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Female mice with deletion of Type One 5α-reductase have reduced reproductive responding during proestrus and after hormone-priming

Carolyn J. Koonce[1,5,6] and **Cheryl A. Frye**[1,2,3,4,5,6]

[1]Department of Psychology, University at Albany-SUNY, Albany, NY

[2]Department of Biological Sciences, University at Albany-SUNY, Albany, NY

[3]The Centers for Neuroscience and Life Sciences Research; University at Albany-SUNY, Albany, NY

[4]Department of Chemistry, University of Alaska-Fairbanks, Fairbanks, AK

[5]Institute of Arctic Biology, University of Alaska-Fairbanks, Fairbanks, AK

[6]IDeA Network of Biomedical Excellence (INBRE), University of Alaska–Fairbanks, Fairbanks, Alaska

Abstract

Capacity to form progesterone (P_4)'s 5α-reduced metabolite, 5α-pregnan-3α-ol-20-one (3α,5α-THP; a.k.a. allopregnanolone) in the brain may be related to facilitation of lordosis among estrogen–primed (E_2) mice. We investigated this idea further by comparing effects of endogenous and exogenous progestogens in mice that are deficient in the Type One 5α-reductase enzyme (5α-reductase knockout mice; 5α-RKO), and their wildtype counterparts for sexual behavior. Comparisons were made following administration of progestogens that are expected to increase 3α,5α-THP or not. Sexual receptivity of 5α-RKO mice and their wildtype counterparts was examined when mice were naturally-cycling (Experiment 1); ovariectomized (OVX), E_2-primed (10 μg, subcutaneous; SC) and administered P_4 (0, 125, 250, or 500 μg SC; Experiment 2); and OVX, E_2-primed and administered P_4, medroxyprogesterone acetate (MPA, 4 mg/kg, SC, which does not convert to 3α,5α-THP) or 3α ,5α-THP (4 mg/kg, SC; Experiment 3). The percentage of mounts that elicited lordosis (lordosis quotient) or aggression/rejection behavior (aggression quotient), as well as the quality of lordosis (lordosis rating), was scored. Wildtype, but not 5α-RKO, mice in behavioral estrus demonstrated significantly greater lordosis quotients and lordosis ratings, but similar aggression quotients, compared to their diestrous counterparts. Among OVX

Corresponding Author: Cheryl Anne Frye, Ph.D. Director, Alaska INBRE Professor of Neuroscience Department of Chemistry University of Alaska-Fairbanks P.O. Box 757040 Fairbanks, AK 99775-7040 907-474-5492 (office) 518-322-8058 (cell phone) cafrye@alaska.edu.

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and E_2-primed mice, P_4 facilitated lordosis of wildtype, but not 5α-RKO, mice. MPA neither facilitated lordosis of wildtype, nor 5α-RKO mice. 3α,5α-THP administered to wildtype or 5α-RKO mice increased lordosis quotients and lordosis ratings and decreased aggression quotients. 3α,5α-THP levels in the midbrain, one brain region important for sexual behavior, were increased during behavioral estrus, with P_4 administered to WT, but not 5α-RKO mice, and 3α,5α-THP administered to WT and 5α-RKO mice. MPA did not increase 3α,5α-THP. Thus, deletion of Type One 5α-reductase among female mice may attenuate reproductive responding during the estrous cycle and after hormone-priming.

Keywords

allopregnanolone; medroxyprogesterone acetate; sexual behavior; midbrain; lordosis

1. Introduction

Reproductive behaviors of female rodents depend, in part, upon ovarian hormones, such as 17β-estradiol (E_2) and progesterone (P_4). Cyclic increases in E_2 followed by P_4 over the estrous cycle (behavioral estrus) are associated with lordosis behavior to sexually-relevant stimuli (Allen, 1922). Removal of the ovaries (ovariectomy; OVX), the primary endogenous source of E_2 and P_4, attenuates cyclic increases in these hormones and sexual receptivity of mice (Ring, 1944), which can be reinstated with sequential administration of E_2 and P_4 (Edwards, 1970; Ring, 1944). In general, low lordosis response levels are observed in OVX, E_2-primed mice. Administration of a P_4 regimen that produces circulating concentrations, akin to that observed during behavioral estrus (or proestrus), reliably reinstates sexual receptivity of OVX, E_2-primed mice commensurate to that which can be observed during behavioral estrus (Corpechot et al., 1997; Guttenberg, 1961; Frye and Vongher, 2001a). Thus, P_4 has important facilitatory effects on sexual receptivity of mice.

An important question is whether some of P_4's effects for reproduction occur via its neuroactive metabolites. Some effects of P_4 to facilitate sexual receptivity may be due to *de novo* production of P_4 in the brain, or its actions as a pro-hormone to form metabolites in the brain. Cells that express steroidogenic acute regulatory (StAR) protein also express $P450_{scc}$, a key enzyme involved in the production of P_4 and its metabolites, and may play a role in the hypothalamus to mediate sexual responses (King et al., 2002, 2004). As a pro-hormone, P_4 in the brain is metabolized by the 5α–reductase enzyme to form dihydroprogesterone, which is then converted by the 3α-hydroxysteroid dehydrogenase enzyme to 5α-pregnan-3α-ol-20-one (3α,5α-THP). Expression of these steroidogenic enzymes varies across brain regions, but is high in areas, such as the midbrain ventral tegmental area (VTA). Greater 5α-reductase activity has been demonstrated in the midbrain versus preoptic area of the hypothalamus, hippocampus and cortex of mice (Roselli and Snipes, 1984), and a similar pattern of high 5α-reductase in the midbrain of rats has been reported (Li et al., 1997). The midbrain VTA has been our focus for the non-genomic, rapid effects of P_4 and 3α,5α-THP for lordosis of mice (Frye and Vongher, 1999) and other rodent species (reviewed in Frye, 2011). Lordosis responses of sexually-naïve mice correlate more directly with 3α,5α-THP levels in the midbrain than with P_4 levels (Frye and Vongher, 2001a). Administration of

pharmacological agents that inhibit 5α-reductase to OVX, E_2 and P_4-primed mice or rats attenuates 3α,5α-THP formation in the midbrain and lordosis (Frye and Vongher, 1999, 2001b; Frye et al., 2004, 2008; Petralia et al., 2005). These findings suggest that metabolism to 3α,5α-THP is important for lordosis, and that the midbrain may be one central nervous system target for these effects. The importance of this distinction is related to the well-characterized differences in the mechanisms of action of P_4 and 3α ,5α -THP in the midbrain and beyond (e.g. hypothalamus) for reproductive behavior of rodents (which is beyond the scope of this work, but please see reviews on this topic; Caldwell, 2002; Delville, 1991; Frye, 2007; 2011).

One concern about using pharmacological techniques targeted against a basic regulatory factor, such as 5α-reductase, is that they can be non-specific, and endocrine factors other than those intended to be inhibited may be manipulated. In order to address the role of P_4's metabolism to 3α,5α-THP for lordosis, we have begun to examine the effects of P_4 when administered to mice that are knockouts for the Type One 5α-reductase gene. There are two characterized forms of the 5α-reductase enzyme, referred to as Type One and Type Two, which may play complementary roles in physiological processes. These isoforms have limited homology. The Type One enzyme is expressed throughout the body, being highly expressed in the liver and kidneys, tissues that catabolize steroids. There is a micromolar affinity of 5α-reductase Type One enzyme for steroid substrates (Normington and Russell, 1992). Type Two 5α-reductase is highly expressed in the reproductive tract and has a nanomolar affinity for steroid substrates (Normington and Russell, 1992). In most mammalian species, two different 5α-reductase genes encode for the Type One and Type Two 5α-reductase enzymes. People with mutations in the 5α-reductase Type Two gene are hermaphrodites with normal 5α-reduced androgen and estrogen ratios (Wilson et al., 1993; Milewich et al., 1995). This suggests that Type One 5α-reductase may compensate for the absence of the Type Two enzymes. In mice, very little 5α-reductase Type Two enzyme is normally expressed, compared to expression patterns of Type One enzyme (Mahendroo et al., 1996; Russell and Wilson, 1994; Normington and Russell, 1992). Mice with a deletion of the 5α-reductase Type One gene have been developed to explore the physiological role of 5α-reduced steroids. In the current study, we use this mouse model to examine the extent to which 5α-reduction of progestogens may play a critical role in mediating sexual receptivity of female mice. Sexual responding 5α-RKO mice and their wildtype counterparts were assessed when naturally cycling (Experiment 1), OVX, E_2-primed and administered different dosages of P_4 (0, 125, 250, or 500 μg subcutaneously; Experiment 2), and OVX, E_2-primed and administered P_4, medroxyprogesterone acetate (MPA, a synthetic progestin clinically used in hormone replacement therapies), or 3α,5α-THP (Experiment 3). Sexual responding was defined by the percentage of mounts by males in which females displayed lordosis (lordosis quotients) or aggression/rejection (aggression quotients) and qualitative ratings of the lordosis response (lordosis ratings). We hypothesized that if 5α-reduction of P_4 is essential for mediating sexual receptivity of mice, 5α-RKO mice will show decrements in progestogen-facilitated lordosis and 3α,5α-THP formation in the midbrain, compared to their wildtype counterparts. Although the circuitry involved in reproductive responding of rodents involves areas outside of the midbrain (e.g. hypothalamic regions), a focus in these studies was on the midbrain VTA, as supported by previous studies described above.

2. Methods and Materials

These methods, utilizing animal subjects, were pre-approved by the Institutional Animal Care and Use Committee at the University at Albany.

2.1 Animals and Housing

Subjects were adult (8-10 weeks old), female mice (N=271). Mice were group-housed (4-5 per cage) in polycarbonate cages (26 × 16 × 12 cm) in a temperature-controlled room (21 ± 1 °C) in the Laboratory Animal Care Facility. Mice were maintained on a 12/12-hour reversed light cycle (lights off at 8:00 am) with continuous access to Purina Mice Chow and tap water in their home cages. Three experiments using mouse subjects were completed. Mice in each experiment were only tested on one occasion.

2.2 Mouse Strain, and Genotyping

Male and female Type One 5α-RKO mice were originally developed at the University of Texas (Mahendroo et al., 1996) and are available as cryogenically preserved resources at Jackson Laboratories (Bar Harbor, ME), which is where we obtained breeder pairs for this study (heterozygotes and wildtypes). Female 5α-RKO mice that lack the Type One enzyme are fertile and have normal oogenesis, fertilization, implantation, and placental morphology, and have higher levels of E_2 than other strains of mice (Mahendroo et al., 1997). Previous studies have shown that the female 5α-RKO mice have normal 4–5 day estrous cycles and general locomotor activity (Koonce et al., 2012). However, the absence of the Type One enzyme causes some parturition defects, likely due to high E_2 levels, which result in smaller litter size and even fetal death (Mahendroo et al., 1997, 1996). In this study, subjects were wildtype (+/+) or homozygous (-/-) 5α-RKO knockout mice, congenic on C57BL/6 background and derived from heterozygous (+/-) breeder pairs, and from a colony maintained at the University at Albany in the Life Sciences Research Building. Of note, heterozygous (+/-) mice were not included as experimental subjects in this study because observations of their fertility and fecundity as breeders suggested little difference from wildtype controls as has been reported (Mahendroo et al 1997). Data from small groups of heterozygous mice in conditions in Experiments 1 (n=12) and 2 (n=16) confirmed this. Lordosis responding, as measured by lordosis quotients, was 0.0 (mean) + 0.0 (sem) in diestrous mice and 71.3+3.3 among proestrous mice. OVX, E_2-primed heterozygous mice administered different dosages of P_4 showed a similar pattern of effects as observed in wildtype mice in Experiment 2 (25.0+15.0 for 0 μg/vehicle, 61.1+16.7 for 125 μg, 68.9+8.9 for 250 μg, and 50.0+5.6 for 500 μg of P_4).

Typical polymerase chain reaction (PCR) methods, modified from Jackson Laboratory's published protocol, were utilized to determine the genotype of mice (Frye et al., 2004, Koonce et al., 2012; Sambrook et al., 1989). Genomic DNA was isolated from tails. Briefly, DNA was denatured at 94 °C for 3 min, followed by 35 cycles of amplification: 94 °C for 1.5 mins, 94 °C for 20 s, 55 °C for 30 s and a final primer extension step at 72 °C for 2 min. Specific primers used were: oIMR0044 (5′-gat tgg gaa gac aat agc agg cat gc-3′, which amplifies the mutant allele that is located in 3′ UTR of Neo), 0IMR0970 (5-cca gac acg aac ttc cac gct tct g-3′, which amplifies the heterozygote allele), and oIMR0971 (5′-atg gag ttg

gat gag ttg tgc-3′, which amplifies the wildtype allele). Primers were obtained from Integrated DNA Technologies (Coralville, IL).

2.3 Estrous Cycle

In Experiment 1, to determine the stage of the estrous cycle for each individual mouse, vaginal epithelium of experimental mice was obtained by lavage and examined under a light microscope daily between 0700 and 0900. After two weeks of regular, 4-5 day cycles, mice were tested when in proestrus/behavioral estrus or diestrus. Mice were considered in proestrus when their vaginal epithelium had characteristic nucleated cells, 4-5 days following the previous sample of this type. Mice in diestrus had heterogeneous cell types in their vaginal smears for two consecutive days and nucleated cells were absent.

2.4 Ovariectomy and Steroid Administration

For Experiments 2-3, adult mice were OVX under sodium pentobarbital anesthesia (80 mg/kg, IP or to effect). Seven to ten days later, mice were E_2-primed (0.09 mg/kg in 0.2cc oil), 44 hours before sex testing and then randomly-assigned to receive a regimen of P_4 (0, 125, 250 or 500 μg, Experiment 2) or administered P_4 (the most effective P_4 dosage identified in Experiment 2, 500 μg), MPA (synthetic progestin, 4 mg/kg; Sigma, Experiment 3), or 3α,5α-THP (4 mg/kg, Sigma, Experiment 3). Progestogens were administered 6 hours before behavioral testing. This hormone priming regimen was utilized to produce physiological levels of these steroids and to mimic estrous cycle increases in E_2 and progestogens during behavioral estrous.

2.5 Sexual behavior testing

Sexual behavioral testing was conducted per previously described methods (Frye and Vongher, 1999) in a round glass container, 20.5cm in diameter and 21.5 in depth, with 5 cm of sawdust bedding. Sexual behavior was assessed by pairing experimental, sexually-naive female mice with sexually-experienced male mice for 10 minutes or 10 mounts by the male, whichever occurred first. The percentage of mounts that elicited a lordosis response by a female (lordosis quotient, LQ) as well as the quality of lordosis (lordosis rating, LR) was scored. The quality of each lordosis response was rated as a 1 if the female allowed the male to mount, but she was not immobile; 2 if female was transiently immobile; and 3 if lordosis was maintained for more than three seconds. Aggression/rejection quotient (AQ) was also scored for each mouse. Aggressive/rejection behaviors in response to male approach included those classified as challenge behaviors (in face posturing, standing, kicking, fighting, and/or biting) and mild avoidance behaviors (kicking, jumping upward, and fleeing).

2.6 Tissue collection and dissection

Immediately after testing, mice were euthanized by cervical subluxation followed by decapitation. Whole brain and trunk blood were collected to measure steroid hormone levels in brain and plasma, and stored at −80°C until processed for radioimmunoassay. Midbrains were dissected from the whole brain. For dissection, each brain was positioned ventral side up on a clean glass beaker turned upside down and filled with ice so that the surface (and

brain) remained cold during dissection. The midbrain area dissected out was posterior to the cerebral aqueduct and above the pontine region, and the right and left lateral borders were approximately 1.5 mm from the midline of the brain (see Figure 1). Immediately after dissection, tissue sample weight was determined on a scale (in milligrams), and this wet weight measurement was utilized to approximate the protein levels in the midbrain (in pg/mg of tissue for E_2, and in ng/mg of tissue for P_4 and 3α,5α-THP).

2.7 Radioimmunoassay for steroid hormones

For each Experiment, separate radioimmunoassays were run for corticosterone in plasma, and E_2, P_4, and 3α,5α-THP in midbrain samples, using previously reported methods (Frye, Paris, and Rhodes, 2007, Frye and Vongher, 1999, Frye and Bayon, 1999). Standard curves were run in duplicate in each assay, and intra-assay variance determined for each and averaged. Data from the curves was compared across the three assays run for each steroid for each experiment to determine inter-assay variance. Both intra- and inter-assay variance for all steroids were low, less than 10%. The intra- and inter- assay variance for corticosterone was 0.04 and 0.05, respectively. The intra- and inter- assay variance for E_2 was 0.06 and 0.08, respectively. The intra- and inter- assay variance for P_4 was 0.02 and 0.05, respectively. The intra- and inter- assay variance for 3α,5α-THP was 0.04 and 0.05, respectively. Specific details about extraction of steroids, radioactive probes and antibodies utilized, as well as data analyses are as follows.

2.7.1 Extraction of steroids from samples—Corticosterone was extracted by heating the plasma samples at 60°C for 30 minutes. For E_2, P_4, and 3α,5α-THP extraction from midbrain samples, samples were first homogenized in 50% methanol (MeOH) and 1% acetic acid with six strokes of a glass/glass homogenizer. Homogenized tissue samples had 800 cpms of tritiated steroid added to the tubes and then they were centrifuged at 3,000 x g at 4 °C. Chromatography was performed on the supernatant using Sep-Pak C18 cartridges (Waters Corp., Milford, MA, USA), which had been equilibrated with 50% MeOH, 1% acetic acid. Steroids were extracted with increasing concentrations of MeOH (50% MeOH followed by 100% MeOH). Solvents were then removed from the test tubes by using a speed drier (Savant). Samples were reconstituted in 350 μl assay buffer.

2.7.2 Radioactive Probes and Antibodies—Tritiated probes were purchased from PerkinElmer (Boston, MA): [^{3}H]corticosterone (NET 182: specific activity = 48.2), [^{3}H]E_2 (NET-317: specific activity = 51.3 Ci/mmol), [^{3}H]P_4 (NET-208: specific activity = 47.5 Ci/mmol), and [^{3}H]3α,5α-THP (NET-1047: specific activity = 65.0 Ci/mmol). The corticosterone antibody (#B3-163, Endocrine Sciences) was used at a 1:20,000 dilution. This antibody has low cross-reactivity with deoxycorticosterone (4%) and negligible (< 1%) cross-reactivity with cortisol, aldosterone, and P4 (McCormick et al., 2005). This antibody generally binds 40-60% of [^{3}H]corticosterone and bound 52% for Experiment 1, 37% for Experiment 2, and 48% for Experiment 3. The E_2 antibody (E#244, Dr. G.D. Niswender, Colorado State University, Fort Collins, CO) was used at a 1:40,000 dilution and has negligible (<1%) cross-reactivity with other estrogens (estrone, 17α-E_2) or progestogens (P_4, 17-hydroxyprogesterone; England et al., 1974) This E_2 antibody generally binds 40%-60% of [^{3}H]E_2 and bound 50% for Experiment 1, 46% for Experiment 2, and 56% for

Experiment 3. The P_4 antibody (P#337 from Dr. G.D. Niswender) was used at a 1:30,000 dilution and This antibody has very low (<4%) cross-reactivity with dihydroprogesterone and 3α,5α-THP (Niswender et al., 1973). This antibody generally binds 30%-50% of [^{3}H]P_4 and bound 40% for Experiment 1, 35% for Experiment 2, and 48% for Experiment 3. The 3α,5α-THP (#921412-5) antibody was purchased from Dr. Robert Purdy (Veterans Medical Affairs, La Jolla, CA) and used at a 1:5000 dilution, with cross-reactivity with 3α-hydroxypregn-4en-20-one (84%), but very low cross-reactivity with dihydroprogesterone (11%), 3β,5β-THP (7%), P_4 (6%), and pregnenolone (< 2%; Finn and Gee, 1994; Purdy et al., 1990). This antibody generally binds 40-60% of [^{3}H]3α,5α-THP and bound 58% for Experiment 1, 45% for Experiment 2, and 52% for Experiment 3.

2.7.3 Set-up and Incubation of Radioimmunoassays—All set-up of assays occurred with antibodies, tritiated probes, and samples on ice baths, and using disposable 12 × 75 borosilicate glass test tubes. Tubes containing the standard curves for each assay were prepared. The standard curve ranged from 0–1000 pg for E_2, with the minimum detectable level of 12.5 pg. The standard curve ranged from 0–4 ng for corticosterone, with the minimum detectable level of 0.01 ng. The standard curve for the P_4 assays ranged from 0-8000pg, with the minimum detectable level of 50 pg. The standard curve for the 3α,5α-THP assays ranged from 0-8000 pg, with the minimum detectable level of 100 pg for 3α,5α-THP. Standards were run in duplicate and added to assay buffer followed by addition of the appropriate antibody and [^{3}H]steroid. Samples were run in singlicate and 100 µl of sample were added to test tubes and containing assay buffer, and then appropriate antibody and [^{3}H]steroid were added. Tubes were vortexed immediately before incubation. Total assay volumes were 950 µl for corticosterone, 800 µl for E_2, 800 µl for P_4, and 950 µl for 3α,5α-THP. Corticosterone and E_2 assays were incubated for 60 mins at room temperature and P_4 and 3α,5α-THP assays were incubated overnight at 4 °C. Following incubation, dextran-coated, ice cold charcoal buffer was rapidly added to assay tubes in order to separate bound and free steroid. Samples were incubated with charcoal buffer for 30 minutes. Following incubation with charcoal, samples were centrifuged at 3000 x g for 10 minutes and the supernatant was decanted into a glass scintillation vial with 5 ml scintillation cocktail (Scintiverse BD, Fisher Scientific). Steroid concentrations were calculated using the logit-log method of Rodbard and Hutt, interpolation of standards, and correction for recovery with the Macintosh version of AssayZap (Biosoft, Cambridge, UK). Genotype and hormone condition did not influence corticosterone levels. All levels were basal and less than 2 µg/dl (data not shown).

4.0 Statistical Analyses

Two-way ANOVAs were used to determine effects of hormonal status and genotype (wildtype vs. 5α-RKO). When the α level for statistical significance reached $p \leq 0.05$, or a trend was observed $p \leq 0.10$, Fisher's *post-hoc* test were used to examine group differences. Power analyses for ANOVA were performed on pilot data and informed by previous investigations comparing steroid target knockouts and different progestogen administration to ovariectomized mice for reproductive responding. For these, lordosis quotients were the measure utilized. The minimum number of mice needed per condition based on predicted effect size, power of 80% or greater, and p value of 0.05 ranged from 10-18 for reliable

statistical analyses and interpretation of data from Experiments 1-3. Additionally, multiple regression were run for each experiment to determine the relationship between behavioral measures and steroids levels analyzed; there was no significant effect observed.

3. Results

3.1 Experiment 1: Proestrous, wildtype mice, had higher LQs, LRs, and lower AQs, and higher P_4 and 3α,5α-THP levels, than did their diestrous counterparts or 5α-RKO mice

For LQs, there was a significant interaction between estrous phase and genotype for LQs ($F [1, 48] = 6.120$, $P < 0.02$). Wildtype, but not 5α-RKO, proestrous mice had higher LQs than did their diestrous counterparts. There was also a significant main effect of estrous phase ($F [1, 48] = 15.1$, $P < 0.01$). As expected, mice in proestrus had higher LQs than did mice in diestrus. There was no main effect of genotype on LQs. See Figure 2.

For LRs, there was a significant main effect of estrous phase ($F [1,48] = 5.0$, $P < 0.03$). Mice in proestrus, compared to diestrus, had higher LRs. There was neither a main effect of genotype, nor an interaction between estrous phase and genotype. See Table 1 (top).

For AQs, there was a significant interaction between estrous phase and genotype ($F [1,48] = 8.8$, $P < 0.01$). Proestrous wildtype, but not 5α-RKO, mice had lower AQs than did their diestrous counterparts. There was a significant main effect of estrous phase ($F [1,48) = 4.2$, $P < 0.04$). Proestrous mice had lower AQs than did diestrous mice. There was no main effect of genotype. See Table 1 (top).

For midbrain 3α,5α-THP levels, there was a significant main effect of estrous phase ($F [1,42] = 8.1$, $P < 0.01$). Proestrous mice had significantly higher midbrain 3α,5α-THP levels than did diestrous mice. There was neither a main effect of genotype, nor an interaction between genotype and estrous phase.

For midbrain P_4, there was a tendency for an interaction between estrous phase and genotype ($F [1,42] = 3.09$, $P < 0.08$), with wildtype mice in proestrus having higher P_4 levels compared to 5α-RKO mice. there was a significant main effect of estrous phase ($F [1,42] = 32.5$, $P < 0.01$). Mice in proestrus had significantly higher midbrain P_4 levels than did mice in diestrus. There was a significant main effect of genotype ($F [1,42] = 14.1$, $P < 0.01$). Wildtype mice had significantly higher midbrain P_4 levels compared to 5α-RKO mice. See Table 1 (bottom).

For midbrain E_2 levels, there was a significant main effect of estrous phase ($F [1,42] = 4.1$, $P < 0.05$). Proestrous mice had significantly higher midbrain E_2 levels compared to diestrous mice. There was a significant main effect of genotype ($F [1,42] = 6.6$, $P < 0.01$) on midbrain E_2 levels. WT mice had lower midbrain E_2 levels than did 5α-RKO mice. There was no interaction between estrous phase and genotype. See Table 1 (bottom).

3.2 Experiment 2: Progesterone facilitated lordosis, inhibited aggression/rejection behaviors, and increased P_4 and 3α,5α-THP levels among wildtype, but not 5α-RKO mice

For LQs, there was a significant main effect of P_4 condition ($F [3,100] = 4.8$, $P < 0.01$). All dosages of progesterone (125 < 250 < 500 μg) increased LQs compared to vehicle. There was a significant main effect of genotype ($F [1,100] = 5.7$, $P < 0.01$) for LQs. Wildtype mice had higher LQs than did 5α-RKO mice. There was no interaction between genotype and P_4 condition for LQs. See Figure 3.

For LRs, there was a significant main effect of genotype ($F [1,100] = 6.0$, $P < 0.01$). Wildtype mice had higher LRs than did 5α-RKO mice. There was neither a main effect of P_4, nor an interaction between P_4 condition and genotype, for LRs. See Table 2 (top).

For AQs, there was a significant main effect of P_4 condition ($F [3,100] = 9.2$, $P < 0.05$). Progesterone lowered AQs compared to vehicle. There was a significant main effect of genotype ($F [1,100] = 5.1$, $P < 0.02$) for AQs. Wildtype mice had lower AQs than did 5α-RKO mice. There was no interaction between P_4 condition and genotype. See Table 2 (top).

For midbrain 3α,5α-THP levels, there was a significant interaction of P_4 condition and genotype ($F [3,74] = 8.1$, $P < 0.02$). When administered 500 μg of P_4, wildtype mice had higher 3α,5α-THP levels than did 5α-RKO mice. There was a significant main effect of P_4 condition ($F [3,74] = 5.2$, $P < 0.01$). Levels of 3α,5α-THP were higher among those administered P_4 compared to vehicle. There was a significant main effect of genotype for midbrain 3α,5α-THP levels ($F [1,74] = 19.7$, $P < 0.01$), wherein wildtype mice had higher levels compared to 5α-RKO mice. See Table 2 (bottom).

For midbrain P_4 levels, there was a significant interaction between P_4 condition and genotype ($F [3,74] = 2.5$, $P < 0.05$). When administered 250 or 500 μg of P_4, compared to vehicle, wildtype mice had higher P_4 levels than did 5α-RKO mice. There was a significant main effect of P_4 condition ($F [3,74] = 10.8$, $P < 0.01$). Progesterone levels were higher among mice administered P_4 compared to vehicle. There was no main effect of genotype for midbrain P_4 levels. See Table 2 (bottom).

For midbrain E_2 levels, there was a significant main effect of hormone condition ($F [3,74] = 2.7$, $P < 0.05$). Progesterone administration decreased midbrain E_2 levels compared to vehicle. There was neither a main effect of genotype, nor an interaction between genotype and P_4 condition for E_2 levels. See Table 2 (bottom).

3.3 Experiment 3: 3α,5α-THP increases LQs, LRs and midbrain 3α,5α-THP, and decreases AQs, of wildtype and 5α-RKO mice

For LQs, there was a significant interaction between progestogen condition and genotype ($F [3,123] = 17.7$, $P <0.01$) for LQs. Both wildtype and 5α-RKO mice had increased LQs with administration of 3α,5α-THP; however, only wildtype mice had increased LQs with P_4 administration. Neither wildtype, nor 5α-RKO, mice had LQs that differed from vehicle following MPA administration. There was a significant main effect of progestogen condition ($F [3,123] = 21.0$, $P < 0.01$). Progesterone and 3α,5α-THP increased LQs compared to

vehicle administration. There was a significant main effect of genotype ($F [1,123] = 4.2$, $P < 0.04$). Wildtype mice had higher LQs than did 5α-RKO mice. See Figure 4.

For LRs, there was a significant interaction between progestogen condition and genotype ($F [3,123] = 12.4$, $P < 0.01$) for LRs. Both wildtype and 5α-RKO mice had increased LRs with 3α,5α-THP administration; however, only wildtype mice had increased LRs with P_4 administration. Neither wildtype nor 5α-RKO mice had increased LRs with MPA administration. there was a significant main effect of progestogen condition ($F [3,123] = 13.9$, $P < 0.01$). Progesterone and 3α,5α-THP administration increased LRs compared to vehicle administration. There was no main effect of genotype. See Table 3, top.

For AQs, there was a significant interaction between progestogen condition and genotype ($F [3,123] = 7.2$, $P < 0.01$). Both wildtype and 5α-RKO mice had decreased AQs with 3α,5α-THP administration; however, only wildtype mice had decreased AQ with P_4 administration. There was a significant main effect of progestogen condition ($F [3,123] = 10.4$, $P < 0.01$). Progesterone and 3α,5α-THP administration decreased AQs compared to vehicle administration. Neither wildtype nor 5α-RKO mice had decreased AQs with MPA administration. There was no main effect of genotype for AQs. See Table 3 (top).

For midbrain 3α,5α-THP levels, there was a significant main effect of progestogen condition ($F [3,93] = 8.2$ $P < 0.01$). Progesterone and 3α-5α-THP administration increased 3α,5α-THP levels compared to vehicle administration. There was a significant main effect of genotype ($F [1, 93] = 16.0$ $P < 0.01$) on midbrain 3α-5α-THP levels. Wildtype mice overall had higher levels of midbrain 3α,5α-THP. There was no interaction between progestogen condition and genotype for 3α,5α-THP levels in the midbrain. See Table 3 (bottom).

For midbrain P_4, there was a significant interaction between progestogen condition and genotype ($F [3,93] = 3.5$ $P < 0.01$) for P_4 levels in the midbrain. Wildtype mice had higher levels of P_4 in the midbrain with administered P_4 or 3α,5α-THP, compared to 5α-RKO mice. There was no main effect of progestogen condition. There was a tendency for a genotype effect ($F [1,93] = 3.1$ $P < 0.08$) for P_4 levels, with wildtype mice having higher levels than 5α-RKO mice. See Table 3 (bottom).

For E_2 levels in midbrain, there was a tendency for an interaction between progestogen condition and genotype ($F [3,93] = 2.5$, $P < 0.06$) for E_2 levels in the midbrain. 5α-RKO mice, but not wildtype mice, administered 3α,5α-THP tended to have higher levels of E_2. There was a significant main effect of progestogen condition ($F [3,93] = 2.9$, $P < 0.03$). Mice administered 3α,5α-THP had higher levels of E_2. There was a significant main effect of genotype ($F [1,93] = 3.9$, $P < 0.05$). Wildtype mice had higher midbrain E_2 levels in than did 5α-RKO mice. See Table 3 (bottom).

4. Discussion

Our hypothesis that mice deficient in 5α-reductase would have decrements in progestogen-facilitated reproductive responding, related to capacity to produce 3α,5α-THP in the midbrain, was partially supported. In support, wildtype, but not 5α-RKO, mice in proestrus

demonstrated significantly greater lordosis responding and lower aggression towards a male than did their diestrous counterparts. When mice were OVX and E_2-primed, P_4 facilitated lordosis of wildtype more so than 5α-RKO, mice; yet, both genotypes responded to 3α,5α-THP, but not MPA, administration with increased lordosis and decreased aggression responding. The hormone measurements in the midbrain are the results that do not fit particularly well with the hypothesis across all experiments. Over the estrous cycle, wildtype and 5α-RKO mice both had higher 3α,5α-THP levels in the midbrain, and wildtype mice in proestrous had higher P_4, but lower E_2, levels in the midbrain compared to the 5α-RKO mice. When mice were OVX and E_2-primed, and administered different dosages of P_4 in experiment 2, there was a pattern for P4 or 3α,5α-THP levels of wildtype mice to be increased more so than in 5α-RKO mice; a similar pattern was observed in Experiment 3 with P_4, but not 3α,5α-THP, levels following P_4 or 3α,5α- THP administration. In Experiment 3, 5α-RKO mice overall had lower 3α,5α-THP levels; however, 3α,5α-THP, and to a lesser extent P_4, administration increased midbrain 3α,5α-THP levels in wildtype and 5α-RKO mice. Thus, lifelong knockout of Type One 5α-reductase may influence reproductive responding of mice over the estrous cycle or with exogenous hormone replacement.

The present findings extend previous work investigating the mechanisms of steroids for effects of lordosis across the estrous cycle. Here, proestrous mice, irrespective of genotype, demonstrated significant increases in lordosis and similar effects of greater levels of 3α,5α-THP in the midbrain, compared to diestrous mice. There was a significant interaction between estrous phase and genotype, showing that wildtype, but not 5α-RKO, proestrous mice had significantly increased lordosis compared to their diestrous counterparts. This same pattern did not occur with midbrain 3α,5α-THP, which was not predicted given the role of 5α-reductase for the metabolism of ovarian sources of P_4. One interpretation of these effects is that they were due in part to increased levels of E_2 among the 5α-RKO mice compared to the wildtype mice. Other mouse models have shown that responsiveness to E_2 varies based with strain and genotype (Rissman et al., 1999; Dominguez-Salazar et al., 2004). In the present study, the 5α-RKO mice had significantly higher levels of midbrain E_2, and a more modest reduction in midbrain 3α,5α-THP levels than was expected. It is known that E_2 and P_4 co-vary, can facilitate lordosis, and are necessary for mice to show mating behavior (Christian, 1964). Whether this effect is mainly due to E_2 or to effects of 3α,5α-THP is not well-established. Steroidogenic enzymes necessary for the formation of brain progestogens can be increased by E_2 (Cheng and Karavolas, 1973; Frye and Vongher, 1999; Micevych et al., 2008). Moreover, studies in 5α-RKO mice have shown increased sensitivity and levels of E_2 during development (Mahendroo et al., 1997), which may account for the increase in lordosis behavior observed throughout the three experiments in response to natural E_2 and administration of E_2. Mice do not show a lordosis response without E_2-priming, so it is not possible to parse out effects of E_2 versus progestogens by having an OVX, placebo control group. Another consideration is that the sexually-naïve mice in this study had robust lordosis responses. It has been reported that sexually-naïve mice show a 25% lordosis response to male mounting, which increases over repeated testing sessions; yet, there are strain differences in the initial receptivity of sexually-naïve females (Thompson and Edwards 1971; Gorzalka and Whalen 1974; Frye and Vongher 2001a, Frye

et al., 2012), which is a factor to consider in the present study. Indeed, we have previously observed that among sexually-naïve mice, greater lordosis response during the first pairing with a male was associated with greater levels of progestogens in plasma and the brain (Frye and Vongher, 2001a). A consideration is that an even higher baseline would have facilitated the observation of stronger behavioral and endocrine effects. This may be particularly relevant given that the 5α-RKO mice have higher anxiety responding than do the wildtypes (Frye et al., 2004; Koonce and Frye, 2013), and one could argue that habituation would mitigate potential experiential confounds. However, based upon previous studies, we were interested in the response of the mice acutely, after a novel, socially-relevant experience of mating. 3α,5α-THP in the midbrain can be dynamically and rapidly changed with mating experience alone, as we have demonstrated in female rats (Frye, 2011). Multiple regressions to understand the relationship between steroids measured and behavioral responses in the present study did not suggest variability in steroids related to lordosis responding; however, clearer effects may have been observed in comparing mice with differential experience with mating. Thus, experiential effects and the role that E_2 may have as a pro-hormone for 3α, 5α-THP's effects in the midbrain on sexual responding of mice is of continued interest.

The present findings, which compared the effects of endogenous levels and different progestogen administration to wildtype and 5α-RKO Type One mice, extends the literature on the effects of pharmacological blockade of 3α,5α-THP synthesis in the brain for reproductive responding. Administration of P_4 was only effective in increasing lordosis and midbrain 3α,5α-THP levels of OVX, E_2-primed wildtype mice, suggesting 5α-reductase is necessary for a lordosis response. These results confirm previous reports, which indicate that inhibiting formation of 3α,5α-THP with pharmacological agents disrupts enhancement of lordosis. In support, both epostane (a P_4 biosynthesis inhibitor) and finasteride (a 5α-reductase inhibitor) reduce LQs of hormone-primed C57BL/6, C57BL/6X129 and PRKO mice when administered systemically (Frye and Vongher, 2001a). In the present study, administration of 3α,5α-THP to both wildtype and 5α-RKO mice decreased aggression/ rejection responses, and increased lordosis responses. This is important owing to the reported effects of progestogens to reduce aggression among mice (Davis and Marler, 2003; 2004), as well as showing effects beyond lordosis. These results also extend the findings of the role of 5α-reductase for testosterone-facilitated aggression in 5α-RKO mice and alterations in 5α-reductase and 3α,5α-THP with social isolation and aggression of mice (Agís-Balboa et al., 2004; Frye et al., 2002). This is also notable because it shows a normative response to this progestogen among mice with some deficiencies in its formation. As such, lower lordosis, or higher aggression, responding is not necessarily due to reduced response to 3α,5α-THP administration in the present study.

The present study adds to the literature on the effects of MPA for hormone-sensitive behaviors and brain hormone levels. We and others have demonstrated in rat and mouse models that systemic administration of MPA is associated with lower 3α-5α-THP levels, poorer responses in anxiety or learning tasks, and less neuroprotection following a brain insult among rats (Braden et al., 2010, Braden et al., 2011; Ciriza et al., 2006; Frye et al., 2010; 2013). However, MPA has been used successfully to facilitate female sexual receptivity in rats (Pazol et al., 2006). Here, we do not see that dosage of MPA utilized altered 3α,5α-THP in midbrain of mice. We have previously shown similar effects in other

brain regions, such as the hippocampus and frontal cortex of mice (Frye et al., 2013); yet, a consideration is that MPA does not unequivocally prevent the formation of 5α-reduced compounds, and may do so only in very high dosages (Jarrel, 1984; Penning et al., 1985). The present results add to this literature by examining sexual responding and showing that MPA neither increases midbrain 3α,5α-THP levels, nor the lordosis response of OVX, E_2-primed mice. In the present study, and related studies in mice (Frye et al., 2013; Koonce & Frye, 2013), we have only utilized a single dosage of MPA so it is not known whether there would be a different pattern of effects with other dosages. Indeed, there is evidence of dose-responsive effects of MPA for lordosis facilitation of rats, with the greatest effects occurring at the lower dosage (62 μg, as compared to 615or 1850 μg) utilized in this study (Pazol et al., 2006). Moreover, in addition to species and dosage effects, MPA has many other steroid targets to consider for its mechanisms of effects that may influence lordosis beyond actions solely through 3α,5α-THP.

Of interest are the brain targets associated with 3α,5α-THP's effects for reproductive behavior. The present study adds to the literature that progestogens have effects in the midbrain VTA for lordosis, as supported by estrous cycle comparisons across species (e.g. rats, mice, hamsters) and systemic and direct administration of progestogens to the midbrain VTA of OVX rodents (Frye, 2001; Frye and DeBold, 1993, Frye and Gardiner, 1996; Frye and Vongher, 2001b). These findings support previous work on the midbrain of female mice having greater 5α-reductase activity than other regions involved in reproduction (e.g. preoptic area of the hypothalamus) and affective behaviors supporting reproduction (e.g. the hippocampus and cortex) (Roselli and Snipes, 1984). A similar pattern has been demonstrated in rat brain, with high levels of 5α-reductase activity in the midbrain (Li et al., 1997). The present study extended these findings by demonstrating patterns of progestogen and E_2 levels in the midbrain of 5α- RKO mice and their wildtype counterparts over the estrous cycle and with hormone-priming. However, we did not directly examine other brain regions likely to be involved in reproductive responding. As well, there are a few results that suggest that there are regions beyond the midbrain involved for effects of 3α,5α-THP. For example, levels of 3α,5α-THP in the 5α-RKO and wildtype mice are comparable during both diestrous and proestrous. Similar results are also seen in Experiment 2. It may be that 3α,5α-THP is acting on other brain circuits, or is produced elsewhere and released into midbrain. **T**here is a precedent for these hormones also playing a role in sexual behavior mediated through the hypothalamus (King et al., 2002, 2004), which would be a target of interest in future studies. Of note, we have previously demonstrated a reduced capacity for 3α,5α-THP formation in the hippocampus and cortex of 5α-RKO mice with increased anxiety-like responding, compared to proestrous, wildtype controls or after hormone-priming (Frye & Koonce, 2013; Koonce et al., 2012). Although a focus herein was on the midbrain VTA, present studies substantiate further investigation of other potential regions involved in progestogen-facilitated lordosis of mice and the role of 5α-reductase.

Differences in progestogen levels between Experiment 2 and 3 among OVX mice administered vehicle or the highest dosing of P_4 need to be considered. Mice were tested in different cohorts for each experiment, and experiments were run serially. Although all efforts were made to minimize potential cohort differences (i.e. keeping age, duration in an

ovariectomized state, drug administration, time of day testing that took place, testing room constant), one explanation for differences across these experiments is that there may have been a developmental or epigenetic effect of noise exposures among mice raised in the new building that differed between generations (reviewed in Blaustein, 2012; Ho and Burggen 2010; Kappeler and Meaney, 2010). This could not be systemically examined in the present study; however, we have noted in other studies that such environmental exposures can alter behaviors of mice (Walf and Frye 2009). As such, it is impressive in the current study that robust differences in reproductive responding, albeit less so for measurements of 3α,5α-THP, were noted for responses to hormones in these different strains of mice, despite potential developmental and epigenetic effects to consider. There was no evidence of variability for behavioral measures assessed within each small cohort tested in each experiment. Of note, in running concurrent analyses of these steroids in mice in similar experiments, differences between experiments were not noted in other brain regions, such as the hippocampus or frontal cortex (Koonce and Frye 2013). It may be that the midbrain is differentially sensitive to environmental effects; this is an ongoing question in the laboratory that could not be directly assessed here, but is one consideration for differences in levels across these experiments.

Another major consideration in the present study is the role of potential developmental effects of the 5α-reductase knockout and even compensatory processes in the mutant mice that were utilized. It is well-recognized that 5α-reduction of androgens are critical for sexual differentiation of brain and body *in utero* and have activational effects for reproduction and other behaviors. We do not know the extent to which differences in responding are due to these differences in early development, or for the more confined effects on steroid metabolism at the time of testing in the adults in this study. To address this, it would be useful to compare conditional 5α-RKO mice, or the effects of pharmacological blockade of steroid targets in these mice as adults; however, these types of comparisons were beyond the scope of the present study. It must be noted that levels of 3α,5α-THP were very low in the 5α-reductase Type One knockout mice (similar to low physiological levels in diestrous wildtype mice), but they were not completely absent. This may be because there can be *de novo* synthesis of 3α,5α-THP in the brain itself, in addition to metabolism of circulating precursors, such as P_4. Indeed, it may be that these levels of 3α,5α-THP were produced via actions of 5α-reductase Type Two in the midbrain, and/or other regions in this circuit. Another consideration is that the behavioral effects of knockout of 5α-reductase Type One are less related to effects on production of 3α,5α-THP or even other steroids that can be derived in the periphery or brain (P_4, E_2), but may also relate to different rates of clearance of neuroactive steroids. There are differences in distribution of these enzymes throughout development and their affinity for precursor steroids (e.g. testosterone and progesterone). For instance, the 5α-reductase Type One enzyme demonstrates ubiquitous expression throughout the body, with very high expression in the liver and the brain throughout life (namely, regions such as the hypothalamus and midbrain and other structures rich in myelin), even in early development where its expression does not seem to be modified by androgens (Celotti et al., 1992; Melcangi et al., 1998; Poletti et al., 1998). Specifically, mouse studies have demonstrated that 5α-reductase Type One mRNA expression in the hypothalamus was not different across early developmental periods, with androgen

manipulations during these early periods, or between males and females (Karolczak et al., 1998). However, the Type Two enzyme has a more specific pattern of expression in androgen-dependent structures, like reproductive tissues (e.g. prostate) and the brain (reviewed in Poletti and Martini, 1999; Poletti et al., 1998). Particularly among males, this expression in the brain is modified by androgens and expression is highest during early developmental periods throughout the brain, but showing specific expression in adulthood in regions, such as the hypothalamus and hippocampus (reviewed in Poletti and Martini, 1999; Poletti et al., 1998). Among male rats, administration of testosterone or dihydrotestosterone increased mRNA expression of Type Two, but not Type One, 5α -reductase in the cortex (Torres and Ortega, 2003). Type One 5α-reductase has lower affinity for steroid substrates than does Type Two, supporting the notion that the Type One enzyme may be involved in catabolism of precursor steroids at myelin to mitigate any potential neurotoxic effects of these steroids when they are outside a low, physiological range. An example in support of this is the demonstrated high levels of fetal death in 5α-reductase Type One knockout mice due to high levels of E_2, aromatized from testosterone during development that cannot be reduced to dihydrotestosterone (Mahendroo et al., 1997). Alternatively, Type Two 5α-reductase may play a larger role when levels of steroids are particularly high (e.g. with stress and/or pregnancy), suggesting increased rates of P_4's conversion 3α,5α-THP, which is known to increase following environmental stressors (e.g. cold water swim, restraint) as well as social challenges (e.g. mating) and may mitigate stress responding (reviewed in Frye, 2007; Maguire and Mody, 2007; Sarkar et al., 2011). In support, mRNA and protein levels of 5α-reductase Type Two were increased in the prefrontal cortex of male and female rats following environmental stressor exposure (consisting of excessive heat, artificial light, and restraint); a decrease in 5α-reductase Type One was observed among females following stress (Sánchez et al., 2009). Progesterone administration to adult female mice, which would be expected to produce supraphysiological P_4 levels in the brain, increased Type Two enzyme expression in the hippocampus (Matsui et al., 2002). In the present study, the expression and activity of 5α-reductase Type Two enzymes are unknown. In light of these findings from the literature and the present study in mice developmentally exposed to stressors and the social challenge of mating, provide a compelling case that a possible compensatory mechanism in these mice may be upregulation of 5α-reductase Type Two. The role of 5α-reductase Type Two activity is an important question for future studies.

In conclusion, 5α-reductase Type One may be important for sexual responding of female mice. A focus in the present paper was reproductive responding and actions of 5α-reductase Type One in the midbrain; however, these factors may play a key role for social/ reproductive, cognitive, and affective behaviors in brain regions that were not examined here, such as the hypothalamus and hippocampus. Further understanding this neural circuitry is of continued interest.

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Highlights

- Proestrous mice had greater lordosis and midbrain 3α,5α-THP than diestrous mice
- Progestogens (P4 and 3α,5α-THP, not MPA) that increase 3α,5α-THP enhance lordosis
- There were deficits in lordosis and 3α,5α-THP of mice lacking 5α-reductase
- 5α-reduction of progestogens in the midbrain may be required for lordosis of mice

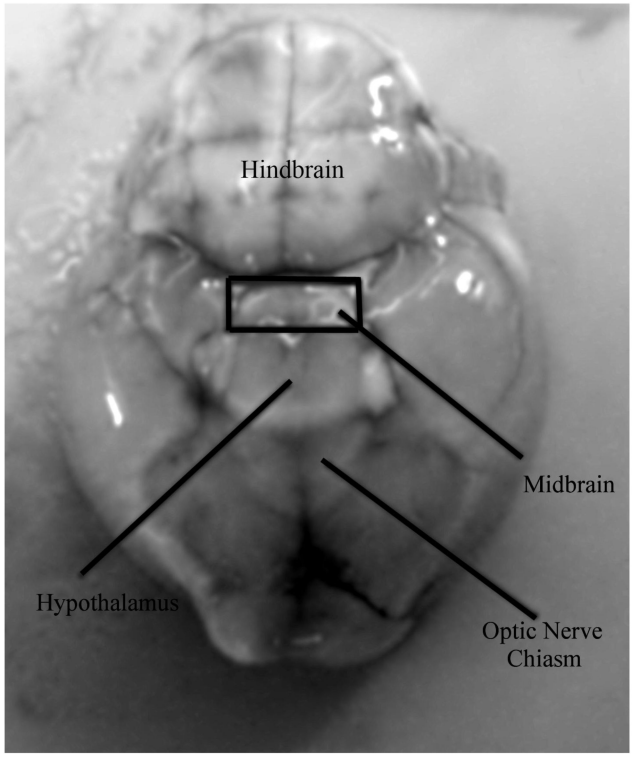

Figure 1.
Representative picture of ventral side of mouse brain with optic nerve, hypothalamus, and midbrain area that is dissected out indicated by a square.

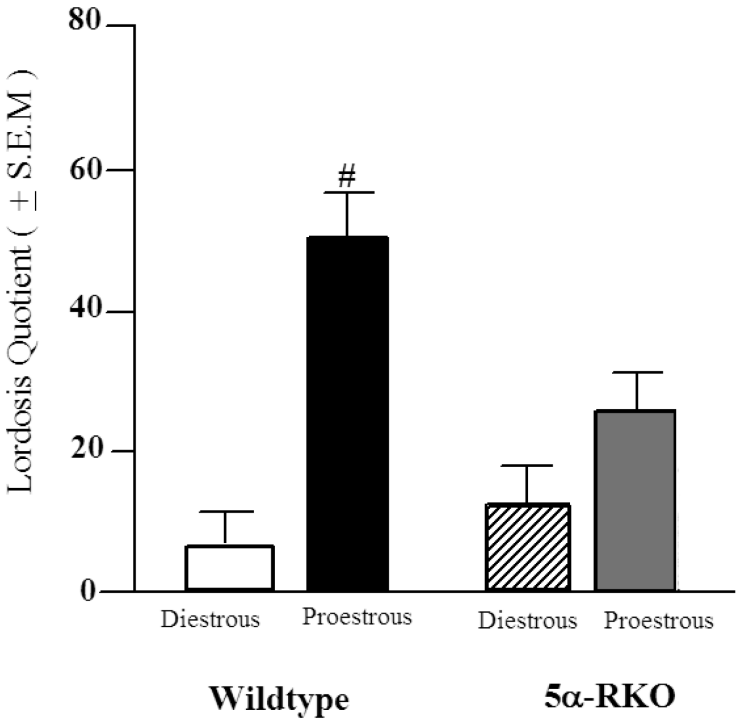

Figure 2.

Mean lordosis quotients (LQs) (+S.E.M.) of wildtype mice in diestrus (n=16) or proestrus (n= 10, left side) or 5α-RKO mice in diestrus (n=16) or proestrus (n=10, right side), Wildtype mice in proestrus had increased LQs compared to 5α-RKO mice and mice in diestrus. A significant interaction of genotype and condition is indicated by (#) above bar ($p < 0.05$).

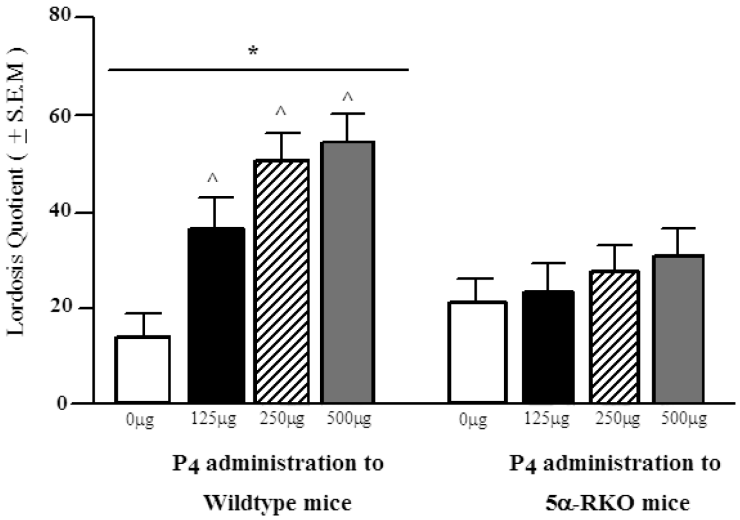

Figure 3.

Mean lordosis quotients (LQs) (+S.E.M.) of wildtype mice administered different concentrations of progesterone (P_4); 0μg (n=16), 125μg (n=11), 250μg (n=13), or 500μg (n=15, left side) or 5α-RKO mice administered different concentrations of progesterone (P_4); 0μg (n=16), 125μg (n=12), 250μg (n=10), or 500μg (n=15, right side), WT mice compared to 5α-RKO mice dose-dependently increased LQ's. A significant difference between genotype is indicated by (*) above line. A significant difference of progesterone condition is indicated by a (^) above bar ($p < 0.05$).

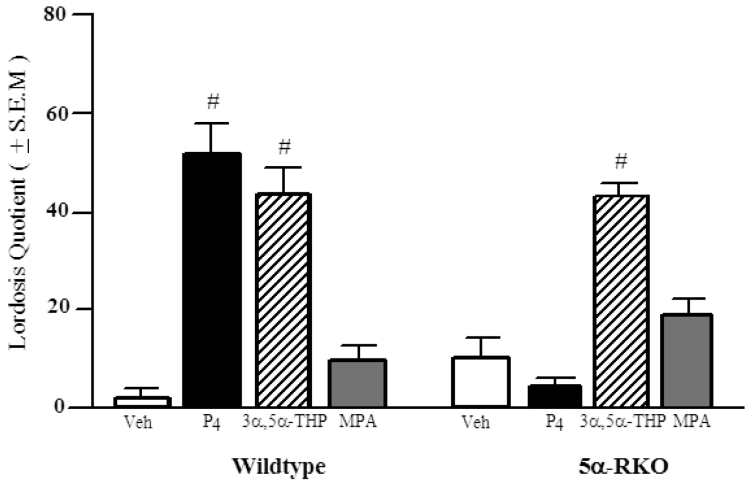

Figure 4.

Mean lordosis quotients (LQs) (+S.E.M.) of wildtype mice administered vehicle (veh, n=17), progesterone (P_4, 4 mg/kg, n=17), 3α,5α-THP (4 mg/kg, n=12), or medroxyprogesterone acetate (MPA 4 mg/kg, n=18, left side) or 5α-RKO mice administered vehicle (veh, n=16), progesterone (P_4, 4 mg/kg, n=8), 3α,5α-THP (4 mg/kg, n=125, or medroxyprogesterone acetate (MPA 4 mg/kg, n=18, right side). P_4 increased LQs of wildtype, but not 5α-RKO, mice and administration of 3α-5α-THP to both WT and 5α-RKO mice increased LQs. A significant interaction of genotype and condition is indicated by (#) above bar ($p < 0.05$).

Table 1

Experiment 1: Lordosis ratings (LR), aggression quotients (AQ), midbrain levels of 3α-5α-THP, progesterone (P_4), and estrogen (E_2) in wildtype and 5α-reductase knockout (5αRKO) mice for Experiment 1. A significant effect of cycle condition is indicated by (^), a significant effect of genotype is indicated by (*), and a significant interaction of genotype and condition is indicated by (#).

Genotype	**Wildtype**		**5α-RKO**	
Condition	**Diestrus**	**Proestrus**	**Diestrus**	**Proestrus**
N	n=16	n=10	n=16	n=10
LR (±s.e.m.)	0.3 ± 0.1	1.0± 0.3^	0.7± 0.2	0.9± 0.3^
AQ (±s.e.m.)	93.8± 4.1	51.0± 14.9#	80.9± 7.7	88.5± 6.9
Hormone Levels				
3α,5α-THP (ng/g)	2.0± 0.4	3.7± 0.3^	2.2 ± 0.4	2.9± 0.4^
P_4 (ng/g)	7.0± 1.2*	10.9± 0.7*^	1.5 ± 1.0#	8.9± 0.2^
E_2 (ng/g)	1.1 ± 0.4	1.4± 0.4^	1.8 ± 0.6*	3.9± 0.9#

Table 2

Experiment 2: Lordosis ratings (LR), aggression quotients (AQ), midbrain levels of 3α-5α-THP, progesterone (P_4), and estrogen (E_2) in wildtype and 5α-reductase knockout (5α-RKO) mice for Experiment 2. A significant effect of condition is indicated by (^), a significant effect of genotype is indicated by (*), and a significant interaction of genotype and condition is indicated by (#).

Genotype								
	Wildtype				**5α-RKO**			
Condition	**0 μg P_4**	**125 μg P_4**	**250 μg P_4**	**500 μg P_4**	**0 μg P_4**	**125 μg P_4**	**250 μg P_4**	**500 μg P_4**
N	n=16	n=11	n=13	n=15	n=16	n=12	n=10	n=15
LR (±s.e.m.)	0.9* ± 0.2	1.2* ± 0.2	1.2* ± 0.2	1.3* ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	1.0 ± 0.2
AQ (±s.e.m.)	98.1 ± 1.4	44.2^ ± 12.5	45.4^ ± 11.8	48.1^ ± 9.9	91.2 ± 6.5	68.4 ± 9.9	68.2 ± 12.6	67.6 ± 8.9
Hormone Levels								
3α,5α-THP (ng/g)	5.1*^ ± 0.3	4.5*^ ± 0.2	4.4*^ ± 0.3	6.0*^# ± 0.3	4.4 ± 0.3	3.6 ± 0.2	4.2 ± 0.2	4.1 ± 0.3
P_4 (ng/g)	0.7 ± 0.3	1.7^ ± 0.4	3.2^ ± 0.4	3.6^ ± 0.4	0.8 ± 0.2	2.2^ ± 0.3	2.2^ ± 0.5	2.1^ ± 0.6
E_2 (ng/g)	5.9 ± 1.2	2.7^ ± 1.2	4.2^ ± 0.6	4.5^ ± 0.7	5.5 ± 1.9	2.6^ ± 0.5	4.5^ ± 0.5	4.3^ ± 0.5

Table 3

Experiment 3: Lordosis ratings (LR), aggression quotients (AQ), midbrain levels of 3α-5α-THP, progesterone (P_4), and estrogen (E_2) in wildtype and 5α-reductase knockout (5αRKO) mice for Experiment 3. A significant effect of condition is indicated by (^), a significant effect of genotype is indicated by (*), and a significant interaction of genotype and condition is indicated by (#).

Genotype	**Wildtype**				**5α-RKO**			
Condition	**Veh**	**3α,5α-THP**	**P_4**	**MPA**	**Veh**	**3α,5α-THP**	**P_4**	**MPA**
N	n=17	n=12	n=17	n=18	n=16	n=15	n=18	n=18
LR (±s.e.m.)	0.2 ± 0.1	1.6^# ± 0.2	1.2^# ± 0.2	0.6 ± 0.2	0.6 ± 0.1	1.5^# ± 0.2	0.4 ± 0.1	0.9 ± 0.1
AQ (±s.e.m.)	98.8 ± 0.8	75.0^# ± 7.5	65.4^# ± 8.9	97.7 ± 2.2	97.3 ± 1.5	66.7^# ± 9.3	100.0 ± 0.0	93.3 ± 4.6
Hormone Levels								
3α,5α-THP (ng/g)	2.5 ± 0.2	4.2^ ± 0.4	3.8^ ± 0.3	3.3 ± 0.1	2.0 ± 0.3	3.4^ ± 0.3	2.3^ ± 0.4	2.4 ± 0.4
P_4 (ng/g)	1.9 ± 0.5	3.4# ± 0.5	2.9# ± 0.6	1.5 ± 0.7	1.6 ± 0.4	1.0 ± 0.1	2.2 ± 0.4	2.3 ± 0.3
E_2 (ng/g)	5.2* ± 0.8	4.9* ± 0.5	5.2* ± 0.7	4.6* ± 0.9	4.9^ ± 0.8	5.8^ ± 1.2	2.4 ± 0.7	1.9# ± 0.2