**Methods**

**Animals**

All experimental procedures were approved by the University of Florida Institutional Animal Care and Use Committee. The study was conducted and data is reported in accordance with the ARRIVE guidelines. Sprague–Dawley rats [120 males and 72 females (generation F0)] were purchased from Charles River (Wilmington, MA). The F0 female rats were used as breeders only to generate offspring (generation F1). All rats were housed under controlled illumination (12-h light/12-h dark, lights on at 7:00 am) and temperature (23–24 °C) with free access to food and water. Experimental data in this study are from 192 male and 72 female rats. One rat in injury group died immediately after the traumatic brain injury induction and two other rats in the surgery group were removed from the study because of the suture failure during the recovery period. All three rats were excluded from all analyses.

**Treatment Groups**

Figure 1 shows an overview of the study design. F0 male rats were randomized into four treatment groups (n = 30/group) using a randomization plan with a web-based generator, and the investigators were blinded to group assignments. Rats in the injury group were subjected to all interventions: 1) surgery under 3% SEVO anesthesia for 40 min on postnatal day 60 to conduct a craniectomy and implant an injury hub; 2) a midline fluid percussion injury-inflicted moderate traumatic brain injury1,2 on the same day; and 3) exposure to 2.1% sevoflurane for 3 h on postnatal days 62, 64, and 66,3 to model anesthesia/sedation needed for treatment of conditions associated with traumatic brain injury or unrelated ones. Rats in the sevoflurane group had only sevoflurane exposure on postnatal days 60, 62, 64, and 66. Rats in the surgery (sham) group had a craniectomy and injury hub implantation, but not traumatic brain injury, on postnatal day 60. These rats were exposed to 2.1% sevoflurane for 3 h on postnatal days 62, 64, and 66. Rats in the control group were placed in a new cage and housed one per cage for an equivalent time on postnatal days 60, 62, 64, and 66.

A subset of F0 male rats from all groups (n = 12/group) was sacrificed 1 h after recovery from sevoflurane anesthesia on postnatal day 66 or at an equivalent timepoint in the control group to study acute effects. The remaining F0 male rats (n = 18/group) were mated on postnatal day 90 with F0 control female rats to produce offspring (generation F1). A cohort of 18 breeding pairs of F0 male and female rats were used to produce F1 offspring for a given experimental group (18 F1 rats/group/sex). A given F1 offspring experimental group included 1 to 2 rats from a given F1 litter. Within 24 h of delivery, F1 litters were culled to 12 pups. The F0 females were housed individually throughout the entire gestation and postpartum rearing periods. At the age of 21 days, pups were weaned and housed in sex-matched pairs for the rest of the study. F1 rats were not exposed to any treatment and were subjected to animal facility rearing only.

The F0 sires were sequentially evaluated in the elevated plus maze4 on postnatal day 105, for prepulse inhibition of the acoustic startle response4,5 on postnatal day 115, and in the Morris water maze6,7 between postnatal days 125 and 130. Ten days later, stress-induced release of corticosterone was studied after exposing the animals to physical restraint for 30 min. The serum levels of corticosterone were measured before physical restraint and 10, 60, and 120 min after the restraint. Blood sampling (~300 µL) was done using the “tail clip” method, as previously described.3 The F1 male and female offspring were evaluated in the elevated plus maze on postnatal day 60, for prepulse inhibition of the acoustic startle response on postnatal day 70, and in Morris water maze tests between postnatal days 90 and 95. Blood samples were collected using the “tail clip” method 30 min after completion of the prepulse inhibition test to measure stress-induced serum corticosterone levels. Ten days after completing the *in vivo* studies, the F0 and F1 rats were anesthetized and euthanized through decapitation to collect trunk blood and brain tissue samples for enzyme-linked immunosorbent assay,3 reverse transcription-polymerase chain reaction,3,6 immunohistochemistry,3 and targeted next-generation bisulfite sequencing studies.8

**Midline Fluid Percussion Injury Model of Traumatic Brain Injury**

The fluid percussion injury device (Model 01-B, Custom Design & Fabrication, Inc., Sandston, VA, USA) was used to induce the midline fluid percussion injury model of moderate traumatic brain injury.1,2 Anesthesia was induced with 6% sevoflurane for 3 min in an anesthesia chamber and then a rat was placed in a stereotaxic frame with a continuous supply of 3% sevoflurane for 37 min via a nose cone. Sevoflurane was delivered in a mixture of ~50% O2 inair (1.5 L/min). To apply a uniform sevoflurane anesthesia duration and depth to all experimental animals, anesthesia in all rats was induced with 6% sevoflurane for 3 min and maintained with 3% sevoflurane for 37 min, which was slightly longer than the time needed for the craniectomy and injury hub implantation procedure. The rats’ rectal temperature during surgery was monitored and maintained at ~+37.0 ºC using a homeothermic monitoring system (50-7220F, Harvard Apparatus, St. Laurent, Quebec, Canada). After a ~15-mm midline incision and fascia removal from the skull, a craniectomy was centrally made over the sagittal suture between bregma and lambda by using a trephine with an outer diameter of 4.8 mm (Integra Miltex, Princeton, NJ, USA). The dura was kept intact. The female hub of a 20-gauge Luer-Loc needle was placed over the craniectomy with cyanoacrylate gel first and then methyl-methacrylate dental cement was added to secure the hub (Henry Schein, Melville, NY, USA). The dura was visually inspected to ensure it was intact with no debris. The inducer was placed over the hub, filled with normal saline, and attached to the male end of the fluid percussion injury device, avoiding air bubbles at the junction. A pressure wave averaging 2.0 ± 0.2 atm was delivered by releasing the pendulum onto the fluid-filled cylinder. The righting reflex time (the time from the initial impact until the rat spontaneously rights itself from a supine position) was 8.97 ± 1.82 min. The injury site was inspected for bleeding, herniation, hematomas, and dura integrity before the wound was sutured.

**Post-injury Anesthesia Exposure**

Rats in the sevoflurane, surgery, and injury groups were also exposed to sevoflurane on postnatal days 62, 64, and 66, as we previously described.3 During sevoflurane exposure, rats were held in a temperature-controlled chamber to maintain body temperature at ~+37 ºC. The chamber was continuously supplied with 50% O2 in air (1.5 L/min) during anesthesia. Anesthesia with sevoflurane was induced with 6% sevoflurane for 3 min followed by 2.1% sevoflurane for 177 min. Gas monitoring was performed using a calibrated Datex side stream analyzer (Datex-Ohmeda, Helsinki, Finland), which sampled from the animal chamber interior. This level of anesthesia was sufficient to induce loss of the righting reflex, but not sufficient to induce loss of the withdrawal reflex to a noxious stimulus. F0 male rats in the control group were housed alone in a new cage for a time equivalent to the time that the rats in the sevoflurane, surgery, and injury groups were housed alone on postnatal days 60 to 66.

**The Elevated Plus Maze Test**

The elevated plus maze studies were performed using an elevated plus maze apparatus and BIO-EPM 3C video-tracking software (EB Instruments, Pinellas Park, FL, USA) during the light phase of the dark–light cycle, as we previously described.4 The maze consists of two opposing open (50 × 10 × 0.5 cm) and two enclosed (50 × 10 × 45 cm) arms elevated 75 cm above the floor, with a 0.5-cm edge on the open arms. Animals were placed in the center square facing an open arm and were allowed to explore the maze for 5 min. The percentage of time spent in the open arms, the percentage of entries to the open arms, and the total distance traveled during the elevated plus maze were compared. If a fall occurred, the animal was removed from the study. No animal falls occurred in this study.

**Measurements of the Prepulse Inhibition of Startle**

We performed prepulse inhibition of acoustic startle tests in rats using the SR-Lab startle apparatus (San Diego Instruments, San Diego, CA, USA), as previously described.4,5 Testing occurred during the light phase of the dark–light cycle. The entire test for a given animal lasted 28 min. The animal enclosure (20 cm in length, 9 cm in interior diameter) permitted the animal to turn around in the enclosure. At the beginning of every testing session, each animal was placed in the cylindrical animal enclosure and exposed to a 75-dB white noise background for a 5-min acclimation period. The acclimation period was followed by a test session consisting of five types of trials: a 120-dB 40-ms pulse only; a 120-dB 40-ms pulse preceded by a 20-ms prepulse at 3, 6, and 12 dB above background; and a no-stimulus trial of background noise. The delay between the onset of the prepulse and the onset of the pulse was 100 ms. The trials were presented in pseudorandom order with variable inter-trial intervals averaging 15 s. The first four trials and the last three trials consisted of 120-dB pulse-only trials. All five types of trials were presented eight times, each in pseudorandom order after the first four and before the last three pulse-only trials. The percent prepulse inhibition for each prepulse inhibition was calculated using the following formula: % prepulse inhibition = 100 × [(pulse alone) – (prepulse + pulse)]/pulse alone.9 Data were collected as Vmax amplitude (the highest voltage during the response window).

**The Morris Water Maze Test**

To evaluate spatial learning and memory in rats, we used the Morris water mazebehavioral assay, as previously described.6,7 The Morris water maze apparatus consisted of a circular tank (183 cm in diameter, 58 cm in height). The maze was filled with water (23 ± 1 °C) made opaque with the addition of nontoxic paint (Blick Art Materials, Highland Park, IL, USA). The pool was surrounded with black curtains on which were affixed large white geometric shapes (extramaze cues). The tank was divided into four equal quadrants, labeled NW, NE, SE and SW (NW – northwest; NE – northeast; SE – southeast; SW – southwest;). A 12-cm diameter platform (the escape platform) was submerged 2 cm below the surface of the water at a constant location in one of the quadrants. Data were recorded using a computer-based video tracking system (ANY-maze, Stoelting Co., Wood Dale, IL, USA). Rats received four trials of daily training over 5 consecutive days. In each trial, rats were placed into the water facing the wall of the maze at one of the four quadrants. The start positions were varied in a pseudorandom manner. Once in the water, rats were allowed to swim until they found the hidden platform or until 60 s elapsed, at which time the rats were gently guided to the platform. Rats remained on the platform for 15 s before the next trial. Twenty-four hours later, the spatial memory test was performed. The spatial memory test lasted 60 s, during which time the escape platform was removed. The rat was placed in the tank in the contralateral quadrant (the entrance quadrant, SE) relative to the original location of the escape platform (the target quadrant, NW). The time spent in each quadrant and the number of times the rat crossed the previous location of the escape platform were recorded.

**Stress-Induced Serum Corticosterone Levels**

To measure stress-induced serum levels of corticosterone, F0 male rats were physically restrained for 30 min. Physical restraint was administered using rodent holders (Kent Scientific Corporation, Torrington, CT, USA). Blood samples (~300 µL) were collected immediately before physical restraint and 10, 60, and 120 min after the restraint. Blood sampling was done using the “tail clip” method, as previously described.3 Specifically, the distal 0.5 mm of the tail was removed using a sterile scalpel blade, and blood was allowed to drain directly into a microcentrifuge tube. To measure stress-induced serum levels of corticosterone in F1 offspring, the tail blood samples were collected 30 min after completion of the prepulse inhibition of acoustic startle test, using the same technique as described above.

**Tissue Collection**

Rats were anesthetized with sevoflurane and decapitated. The trunk blood samples were collected and centrifuged at 4 °C, 1000 g for 15 min, and then kept at −80 °C for resting corticosterone and proinflammatory cytokine interleukin-1β and interleukin-6 assays. The hypothalamus was isolated by making an anterior cut at the level of the optic chiasm, a posterior coronal section anterior to the mammillary bodies, two sagittal cuts parallel to the lateral ventricles, and a dorsal horizontal cut at the level of the anterior commissure.3 The hippocampus was isolated from the respective blocks. All tissue samples were placed in vials filled with *RNAlater* solution (Invitrogen, Carlsbad, CA, USA). Sperm were isolated from the caudal epididymis of adult males and placed into phosphate-buffered saline with 1% bovine serum albumin at 37 °C using a swim-up assay. After settling for 30 min, sperm-containing supernatant was centrifuged for 5 min at 4000 rpm. Sperm pellets were stored at –80 °C.3,10

**Measurements of Serum Concentrations of Corticosterone, Interleukin-1β and Interleukin-6**

Enzyme-linked immunosorbent assay kits were used to measure serum concentrations of interleukin-1β(BMS630, Invitrogen), interleukin-6 (BMS625, Invitrogen) and corticosterone (501320, Cayman Chemical Company, Ann Arbor, MI, USA) by following the manufacturers’ instructions.

**Measurement of Messenger RNA Levels**

The messenger RNA (mRNA) levels in the hypothalamus and hippocampus were analyzed via reverse transcription-polymerase chain reaction in a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), as previously described.3,6 RNA was extracted from the samples using an RNeasy Plus Kit (Qiagen, Valencia, CA, USA), reverse transcribed with a high-capacity cDNA reverse transcription kit (Bio-Rad Laboratories, Hercules, CA, USA), and then analyzed via reverse transcription-polymerase chain reaction. Taqman probes specific for corticotropin-releasing hormone (*Crh*), glucocorticoid (*Gr*) and mineralocorticoid (*Mr*) receptors, and brain-derived neurotrophic factor (*Bdnf*) were obtained from Applied Biosystems: *Crh* (Rn01462137\_m1)*, Gr* (Rn00561369\_m1)*, Mr* (Rn00565562\_m1)and *Bdnf* (Rn02531967\_s1). Data were normalized to glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) mRNA (Rn01775763\_g1). Gene expression was calculated using the ΔΔCT method and data are presented as relative fold change from that of control animals.

**Immunohistochemistry**

Rats were anesthetized with sevoflurane and transcardially perfused with saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were collected and fixed in the 4% paraformaldehyde overnight and then dehydrated in 30% sucrose solution in phosphate-buffered saline at 4 °C for 2 days. The brains were cut into 40-µm-thick coronal sections using a vibratome (VT1000 S, Leica, Wetzlar, Germany). After blocking with 10% normal goat serum for 1 h at room temperature, the slices were incubated with the primary antibody rabbit anti- ionized calcium binding adaptor 1 (microglia/macrophage-specific protein marker; 1:500, Wako Pure Chemical Industries, Osaka, Japan) in 1% bovine serum albumin at 4 °C overnight. After washing three times with phosphate-buffered saline for 10 min, the slices were exposed to the secondary antibody Alexafluor 549 goat anti-rabbit (Invitrogen) for 1 h at room temperature. After washing out the secondary antibodies, the sections were incubated with 4´,6-diamidine-2´-phenylindole dihydrochloride (DAPI) for nuclear staining. A fluorescence microscope (BZ-X810E, Keyence, Osaka, Japan) was used to capture the images. Six sections of the hippocampus per rat were analyzed by ImageJ (National Institutes of Health, Bethesda, MD, USA). Three nonoverlapping fields of each section in the hippocampal cornu ammonis 1 area were randomly acquired. The immunoreactivity of a protein was quantified by the percentage of area with positive staining out of the total area of the imaged field.3,11 All quantitative analyses were performed by an experimenter blinded to the group of each sample.

**Targeted Next-Generation Bisulfite Sequencing**

Targeted next-generation bisulfite sequencing was performed by EpigenDx Inc. (Worchester, MA, USA). In total, 29 CpG sites for the *Gr* promoter were analyzed. Genomic DNA was extracted from the hippocampus and sperm pellets of adult F0 rats and from hippocampal tissues of F1 rats using the Quick-DNA™ Microprep Kit (ZymoResearch, Irvine, CA, USA). Sodium bisulfite conversion was performed with EZ-96 DNA Methylation-Direct Kit™ (ZymoResearch) following the manufacturer’s instructions. Multiple polymerase chain reaction amplification was performed with HotStarTaq (Qiagen, Hilden, Germany). Polymerase chain reaction products from the same sample were pooled and purified using the QIAquick PCR Purification Kit plates (Qiagen). Libraries were prepared using a custom library preparation method created by EpigenDx. Barcoded samples were then pooled in an equimolar fashion before template preparation and enrichment were performed on the Ion Chef™ system using Ion 520™ & Ion 530™ ExT Chef reagents (Thermo Fisher, Waltham, MA, USA). Afterward, enriched, template-positive library molecules were sequenced on the Ion S5™ sequencer using an Ion 530™ sequencing chip. FASTQ files from the Ion Torrent S5 server were aligned to a local reference database using the open-source Bismark Bisulfite Read Mapper program v0.12.2 (Babraham Bioinformatics, Cambridge, UK) with the Bowtie2 alignment algorithm. Methylation levels were calculated in Bismark by dividing the number of methylated reads by the total number of reads. The DNA methylation level at each CpG sites were reported by EpigenDx.

**Statistical Analyses**

The primary outcomes in this study were the neuroendocrine and behavioral changes in F0 sires and in their F1 offspring. All other outcome measurements were secondary outcomes. Sample size calculations were done, assuming a range of anticipated differences in mean outcomes and standard error based on background data and past experience with similar measurements in Sprague-Dawley rats.3,8 This analysis indicated that sample sizes of at least n = 16 rats/group for behavioral studiesandn = 5 rats/group formeasurements in tissue samples were required to detect differences between treatment groups, with effect sizes of ≥0.8, assuming an α level of 0.05. This translated to a mean difference of 10% in the time spent in the open arms (elevated plus maze), prepulse inhibition of startle, the time spent in the each quadrant (Morris water maze), serum levels of corticosterone, interleukin-1β and interleukin-6, messenger RNA (mRNA)levels, and DNA methylation levels of *Nr3c1*. To account for possible attrition over the 6-mo study (estimated at 5%–10% due to unexpected disease, removal of animals from behavioral tests because of housing incidents such as cage flooding, incidents during behavioral tests such as a fall from the maze during the elevated plus maze test, and removal of outliers, among other factors, based on our previous experience with similar studies), group sizes for some comparisons were larger than those calculated from the power analyses. Values are reported as mean ± SD. Boxplots were used to identify outliers. No outliers were detected that were not in the plausible range of values for the outcomes; therefore, all data were maintained in analyses. One-way ANOVA was used to assess F0 data for acute serum corticosterone, interleukin-1β and interleukin-6 levels, acute ionized calcium binding adaptor 1 (microglia/macrophage-specific protein marker) expression, long-term resting corticosterone, total corticosterone concentrations before and after the restraint, interleukin-1β and interleukin-6 levels, changes in gene expression, time spent in and number of entries to the open arms and total distance traveled during the elevated plus maze test, and number of crossings over the former platform during the Morris water maze probe test. Two-way repeated measures ANOVA with experimental groups and time as the independent variables was run to analyze changes in serum corticosterone levels before and at three time points after the restraint. Two-way repeated measures ANOVA was used to analyze the F0 prepluse inhibition data, with treatment and prepulse intensity as independent variables. Two-way ANOVA with experimental groups and days of training as the independent variables was used to analyze changes in escape latencies to the escape platform during the Morris water maze test in F0 rats. Two-way ANOVA was used to analyze the time spent in each quadrant during the MWM probe test in F0 rats, with the treatment and quadrant as independent variables. Two-way ANOVA with treatment and sex as the independent variables were used to assess F1 data for changes in serum corticosterone levels at rest and after the prepluse inhibition test, changes in serum interleukin-1β and interleukin-6 levels, changes in gene expression, time spent in and number of entries to the open arms and total distance traveled during the elevated plus maze test, and Morris water maze platform location crossing times. For F1 prepluse inhibition, Morris water maze escape latency and time in each quadrant, linear mixed models for repeated measures were used, with prepluse inhibition intensity, days of training, and quadrant modeled as repeated measures, respectively. These analyses account for within-subject corrections across repeated measurements. The models also included treatment and sex as main effects, as well as interaction terms. An independent t test was used to analyze methylation level at each CpG site and overall CpG sites of *Nr3c1* gene. Analyses were conducted in SigmaPlot 14.0 software and SPSS v27 (IBM Corp., Armonk, NY, USA). Multiple pairwise comparisons were done with the Holm-Sidak method. *P* < 0.05 was considered significant.

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**Supplemental Table 1.** The results of the statistical analyses (the F and *P* values) of experimental findings in F0 Rats. The figure numbers in the table are given to link the results of the statistical analyses in the table with the respective data in the text and figures in the manuscript.

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| Measured variables | The results of the statistical analyses |
| Acute serum corticosterone (fig. 2A) | F(3,20) = 12.23, *P* < .001 |
| Acute serum interleukin-1β (fig. 2B) | F(3,20) = 25.94, *P* < .001 |
| Acute serum interleukin-6 (fig. 2C) | F(3,20) = 77.23, *P* < .001 |
| Acute expression of ionized calcium binding adaptor 1 (fig. 2D,E) | F(3,428) = 201.36, *P* < .001 |
| Resting serum corticosterone (fig. 3A) | F(3,25) = 13.93, *P* < .001 |
| Total serum corticosterone during physical restraint (area under the curve) (fig. 3B) | F(3,25) = 11.61, *P* < .001 |
| Respective levels of corticosterone during physical restraint (fig. 3C) | F(3,75) = 12.03, *P* < .001 (treatment) |
| F(3,75) = 562.75, *P* < .001 (time) |
| F(9,75) = 6.85, *P* < .001 (treatment × time interaction) |
| Hypothalamic *Crh* Mrna (fig. 3D) | F(3,20) = 7.11, *P* = .002 |
| Hippocampal *Crh* mRNA (fig. 3E) | F(3,20) = .50, *P* = .689 |
| Hypothalamic *Nr3c1* mRNA (fig. 3F) | F(3,20) = 5.53, *P* = .006 |
| Hippocampal *Nr3c1* mRNA (fig. 3G) | F(3,20) = 9.79, *P* < .001 |
| Hypothalamic *Nr3c2* mRNA (fig. 3H) | F(3,20) = 6.39, *P* = .003 |
| Hippocampal *Nr3c2* mRNA (fig. 3I) | F(3,20) = 12.14, *P* < .001 |
| Serum interleukin-1β (fig. 3J) | F(3,28) = 16.72, *P* < .001 |
| Serum interleukin-6 (fig. 3K) | F(3,28) = 25.35, *P* < .001 |
| Hypothalamic *Bdnf* mRNA (fig. 3L) | F(3,20) = 10.71, *P* < .001 |
| Hippocampal *Bdnf* mRNA (fig. 3M) | F(3,20) = 5.14, *P* = .009 |
| Time spent in open arms of elevated plus maze (fig. 4A) | F(3,68) = 11.44, *P* < .001 |
| Entries to open arms of elevated plus maze (fig. 4B) | F(3,68) = 5.49, *P* = .002 |
| Total distance during elevated plus maze (fig. 4C) | F(3,68) = 1.08, *P* = .363 |
| Prepulse inhibition of acoustic startle response (fig. 4D) | F(3,134) = 5.13, *P* = .003 (treatment) |
| F(2,134) = 130.63, *P* < .001  (prepulse intensity) |
| F(6,134) = 1.27, *P* = .276 (treatment × prepulse intensity interaction) |
| Escape latency of Morris water maze (fig. 4E) | F(3,272) = .38, *P* = .768 (treatment) |
| F(4,272) = 140.04, *P* < .001  (day of training) |
| F(12,272) = .83, *P* = .615 (treatment × day of training interaction) |
| Crossing times over the platform of Morris water maze (fig. 4F) | F(3,69) = 6.89, *P* < .001 |
| Time spent in each quadrant of Morris water maze (fig. 4G,H) | F(3,204) = .39, *P* = .758 (treatment) |
| F(3,204) = 43.11, *P* <.001 (quadrant) |
| F(9,204) = 3.38, *P* <.001 (treatment × quadrant interaction) |

mRNA: messenger RNA; *Crh:* Corticotropin-releasing hormone; *Nr3c1:* glucocorticoid receptor; *Nr3c2:* Mineralocorticoid receptor; *Bdnf*: Brain-derived neurotrophic factor.

**Supplemental Table 2**. The results of the statistical analyses (the F and *P* values) of experimental findings in F1 Rats. The figure numbers in the table are given to link the results of the statistical analyses in the table with the respective data in the text and figures in the manuscript.

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| Measured variables | Effect of | The results of the statistical analyses |
| Resting serum corticosterone levels (fig. 5A) | Paternal treatment | F(3,46) = 1.04, *P* = .386 |
| F1 Sex | F(1,46) = 30.99, *P* < .001 |
| Patern. treatment × F1 sex interaction | F(3,46) = .38, *P* = .772 |
| Stress serum corticosterone levels 30 min after prepulse inhibition of startle test (fig. 5B) | Paternal treatment | F(3,47) = 10.53, *P* < .001 |
| F1 Sex | F(1,47) = 48.45, *P* < .001 |
| Patern. treatment × F1 sex interaction | F(3,47) = 9.14, *P* < .001 |
| Hypothalamic *Crh* mRNA (fig. 5C) | Paternal treatment | F(3,39) = .20, *P* = .894 |
| F1 Sex | F(1,39) = .35, *P* = .559 |
| Patern. treatment × F1 sex interaction | F(3,39) = .23, *P* = .872 |
| Hippocampal *Crh* mRNA (fig. 5D) | Paternal treatment | F(3,40) = .65, *P* = .585 |
| F1 Sex | F(1,40) = .02, *P* = .891 |
| Patern. treatment × F1 sex interaction | F(3,40) = .46, *P* = .710 |
| Hypothalamic *Nr3c1* mRNA (fig. 5E) | Paternal treatment | F(3,39) = 1.12, *P* = .355 |
| F1 Sex | F(1,39) = 20.54, *P* < .001 |
| Patern. treatment × F1 sex interaction | F(3,39) = 3.12, *P* = .037 |
| Hippocampal *Nr3c1* mRNA (fig. 5F) | Paternal treatment | F(3,40) = 4.58, *P* = .008 |
| F1 Sex | F(1,40) = 34.30, *P* < .001 |
| Patern. treatment × F1 sex interaction | F(3,40) = 4.93, *P* = .005 |
| Hypothalamic *Nr3c2* mRNA (fig. 5G) | Paternal treatment | F(3,37) = .54, *P* = .660 |
| F1 Sex | F(1,37) = 5.67, *P* = .023 |
| Patern. treatment × F1 sex interaction | F(3,37) = 2.23, *P* = .102 |
| Hippocampal *Nr3c2* mRNA (fig. 5H) | Paternal treatment | F(3,40) = 5.11, *P* = .004 |
| F1 Sex | F(1,40) = 1.20, *P* = .280 |
| Patern. treatment × F1 sex interaction | F(3,40) = .21, *P* = .890 |
| Serum interleukin-1β (fig. 5I) | Paternal treatment | F(3,56) = 14.63, *P* < .001 |
| F1 Sex | F(1,56) = 4.26, *P* = .044 |
| Patern. treatment × F1 sex interaction | F(3,56) = 6.10, *P* = .001 |
| Serum interleukin-6 (fig. 5J) | Paternal treatment | F(3,56) = 17.63, *P* < .001 |
| F1 Sex | F(1,56) = 40.35, *P* < .001 |
| Patern. treatment × F1 sex interaction | F(3,56) = 6.94, *P* < .001 |
| Hypothalamic *Bdnf* mRNA (fig. 5K) | Paternal treatment | F(3,39) = 6.73, *P* < .001 |
| F1 Sex | F(1,39) = 30.07, *P* < .001 |
| Patern. treatment × F1 sex interaction | F(3,39) = 3.85, *P* = .017 |
| Hippocampal *Bdnf* mRNA (fig. 5L) | Paternal treatment | F(3,40) = 4.79, *P* = .006 |
| F1 Sex | F(1,40) = 19.93, *P* < .001 |
| Patern. treatment × F1 sex interaction | F(3,40) = 3.94, *P* = .015 |
| Time spent in open arms of elevated plus maze (fig. 6A) | Paternal treatment | F(3,136) = 3.92, *P* = .010 |
| F1 Sex | F(1,136) = 21.62, *P* < .001 |
| Patern. treatment × F1 sex interaction | F(3,136) = 1.24, *P* = .298 |
| Entries to open arms of elevated plus maze (fig. 6B) | Paternal treatment | F(3,136) = 4.77, *P* = .003 |
| F1 Sex | F(1,136) = 1.16, *P* = .282 |
| Patern. treatment × F1 sex interaction | F(3,136) = .49, *P* = .688 |
| Total distance during elevated plus maze (fig. 6C) | Paternal treatment | F(3,136) = 3.59, *P* = .015 |
| F1 Sex | F(1,136) = 4.97, *P* = .027 |
| Patern. treatment × F1 sex interaction | F(3,136) = .27, *P* = .846 |
| Prepulse inhibition of acoustic startle response (fig. 6D) | Paternal treatment | F(3,408) = 12.08, *P* < .001 |
| Prepulse intensity | F(2,408) = 51.53, *P* < .001 |
| F1 Sex | F(1,408) = 5.81, *P* = .016 |
| Patern. treatment × prepulse intensity × F1 sex interaction | F(6,408) = .61, *P* = .719 |
| Escape latency of Morris water maze (fig. 6E) | Paternal treatment | F(3,520) = 2.41, *P* = .066 |
| Day of training | F(4,520) = 100.06, *P* < .001 |
| F1 Sex | F(1,520) = 0.12, *P* = .734 |
| Patern. treatment × day of training × F1 sex interaction | F(12,520) = .95, *P* = .500 |
| Crossing times over the platform of Morris water maze (fig. 6F) | Paternal treatment | F(3,104) = 3.50, *P* = .018 |
| F1 Sex | F(1,104) = 1.74, *P* = .190 |
| Patern. treatment × F1 sex interaction | F(3,104) = .73, *P* = .535 |
| Time spent in each quadrant of Morris water maze (fig. 6G,H) | Paternal treatment | F(3,416) = .02, *P* = .997 |
| Quadrant | F(3,416) = 28.80, *P* < .001 |
| F1 Sex | F(3,416) = .02, *P* = .899 |
| Paternal treatment × quadrant × F1 sex interaction | F(9,416) = 1.74, *P* = .079 |

mRNA: messenger RNA; *Crh:* Corticotropin-releasing hormone; *Nr3c1:* glucocorticoid receptor; *Nr3c2:* Mineralocorticoid receptor; *Bdnf*: Brain-derived neurotrophic factor.