Supporting Information

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SI Materials and Methods

Spatial Learning and Memory. As our preliminary studies revealed that deer mice are incredible jumpers, a modified version of the Barnes maze was constructed to prevent them from escaping the maze. The maze (95-cm diameter), with 12 escape holes (5-cm diameter) placed every 30°, was surrounded with a 50-cm-high barrier of flexible aluminum to prevent the animals jumping out of the maze (Fig. S1). A small ramp was attached to the correct exit hole that led to the home cage of the animal. A black cloth curtain surrounded the apparatus to prevent the animals from using visual cues situated outside of the maze. To aid the orientation of the animal within the maze, four visual cues (triangle, square, circle, vertical bar) were placed every 90° inside the maze wall. At the beginning of each training day, the maze was rotated 90° to eliminate odor cues for consecutively tested mice, but the location of the exit hole and visual cues remained constant for any individual mouse across all trials. Each trial was recorded by using EthoVision XT video camera (Noldus), and latency to enter exit hole and distance traveled tracked by using accompanying automated tracking EthoVision XT software. For each trial, errors and spatial strategy were scored from a tracking image composited by EthoVision XT (Fig. 2*A*, main text). The random search strategy (coded 1) was operationally defined as localized searches of holes separated by maze center crosses (Fig. 2*A*, main text). Serial strategy (coded 2) was defined as a systematic search of consecutive holes in a clockwise or counterclockwise direction. Finally, spatial strategy (coded 3) was defined as navigating directly to exit hole without crossing the center of the maze more than once and with three or fewer errors (Fig. 2*A*, main text).

Exploratory and Anxiety-Like Behavior. The elevated-plus maze (EPM) was constructed of black polypropylene in a plus configuration with two opposite open arms (30 cm), a middle platform (5 × 5 cm), and two opposing closed arms (30 cm). The maze was supported 100 cm above the floor by a stand constructed of polypropylene. Each mouse was placed on the center of the platform and allowed to explore the maze for 5 min. After each test, the apparatus was cleaned with 70% EtOH. Each trial was recorded with EthoVision XT, which automatically scores total time spent in open, center, closed arms, number of closed and open arm entries and center entries, as well as proportion of total time spent in open arms [open/(open + closed)]. Arm entry was defined as both front paws and shoulders placed into the area. On the occasion a mouse jumped off the maze, it was gently placed back on the center, and the trial was continued.

Mate Choice Experiment. Females were hormonally treated with an s.c. injection of 5 μg of estradiol benzoate at 2300 hours, 48 h before behavioral testing. In contrast to *Rattus norvegicus* and *Mus musculus*, pilot tests indicated that progesterone injection suppressed rather than potentiated lordosis (1). Females were habituated to the testing apparatus for 2 consecutive days (Fig. S2). Each female was tested twice with a stimulus bisphenol A (BPA)-exposed and control male, and the placement of order of males on one side or the other of the arena was counterbalanced to control for any potential side preference. Before each trial, females were placed in the cage of a sexually experienced but otherwise behaviorally naïve male to confirm lordosis, and females that exhibited this response were used in the behavioral testing. Two females failed to exhibit lordosis and were excluded. A total of 9 control and 9 BPA-exposed females and 10 control and 10 BPA-exposed males were tested.

The mate preference test arena was constructed from glass and measured 122 × 46 × 54 cm, and the apparatus was evenly divided into three chambers measuring 40.6 × 15.3 × 18 cm (Fig. S2). Two removable walls divided the chambers. At the beginning of each trial, BPA and control males were placed on opposing sides (location of males was counterbalanced across trials) of the testing arena in small wire-bar cups (Galaxy Cup, Spectrum Diversified Designs) that allowed 360° exchange of olfactory cues with the experimental female. In addition, the space between the wire-bars allowed animals to exchange physical contact. The experimental female was placed in the center chamber; after a 2-min habituation period the dividers were lifted, and behavioral recording began after the observer left the room. All trials lasted 10 min and were conducted 1 h after onset of the dark phase of the light cycle, beginning at 2300 hours, in a room illuminated with a low-wattage, 12-W red light so as not to disrupt the light/dark cycle of the animals. At the end of each trial all animals were removed, and the testing arena was wiped down with 70% ethanol to eliminate any scent marks.

The videotaped trials were scored by using JWatcher v1.0 (www.ucla.edu/JWatcher) and two observers blind to the treatment of the mice. Time spent in stimulus animal chamber was measured once all four paws crossed the boundary of the center chamber and the stimulus animal's chamber, and as soon as one paw crossed this boundary, time spent in the stimulus animal's chamber was stopped. Two preferential behaviors were defined and recorded: duration of nose-to-nose facial inspection (Inspection time) and duration of contact with the stimulus male other than nose-to-nose inspection, such as sniffing around the wire-bar cup, engaging in ano-nasal sniffing, and nose pokes and physical exchange through the wire-bar cup (Contact time). Other nonpreferential behaviors were also scored: time spent grooming (Grooming), time spent standing on hind legs (Standing), duration of immobility (Still), and time spent in the center chamber (Center). Interrater agreement was high across all categories, with an average interrater reliability > 0.8 (κ α).

Serum Testosterone and Corticosterone Concentrations. Twenty-four hours after mate preference tests, selected male mice [20 control, 18 ethinyl estradiol (EE), and 19 BPA] were deeply anesthetized with 0.015 mL/g (≈0.4–0.5 mL per mouse) tribromoethanol (Avertin), followed by cardiac serum collection, and killed by rapid decapitation. Serum concentrations of testosterone and corticosterone were measured, in duplicate, by routine methods using a solid phase [^{125}I]-RIA as per the manufacturer's instructions (TKTT2, Coat-a-Count, Siemens Healthcare Diagnostics, and ICN Biomedicals for testosterone and corticosterone, respectively).

Statistical Analyses. For EPM, frequency of open arm entries was a covariate in analyses of total and proportional time in open arms, and time immobile was a covariate for time in open and closed arms and proportion of time in open arms. An α of 0.007 (0.05/7) was adopted for sex, diet, and interaction effects to control for multiple analyses. Barnes maze strategies and errors were also analyzed with a 2 (sex) × 3 (diet) × 7 (day) repeated-measures ANCOVA; number of trials and proportion of time in open arms on EPM were covariates. Two additional analyses were conducted to analyze the latency of each animal in the Barnes maze. The first analysis was to confirm that our main finding of disrupted spatial behavior in males was not due to total distance traveled in the maze; specifically, total distance traveled was used

as a covariate, along with trial, in a 3 (diet) × 7 (day) analysis of covariance (ANCOVA) (only male data were analyzed). The second analysis was to control for exposure to an owl screech (below) during testing; specifically, the 2 × 3 × 7 ANCOVA was conducted by using only latencies >30 s (onset of screech), with trial and time spent immobile (measure of freezing when anxious) from EPM as covariates. Least-squares means *t* tests were used to decompose significant effects, with Bonferroni corrections for number of comparisons ($\alpha = 0.05/3 = 0.0167$ for within-sex contrasts across diets; $\alpha = 0.05/9 = 0.0056$ for between-sex contrasts across diets).

SI Results

Spatial Learning and Memory. Fig. 1 *A* and *C* (main text) illustrate the significant interactions among sex, diet, and latency to find the exit hole ($F_{12,1116} = 5.79$, $P = 0.0001$). Fig. 1 *B* and *D* (main text) demonstrate the frequency of entering the wrong exit hole for males (Fig. 1*B*) and females (Fig. 1*D*). These errors decreased across days ($F_{6,624} = 7.27$, $P = 0.0001$), differed across diets ($F_{2,104} = 7.38$, $P = 0.001$), and diet and sex interacted ($F_{2,104} = 38.58$, $P = 0.0001$). The interactions involving day and diet, day and sex, and the three-way were not significant (all $P < 0.0627$). Control males and females exposed to EE through maternal diet did not differ in number of committed errors ($t = -1.36$, $P = 0.1767$), but both groups committed fewer errors than males exposed to BPA ($t = -7.18$, $P = 0.0001$; $t = -4.81$, $P = 0.0001$, respectively) and EE ($t = -8.21$, $P = 0.0001$; $t = -4.42$, $P = 0.0001$, respectively) and fewer errors than control females ($t = -7.11$, $P = 0.0001$; $t = -4.51$, $P = 0.0001$, respectively) and BPA-exposed females ($t = -5.02$, $P = 0.0001$; $t = -3.11$, $P = 0.0024$, respectively).

For males, distance traveled did not differ across days ($F_{6,588} = 0.37$, $P = 0.8962$) but did differ across diets ($F_{2,98} = 25.99$, $P = 0.0001$), and the effect of diet interacted with day ($F_{12,588} = 2.09$, $P = 0.016$). Comparison of pairwise least-squares means, covarying distance, confirmed that control males had shorter latencies than males exposed to EE or BPA for trial days 2–7, inclusive (all $P < 0.0096$). Latencies for the two latter groups did not differ on any day (all $P > 0.1746$) except day 4, for which males exposed to EE had shorter latencies than males exposed to BPA ($P < 0.0056$).

Because BPA-exposed males exhibited higher anxiety-like behaviors in the EPM, the recording of the barn owl screech may have contributed to diet-related differences in Barnes maze latencies (e.g., the screech might have resulted in the animal becoming immobile). As outlined in *Materials and Methods* (main text), the barn owl recording was played if the animal failed to enter the exit hole within 30 s. For Barnes latencies >30 s, the ANCOVA confirmed the significant diet ($F_{2,61} = 5.73$, $P = 0.005$), sex × diet ($F_{2,61} = 12.91$, $P = 0.0001$), day × diet ($F_{12,366} = 3.07$, $P = 0.0004$), and day × sex × diet ($F_{12,366} = 3.34$, $P = 0.003$) interactions. As reported in *Results* (main text), a stable pattern emerged by day 3 of acquisition training. Control males had shorter latencies than males exposed to EE or BPA on days 3–7, inclusive (all $P < 0.0209$), and the latencies for the two latter groups did not differ on any of these days (all $P > 0.4036$). Control males also had shorter latencies than control females (all $P < 0.0008$) and females exposed to BPA (all $P < 0.0233$) on days 3–7, inclusive; the two latter groups did not differ on any of these days (all $P < 0.1941$; $P = 0.0768$ for day 7). Females exposed to EE did not differ from control males across these days (all $P > 0.1532$) and had shorter latencies than control females and females exposed to BPA on days 4, 6, and 7 (all $P < 0.0033$).

Spatial Navigational Search Strategies. Fig. 2*B* (main text) demonstrates the significant interactions among sex, diet, and day in use of random, serial, and spatial strategies ($F_{12,624} = 2.40$, $P = 0.0049$). On day 1 of acquisition, females exposed to EE used the cognitively sophisticated search strategy (i.e., spatial) more often than control females ($t = 6.59$, $P = 0$ 0.0001) and males ($t = 6.174$, $P = 0.0001$), females ($t = 4.78$, $P = 0.0001$) and males ($t = 6.70$, $P = 0.0001$) exposed to BPA, and males exposed to EE ($t = 7.23$, $P = 0.0001$); no other groups differed. On day 2, males exposed to EE or BPA had lower scores (as coded by 1, 2, and 3 for random, serial, and spatial search strategies, respectively) than control males ($t > -6.16$, all $P = 0.0001$) and females in all diet groups (all $t > -4.03$, all $P = 0.0001$). Females exposed to EE had higher scores than control females ($t = 2.86$, $P = 0.0052$), but there were no other group differences. Differences in strategy emerged by day 3. From this day forward, scores of control males were higher than those of males exposed to EE (all $t > 3.05$, all $P < 0.0029$) or BPA (all $t > 3.18$, all $P < 0.0019$). Scores of females exposed to EE were higher than those of control females (all $t > 2.90$, all $P < 0.0046$) and females exposed to BPA (all $t > 2.69$, all $P < 0.0084$). Control males had higher scores than those of control females (all $t > 3.79$, all $P < 0.0003$) and females exposed to BPA ($t = 2.66$, $P = 0.0089$ for day 3; all $t > 3.39$, all $P < 0.001$, days 4–7, inclusive) but did not differ from those of females exposed to EE (all $P > 0.20$). Females exposed to EE had higher scores than males exposed to EE (all $t > 3.29$, all $P < 0.0014$) and BPA ($t = 2.59$, $P = 0.011$ for day 7; all $t > 3.74$, all $P < 0.0003$, days 3–6, inclusive).

Exploratory and Anxiety-Like Behavior. Exploratory behavior and anxiety-like behavior were assessed by using the EPM. There were significant diet ($F_{2,83} = 12.97$, $P = 0.0001$) and sex × diet ($F_{2,83} = 6.07$, $P = 0.0035$) effects for time spent in open arms. There were no significant diet effects for females (all $P > 0.20$), but control males spent more time in the open arms than EE- ($t = 2.51$, $P = 0.014$) and BPA-exposed males ($t = 6.02$, $P = 0.0001$), and EE males spent more time in the open arms than BPA males ($t = 3.38$, $P = 0$ 0.001). Control males also spent more time in the open arms than control females ($t = 2.97$, $P = 0.0039$) and EE-exposed ($t = 4.02$, $P = 0.0001$) and BPA-exposed ($t = 3.19$, $P = 0.002$) females. There were significant diet effects for time spent immobile ($F_{2,83} = 3.64$, $P = 0.03$), but no sex differences ($F_{1,83} = 0.44$, $P = 0.51$) or sex × diet effects ($F_{2,83} = 1.53$, $P = 0.22$). BPA-exposed mice (mean 158 s, SD 51 s) spent more time immobile than either control (mean 132 s, SD 34 s) or EE-exposed (mean 125 s, SD 51 s) mice ($t = 1.91$, $P = 0.0591$; $t = 2.61$, $P = 0.0107$, respectively). No other across-sex comparisons met the Bonferroni α value (i.e., $P = 0.0056$). There were significant sex ($F_{1,83} = 5.91$, $P = 0.0172$), diet ($F_{2,83} = 3.28$, $P = 0.0425$), and interaction ($F_{2,83} = 3.16$, $P = 0.0478$) effects for number of entries into open arms. Control males entered open arms more frequently than EE-exposed males ($t = 2.93$, $P = 0.0044$, 0.0006), BPA-exposed males ($t = 3.14$, $P = 0.0024$), and BPA-exposed females ($t = 3.14$, $P = 0.0024$), as well as more entries than control females ($t = 3.55$, $P = 0.0006$).

ANCOVA, with open entries as the covariate, confirmed the significant diet ($F_{2,82} = 9.74$, $P = 0.0002$) and interaction ($F_{2,82} = 5.27$, $P = 0.007$) effects for time in open arms. Males exposed to BPA spent less time in open arms than control ($t = 5.14$, $P = 0.0001$) and EE-exposed ($t = 3.41$, $P = 0.001$) males, but the contrast of control males and EE males was no longer significant ($t = 1.82$, $P = 0.0722$). Control males spent more time in open arms than females in all diet groups, but only the contrast with EE-exposed females ($t = 3.42$, $P = 0.001$) survived the Bonferroni correction (control, $t = 2.09$, $P = 0.0399$; BPA, $t = 2.42$, $P = 0.0176$).

The same pattern emerged for total time in closed arms; sex ($F_{1,83} = 5.65$, $P = 0.0198$), diet ($F_{2,83} = 12.29$, $P = 0.0001$), and interaction ($F_{2,83} = 7.59$, $P = 0.0009$). There were no significant diet effects for females (all $P > 0.25$), but control males spent less time in closed arms than EE- ($t = -2.63$, $P = 0.01$) and BPA-exposed males ($t = -6.19$, $P = 0.0001$), and EE males

spent less time in the closed arms than BPA males ($t = -3.43$, $P = 0.0009$). Control males also spent less time in closed arms than control females ($t = -3.73$, $P = 0.0003$) and less time than EE-exposed ($t = -4.44$, $P = 0.0001$) and BPA-exposed ($t = -3.42$, $P = 0.001$) females; no other across-sex contrasts met the Bonferroni α value. The number of entries into closed arms did not differ for sex ($F_{1,83} < 1$, $P = 0.654$), diet ($F_{2,83} < 1$, $P = 0.717$), or sex × diet interaction ($F_{2,83} = 1.75$, $P = 0.1797$).

A similar pattern surfaced for proportion of time in open arms; sex ($F_{1,83} = 4.55$, $P = 0.036$), diet ($F_{2,83} = 13.75$, $P = 0.0001$), and interaction ($F_{2,83} = 7.09$, $P = 0.0014$). There were no significant diet effects for females (all $P > 0.25$), but control males spent proportionally more time in open arms than EE- ($t = 2.79$, $P = 0.0066$) and BPA-exposed males ($t = 6.34$, $P = 0.0001$), and EE males spent proportionately more time in open arms than BPA males ($t = 3.42$, $P = 0.001$). Control males also spent proportionately more time in the open arms than control females ($t = 3.54$, $P = 0.0007$) and more time than EE- ($t = 4.38$, $P = 0.0001$) and BPA-exposed ($t = 3.53$, $P = 0.0007$) females. No other across-sex comparisons met the Bonferroni α standard.

There were no sex ($F_{1,83} = 2.70, 3.02$, $P = 0.104, 0.086$), diet ($F_{2,83} < 1, <1$, $P = 0.765, 0.505$), or interaction ($F_{2,83} = 1.62, < 1$, $P = 0.204, 0.527$) effects for time spent in the center or number of center entries, respectively. ANOVAs, with time immobile as the covariate, confirmed that the diet and diet × sex effects remained significant for time in open arms ($F_{2,82} = 7.36$, $P = 0.0012$; $F_{2,82} = 5.39$, $P = 0.0063$, respectively), closed arms ($F_{2,82} = 9.48$, $P = 0.0002$; $F_{2,82} = 6.72$, $P = 0.002$, respectively), and proportion of time in open arms ($F_{2,82} = 7.59$, $P = 0.0009$; $F_{2,82} = 6.25$, $P = 0.003$, respectively).

Mate Choice Experiment. Females spent more time engaged in nose-to-nose inspection of control males (least-squares mean 29.5 s, SE 5.86 s) than males exposed to BPA (mean 14.3 s, SE 5.76 s) ($F_{1,33} = 7.19$, $P = 0.0114$). Inspection time did not vary with maternal diet of the females ($F_{1,33} = 1.24$, $P = 0.2727$), nor did maternal diet of the females interact with maternal diet of the males ($F_{1,33} < 1$, $P = 0.7458$). Neither contact time nor time spent in the male's side of the arena varied across male or female diet, nor was there an interaction (all $P < 0.1336$). Finally, time spent in the center of the arena, immobile, and grooming did not differ across females from the various maternal diet groups ($P > 0.1009$).

1. Dluzen DE, Carter CS (1979) Ovarian hormones regulating sexual and social behaviors in female prairie voles, *Microtus ochrogaster*. *Physiol Behav* 23:597–600.

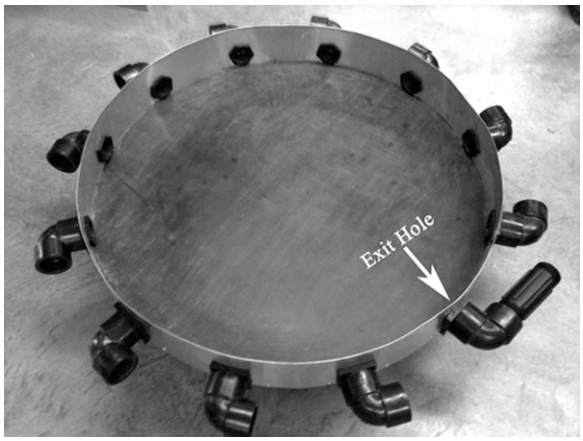


Fig. S1. Picture of modified Barnes maze used to quantify spatial learning in male and female deer mice.

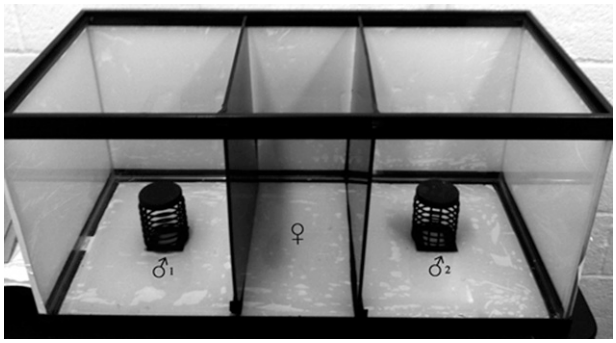


Fig. S2. Picture of three-chambered mate preference apparatus used in the mate-choice experiment.

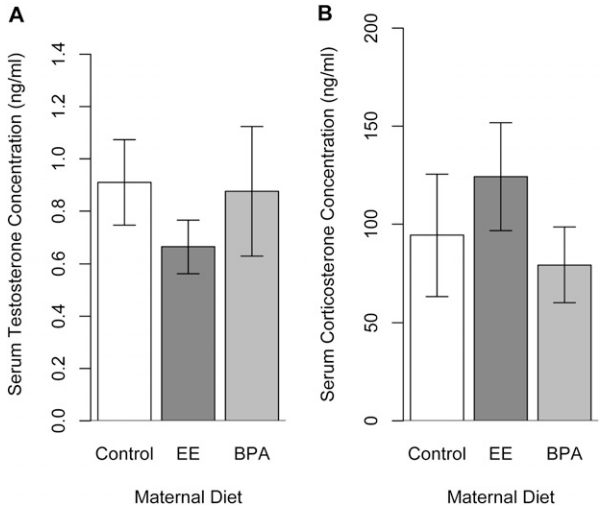


Fig. S3. Serum testosterone and corticosterone concentrations in adult male deer mice (mean ± SEM). There was no difference in serum testosterone (*A*) or corticosterone (*B*) concentrations between any of the diet group comparisons ($P > 0.05$).

Table S1. Effects of endocrine-disrupting compounds on litter composition of *Peromyscus maniculatus*

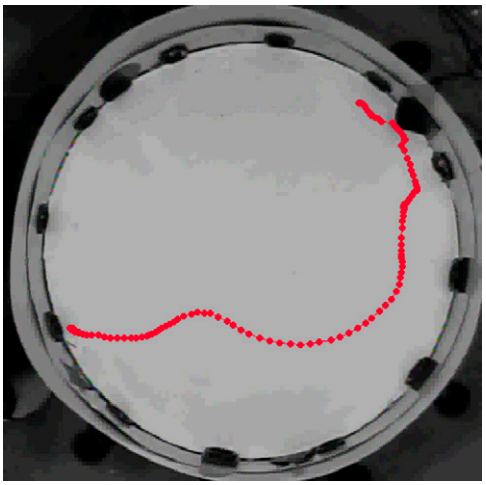
Diet of dam	No. of litters	No. of offspring	Average litter size (±SEM)	Sex ratio
Control	24	47 ♂ 24 ♀	2.95 ± 0.28	0.62
EE	25	30 ♂ 39 ♀	2.76 ± 0.31	0.41*
BPA	24	43 ♂ 26 ♀	2.85 ± 0.20	0.65

*$P < 0.05$, compared with dams on the control and BPA-supplemented diets. Binomial tests indicated that the sex ratios for dams on the control ($P = 0.009$) and EE-supplemented ($P = 0.001$) diets differed from 50%, with a similar trend for dams on the BPA-supplemented diet ($P = 0.053$). However, no sex ratio differences were evident in litters born to dams on the control vs. BPA-supplemented diet ($P = 0.83$).

Table S2. Functional sensory battery tests administered to postnatal day 25 offspring

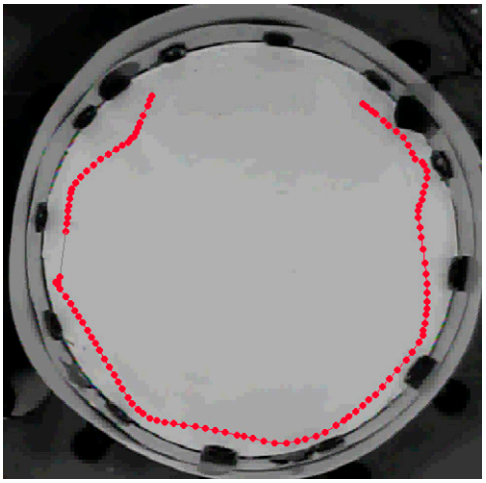
Treatment group (*n*)	Olfaction: Time to find treat (s)	Neuromuscular: Time to hold onto wire bar (s)	Vision: Ability to see wire bar (%)	Auditory: Startled by noise (%)
Control males (19)	237 ± 57.14	51 ± 4.76	100	100
BPA males (20)	299 ± 65.71	53 ± 4.17	100	100
EE males (18)	178 ± 60.65	49 ± 4.77	100	100
Control females (13)	326 ± 97.07	55 ± 5.08	100	100
BPA females (9)	190 ± 64.83	52 ± 4.48	100	100
EE females (10)	368 ± 75.72	36 ± 9.05	100	100

Means ± SEM for sensory measures.



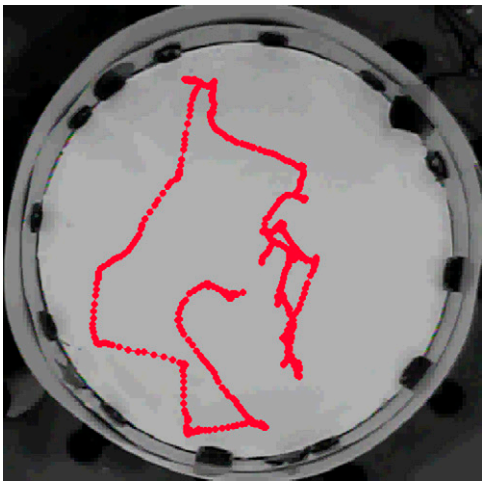
Movie S1. Example automated tracking in the Barnes maze for an average performance of control male deer mouse on day 4 of acquisition training.

Movie S1



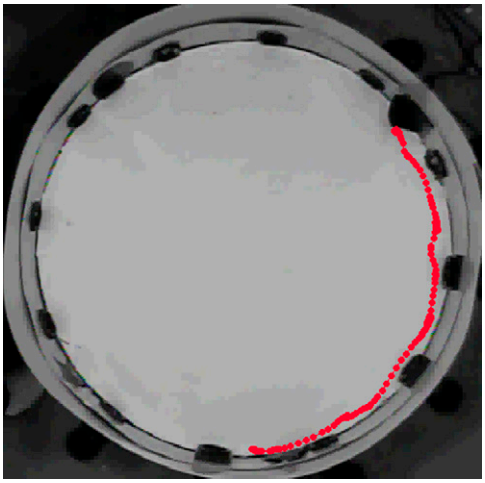
Movie S2. Example automated tracking in the Barnes maze for an average performance EE-exposed male deer mouse on day 4 of acquisition training.

Movie S2



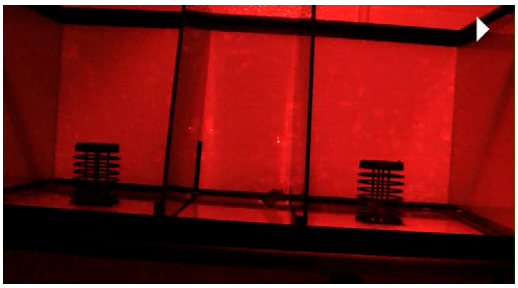
Movie S3. Example automated tracking in the Barnes maze for an average performance BPA-exposed male deer mouse on day 4 of acquisition training.

Movie S3



Movie S4. Example automated tracking in the Barnes maze for an average performance EE-exposed female deer mouse on day 4 of acquisition training, which resembles the performance of a control male.

Movie S4



Movie S5. Example video of female preference test. Male exposed to BPA is in the right cup (from the viewer's point of view), whereas control male is in the left cup in the mate preference chamber. The experiment was performed during the dark phase, and thus a 12-W red light that does not interfere with the light/dark cycle of the animals was used.

Movie S5