Supplemental Methods.

Animals and developmental manipulations. Animals were maintained on a reverse 12:12 hour light cycle and provided free access to food and water. Cages were checked regularly for the presence of pups to determine the day of birth (PN0) and four litters found the morning of delivery were used for the initial behavior experiment. Intracerebroventricular injections were performed on cold-anesthetized pups. Under bright light, the cranial landmark Bregma is visible through the skin and was used to locate the lateral ventricles. Injections were performed by penetrating the skin and skull with a 23-gauge 1cc Hamilton syringe stereotaxically lowered to a predetermined depth. Infusion volume was 1 µl and extended for a 60sec period. Animals received a sc injection of ink to the paw for group identification and were distributed randomly among the four mothers. Animals were weaned at PN21 and housed randomly in sets of three with members of the same sex.

Behavioral analyses. Male sex behavior tests were conducted under dim red light during the dark phase once a week for three consecutive weeks beginning on PN52. Subjects were placed in a 50x38x25cm Plexiglas chamber for 15min prior to introducing a sexually receptive female. These stimulus females were ovariectomized, and estrogen and progesterone primed. The hormonal regime used to reliably induce female sexual receptivity consisted of sc injections of 10µg 17-β-estradiol-3-benzoate/0.2ml sesame oil 48 and 24hrs before testing and one sc injection of 1mg progesterone/0.2ml sesame oil 4hrs before testing. All subjects were observed for the display of male-typic sex behavior over a 20min session, with the introduction of the stimulus female defining the onset of testing. Pairing of the test subjects with stimulus
females was random and during each session, a novel stimulus female was presented to the test animal at 10min. The observer was blind to treatment group until after the third trial. Frequency of mounts and intromissions, and latencies to first mount, intromission and ejaculation were measured for each 20min bout. Mounting was distinguished as the placement of the test subjects forelimbs on the female’s flanks inducing the typical lordosis behavior in the stimulus female. Since penile insertion was not directly visualized in males, and impossible to test in females, we characterized an intromission as a mount followed immediately by rhythmic pelvic movements. Ejaculation was characterized as an intromission followed by a deep pelvic thrust, slow dismounting behavior and a prolonged refractory period.

**Radioimmunoassays.** Briefly, 50µl of standard, testosterone control or sample, 500µl of testosterone $^{125}$I reagent, and 100µl of testosterone antiserum was added to a tube, vortexed and incubated at 37°C for 60min. For nonspecific counts, 150µl of testosterone calibrator substituted for the sample and antiserum. To these tubes, 1ml of the precipitating reagent was added, the tube vortexed and incubated at room temperature for 15min. The mixtures were then centrifuged at 1500g for 20min, decanted, and the tubes counted in a gamma counter for one minute. Similar methods were used to obtain total counts. Standard curves were generated allowing for steroid level determination. The sensitivity of the assay was 15ng/dl and the intra- and interassay coefficients of variation were below 6.5% and 14.5%, respectively.

**Western immunoblots.** Western analysis was utilized to quantify COX-2 and spinophilin protein in neonatal and adult tissue, respectively. Neonatal brains were collected on PN2, approximately 24 hours after the second of two daily hormonal
treatments with the first within 6 hours of birth. Both males and females received sc injections of 0.1cc sesame oil and another group of females received sc injections of 100µg E₂ in 0.1cc sesame oil (n=4/group). For measurement of spinophilin by Western blot, adult brains were removed two days subsequent to the completion of behavioral testing. The preoptic area (POA) and rostral hippocampal formation (including the rostral portions of the differentiating, CA1 and CA3 fields, as well as the dentate gyrus) were dissected using the guidelines described below. Briefly, the optic chiasm appears near the rostral portion of the diencephalon and was used as a landmark to guide the rostral-to-caudal boundaries. The POA was then dissected from the section (1mm thick in neonates, 2mm in adults) using the perimeter of the anterior commissure for both the dorsal and lateral incisions. For adult tissue, another 2mm slice was taken immediately posterior to the section containing the POA. This segment contained the rostral portion of the hippocampal formation which was dissected using the lateral ventricle to delineate the lateral limits, the corpus callosum the dorsal limit and the dorsal portion of the third ventricle the ventral limit. These blocks of tissue were immediately flash frozen in isopentane and stored at -70°C until being homogenized in 400µl lysis buffer containing 0.8% Tris-HCl, 0.9% NaCl, 0.5% Triton X-100 (Sigma), 1mM phenyl methyl sulfonyl fluoride and peptidases including leupeptin, aprotinin, and pepstatin (1µg/ml) at 4°C. The homogenate was then subjected to a Bradford protein assay to determine and standardize protein levels in subsequent Western analyses. Protein (10µg) was electrophoresed in separate lanes on an 8-16% precast SDS-polyacrylamide gel (Novex, San Diego, CA) and transferred to a polyvinyl difluoride membrane (BioRad, Hercules, CA). Membranes for spinophilin experiments were blocked in 5% non-fat milk
in 0.1% tween TBS (M-TTBS) for one hour at room temperature. They were then incubated in anti-COX-2 goat polyclonal IgG (Santa Cruz Biotechnology, CA) at 2µg/ml in M-TTBS or anti-spinophilin/neurabin II rabbit polyclonal IgG (Upstate Biotechnology) at 1µg/ml in M-TTBS for three hours at room temperature. A 30 minute incubation of a rabbit anti-goat or goat anti-rabbit HRP (New England BioLabs, Beverly, MA) conjugated IgG at a 1:5,000 dilution followed.

**Culture conditions.** Tissue blocks were placed in HBSS+ (Hanks balanced salt solution, Hepes Buffer, anitbiotic/antimycotic (10,000 U penicillin G sodium, 10,000µg streptomycin sulfate, 25µg Amphotericin B; Life Technologies, Inc., Grand Island, NY) and 0.25% trypsin. After 15min at 37°C, 1ml of 10% dextran-treated charcoal-stripped fetal bovine serum (Life Technologies, Inc.) and 0.5ml antibiotic/antimycotic [FBS+] was added to deactivate the trypsin. The solution was dissociated by mechanical trituration and centrifuged at 1000rpm for 5min, the supernatant discarded, and the pellet resuspended in phenol red-free, sterile culture medium (SCM) [DMEM-F12 (Life Technologies, Inc.) and FBS+]. Density was established and cells were seeded onto poly-L-lysine (MW 70,000; Sigma, St. Louis, MO) coated glass coverslips (#1, 25mm diameter; Fischer Scientific, Pittsburgh, PA) at a density of 150,000cells/100µl medium.

**Immunocytochemistry for spinophilin.** Coverslips with adhered cells were immersed in 4% paraformaldehyde and 5% sucrose in 0.1M phosphate buffered saline (PBS; ph 7.5) warmed to 37°C for 10 minutes, washed three times in PBS, and permeabilized with 50% ETOH for 60min at 4°C. The cells were then washed three times in PBS and incubated in 10% normal goat serum in 0.4% Triton X-100 PBS (PBST) for 60min at room temperature with agitation. After blocking, a rabbit polyclonal
anti-spinophilin primary antibody (Upstate) [1:8000] was applied in 10% normal goat serum in PBST, and the cultures were incubated overnight at 4°C. The coverslips were washed three times in PBST and incubated in biotinylated goat anti-rabbit secondary antibody (Vector Laboratories, Burlingame, CA) [1:500] for 60min at room temperature with agitation, followed by three washes in PBST. The cultures were then incubated with an avidin-biotin horseradish-peroxidase complex (Vectastain ABC, Elite Kit; Vector Laboratories) for 60min at room temperature, washed twice with PBST, and visualized with 0.05% 3,3′-diaminobenzidine tetrahydrochloride (Polysciences, Warrington, PA) and 0.005% H2O2. After visualization, the coverslips were mounted on 2% gelatin-coated glass slides.

Golgi-Cox impregnation. Briefly, neonates were transcardially perfused with 0.9% saline and their brains removed and placed in 30ml of Golgi-Cox Solution (1:1 solution of 5% K2Cr2O7 and 5% HgCl2, which is then added to 5% K2CrO4 in a 2:5 ratio). After six days of impregnation, brains were transferred to the cryoprotectant solution from an FD Rapid GolgiStain Kit (FD NeuroTechnologies, Ellicott City, MD) and stored at 4°C for 48 hours. The brains were then cut into 100µm section on a cryostat at -20°C and mounted on 2% gelatin-subbed slides. The sections were allowed to dry and were then stained with Rapid GolgiStain solutions D and E for 10min. Tissue was rinsed, dehydrated, and cleared before coverslipping with Permount.

Quantitative PCR. Real-time quantitative PCR was performed using the DNA Engine Opticon continuous fluorescence detection system (MJ Research, Waltham, MA) according to the manufacture’s protocol. In this apparatus, PCR occurs in thin wall polypropylene sample blocks consisting of 96 V-shaped wells allowing optimal thermal
transfer and recovery. The DyNAmo qPCR kit (MJ Research) was utilized for the reactions and includes both a highly specific double-stranded (ds) DNA-binding dye, SYBR green I and a modified, very sensitive *Thermus brockianus* DNA polymerase. The fluorescence emitted by SYBR green greatly enhances when bound to the minor groove of dsDNS, thus during the cycles of PCR, various intensities of fluorescent signals can be detected dependent on the amount of dsDNA present. At the completion of each cycle, samples were excited (470nm) and fluorescence recorded (530nm). Each 30µl sample consisted of 15µl DyNAmo master mix, 0.3µM forward and reverse primer and 12µl template. Specific primers were as follows: cyclooxygenase-2 (GenBank accession number S67722), forward primer position 534 and reverse primer position 952 and prostaglandin-E synthase (GenBank accession number AB041998), forward primer number 379 and reverse primer number 674. Primers were developed using Oligo software (Molecular Biology Insights, Cascade, CO) and synthesized by GibcoBRL (Rockville, MD) with standard purity. All samples were amplified to 40 cycles, more than 10 cycles beyond onset of the linear phase of fluorescence. Specifically, an initial denaturing step was done for 10 minutes at 95°C, followed by 40 cycles of a 95°C melting step for 10s, an annealing step at 57.5°C for 10sec, and a extension step at 72°C for 10sec. Fluorescence data was obtained at the end of each elongation step and the cycle repeated. Opticon Monitor (MJ Research) data acquisition and analysis software graphed the intensity of fluorescence for each sample as a function of the cycle time. The software plots a crossing-line intercept parallel to the x-axis at a point where the template amplification for all of the samples enters the logarithmic-linear phase of the curve. Therefore, samples with higher concentration of initial cDNA enter
the linear phase at a lower cycle time than those with less. To determine the absolute concentration of the target transcript, a dilution series of the cDNA generated from untreated POA and hippocampus was amplified simultaneously using qPCR, providing standard curves with slope of approximately -0.3. These data are reported as genomic equivalents, or the amount of DNA necessary to be present in a purified sample to guarantee that all genes will be present. The crossing-line intercept for each unknown sample was then compared with the standard curve to generate a quantitative comparison.