioral activities in rodents are organized and possibly sexually differentiated under the influence of perinatal gonadal hormones (1,22,23). Social and nonsocial behaviors are masculinized in female rats by neonatal androgenization of females [see (36)]. This could be mediated by the aromatization of testosterone into estradiol.

Bisphenol A (BPA) is a particularly important environmental estrogen. Not only is it widespread in the environment, but it is commonly ingested by humans, being released by polycarbonate plastics, the lining of food cans, and dental sealants (2,19,27). BPA has a weak estrogenic activity in vitro and in vivo (18,31), but recent evidence indicates that this substance is able to interact with the estrogen receptor alpha (ERa) in a unique manner, somewhat different from estradiol (12).

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BPA is able to affect in mice the development of reproductive organs and their function in the male offspring of mothers fed with this chemical during pregnancy (32). The same authors report that exposure during fetal life to other estrogenic chemicals can influence the development of territorial behavior (33).

In the present work, we study the effects of precocious exposure to BPA, at concentrations within the range of human exposure and not patently teratogenic (2,24,27), on forms of nonsocial behavior sensitive to the action of perinatal gonadal hormones (21,35). To this purpose, we administered BPA orally to female rats by two modalities: (a) a low dosage (40 μg/kg) from before mating with stud males until weaning of the pups, to analyze the behavioral effects in offspring of chronic exposure to BPA; and (b) a high dosage (400 μg/kg) from day 14 of gestation until day 6 after delivery, to analyze the effects in the offspring of acute exposure during a period that is critical for brain organization.

METHOD

Subjects

We used 84 Sprague–Dawley rats (42 females and 42 males) that were 85 days old at the time of testing, the offspring of the treated mothers, and born and bred in the Department of Animal Biology, University of Florence. Rats were housed in groups of six per cage (42 × 26 × 15 cm), all of the same sex and treatment, in a room adjacent to the testing rooms, under a reversed light–dark cycle (lights off from 0900 to 1900 h). Food and water were freely available.

During the fetal and neonatal period, the animals were exposed to Bisphenol A, which was administered to their mothers, as subsequently reported.

Experimental procedures followed the regulations of the European Communities Council Directive 86/609/EEC.

Treatment Procedure

Two dosages of Bisphenol A (FLUKA Ltd.), 40 and 400 μg/kg body weight, were administered daily to two groups of mothers during pregnancy and lactation, for periods of different duration. Thirty-one females in reproductive age were randomly allocated to three groups: low dosage ($n = 11$), receiving 40 μg/kg Bisphenol A, administered from day 10 preceding conception until the weaning of the pups; high dosage ($n = 11$), receiving arachis oil from day 10 preceding conception until day 13 of gestation, followed by 400 μg 7 kg Bisphenol A until day 6 after delivery, and then arachis oil again until the weaning of the pups; control ($n = 9$), receiving arachis oil from day 10 preceding conception until the weaning of the pups. The substance, dissolved in arachis oil at the concentration of 5.32 and 53.2 μg/ml, for the low and high dosages, respectively, was administered orally by micropipette, in a variable volume according to body weight. Because the animals were trained to receive the oil, the procedure was not stressful.

The litters were weaned on day 21. For each treatment group, the animals were randomized and housed in mixed-sex groups of three males and three females. The three treatment groups consisted of: low dosage, 30 pups (15 males and 15 females), high dosage, 24 pups (12 males and 12 females), and control, 30 pups (15 females and 15 males). Three weeks before testing, the animals were housed as groups of six per cage, of the same sex and treatment.

Behavioral Testing

Behavioral testing started at day 85 after birth and was completed in 3 days. Animals belonging to the same treatment and sex were tested in separate sessions. Animals of the same cage were tested in sequence. Each subject performed the two tests in sequence, the holeboard (5 min) being followed immediately by the elevated plus-maze (5 min). The tests were carried out during the dark phase under dim red light, combined with a low, indirect white light. All animals were unfamiliar with the two apparatuses. Both apparatuses were thoroughly cleaned at the end of each test. All sessions were recorded with a videocamera (Sony HI8) positioned above each apparatus. We subsequently scored the videotapes with software that allowed analysis of the frequency and duration of the behavioral parameters (Observer software, Noldus Information Technology).

The holeboard was a black Perspex box (63 × 63 × 43 cm) with four holes (3.8 cm diameter) equally spaced on the floor. The floor was divided into 36 squares. Frequency and duration of head dipping, self-grooming, and rearing were recorded, while locomotor activity was recorded as the number of squares crossed. In addition, the percentage of internal crosses [no. internal/total crosses × 100] was calculated. The number of boluses was recorded at the end of the test. The holeboard apparatus provides separate measures of motor activity and exploration (6,10).

The elevated plus-maze was made of black Perspex, and consisted of 2 open arms (50 × 10 cm) and 2 closed arms (of the same size but with 34 cm high walls) extending from a central square platform (10 × 10). The arms were arranged so that those of the same type were opposite each other. The apparatus was elevated 60 cm above the floor. Each animal was placed in the central square facing an open arm. We then recorded standard spatiotemporal measures: the numbers of entries into the open and closed arms (arm entry defined as $^3/_4$ of body into an arm), and the time spent in both types of arms. In addition to these parameters, the time spent in the central square was also recorded. We also calculated the percentage of time spent in each of the three sections of the plus-maze (time/300 s × 100), the number of total entries (open + closed entries), and the percentage of open entries (open entries/total entries × 100).

In addition to these traditional spatiotemporal measures, giving separate measures of anxiety and locomotion (15,20), we also recorded some behavioral parameters that have recently been included in an enlarged version of the elevated plus-maze test in mice (28) and rats (6) to supplement and strengthen the traditional measures. In our experiments, these were confined to the frequency of head dips (protruding the head over the edge of the apparatus) and of stretched-attend posture (stretching the body forward without movement of the paws and then returning it to the original position). The frequency and duration of self-grooming and rearing were also scored.

Statistical Analysis

The holeboard and elevated plus-maze data were tested by two-way ANOVA with the factors sex (males and females) and treatment (low dosage, high dosage, control). One-way ANOVA (treatment) was later applied to males and females. Post hoc analysis was used for comparisons between groups (Fisher LSD test; level of significance required 0.05).

A factor analysis was applied to the data from both tests for all groups of animals; the principal components method

with an orthogonal rotation (varimax) of the factor matrix was employed. To determine the number of factors, we used a combination of two criteria: the criterion of 75% variance rule (11) and the root curve criterion (4). The factor loading of each variable indicates how the variable is correlated with the factor; only factor loadings higher than 0.5 are reported. To avoid redundance, we preliminarily selected the measures to be entered in the analysis, taking into account the matrix sampling adequacy, both totally and for each variable (17).

RESULTS

There were no evident signs of maternal toxicity or fetal malformations.

Tables 1 and 2 report the results of the two-way ANOVA (treatment and sex) of the data from holeboard and elevated plus-maze tests, respectively. The means and SE of the behavioral parameters for both sexes are reported in Table 3 for the holeboard test, and in Table 4 for the elevated plus-maze test.

Holeboard Test

The two-way ANOVA (Table 1) revealed significant effects of the factor treatment: independently of sex, self-grooming (frequency and duration) was increased by maternal treatment with BPA, while head dipping (frequency and duration), number of crosses, and the percent of internal crosses were decreased.

The factor sex significantly affected the percent of internal crosses, with higher levels in males than females, and marginally affected the number of boluses, higher in males than females.

To analyze more specifically the effects in both sexes of the two modalities of maternal treatment with BPA, we tested the male and female data separately with one-way ANOVA (treatment). This was followed by the appropriate post hoc comparisons (Fisher LSD test). The results are reported in Table 3.

In females, the duration of head dipping showed a significant decrease with both dosages ($F = 5.57$, $p < 0.01$), while the frequency of head dipping ($F = 5.16$, $p < 0.01$) was reduced only with the high dosage. In contrast, the number of crosses ($F = 4.71$, $p < 0.01$) was significantly reduced with the low dosage. The effect on the percent of internal crosses was only marginally significant ($F = 2.68$, $p < 0.1$).

In males, there was a significant effect of treatment only on the frequency of head dipping ($F = 3.13$, $p = 0.05$), due to a decrease in the high dosage group. As in the females, the effect on percent of internal crosses was only marginally significant ($F = 2.96$, $p < 0.1$).

Elevated Plus-Maze Test

The two-way ANOVA (Table 2) revealed various significant effects on the spatiotemporal measures and behavioral parameters recorded in the plus-maze test.

Spatiotemporal measures. Both the factor treatment and the interaction treatment × sex had significant effects on the number of open arm entries, which indicates a different effect of maternal BPA treatment in the two sexes. The same was true for the percent of open-arm entries. There was a significant effect of treatment on the number of closed-arm entries and a significant effect of the interaction treatment × sex on the percent of time spent in the closed arms. The effect on the percent of time spent in the central square was significant for the factor treatment and marginally significant for the interaction treatment × sex.

Behavioral parameters. The factor treatment had a significant effect only on the frequencies of self-grooming and stretched-attend posture.

These findings indicate that most of the plus-maze parameters are affected differently in the two sexes by maternal treatment with BPA. To obtain more information about the two modalities of treatment in each sex, we applied one-way ANOVA followed by the appropriate post hoc comparisons (Fisher LSD). In females, treatment had marginally significant effects on the number of closed-arm entries ($F = 3.09$, $p < 0.057$) and the number of total entries ($F = 2.56$, $p < 0.09$), both parameters being decreased by the high dosage treatment (Table 4). The percent of time spent in the central square was significantly decreased ($F = 3.50$, $p < 0.05$) by both dosages. Among the behavioral parameters, only frequency of self-grooming was significantly affected ($F = 3.41$, $p < 0.05$), with an increase in the low-dosage group.

In males, treatment significantly affected the number of open-arm entries ($F = 4.8$, $p < 0.01$), the percent of open-arm entries ($F = 12$, $p < 0.0001$), and the percent of time spent in the open arms ($F = 3.4$, $p < 0.05$); the low-dosage maternal treatment significantly increased all these parameters, while the high dosage treatment increased the percent of open-arm entries. The only behavioral parameter significantly affected

TABLE 1

TWO-WAY ANOVA FOR MEASURES OBTAINED IN THE HOLEBOARD TEST

	Treatment (*df* = 2.76)		Sex (*df* = 1.76)		Treatment × Sex (*df* = 2.76)	
	F	*p*	*F*	*p*	*F*	*p*
Self-grooming (f)	3.12	<0.05	0.02	NS	0.48	NS
Self-grooming (d)	3.17	<0.05	2.51	NS	0.14	NS
Rearing (f)	0.46	NS	0.30	NS	0.63	NS
Rearing (d)	0.57	NS	0.02	NS	0.47	NS
Head dipping (f)	7.69	<0.001	1.95	NS	0.08	NS
Head dipping (d)	4.65	<0.01	1.30	NS	0.07	NS
No crosses	6.58	<0.002	0.87	NS	0.46	NS
% internal/total crosses	5.05	<0.01	5.27	<0.02	0.67	NS
No boluses	0.45	NS	3.27	<0.1	1.33	NS

df: degrees of freedom; NS: not significant; (f): frequency; (d) duration.

TABLE 2

TWO-WAY ANOVA FOR MEASURES OBTAINED IN THE ELEVATED PLUS-MAZE TEST

	Treatment ($df = 2.75$)		Sex ($df = 1.75$)		Treatment × Sex ($df = 2.75$)	
	F	p	F	p	F	p
Open-arm entries	3.07	<0.05	2.02	NS	2.95	<0.05
% time in open arms	2.14	NS	2.00	NS	1.85	NS
Closed-arm entries	3.84	<0.03	0.00	NS	0.44	NS
% time in closed arms	0.72	NS	0.00	NS	3.26	<0.05
% time in center	3.09	<0.05	1.03	NS	2.79	<0.1
Total entries	1.57	NS	0.59	NS	1.73	NS
% open/total entries	5.66	<0.005	1.92	NS	3.53	<0.03
Rearing (f)	2.20	NS	0.34	NS	0.09	NS
Rearing (d)	1.89	NS	1.06	NS	0.07	NS
Self-grooming (f)	4.14	<0.02	0.00	NS	0.72	NS
Self-grooming (d)	0.37	NS	0.09	NS	2.10	NS
Head dips (f)	0.31	NS	0.02	NS	1.88	NS
Stretched-attend posture (f)	6.82	<0.002	3.56	<0.1	1.09	NS

df: degrees of freedom; NS: not significant; (f): frequency; (d) duration.

was the frequency of stretched-attend posture ($F = 8.6$, $p < 0.001$) which was decreased by both dosages (Table 4).

Principal components analysis was applied to the data from both tests for all groups of animals. The measures used for the analysis were preliminarily selected to avoid redundance (see the Methods section). Table 5 shows the loading of each measure on five independent factors, accounting for 80% of the total variance. Only loadings higher than 0.5 were selected.

The measures loading on factor 1 were all from the elevated plus-maze test: number of closed-arm entries, number of total entries, percent of time spent in the central square, and self-grooming, with a negative sign and a poor loading. This factor can be interpreted as general activity in the elevated plus-maze.

The measures loading on factor 2 were also from the elevated plus-maze test: percent of open-arm entries, percent of time spent in the open arms, frequencies of head dips, and stretched-attend posture, the latter with a negative sign. This factor seems to be related to anxiety.

The measures loading on factor 3 were from the holeboard test: head dipping (frequency and duration). This factor represents motivation to explore.

The measures loading on factor 4 were also from the holeboard test: rearing (frequency), number of crosses, and self-grooming (duration), the latter with a negative sign. This factor likely represents the motor activity, both horizontal and vertical, in this test.

The only measure loading (highly) on factor 5 was from the elevated plus-maze test: rearing (frequency).

When the factor analysis was applied separately to males and females (Table 6), differences between the sexes were found in the number and position of factors. Five factors were extracted in females, but only four in males.

In males, factor 1 (high loading of number of open-arm en-

TABLE 3

EFFECTS OF PRECOCIOUS BISPHENOL A EXPOSURE IN FEMALE AND MALE RATS [ACTIVITY IN THE 5-MIN HOLEBOARD TEST (MEANS ± SE)]

	Females			Males		
	Control (14)	Low Dosage (15)	High Dosage (12)	Control (14)	Low Dosage (15)	High Dosage (12)
Self-grooming (f)	5.7 ± 1.0	8.5 ± 1.3	8.1 ± 1.2	6.5 ± 1.1	7.6 ± 0.7	8.5 ± 0.7
Self-grooming (d)	25.8 ± 5.9	36.2 ± 6.7	41.6 ± 7.4	34.4 ± 6.4	41.7 ± 6.7	54.7 ± 8.9
Rearing (f)	20.8 ± 2.2	16.9 ± 2.2	18.0 ± 1.8	17.6 ± 1.9	18.1 ± 1.6	17.2 ± 2.5
Rearing (d)	43.1 ± 4.8	37.3 ± 6.2	35.0 ± 4.1	37.4 ± 5.2	41.7 ± 5.4	34.6 ± 5.9
Head dipping (f)	5.8 ± 0.6	3.9 ± 0.9	2.4 ± 0.6*	6.6 ± 1.3	5.3 ± 0.6	3.2 ± 0.7*
Head dipping (d)	16.7 ± 3.5	9.2 ± 2.2*	4.3 ± 1.3*	18.8 ± 7.1	14.1 ± 2.1	7.8 ± 2.5
No. crosses	156.2 ± 11.0	107.3 ± 12.8*	125.4 ± 10.3	141.0 ± 11.6	111.0 ± 8.4	110.2 ± 15.2
% internal/total crosses	18.9 ± 1.8	19.7 ± 2.1	13.4 ± 2.3	20.9 ± 2.8	27.1 ± 2.8	17.7 ± 2.7
No. boluses	3.9 ± 1.4	4.1 ± 0.8	2.4 ± 0.8	6.0 ± 1.8	3.7 ± 0.6	5.8 ± 0.9

Number of rats in each group in parentheses; (f): frequency; (d) duration.
Post hoc comparisons (Fisher LSD test): *$p < 0.05$ compared to control group of the same sex.

TABLE 4

EFFECTS OF PRECOCIOUS BISPHENOL A EXPOSURE IN FEMALE AND MALE RATS
[ACTIVITY IN THE 5-MIN ELEVATED PLUS-MAZE TEST (MEANS ± SE)]

	Females			Males		
	Control (14)	Low Dosage (15)	High Dosage (11)	Control (14)	Low Dosage (15)	High Dosage (12)
Open-arm entries	1.4 ± 0.4	1.5 ± 0.4	0.8 ± 0.2	0.9 ± 0.3	2.5 ± 0.4*	1.7 ± 0.4
% time in open arms	5.5 ± 1.8	5.7 ± 1.4	5.0 ± 1.1	4.0 ± 1.6	10.3 ± 2.0*	7.7 ± 1.5
Closed-arm entries	4.2 ± 0.7	2.9 ± 0.4	2.5 ± 0.3*	3.7 ± 0.5	3.1 ± 0.4	2.8 ± 0.5
% time in closed arms	79.0 ± 6.0	87.9 ± 2.5	90.5 ± 1.7	89.1 ± 2.2	82.7 ± 3.1	85.9 ± 2.6
% time in center	15.6 ± 4.8	6.4 ± 1.6*	4.5 ± 1.0*	6.9 ± 0.9	7.0 ± 1.6	6.4 ± 1.8
Total entries	5.7 ± 0.9	4.3 ± 0.7	3.3 ± 0.4*	4.6 ± 0.8	5.6 ± 0.7	4.5 ± 0.7
% open/total entries	21.9 ± 5.5	26.1 ± 6.3	23.2 ± 5.2	12.0 ± 4.4	40.3 ± 3.9*	36.7 ± 5.4*
Rearing (f)	14.1 ± 1.9	11.5 ± 1.3	13.2 ± 2.1	13.9 ± 1.5	10.1 ± 0.8	12.5 ± 2.0
Rearing (d)	26.4 ± 4.4	20.9 ± 3.4	21.1 ± 4.3	23.7 ± 4.0	16.3 ± 1.5	19.2 ± 3.1
Self-grooming (f)	5.9 ± 0.5	8.7 ± 1.1*	6.2 ± 0.6	6.6 ± 0.8	7.7 ± 0.7	6.4 ± 0.4
Self-grooming (d)	52.7 ± 8.5	70.4 ± 10.9	84.7 ± 13.1	78.3 ± 9.9	72.7 ± 11.5	64.9 ± 10.2
Head dips (f)	7.0 ± 1.8	4.7 ± 0.9	5.5 ± 0.9	4.5 ± 09	5.9 ± 1.1	7.0 ± 1.1
Stretched-attend posture(f)	4.6 ± 0.7	3.3 ± 0.6	3.8 ± 0.7	4.6 ± 0.6	1.7 ± 0.4*	2.6 ± 0.5*

Number of rats in each group in parentheses; (f): frequency; (d) duration.
Post hoc comparisons (Fisher LSD test): *$p < 0.05$ compared to control group of the same sex.

tries, percent of time spent in the open arms, head dips, and stretched-attend posture) reflects anxiety. Factor 2 (number of total and closed-arm entries, rearing, percent of time spent in the central square) reflects motor activity in the plus-maze; self-grooming loaded negatively on this factor.

Factors 3 and 4 reflect, respectively, exploration and motor activity in the holeboard; self-grooming loaded negatively on factor 4.

In females, factor 1 represents activity in the plus-maze, with additional loading of stretched-attend posture and self-grooming, the latter with a negative sign. Factor 2 represents anxiety and includes head dips. Factors 3 and 4 represent, respectively, motor activity and exploration in the holeboard. Rearing in the elevated plus-maze loaded on a single factor, Factor 5.

DISCUSSION

Our data show that various aspects of nonsocial behavior are affected by perinatal exposure to Bisphenol A. This confirms the hypothesis that exposure to an environmental estrogen in the critical period of sexual differentiation can permanently influence the neural systems involved in the organization of be-

TABLE 5

ORTHOGONAL FACTOR LOADINGS FOR HOLEBOARD AND ELEVATED PLUS-MAZE MEASURES
(ACCOUNTING FOR 80% OF THE TOTAL VARIANCE)

	Factor 1 (30%)	Factor 2 (21%)	Factor 3 (13%)	Factor 4 (9%)	Factor 5 (7%)
PM Closed-arm entries	0.86	–	–	–	–
PM Total entries	0.81	–	–	–	–
PM % time in center	0.75	–	–	–	–
PM Self-grooming (d)	−0.55	–	–	–	–
PM % open/total entries	–	0.89	–	–	–
PM % time in open arms	–	0.83	–	–	–
PM Stretched-attend posture (f)	–	−0.70	–	–	–
PM Head dips (f)	–	0.68	–	–	–
HB Head dipping (d)	–	–	0.96	–	–
HB Head dipping (f)	–	–	0.94	–	–
HB Rearing (f)	–	–	–	0.87	–
HB No. crosses	–	–	–	0.77	–
HB Self-grooming (d)	–	–	–	−0.76	–
PM Rearing (f)	–	–	–	–	0.87

Only factors loading >0.5 are reported; percentage of total variance accounted for by each factor in parentheses; HB: holeboard; PM: elevated plus-maze; (f): frequency; (d): duration.

TABLE 6

ORTHOGONAL FACTOR LOADINGS FOR HOLEBOARD AND ELEVATED PLUS-MAZE MEASURES IN FEMALE AND MALE RATS (ACCOUNTING FOR 81% OF THE TOTAL VARIANCE IN FEMALES AND 75% IN MALES)

	Females					Males			
	Factor 1 (38%)	Factor 2 (17%)	Factor 3 (11%)	Factor 4 (8%)	Factor 5 (7%)	Factor 1 (27%)	Factor 2 (24%)	Factor 3 (15%)	Factor 4 (9%)
PM Closed-arm entries	0.79	–	–	–	–	–	0.91	–	–
PM Total entries	0.67	–	–	–	–	–	0.81	–	–
PM % time in center	0.61	–	–	–	–	–	0.27	–	–
PM Self-grooming (d)	−0.57	–	–	–	–	–	−0.61	–	–
PM % open/total entries	–	0.90	–	–	–	0.84	–	–	–
PM % time in open arms	–	0.93	–	–	–	0.81	–	–	–
PM Stretched-attend posture (f)	0.68	–	–	–	–	−0.80	–	–	–
PM Head dips (f)	–	0.74	–	–	–	0.77	–	–	–
HB Head dipping (d)	–	–	–	0.86	–	–	–	0.96	–
HB Head dipping (f)	–	–	–	0.91	–	–	–	0.95	–
HB Rearing (f)	–	–	0.93	–	–	–	–	–	0.89
HB No. crosses	–	–	0.83	–	–	–	–	–	0.75
HB Self-grooming (d)	–	–	−0.55	–	–	–	–	–	−0.79
PM Rearing (f)	–	–	–	–	0.88	–	0.57	–	–

Only factors loading >0.5 are reported; percentage of total variance accounted for by each factor in parentheses; HB: holeboard; PM: elevated plus-maze; (f): frequency; (d): duration.

havior. In the present experiments, this influence is proved through the use of a simple experimental paradigm, measuring forms of nonsocial behavior (related to anxiety, exploration, and locomotion) known to be sensitive to the actions of perinatal hormones (21,35).

The effects of precocious exposure to BPA were independent of sex for some behaviors and sex dependent for others. However, contrary to our expectation, based on the estrogenic action of BPA and the critical period of administration, a clear masculinization of females was not observed. In the holeboard, locomotion and exploration were on the whole depressed, accompanied by an overall increase of self-grooming (see Table 1): the latter activity has been described as a form of displacement in conflict situations, which thus competes with movement and exploration.

When the sexes were analyzed separately, however, the effects on motor activity and exploration were more evident in females (Table 3).

In the elevated plus-maze test (Table 4), we observed sex-dependent effects of the BPA exposure. In particular, male offspring of treated mothers exhibited more frequent entries into the open arms and more time spent in them, accompanied by a reduction of stretched-attend posture. These modifications are indicative of a reduced level of anxiety. The same parameters remained unchanged in females, although other parameters were affected by the maternal BPA treatment. In particular, there was a reduction in the number of entries into the closed arms and the number of total entries (both indicative of general activity in this test), accompanied by increased levels of self-grooming.

The application of factor analysis to all the data allowed us to identify better the relationship between the two tests. In this respect, the analysis confirmed that the tested variables are of a different nature; thus, the two tests are complementary and provide information about different processes (6,9,20). This was particularly evident for the measures of motor activity in the two tests; their loading on independent factors indicates that the nature of the activity in the two tests is different.

There were sex differences in the structure of the factor analysis applied separately to males and females. In males, four factor were extracted, in the order: anxiety and locomotion in the elevated plus-maze, and exploration and locomotion in the holeboard. In females, five factors were extracted in a different order from that of the males: activity and anxiety in the elevated plus-maze, activity and exploration in the holeboard, and rearing in the plus-maze.

In general, the behavior of females in each test was primarily characterized by activity, whereas in males the strongest behavioral factor was anxiety. This agrees with the results of a basic study of sex differences in the structure of factor analysis applied to the same measures of nonsocial behavior (Wilson et al., this issue). Those authors also describe the prevalence of anxiety in males and of activity in females.

Sex differences were also observed in the distribution among the factors of a behavioral parameter recorded in the plus-maze test, i.e., stretched-attend posture. Stretched-attend posture is regarded as an index of risk assessment in a potentially threatening situation, and in previous factor analysis studies, carried out in males, loaded on the factor anxiety (6,9,28); this has been confirmed in the present study, for males, but in females it loaded positively on the factor motor activity.

Sex differences were also found for rearing, which appeared to be associated in males with other measures of motor activity in each test (factors 2 and 4). This agrees with previous findings (30), and suggests that rearing is a form of vertical motor activity. However, once again, this applies only to males. In females, rearing in the elevated plus-maze test was independent of motor activity. Finally, an inverse association between rearing and self-grooming was found only in males.

A first general conclusion to be drawn is that behaviors (even structurally identical ones) may have different meanings in different contexts, in which it should be underlined, the ani-

mals are artificially confined. In addition, the processes underlying the behaviors may be different in the two sexes. This suggests caution in extrapolating results obtained in males (the case of most studies for the validation of the tests) to females.

The results of the factor analysis allow us to broaden the interpretation of our findings concerning the effects of precocious exposure to BPA on single behaviors.

In females, the parameters of motor activity in both tests were depressed following maternal treatment, as was the motivation to explore. Similarly, in males the motivation to explore was reduced. However, the most relevant finding in this sex was the reduction of anxiety. which was unmodified in the females.

In line with the present findings, it has been shown that the absence of male gonadal hormones in the postnatal period reduces anxiety in males (21) and that testosterone given neonatally to female rats increases anxiety, as measured in the plus-maze test (35). It is known that gonadal hormones in the perinatal period organize the neural mechanisms underlying sex differences in adult behavior. Conversion of androgens to estrogens through aromatization is a key step in this process (29).

The mechanism of action of BPA, which mimics estrogens in many respects (5,31), could be related to its capacity to compete with estradiol for binding with the estrogen receptor alpha (one of the two ER subtypes). However, in vitro and in vivo studies have shown that BPA cannot be regarded merely as a weak estrogen; it has unique ligand properties, which stimulate some responses different from estradiol (12,31).

There are indications that the influence of BPA on the ERa is the basis of the behavioral effects of the substance. In both male and female mice, the integrity of ERa gene expression is necessary for the activation not only of sexual behavior, but also of aggressive and parental behavior (25,26). It has also been suggested that ERa activation during neural development is involved in the regulation of these behaviors.

It cannot be excluded that other mechanisms of action of BPA are the basis of the observed behavioral effects. For example, it has been shown that BPA induces progesterone expression (18), which is important in view of the role played by progesterone in the development of sex differences in the brain and in behavior (34).

Finally, the presence of BPA in the perinatal period could have differential effects in males and females, according to the presence or absence of endogenous aromatizable gonadal hormones.

In the evaluation of such a complex situation, however, it should be borne in mind that the effects of BPA could be due more to an alteration of the neonatal endocrine system than to estrogen mimicry.

In this study, we explored the effects of two modalities of maternal BPA treatment: the first was a prolonged treatment, from before pregnancy until weaning, with a low dosage of the substance; the second was treatment with a high dosage for a shorter period of time, albeit one that is crucial for development and sexual differentiation. It should be emphasized that in both forms of treatment, the offspring received the substance via the placental circulation as well as via the milk. Appropriate experiments will be necessary to explore the contribution of the two forms of transfer from mother to fetus and to newborn.

There were no substantial differences in the effects on the offspring of the two modalities of maternal BPA administration. We suggest that the prolonged treatment with the low dosage compensates for the higher dosage given during the shorter steroid-sensitive period. This may be cause for concern for public health, given the greater incidence of prolonged human exposure to low concentrations released into the environment (8,18).

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