Supporting Information

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SI Text

SI Materials and Methods

Animals and Treatments. BALB/c mice were maintained on a 12:12-h light:dark cycle with ad libitum access to food and water. Before mating, 24 female mice were assigned to each of the four treatment groups, and were orally exposed to bisphenol A (BPA) dissolved in tocopherol-stripped corn oil (2, 20, or 200 μg/kg/d) or only corn oil (vehicle control). This dosing method generated total BPA levels in urine corresponding to a fourfold (2 μg vs. 20 μg) and 14-fold (20 μg vs. 200 μg) difference in BPA levels, suggesting that increasing doses produced increasing internal levels of BPA. Pregnant mice were placed individually in 10.5 × 19 × 6-inch polysulfone cages and were exposed daily to the assigned treatment during the entire gestational period (gestational days 0–19). Offspring used for behavioral testing were housed in same-sex/same-treatment cages ($n = 3$ per cage) at the time of weaning. All procedures were performed with the approval of the Institutional Animal Care and Use Committee at Columbia University.

Maternal Behavior. The procedure for assessing maternal behavior in mice has been previously described (1). Maternal behavior was scored from day 1 through day 6 postpartum during four observation periods lasting 60 min each day. Frequency of maternal behavior was calculated as the total number of observations of the behavior divided by the total number of observations.

Nucleic Acid Isolation. Allprep DNA/RNA mini kit (Qiagen) was used for simultaneous extraction of total RNA and genomic DNA from dissected cortical, hypothalamic, and hippocampal tissue samples.

Quantitative Real-Time PCR. Gene expression was assessed by using reverse transcription (SuperScript III First-Strand Synthesis System; Invitrogen) followed by quantitative real-time PCR with a model 7500 real-time PCR system (Applied Biosystems). By using specific primer sets (Table S4), mRNA levels of the following genes were examined: estrogen receptor (ER) α [estrogen receptor 1 (*Esr1*)] and ERβ [estrogen receptor 2 (*Esr2*)], estrogen-related receptor γ (*Esrrg*), and DNA methyltransferase 1 (*Dnmt1*) and *Dnmt3a*. Relative mRNA expression was calculated using the standard $\Delta\Delta C_T$ method with female control (vehicle) samples as a reference sample and cyclophilin A (*CypA*) as an endogenous reference gene. Analyses of cortical, hypothalamic, and hippocampal tissue samples indicated no BPA-induced effects on levels of *CypA*.

Bisulfite Pyrosequencing. Methylation at specific CpG sites in the *Esr1* gene was analyzed by using a bisulfite-pyrosequencing method. Bisulfite conversion of DNA samples (500 ng) was carried out by using an Epitect Bisulfite Kit (Qiagen). Biotinylated PCR products were obtained by using a PyroMark PCR kit (Qiagen) and primers specific for the *Esr1* gene regions (Table S5). Pyrosequencing was performed on a PyroMark Q24 pyrosequencer using specific pyrosequencing primers (Table S5). Average methylation levels of CpG sites were quantified using PyroMark Q24 2.0.4 software (Qiagen). Representative pyrograms are included in Fig. S3.

Home-Cage Social Behavior. Home-cage behaviors were examined between postnatal day (PND) 30 and PND 40 with 1 h of observation per cage each day (conducted between 2:00 PM and 4:00 PM) and behaviors coded every 3 min within a session. Number of cages observed per treatment ranged from four to six for males and 7 to 11 for females. Cages contained pups from different litters (same treatment, same sex).

Open-Field Test. The open-field apparatus used was a 24 × 24 × 16-inch Plexiglas box. On the day of testing, the mouse was placed directly into one corner of the open field. After a 10-min session, the mouse was returned to its home cage. All testing was conducted under red lighting conditions. Behaviors were video recorded. Behaviors scored by using Ethovision (Noldus) included (*i*) center area exploration (time spent in the inner 12 × 12-inch area) and (*ii*) distance traveled. Numbers of offspring tested per treatment ranged from 8 to 15 for males and 9 to 13 for females.

Social Approach and Aggression. At PND 70, a subject mouse was placed in a 20 × 20 × 16-inch cage with a same-sex stimulus mouse (129Sv) for 15 min. Frequency and duration of sniffing and frequency of aggressive behaviors were coded and social status was classified. Number of offspring tested per treatment ranged from 9 to 15 for males and 9 to 14 for females.

Statistical Analyses. Analyses of gene expression, DNA methylation, and behavioral outcomes were run in Stata 12.1 (2011; StataCorp). Complimentary analyses (conducted with ANOVA; run in SPSS version 19; IBM) is described in *Complementary Data Analysis*. Treating BPA dosage as a continuous predictor on a logarithmic scale (2, 20, 200 μg doses), we used multilevel models to look for evidence of a curvilinear (quadratic) effect of dosage level on offspring gene expression and behavior as well as on maternal behaviors (specifically licking/grooming and arched-back nursing) of BPA-treated dams. Multilevel models have been previously applied across a range of biological fields, including developmental biology (2) and evolutionary biology (3). One of the main strengths of this approach is that multilevel models are a natural extension of regular regression models, but are able to correct for pseudoreplication, that is, the error of treating multiple observations from a single group as independent. This type of error can lead to incorrectly low *P* values and potentially inflated effect sizes. In the present study, our data contained repeated observations within a single litter. Rather than ignore these replications or collapse the data by litter, both of which can lead to estimation errors (4–6), we leveraged the power of multilevel models (also referred to mixed-effects, random-effects, and hierarchical models). These models account for the correlation between repeated observations within a group by fitting what is called a "random intercept"—a typical response level for each group. In the fixed (nonrandom) portion of our model, we examined sex-specific effects by including the interaction terms: sex*linear dose and sex*quadratic dose (in addition to the main effect terms: sex, linear dose, and quadratic dose). We report significant sex*quadratic dose interactions where they existed. If the sex*quadratic dose interaction was not significant ($P > 0.1$), we dropped this term from the model, retaining the main effect quadratic term and the sex*linear dose interaction term. It should be noted that this $P > 0.1$ cutoff was used only to determine whether a term was removed completely from the model. Effects are reported as significant only if they attain the $P < 0.05$ threshold. Any effect that was not significant at the $P < 0.05$ threshold but had a *P* value lower than 0.1 is described as marginal. If there was no

overall quadratic dosage effect, we dropped it from the model, retaining the sex*linear dose interaction. If this interaction was also not significant, we dropped it from the model, retaining the main effects of sex and linear dose. Following this general procedure, we created a final model for each outcome measure and reported the significance of the term with highest priority: sex*-quadratic dose, quadratic dose, sex*linear dose, and linear dose. All gene-expression and behavior models included a random intercept (a multilevel model) for dam to control for multiple observations within the same litter. We used a Poisson error distribution with a log link for all count outcomes (sniffing behavior) and a binomial error distribution with a logit link for all binary outcomes (aggression and dominance). All other outcomes were modeled with a Gaussian error distribution with an identity link. As multilevel models of this kind assume an asymptotic sampling distribution, *z*-statistics were calculated (7). DNA methylation data were also analyzed with a multilevel model to control for repeated observations within litters and are based on *z*-statistics.

To test for a joint effect of dosage and maternal behavior and potential mediation/suppression effects by maternal behavior, for each gene, behavior, and methylation model, we ran an additional model including a composite score of maternal care (arched-back nursing and licking/grooming) and sex*maternal care. If the new interaction term was not significant, we dropped it from the model, retaining only the main effect of maternal behavior.

Complementary Data Analysis

Although the regression models in the manuscript provide evidence for long-term sex-specific and dose-dependent effects of BPA, the analyses described in this section compliment the analytic approach used in the manuscript with a more traditional two-way ANOVA (with sex and treatment as factors), Tukey post hoc analyses of dose effects, and, in some cases, *t* tests to determine group differences. A summary of specific dose effects (compared with vehicle) for gene expression outcomes is provided in Table S1. These analyses, similar to those presented in the main article text, illustrate the sex-specific and dose-dependent effects of BPA (although with more limited ability to describe the overall pattern of BPA effects across doses and within sex).

BPA Effects on ER-Related Gene Expression. We examined the effects of prenatal BPA treatment on the expression of genes encoding ERα (*Esr1*) and ERβ (*Esr2*) as well as estrogen-related receptor γ (*Esrrg*) in the prefrontal cortex, hippocampus, and hypothalamus at PND 28 (Fig. 2 and Fig. S1 *A*–*C*). In the prefrontal cortex, we found a significant main effect of BPA on *Esr1* expression [$F(3,48) = 3.31$; $P < 0.05$; Fig. 2*A*], no effects of BPA or sex on *Esr2* expression (Fig. 2*B*), and a significant BPA-by-sex interaction on *Esrrg* expression [$F(3,48) = 12.96$; $P < 0.001$; Fig. 2*C*]. In the hippocampus, there were no effects of BPA or sex on *Esr1* expression (Fig. S1*A*), whereas there was a significant BPA-by-sex interaction on *Esr2* [$F(3,48) = 5.53$; $P < 0.01$; Fig. S1*B*] and *Esrrg* [$F(3,48) = 2.79$; $P < 0.05$; Fig. S1*C*] expression. In the hypothalamus, there was a significant BPA-by-sex interaction on *Esr1* [$F(3,48) = 8.10$; $P < 0.001$; Fig. 2*D*], *Esr2* [$F(3,48) = 10.52$; $P < 0.001$; Fig. 2*E*], and *Esrrg* [$F(3,48) = 10.87$; $P < 0.001$; Fig. 2*F*]. In summary, our data show that prenatal BPA treatment significantly alters postnatal expression of ER-related genes in a sex-specific, dose-dependent manner that varies by brain region.

BPA Effects on DNMT Gene Expression. To examine whether epigenetic mechanisms may underlie BPA effects on ER-related gene expression, we first examined gene expression of the enzymes that catalyze DNA methylation, DNMT1 and DNMT3A, in all three brain regions at PND 28 (Fig. 3 and Fig. S1 *D* and *E*). In the prefrontal cortex, we found a significant BPA-by-sex interaction on *Dnmt1* [$F(3,48) = 3.43$; $P < 0.05$; Fig. 3*A*] and *Dnmt3a* expression [$F(3,48) = 8.53$; $P < 0.001$; Fig. 3*B*]. No significant effects of BPA on DNMT gene expression were noted in the hippocampus (Fig. S1 *D* and *E*). In the hypothalamus, we found a significant BPA-by-sex interaction on *Dnmt*1 expression [$F(3,48) = 12.70$; $P < 0.001$; Fig. 3*C*] and a trend for this interaction on *Dnmt*3a expression [$F(3,48) = 2.64$; $P = 0.06$; Fig. 3*D*].

BPA Effect on DNA Methylation of ERα (*Esr1*) Gene. To further explore whether epigenetic mechanisms contributed to BPA effects on ER-related gene expression, we examined DNA methylation profiles of the *Esr1* gene in the prefrontal cortex and hypothalamus following 20 μg/kg prenatal BPA treatment. We assessed methylation status of 17 individual CpG sites in two *Esr1* gene regions, untranslated exons A and C (Fig. 4). Prenatal BPA treatment resulted in a significant increase in DNA methylation of exon A in the male cortex [$t(10) = 2.53$; $P < 0.05$; Fig. 4*B*], whereas the methylation levels of this region were decreased in the hypothalamus of BPA-treated females [$t(10) = 4.61$; $P < 0.001$; Fig. 4*E*]. We did not observe any significant changes in the methylation levels of *Esr1* exon A in the female cortex (Fig. 4*C*) and male hypothalamus (Fig. 4*D*). Exon C methylation levels were not found to vary as a function of BPA exposure in males or females (Fig. S4).

BPA Effect on Home-Cage Social Behavior. We examined the effects of prenatal BPA treatment on home-cage behaviors between PND 30 and PND 40. Two-way ANOVA (with sex and treatment as factors) indicated no significant effects of these variables on the frequency of huddling, eating, drinking, hopping, and fighting. However, we found a main effect of BPA on frequency of sniffing [$F(3,56) = 2.80$; $P < 0.05$; Fig. 5*A*] and allogrooming [$F(3,56) = 3.74$; $P < 0.05$; Fig. S5*B*] and a BPA-by-sex interaction on frequency of chasing behavior [$F(3,56) = 5.13$; $P < 0.01$; Fig. 5*B*]. Post hoc analyses indicated increased sniffing (Fig. 5*A*) and allogrooming (Fig. S5*B*) at the 2-μg dose (compared with vehicle) in both sexes ($P < 0.05$), and reduced chasing in males at the 200-μg dose ($P < 0.05$) to a level that mimicked frequency of this behavior in females (Fig. 5*B*).

BPA Effects on Exploratory and Anxiety-Like Behavior. At PND 60, we performed the open-field test to examine whether prenatal BPA treatment altered overall activity (distance traveled) and anxiety-like behavior (time in the center area of the field) in male and female BALB/c mice. We found a significant BPA-by-sex interaction on time spent in the inner area of the open-field apparatus [$F(3,90) = 2.71$; $P < 0.05$; Fig. 5*D*] and total distance traveled during testing [$F(3,90) = 4.30$; $P < 0.01$; Fig. 5*C*]. In females, BPA decreased time spent in the inner area of the open field at the 200-μg dose ($P < 0.05$), with no significant effect in males. In males, prenatal BPA treatment at all three doses was associated with hyperactive phenotype indicated by increased distance traveled ($P < 0.01$). No effect of BPA was observed on this measure in female offspring.

BPA Effects on Social Approach and Aggression. We further examined social behavior at PND 70 by placing a subject mouse in a novel environment with a same-sex stimulus mouse. The following behaviors were coded: frequency and duration of sniffing stimulus mouse and frequency of aggressive behaviors toward stimulus mouse (tail rattling, biting, mounting). Each subject mouse was classified as dominant (aggressor), subordinate, or neutral in social status. We found a significant BPA-by-sex interaction on frequency of sniffing [$F(3,92) = 2.84$; $P < 0.05$; Fig. 5*E*] and a main effect of BPA on frequency of aggressive behaviors [$F(3,92) = 2.76$; $P < 0.05$; Fig. 5*F*]. There were no effects of sex or BPA on duration of sniffing. In males, there was increased frequency of sniffing consequent to prenatal treatment with 20 μg BPA ($P < 0.05$), with no significant effects observed in females (Fig. 5*E*). Prenatal BPA treatment significantly decreased aggression at the 2-μg dose and increased aggression at 200 μg in

both sexes (Fig. 5*F*). BPA also affected social dominance status in a dose-dependent way. In males, there was an increase in dominant status with increasing BPA dose, whereas, in females, dominance was decreased at 2-μg and 20-μg doses and increased at the 200-μg dose (Fig. S6).

BPA Effects on Maternal Behavior. BPA treatment was associated with a significant effect on licking/grooming [$F(3,48) = 3.13$; $P < 0.05$; Fig. 6*A*], and arched-back nursing [$F(3,48) = 2.90$; $P < 0.05$; Fig. 6*B*], and a trend for an effect on self-grooming behavior [$F(3,48) = 2.46$; $P = 0.08$; Table S2]. Compared with vehicle-treated dams, females that received 2 μg/kg BPA throughout pregnancy exhibited reduced maternal care evident as significantly lower frequency of licking/grooming ($P < 0.05$) and arched-back nursing ($P < 0.05$). At the 20-μg/kg BPA dose, there was also a trend toward decreased licking/grooming behavior ($P = 0.09$). Interestingly, the highest BPA dose did not significantly alter maternal behavior. BPA did not have a significant effect on overall frequency of nursing, nest building, eating, and drinking during the postpartum period (Table S2).

1. Champagne FA, Curley JP, Keverne EB, Bateson PP (2007) Natural variations in postpartum maternal care in inbred and outbred mice. *Physiol Behav* 91(2-3):325–334.
2. Yeatman JD, Dougherty RF, Ben-Shachar M, Wandell BA (2012) Development of white matter and reading skills. *Proc Natl Acad Sci USA* 109(44):E3045–E3053.
3. Johnson PTJ, Hoverman JT (2012) Parasite diversity and coinfection determine pathogen infection success and host fitness. *Proc Natl Acad Sci USA* 109(23):9006–9011.
4. Lazic SE (2010) The problem of pseudoreplication in neuroscientific studies: Is it affecting your analysis? *BMC Neurosci* 11:11.
5. Gelman A, Hill J (2006) *Data Analysis Using Regression and Multilevel/Hierarchical Models* (Cambridge Univ Press, Cambridge).
6. Rouder JN, Lu J, Speckman P, Sun D, Jiang Y (2005) A hierarchical model for estimating response time distributions. *Psychon Bull Rev* 12(2):195–223.
7. Rabe-Hesketh S, Skrondal A (2008) *Multilevel and Longitudinal Modeling Using Stata* (Stata Press, College Station, TX), 2nd Ed.

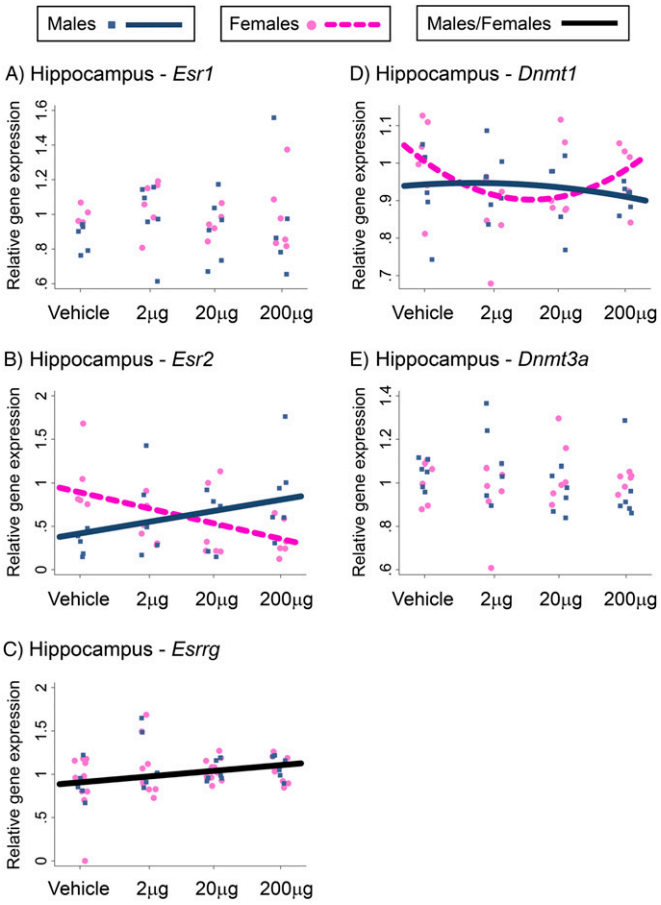
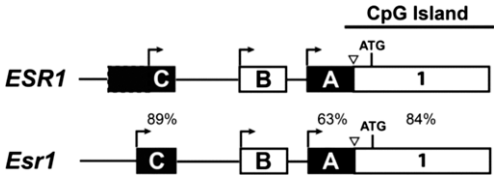


Fig. S1. Prenatal BPA treatment effects on hippocampal gene expression. *Esr1* (*A*), *Esr2* (*B*), *Esrrg* (*C*), *Dnmt1* (*D*), and *Dnmt3a* (*E*) gene expression was analyzed in the PND 28 hippocampus. Graphs include individual data points and the best-fit (linear or curvilinear) model for these data [single black line, significant non–sex-specific effect of BPA; blue (male) and pink (female) line, significant sex-specific effect of BPA] generated by multilevel regression analyses (Stata version 12.1).



Exon C The whole sequence below**: chr10:5,636,197-5,636,496** [reverse strand - UCSC Genome Browser - Mouse July 2007 (NCBI37/mm9) Assembly]:

TTCATCCAGCTCGTGCAGGAGCCCAGCTGCCTTGTCTGCCGGGAGGGGCTGCCAAGTGCCCTGC
CTACTGGCTGCTTCCCGAGAGTCCCTGCCACTCCACATACAAACACATCCACACA**CG**$^{(C1)}$CTCT
GCCTTGATCACACAC**CG**$^{(C2)}$**CG**$^{(C3)}$CCACT**CG**$^{(C4)}$ATCATT**CG**$^{(C5)}$AGCACATTCCTTCCTTC**C**
G$^{(C6)}$TCTTACTGTCTCAGCCCTTGACTTCTACAAACCCATGGAACATTTCTGGAAAGACGCTCTT
GAACCAGCAGGGTAGGATCGGCTTTTGATTTCTCTCTCTCTTTCTCTCTCTGTAGCTTGAG

The ***Exon C*** sequence based on Kos et al. (2001) is marked in the gray box (EMBL-EBI data base – AJ276597). We sequenced the same region in the DNA samples obtained from Balb/C mice used in our experiments. Our sequence completely matched the sequence in the EMBL-EBI data base as well as the sequence provided in the UCSC Genome Browser. Red font – position of the PCR primers; Underlined – position of the pyrosequencing primer; Bold and enlarged font – analyzed CpG sites (C1-C6).

Note: The primers for this region were designed based on the bisulfite-converted sequence of the complementary (+) DNA strand. In addition, although C1 site is in the region adjacent to the Exon C, in this paper we refer to it as if it were in the Exon C to be consistent with Westberry et al. (2010).

Exon A The whole sequence below**: chr10:5,634,091-5,634,387** [reverse strand - UCSC Genome Browser - Mouse July 2007 (NCBI37/mm9) Assembly]:

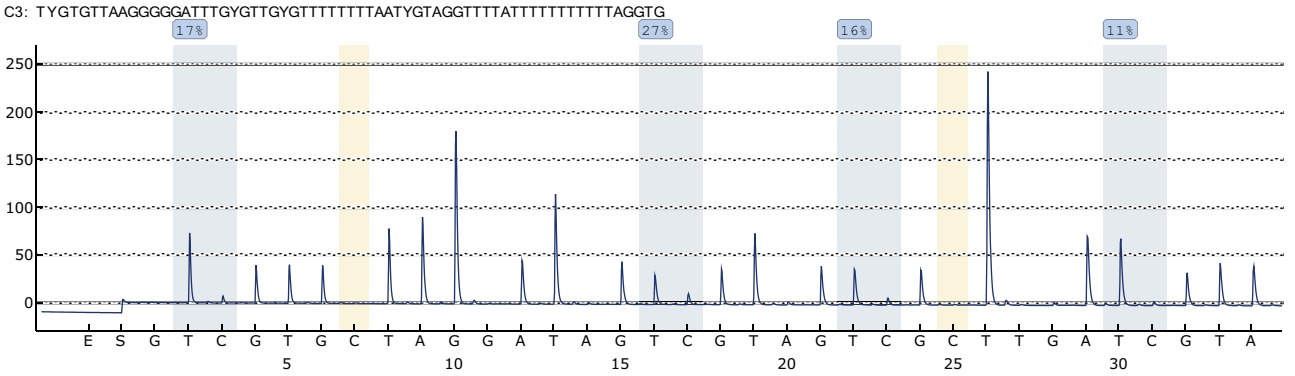
ACCCGGGAGCGTCTGGGTGCGCTCCTTGGAGTTGGGTCACCTGTGTTCTGCAGGATAGCTCTGC
CCCGCAGGGGCAGAGGCAGGGCCAGGGCCAGTACT**CG**$^{(A1)}$TGCCAAGGGGGACTTG**CG**$^{(A2)}$CTG**C**
G$^{(A3)}$CCTTCTCTAAT**CG**$^{(A4)}$CAGGCTCTACTCTTTTTTCCAGGTGGCCCA**CG**$^{(A5)}$**CG**$^{(A6)}$CTGCTG
AGCCCTCTG**CG**$^{(A7)}$TG**CG**$^{(A8)}$**CG**$^{(A9)}$GGGAGCCAGTCTGTAACT**CG**$^{(A10)}$C**CG**$^{(A11)}$GCTGCCACTT
ACCATGACCATGACCCTTCACACCAAAGCCTCGGGAATGGCCTTGCTGCACCAGATCCAAGGGA
ACGAGC

The ***Exon A*** sequence based on Kos et al. (2001) is marked in the gray box (EMBL-EBI data base – AJ276597). The sequence was corrected after sequencing the same region in the DNA samples obtained from Balb/C mice used in our experiments. Although it was slightly different from the *Esr1* sequence in the EMBL-EBI data base, our sequence completely matched the sequence provided in the UCSC Genome Browser. Red font – position of the PCR primers; Underlined – position of the pyrosequencing primers; Bold and enlarged font – analyzed CpG sites (A1-A11); Green box (ATG) – translational start site

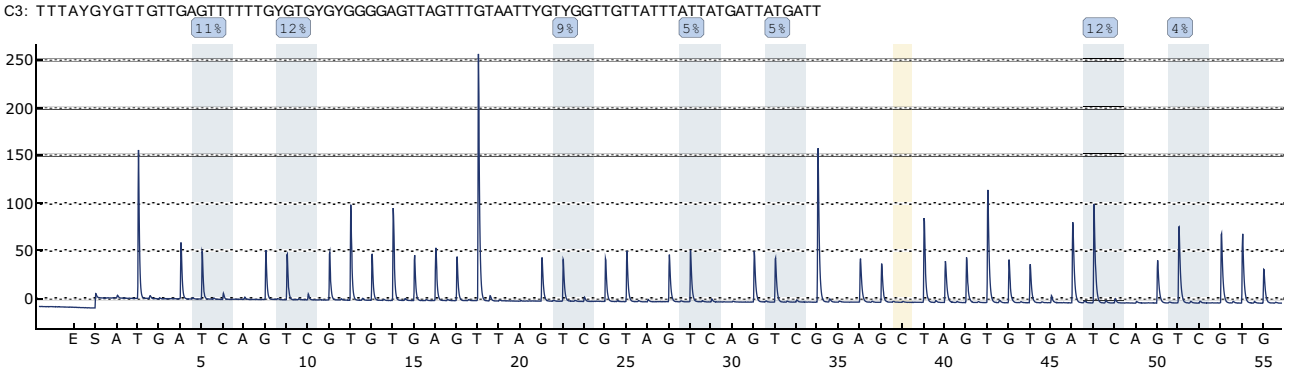
Note: although sites A5-A11 are located in the region adjacent to the Exon A, in this paper we refer to them as if they were in the Exon A to be consistent with Westberry et al. (2010).

Fig. S2. Information about the *Esr1* exon C and A sequences.

ExonA-1 (A1-A4)



ExonA-2 (A5-A11)



Exon C (C1-C6)

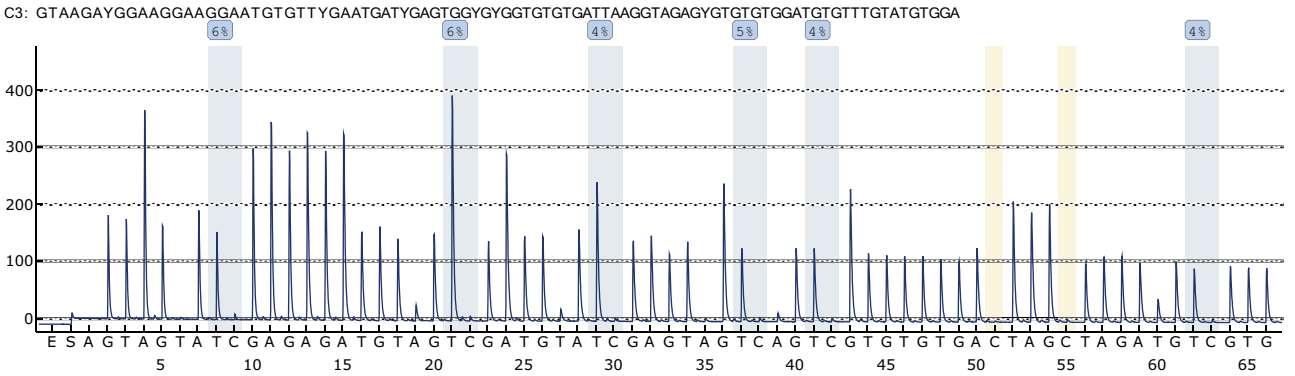


Fig. S3. Representative pyrograms for one of the samples (control female, hypothalamus). Dispensations corresponding to the potentially methylated cytosine (C or T after bisulfite treatment) are highlighted in blue. The percentage of methylation at individual CpG positions is shown above the respective positions. All CpG sites show the blue quality score ("passed") i.e., a perfect correlation between expected and observed pyrograms. Highlighted in red are controls for bisulfate conversion (no signal indicates successful/complete bisulfate conversion).

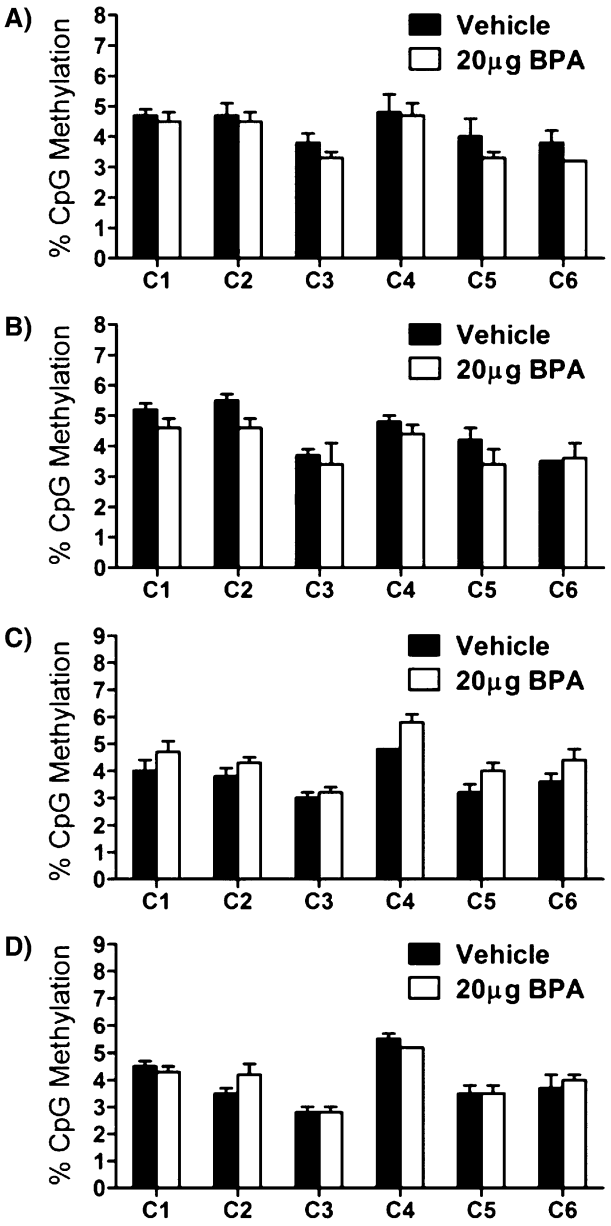


Fig. S4. Effect of gestational BPA exposure on DNA methylation of *Esr1* exon C at PND 28. By using bisulfite-pyrosequencing method, methylation of six CpG sites in exon C (Fig. S2 provides detailed information about the sequence) was examined in the male (*A*) and female (*B*) prefrontal cortex as well as in the male (*C*) and female (*D*) hypothalamus at PND 28 following in utero exposure to vehicle or 20 μg/kg BPA.

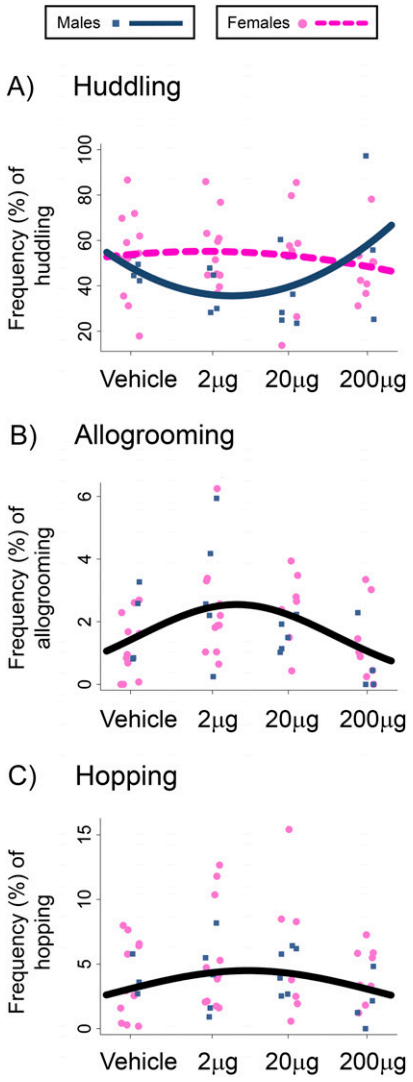


Fig. S5. The effect of prenatal BPA treatment on home-cage social behavior. Following prenatal BPA treatments with vehicle or 2, 20, or 200 μg/kg BPA, male and female offspring (n = 3 per cage) were observed to determine frequency of home-cage behaviors from PND 30 to 40, including (*A*) huddling, (*B*) allogrooming, and (*C*) hopping. BPA dosage was treated as a continuous predictor on a logarithmic scale (x axis). Graphs indicate individual data points and the best-fit model for the data. A single black line indicates significant non-sex–specific effect of BPA; blue (male) and pink (female) lines indicate significant sex-specific effect of BPA.

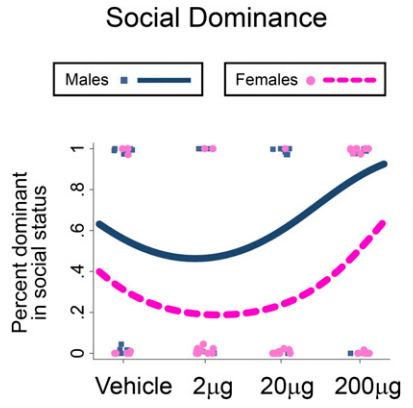


Fig. S6. The effect of prenatal BPA treatment on social dominance. Following prenatal BPA treatments with vehicle or 2, 20, or 200 μg/kg BPA, male and female offspring were observed during dyadic encounters with a stimulus mouse to determine whether offspring were dominant or subordinate in social status. BPA dosage was treated as a continuous predictor on a logarithmic scale (x axis). Graphs indicate individual data points and the best-fit model for the data. Blue (male) and pink (female) lines, significant sex-specific effect of BPA.

Table S1. Dose comparisons in the effect of BPA on gene expression

	Sex specificity		Dose		
Gene/brain region	Significance	Sex	2 μg	20 μg	200 μg
Esr1					
Prefrontal cortex	NS	M, F	NS	↓	NS
Hippocampus	NS				
Hypothalamus	$P < 0.001$	M	NS	↑	↓
		F	↓	↓	NS
Esr2					
Prefrontal cortex	NS				
Hippocampus	$P < 0.01$	M	NS	NS	↑
		F	↓	↓	↓
Hypothalamus	$P < 0.001$	M	↑	NS	↓
		F	↓	↓	↓
Esrrg					
Prefrontal cortex	$P < 0.001$	M	↑	↑	↓
		F	↓	↓	NS
Hippocampus	$P < 0.05$	M	↑	NS	NS
		F	↓	NS	NS
Hypothalamus	$P < 0.001$	M	↑	↑	↓
		F	NS	NS	NS
Dnmt1					
Prefrontal cortex	$P < 0.05$	M	NS	NS	↓
		F	↓	↓	↓
Hippocampus	NS				
Hypothalamus	—	M	↑	NS	↓
		F	↓	↓	NS
Dnmt3a					
Prefrontal cortex	$P < 0.001$	M	↑	↑	NS
		F	↓	↓	NS
Hippocampus	NS				
Hypothalamus	$P = 0.06$	M	↑	NS	↓
		F	NS	NS	NS

Arrows indicate a significant effect of $P < 0.05$ in the indicated direction vs. vehicle. NS, not significant.

Table S2. Frequency of postpartum maternal behavior in BPA-treated dams

Treatment	Nursing	Licking/grooming	Arched-back nursing	Nest building	Self grooming	Eating	Drinking
Vehicle ($n = 13$)	43.54 ± 2.06	4.62 ± 0.55	7.96 ± 1.32	4.18 ± 0.52	4.89 ± 0.42	17.12 ± 1.25	1.08 ± 0.16
2 μg BPA ($n = 14$)	38.75 ± 2.48	3.00 ± 0.45	4.12 ± 0.72	3.24 ± 0.47	3.68 ± 0.30	17.20 ± 1.56	1.68 ± 0.20
20 μg BPA ($n = 12$)	41.78 ± 2.39	3.37 ± 0.40	7.05 ± 1.15	4.15 ± 0.83	5.19 ± 0.50	18.71 ± 1.14	0.95 ± 0.20
200 μg BPA ($n = 10$)	41.01 ± 3.37	4.93 ± 0.73	9.21 ± 2.05	4.13 ± 0.82	4.57 ± 0.54	20.84 ± 2.32	1.14 ± 0.26

Values represent mean frequency of behavior ± SEM.

Table S3. Maternal care effects on gene expression

Gene/brain region	Sex specificity	Effect of elevated maternal care	Statistics
Esr1			
Prefrontal cortex	$P < 0.05$	Male, ↓ mRNA; female, ↑ mRNA	Interaction, $z = 2.09$, $P < 0.05$
Hippocampus	NS	↑ mRNA	$z = 2.19$, $P < 0.05$
Hypothalamus	NS	↑ mRNA	$z = 1.71$, $P < 0.09$
Esr2			
Prefrontal cortex	NS	NS	
Hippocampus	$P < 0.05$	Male, NS; female, ↑ mRNA	Interaction, $z = 2.15$, $P < 0.05$
Hypothalamus	NS	↑ mRNA	$z = 2.71$, $P < 0.01$
Esrrg			
Prefrontal cortex	$P < 0.05$	Male, NS; female, ↑ mRNA	Interaction, $z = 2.12$, $P < 0.05$
Hippocampus	NS	NS	
Hypothalamus	NS	↑ mRNA	$z = 2.97$, $P < 0.01$
Dnmt1			
Prefrontal cortex	NS	NS	
Hippocampus	NS	NS	
Hypothalamus	NS	↑ mRNA	$z = 2.74$, $P < 0.01$
Dnmt3a			
Prefrontal cortex	NS	↑ mRNA	$z = 2.97$, $P < 0.01$
Hippocampus	NS	NS	
Hypothalamus	NS	↑ mRNA	$z = 3.31$, $P < 0.001$

NS, not significant.

Table S4. Primers used for gene expression analysis

Gene	Forward primer	Reverse primer
Esr1	CGTGTGCAATGACTATGCCTCT	TGGTGCATTGGTTTGTAGCTGG
Esr2	GTCAGGCACATCAGTAACAAGGG	ATTCAGCATCTCCAGCAGCAGGTC
Esrrg	CCAAGAGACTGTGCTTAGTGTG	TCTCACATTCATTCGTGGCTGG
Dnmt1	GCCATGTGAACAGGAAGATGAC	GTCCAAGTGAGTTTCCGGTCTT
Dnmt3a	TCTTGAGTCTAACCCCGTGATG	CCTCACTTTGCTGAACTTGGCT
CypA	GAGCTGTTTGCAGACAAAGTTC	GAGCTGTTTGCAGACAAAGTTC

Total RNA samples were treated with DNase I and all primers were designed to cross an intron/exon boundary or to span a long intron to exclude any possibility of genomic DNA contamination.

Table S5. Primers used for DNA methylation analyses of Esr1 (PCR and pyrosequencing)

Esr1 region	Primer
Exon A	
PCR primer: forward	TGGGTTATTTGTGTTTTGTAGGATAG
PCR primer: reverse–biotin	/5Biosg/CTTAAATCTAATACAACAAAACCATTC
Pyrosequencing primer F1 (A1–A4)	GGTAGGGTTAGGGTTAGTAT
Pyrosequencing primer F2 (A5–A11)	AGGTTTTATTTTTTTTTTTAGGTGG
Exon C	
PCR primer: forward	TATGGGTTTGTAGAAGTTAAGGGTTGAG
PCR primer: reverse–biotin	/5Biosg/CCAAATACCCTACCTACTAACTACTTCC
Pyrosequencing primer F1	GAAGTTAAGGGTTGAGATA