Published in final edited form as:

Behav Brain Res. 2012 January 15; 226(2): 376–380. doi:10.1016/j.bbr.2011.09.028.

Type 1 5 α -reductase may be required for estrous cycle changes in affective behaviors of female mice

C.J. Koonce¹, A.A. Walf⁴, and C.A. Frye^{1,2,3,4}

¹Dept Psychology, The University at Albany-SUNY, Albany, NY, 12222

²Dept Biology, The University at Albany-SUNY, Albany, NY, 12222

³Centers for Neuroscience, The University at Albany-SUNY, Albany, NY, 12222

⁴Life Sciences Research, The University at Albany-SUNY, Albany, NY, 12222

Abstract

There are estrous cycle differences in affective behaviors of rodents that are generally attributed to cyclic variations in estradiol, progesterone (P) and its metabolites. A question is the role of the steroid metabolism enzyme, 5 α -reductase, for these estrous cycle differences. To address the requirement of 5 α -reductase, estrous cycle variations in the behavior of wildtype mice and their littermates that are deficient in the 5 α -reductase type 1 enzyme (5 α RKO mice) were examined. The hypothesis was that if some of the estrous cycle differences in exploratory (open field) and anxiety (elevated plus maze) are due to P's 5 α -reduction to 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP), then wildtype mice will have estrous cycle differences in the expression of these behaviors, but 5 α RKO mice will not. Mice were tested in these tasks and then had plasma and brains collected so that steroid levels (estradiol, P, 3 α ,5 α -THP, corticosterone) could be measured in these tissues. Results supported this hypothesis. There were estrous cycle differences among wildtype, but not 5 α RKO, mice. Proestrous wildtype mice made more central entries in the open field and spent more time on the open arms of the plus maze, coincident with higher 3 α ,5 α -THP levels in plasma and brain regions important for these behaviors, such as the hippocampus and cortex, compared to their diestrous counterparts. Variability in the open field and elevated plus maze could be explained by circulating and hippocampus levels of 3 α ,5 α -THP, respectively. Thus, 5 α -reductase may be required for the estrous cycle variations in affective behavior and 3 α ,5 α -THP levels of female mice.

Keywords

allopregnanolone; anxiety; elevated plus maze; open field; hippocampus; cortex

Changes in hormones, such as estradiol (E₂) and progesterone (P), over the estrous cycle are associated with variations in exploratory and affective behaviors of mice. In support, when levels of E₂ and P are higher during the proestrous phase of the estrous cycle, mice exhibit

© 2011 Elsevier B.V. All rights reserved.

Address for correspondence: Cheryl A. Frye, Ph.D., Life Sciences Room 1058, The University at Albany – SUNY, Albany, NY 12222, 518-591-8839, cafrye@albany.edu.

The authors have no conflicts of interest to report.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

more exploratory and anti-anxiety behavior. Mice in estrus or proestrus, compared to diestrus, show increased exploration in the open field and greater anti-anxiety behavior in the elevated plus maze [1,2]. A question is what are some of the brain regions that are targets for these effects of ovarian steroids across the estrous cycle.

There is some indication that these effects may occur through actions of steroids in limbic structures, such as the hippocampus, and other brain regions, such as the cortex. In support, levels of P levels are increased in the hippocampus and cortex, concomitant with greater anti-anxiety behavior, among proestrous mice compared to diestrous mice [2]. Moreover, expression levels of the enzymes required to metabolize P to its neuroactive metabolites, dihydroprogesterone and 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP), are very high in the hippocampus and cortex [3,4]. Indeed, mice in proestrus have higher levels of 3 α ,5 α -THP in circulation, and in the hippocampus and cortex, coincident with sexual receptivity and greater exploration and anti-anxiety behaviors, compared to their diestrous counterparts [2,5,6]. Of interest is the extent to which the 5 α -reductase enzyme is required for these estrous cycle effects.

A question is whether 5 α -reduction is important for the effects of progestogens, such as P and 3 α ,5 α -THP, for exploration and anxiety. Finasteride is a pharmacological tool used to investigate the requirement of 5 α -reduction. There two forms of 5 α -reductase, referred as type 1 and type 2, which play a complementary role in physiological processes [7]. The type 1 enzyme is highly expressed in the liver and kidneys, tissues that catabolize steroids [7]. On the contrary, type 2 5 α -reductase enzyme plays an anabolic role and is highly express in the reproductive tract [8]. Finasteride predominantly inhibits the type 2 isozyme of 5 α -reductase [7,8]. Administration of finasteride to mice produces 3 α ,5 α -THP withdrawal with resulting increases in anxiety-like and depression-like behavior [9,10]. However, in mice, very little 5 α -reductase type 2 is normally expressed [7,8]. Thus, progestogens' effects for exploration, anxiety, and social responding may be due to actions of 3 α ,5 α -THP following 5 α -reduction, but this needs to be investigated in a non-pharmacological model.

In order to further address whether 5 α -reduction to 3 α ,5 α -THP underlies estrous cycle differences in exploration and anxiety, a mutant mouse model was utilized. Mice that are deficient in the 5 α -reductase enzyme type 1 enzyme (5 α RKO) lack the capacity to metabolize P to 3 α ,5 α -THP that their 5 α -reductase- replete wildtype littermates possess [11,12]. In addition to not being able to metabolize 3 α ,5 α -THP from P, 5 α RKO mice also have increased levels of E₂ during development [12]. We investigated estrous cycle differences by comparing wildtype and 5 α RKO mice in a test of exploration (open field) and anxiety (elevated plus maze), and measuring levels of E₂, P, 3 α ,5 α -THP and corticosterone in plasma and/or the hippocampus and cortex. We hypothesized if some of the estrous cycle differences noted are due to P's 5 α -reduction to 3 α -5 α -THP, then proestrous wildtype, but not 5 α RKO, mice will have higher 3 α ,5 α -THP levels, irrespective of E₂ levels, and more exploratory and anti-anxiety behavior than will diestrous mice.

These methods were pre-approved by the Institutional Animal Care and Use Committee at the University at Albany. Adult, gonadally-intact female (N=62) wildtype and 5 α RKO mice that were 8–10 weeks old were utilized in this study. Male and female 5 α RKO mice were developed at the University of Texas [11,12] and are cryogenically preserved at Jackson Laboratories (Bar Harbour, ME). Female 5 α RKO mice are fertile and have normal oogenesis, fertilization, implantation, and placental morphology, and are viable because the type 2 5 α -reductase enzyme, which is essential in development, is expressed. As well, mice in the present study cycled normally with 4–5 day long estrous cycles. However, parturition defects can occur and a smaller litter size is common. Wildtype (+/+; n=30) or 5 α RKO (–/–; n=32) mice utilized in the present study were congenic on C57Bl/6 background and derived

from heterozygous (+/-) breeder pairs and raised in the *vivarium* in the Life Sciences Research Building at University at Albany (original stock from Jackson Lab, Bar Harbor, ME). Mice were housed 3–5/cage, and maintained in a temperature-controlled (~ 21° C) room in The Laboratory Animal Care Facility, that was maintained on a reversed-lighting schedule (lights off at 0800 h). All mice had access to rodent chow and tap water available at all times in their home cages. Typical methods of polymerase chain reaction, modified from Jackson Laboratory's protocol, were utilized to determine the genotype of mice [13,14].

Each day, between 0700 and 0900, vaginal epithelium was obtained from mice by lavage and examined using light microscopy to determine the stage of the estrous cycle. After a mouse had two consecutive, regular, 4–5 day estrous cycles, it was randomly-assigned to be tested when in proestrus (epithelium characterized by nucleated cells, 4–5 days after the previous occurrence) or diestrus (heterogeneous cell types in vaginal epithelium for two consecutive days, which transpired 4–5 days after the previous 2-days of diestrus).

After determining estrous cycle phase, mice were tested in a short battery of two tasks, the open field and elevated plus maze. Each task was 5 minutes long and mice were tested consecutively by a trained observer who manually recorded the measures, described below, in this task, while a video-tracking system (Any-Maze, Stoelting, Wood Dale, IL) was simultaneously utilized. The video-tracking system recorded the behavioral measures that were programmed into it as well as made a digital recording of subjects while they were being tested.

Mice were placed in an open field (39 × 39 × 30 cm) for five minutes. During this time, the number of interruptions in horizontal light beams, an index of activity, were automatically recorded by the Digiscan Optical Animal Activity Monitor (Accuscan Instruments Inc., Columbus, Ohio). The open field has a grid floor so that the number of total (an index of general activity) and central (a measure of anti-anxiety behavior) squares entered could be determined. Methods were utilized as previously described [13,14].

Mice were tested in the elevated plus maze. The elevated plus maze contains four arms (2 open, 2 closed; 30 cm in length and 5 cm in width, Columbus Instruments, Inc., Columbus, OH.). After placement in the center of the maze, the time spent in the open arms during this five-minute test is recorded as an index of anti-anxiety behavior [13,15].

Immediately after testing, mice were euthanized by cervical subluxation. Whole brains and trunk blood were collected from each mouse to measure steroid hormone levels in plasma and brain regions. Hippocampus and cortex were dissected on ice immediately prior to steroid measurement.

Standard steroid extraction and radioimmunoassay techniques used by our laboratory to measure steroid levels in mice were employed and detailed elsewhere [2,16,17]. The methods for steroid extraction and the radioactive probes and antibodies are described as follows. Standard liquid scintillation techniques were utilized to measure radioactivity in samples and sample concentrations were determined as a function of standard curves run in each assay. The inter- and intra-assay reliability coefficients were: E₂ (0.06 and 0.08), P (0.08 and 0.10), and 3 α ,5 α -THP (0.09 and 0.10), corticosterone (0.05 and 0.06), respectively.

E₂, P, and 3 α ,5 α -THP were extracted from plasma with ether following incubation with water and 800 cpms of tritiated steroid. After snap-freezing twice, test tubes containing steroid and ether were evaporated to dryness in a Savant and were reconstituted with phosphate assay buffer to the original plasma volume. E₂, P, and 3 α ,5 α -THP were extracted

from hippocampus and cortex by homogenizing the samples in 50% MeOH, 1% acetic acid with a pestles. Samples were then centrifuged at $3000 \times g$. Supernatant was chromatographed on Sepak-cartridges equilibrated with 50% MeOH and 1% acetic acid. Steroids were eluted with increasing concentrations of MeOH (50% then 100%). MeOH was removed using a speed drier. Brain samples were reconstituted in 300 μ l assay buffer. Corticosterone was extracted from 10 μ l of plasma by heating the sample to 60–70 degrees C.

Tritiated steroids and specific antibodies were utilized for the radioimmunoassays run. Tritiated E₂ (specific activity 51.3 Ci/mmol), P (specific activity 47.5 Ci/mmol), 3 α -5 α -THP (specific activity 65.0 Ci/mmol) and corticosterone (specific activity 48.2 ci/mmol) were from Perkin Elmer (Boston, MA). The E₂ antibody (E#244, Dr. G.D. Niswender, Colorado State University, Fort Collins, CO), was used in a 1:40,000 dilution and bound 54% of tritiated E₂. The P antibody (P#337, Dr. G.D. Niswender, Colorado State University), was used in a 1:30,000 dilution and bound 48% of tritiated P. The 3 α -5 α -THP antibody (#921412–5; Dr. Robert Purdy, Veterans Medical Affairs, La Jolla, CA), was used in a 1:5,000 dilution and bound 47% of tritiated 3 α -5 α -THP. Corticosterone antibody (#B3-163; Endocrine Sciences Calabasas Hills, CA), was used in a 1:20,000 dilution and bound 45% of tritiated corticosterone.

Two-way analyses of variances (ANOVAs) were used to examine effects of cycle condition (diestrous, proestrous) and/or genotype (wildtype, 5 α RKO) on behavioral and endocrine measures. When the α level for statistical significance was reached ($p \leq 0.05$), or a trend was observed ($p \leq 0.10$), *post hoc* tests were used to examine group differences. To determine the extent to which variability in the results were due to levels of particular steroids, regression analyses were conducted. What is reported here are those steroids in which there were significant differences and contributions to variability in the behavioral measures.

There were significant effects of cycle phase [$F(1, 58) = 24.14, p < 0.01$] and genotype [$F(1, 58) = 25.97, p < 0.01$] for plasma E₂ levels. Circulating E₂ levels were significantly higher among proestrous than diestrous mice and higher among 5 α RKO than wildtype mice (Table 1). There were significant effects of cycle phase [$F(1, 58) = 9.38, p < 0.05$], genotype [$F(1, 58) = 17.82, p < 0.05$], and an interaction between these variables [$F(1, 58) = 21.44, p < 0.05$] for hippocampal E₂ levels, such that E₂ levels were lower among wildtype, but not 5 α RKO, mice in diestrus (Table 1). There was a significant effect of cycle phase, but not genotype, for cortical E₂ levels [$F(1, 58) = 107.17, p < 0.01$], such that cortical E₂ levels were higher in proestrous compared to diestrous mice (Table 1).

There were significant cycle phase, but not genotype, effects for circulating P [$F(1, 58) = 12.77, p < 0.01$], such that levels were higher among proestrous, compared to, diestrous mice (Table 1). There were significant effects of genotype, but not cycle phase, for hippocampal [$F(1, 58) = 11.20, p < 0.01$] and cortical [$F(1, 58) = 7.48, p < 0.01$] P levels. Wildtype mice had higher P levels in hippocampus and cortex than did 5 α RKO mice.

There was a significant effect of cycle phase [$F(1, 58) = 13.78, p < 0.01$], but not genotype, and an interaction between variables [$F(1, 58) = 5.76, p < 0.02$] for plasma 3 α ,5 α -THP levels (Figure 1). Circulating 3 α ,5 α -THP levels were significantly higher among proestrous compared to diestrous, wildtype, but not 5 α RKO, mice. There was a similar pattern for brain levels of 3 α ,5 α -THP. There was a significant effect of cycle phase [$F(1, 58) = 10.09, p < 0.01$], but not genotype, and an interaction between variables [$F(1, 58) = 5.15, p < 0.03$] for hippocampus 3 α ,5 α -THP levels (Figure 2). There was a significant effect of cycle phase [$F(1, 58) = 9.89, p < 0.01$], but not genotype, and an interaction between variables [$F(1, 58)$

= 3.99, $p < 0.05$] for cortical 3 α ,5 α -THP levels (Table 1). Levels of 3 α ,5 α -THP in the hippocampus and cortex were significantly higher among proestrous compared to diestrous, wildtype, but not 5 α RKO, mice.

Levels of corticosterone were measured in plasma to ascertain any potential differences in stress-responding among mice. There was a tendency for a cycle difference [$F(1, 58) = 3.51$, $p < 0.07$], but no effect of genotype for corticosterone levels. However, all levels were basal, or less than 2 μ g/dl (Table 1).

Proestrous mice made more central entries than did diestrous mice in the open field [$F(1, 58) = 5.88$, $p < 0.05$]. Wildtype mice made significantly more central entries into the open field than did 5 α RKO mice [$F(1, 58) = 8.47$, $p < 0.01$]. There was no significant interaction between variables. Simple regression revealed that plasma 3 α ,5 α -THP ($R^2=0.12$, $F(1,61)=8.20$, $p < 0.05$) explained a significant proportion of variance for central entries. There were no significant effects of cycle phase or genotype or an interaction for total entries made in the open field (Table 1).

Proestrous mice spent a greater duration of time on the open arms of the plus maze than did diestrous mice [$F(1, 58) = 10.01$, $p < 0.05$] (Figure 2). Wildtype mice tended to spend more time on the open arms of the elevated plus maze than did 5 α RKO mice [$F(1, 58) = 3.54$, $p < 0.06$]. There was no significant interaction between variables. Hippocampal 3 α ,5 α -THP ($R^2=0.20$, $F(1,58)=14.99$, $p < 0.05$) explained a significant proportion of variance for open arm time.

Our hypothesis that P's metabolism to 3 α ,5 α -THP via 5 α -reductase is required for estrous cycle variations in exploratory and anti-anxiety behavior of adult, female mice, was supported. Proestrous wildtype, but not 5 α RKO, mice made more central entries in the open field and spent more time engaging in social interaction, coincident with higher 3 α ,5 α -THP levels in plasma, hippocampus and cortex, compared to their diestrous counterparts. Indeed, variability in the open field and elevated plus maze could be explained by a circulating and hippocampus 3 α ,5 α -THP levels, respectively, but not levels of E₂, P, or corticosterone. Thus, metabolism of P to 3 α ,5 α -THP via 5 α -reduction may be essential for estrous cycle variations in affective behavior of female mice.

The present findings confirm previous reports. It has been previously reported that mice in proestrus spend more time on the open arms of the elevated plus maze and show increased social behavior compared to their diestrous counterparts [1,2,18]. A question is related to environmental effects. Mice in proestrus, compared to diestrus, have heighten exploration, decreased anxiety and increased social behavior, regardless of being grouped- or single-housed [19]. Notably, single-housing of mice is considered a model of depression/affective disorder [20,21] and male mice that are singly-housed have lower central 3 α ,5 α -THP levels [20–21, 4]. In the present study, mice were raised and tested in the same facility and were all group-housed. Thus, enhanced anti-anxiety behaviors occur concomitant with higher 3 α ,5 α -THP of group-housed mice.

Of interest are the brain targets for the observed effects across the estrous cycle. In a rat model, proestrous increases in anti-anxiety and pro-social behavior are associated with higher levels of 3 α ,5 α -THP in the hippocampus and cortex and brain regions involved in motivated behaviors (e.g. ventral tegmental area and striatum) [22]. Infusions of 3 α ,5 α -THP, or its prohormone, P, directly to the hippocampus, cortex or amygdala increase anti-anxiety behavior of rats [23–26]. Additionally, there is improvement in another task that is mediated by these brain structures (object recognition) following systemic administration of P [27,28] or direct infusions of P to the hippocampus [29]. The present study extended these findings by demonstrating that some of the variability in responses in the anxiety tasks were

due to 3 α ,5 α -THP in circulation or hippocampus. Thus, the role of 3 α ,5 α -THP for influencing anxiety behavior of mice may occur through actions in the hippocampus.

The present study extends previous work investigating effects across the estrous cycle to suggest that increased exploration and decreased anxiety may be due to effects of 3 α ,5 α -THP, and not necessarily to effects of E₂. Given that E₂ and progestogens co-vary and both classes of steroids have demonstrated effects on anxiety behavior, it was important to begin to parse out their relative roles. When the ovaries are removed (ovx), cyclic increases in exploratory and anti-anxiety behavior of mice are obviated, and enhancements in these behaviors can be reinstated by systemic E₂+P or P administration that produces proestrous-like levels of steroids in circulation and in the brain [16,30,31]. Given that E₂ to OVX mice dose-dependently reduces anxiety-like behavior [15,32–35], a question is the extent to which the behavioral deficits in 5 α RKO mice may be due to deficiencies in 3 α ,5 α -THP formation, and/or overproduction of E₂. Moreover, E₂ can increase activity of enzymes necessary for formation of P in the brain and its reduction to 3 α ,5 α -THP [36,37]. Systemic administration of E₂ increases levels of 3 α ,5 α -THP in the hippocampus and enhances the behavioral effects of progestogen administration in rats [38,39]. Among ovx mice, E₂ can increase hippocampal 3 α , 5 α -THP levels, but this effect is abolished in estrogen receptor β knockout mice [15], suggesting a novel role of this receptor subtype for 3 α ,5 α -THP synthesis. Importantly, in the present study, despite a trend for higher plasma E₂ in the 5 α RKO mice, we did not see an overall significant effect of E₂ levels to account for all of the behavioral effects observed. This suggests that increased E₂ during development did not produce a compensatory effect that overrode deficits in 3 α ,5 α -THP production, and further supports a role of 3 α ,5 α -THP for these behavioral processes. Thus, among proestrous mice, some of E₂'s anxiolytic effects may occur subsequent to 3 α ,5 α -THP formation for its role in mediating affective behaviors.

The present data confirm and extend previous work investigating the requirement of 5 α -reduction for behavioral endpoints of rats and mice. Administration of finasteride, a 5 α -reductase inhibitor, to the hippocampus or amygdala attenuates proestrous increases in anti-anxiety and anti-depressive behavior [40–42]. Finasteride to mice produces 3 α ,5 α -THP withdrawal and increased anxiety- and depression-like behavior [9,10]. Moreover, the anxiety-reducing effects of music exposure to intact female mice are abrogated with administration of finasteride [43]. These results are similar to what was observed in the present study investigating the effects among 5 α RKO mice. Previously, we have demonstrated that P (1 mg; SC) to OVX wildtype, but not 5 α RKO, mice decreases seizure activity following administration of a chemoconvulsant and increases anti-anxiety and anti-depressant behavior [13,44]. However, a consideration to make regarding the present study are the effects of 5 α -reduced androgen metabolites, which were not measured here, but have been demonstrated to have robust behavioral effects among males [45–50]. Together, these data demonstrate the requirement of 5 α -reductase for affective responding as well as neuroprotection.

In conclusion, P's 5 α -reduced metabolite 3 α , 5 α -THP is important in mediating affective behaviors of mice. Brain targets for these effects include the hippocampus and/or the cortex. These data may be clinically relevant for neuropsychiatric disorders, such as anxiety and depression. It is known that women, compared to men, are more likely to experience mood and affective disorders [51]. Because women have higher levels of E₂ and progestogens, a question is the extent to which this sex differences may be due to these differences in hormonal milieu. Indeed, 3 α ,5 α -THP levels can be decreased in depression and post-traumatic stress disorder, but increased in women, and decreased in men, with panic disorder [52–55]. There is some indication in clinical samples, as well as animal models, that alterations in steroid synthesis and metabolism may underlie neuropsychiatric disorders

[56,57]. Thus, 3 α , 5 α -THP may play a crucial role in mediating sex differences in neuropsychiatric disorders so understanding its mechanisms for these effects are of continued interest.

Acknowledgments

This research was supported by a National Institute of Mental Health supplemental grant associated with MH-06769801, and project RMH067698B.

References

1. Gray P, Cooney J. Stress-induced responses and open-field behavior in estrous and nonestrous mice. *Physiol Behav.* 1982; 29:287–92. [PubMed: 6890692]
2. Walf AA, Koonce C, Manley K, Frye CA. Proestrous compared to diestrous wildtype, but not estrogen receptor β knockout, mice have better performance in the spontaneous alternation and object recognition tasks and reduced anxiety-like behavior in the elevated plus and mirror maze. *Behav Brain Res.* 2009; 196:254–60. [PubMed: 18926853]
3. Li X, Bertics PJ, Karavolas HJ. Regional distribution of cytosolic and particulate 5 α -dihydroprogesterone 3 α -hydroxysteroid oxidoreductases in female rat brain. *J Steroid Biochem Mol Biol.* 1997; 60:311–8. [PubMed: 9219922]
4. Pinna G, Agis-Balboa RC, Pibiri F, Nelson M, Guidotti A, Costa E. Neurosteroid biosynthesis regulates sexually dimorphic fear and aggressive behavior in mice. *Neurochem Res.* 2008; 33:1990–2007. [PubMed: 18473173]
5. Corpéchet C, Collins BE, Carey MP, Tsouros A, Robel P, Fry JP. Brain neurosteroids during the mouse oestrous cycle. *Brain Res.* 1997; 766:276–80. [PubMed: 9359616]
6. Frye CA, Vongher JM. Progesterone and 3 α ,5 α -THP enhance sexual receptivity in mice. *Behav Neurosci.* 2001; 115:1118–28. [PubMed: 11584925]
7. Russell DW, Wilson JD. Steroid 5 α -reductase: two genes/two Enzymes. *Annual Review of Biochemistry.* 1994; 63:25–61.
8. Normington K, Russell DW. Tissue distribution and kinetic characteristics of rat steroid 5 α -reductase isozymes. Evidence for distinct physiological functions. *J Biol Chem.* 1992; 267:19548–54. [PubMed: 1527072]
9. Beckley EH, Finn DA. Inhibition of progesterone metabolism mimics the effect of progesterone withdrawal on forced swim test immobility. *Pharmacol Biochem Behav.* 2007; 87:412–9. [PubMed: 17597197]
10. Smith SS, Ruderman Y, Frye C, Homanics G, Yuan M. Steroid withdrawal in the mouse results in anxiogenic effects of 3 α ,5 β -THP: a possible model of premenstrual dysphoric disorder. *Psychopharmacology.* 2006; 186:323–33. [PubMed: 16193334]
11. Mahendroo MS, Cala KM, Russell DW. 5 α -reduced androgens play a key role in murine parturition. *Mol Endocrinol.* 1996; 10:380–92. [PubMed: 8721983]
12. Mahendroo MS, Cala KM, Landrum DP, Russell DW. Fetal death in mice lacking 5 α -reductase type 1 caused by estrogen excess. *Mol Endocrinol.* 1997; 11:917–27. [PubMed: 9178751]
13. Frye CA, Walf AA, Rhodes ME, Harney JP. Progesterone enhances motor, anxiolytic, analgesic, and antidepressive behavior of wild-type mice, but not those deficient in type 1 5 α -reductase. *Brain Res.* 2004; 116–24. [PubMed: 15033426]
14. Sambrook, J.; Fritsch, EF.; Maniatis, T. 2. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press; 1989. *Molecular cloning: A laboratory manual.*
15. Walf AA, Koonce CJ, Frye CA. Estradiol or diarylproionitrile decreases anxiety-like behavior of wild-type, but not estrogen receptor β knockout, mice. *Behavioral Neuroscience.* 2008; 122:974–981. [PubMed: 18823154]
16. Frye CA, Sumida K, Dudek BC, Harney JP, Lydon JP, O'Malley BW, Pfaff DW, Rhodes ME. Progesterone's effects to reduce anxiety behavior of aged mice do not require actions via intracellular progesterone receptors. *Psychopharmacology.* 2006; 186:312–22. [PubMed: 16538472]

17. Frye CA, Koonce CJ, Walf AA. Mnemonic effects of progesterone to mice require formation of 3 α ,5 α -THP. *Neuroreport*. 2010; 21:590–5. [PubMed: 20431492]
18. Maguire JL, Stell BM, Rafizadeh M, Mody I. Ovarian cycle-linked changes in GABA(A) receptors mediating tonic inhibition alter seizure susceptibility and anxiety. *Nat Neurosci*. 2005; 8:797–804. [PubMed: 15895085]
19. Palanza P, Gioiosa L, Parmigiani S. Social stress in mice: gender differences and effects of estrous cycle and social dominance. *Physiol Behav*. 2001; 73:411–20. [PubMed: 11438369]
20. Agís-Balboa RC, Pinna G, Pibiri F, Kadriu B, Costa E, Guidotti A. Down-regulation of neurosteroid biosynthesis in corticolimbic circuits mediates social isolation-induced behavior in mice. *PNAS*. 2007; 104:18736–18741. [PubMed: 18003893]
21. Pibiri F, Nelson M, Guidotti A, Costa E, Pinna G. Decreased corticolimbic allopregnanolone expression during social isolation enhances contextual fear: A model relevant for posttraumatic stress disorder. *PNAS*. 2008; 105:5567–5572. [PubMed: 18391192]
22. Frye CA, Rhodes ME. Progesterin concentrations are increased following paced mating in midbrain, hippocampus, diencephalon, and cortex of rats in behavioral estrus, but only in midbrain of diestrous rats. *Neuroendocrinology*. 2006; 83:336–47. [PubMed: 17028418]
23. Akwa Y, Purdy RH, Koob GF, Britton KT. The amygdala mediates the anxiolytic-like effect of the neurosteroid allopregnanolone in rat. *Behav Brain Res*. 1999; 106:119–25. [PubMed: 10595427]
24. Crain, C.; Paris, JJ.; Frye, CA. 3 α ,5 α -THP has actions in the hippocampus to modulate exploratory, anti-anxiety, and socio-sexual behavior in female rats. Abstract for the annual meeting of the Society for Neuroscience; 2007.
25. Engin E, Treit D. The anxiolytic-like effects of allopregnanolone vary as a function of intracerebral microinfusion site: the amygdala, medial prefrontal cortex, or hippocampus. *Behav Pharmacol*. 2007; 18:461–70. [PubMed: 17762514]
26. Frye CA, Walf AA. Estrogen and/or progesterone administered systemically or to the amygdala can have anxiety-, fear-, and pain-reducing effects in ovariectomized rats. *Behav Neurosci*. 2004; 118:306–13. [PubMed: 15113256]
27. Frye CA, Walf AA. Progesterone to ovariectomized mice enhances cognitive performance in the spontaneous alternation, object recognition, but not placement, water maze, and contextual and cued conditioned fear tasks. *Neurobiol Learn Mem*. 2008; 90:171–7. [PubMed: 18455450]
28. Frye CA, Walf AA. Progesterone enhances performance of aged mice in cortical or hippocampal tasks. *Neurosci Lett*. 2008b; 437:116–20. [PubMed: 18439758]
29. Orr PT, Lewis MC, Frick KM. Dorsal hippocampal progesterone infusions enhance object recognition in young female mice. *Pharmacol Biochem Behav*. 2009; 93:177–82. [PubMed: 19477194]
30. Büttner D. Climbing on the cage lid, a regular component of locomotor activity in the mouse. *J Exp Anim Sci*. 1991; 34:165–9. [PubMed: 1814462]
31. Galeeva A, Tuohimaa P, Shaliapina VG. Role of sex steroids in development of anxiety in female mice. *Russ Fiziol Zh Im I M Sechenova*. 2001; 87:1680–7. [PubMed: 11840972]
32. Imwalle DB, Gustafsson JA, Rissman EF. Lack of functional estrogen receptor β influences anxiety behavior and serotonin content in female mice. *Physiol Behav*. 2005; 84:157–63. [PubMed: 15642619]
33. Morgan MA, Pfaff DW. Estrogen's effects on activity, anxiety, and fear in two mouse strains. *Behav Brain Res*. 2002; 132:85–93. [PubMed: 11853861]
34. Tomihara K, Soga T, Nomura M, Korach KS, Gustafsson JA, Pfaff DW, Ogawa S. Effect of ER- β gene disruption on estrogenic regulation of anxiety in female mice. *Physiol Behav*. 2009; 96:300–6. [PubMed: 18996135]
35. Walf AA, Frye CA. A review and update of mechanisms of estrogen in the hippocampus and amygdala for anxiety and depression behavior. *Neuropsychopharmacology*. 2006; 31:1097–111. [PubMed: 16554740]
36. Cheng YJ, Karavolas HJ. Conversion of progesterone to 5 α -pregnane-3,20-dione and 3 α -hydroxy-5 α -pregnan-20-one by rat medial basal hypothalamus and the effects of estradiol and stage of estrous cycle on the conversion. *Endocrinology*. 1973; 93:1157–62. [PubMed: 4591653]

37. Micevych P, Sinchak K. Estradiol regulation of progesterone synthesis in the brain. *Mol Cell Endocrinol.* 2008; 13:44–50. [PubMed: 18572304]
38. Frye CA, Rhodes ME. Estrogen-priming can enhance progesterone's anti-seizure effects in part by increasing hippocampal levels of allopregnanolone. *Pharmacol Biochem Behav.* 2005; 81:907–16. [PubMed: 16085296]
39. Vongher JM, Frye CA. Progesterone in conjunction with estradiol has neuroprotective effects in an animal model of neurodegeneration. *Pharmacol Biochem Behav.* 1999; 64:777–85. [PubMed: 10593201]
40. Frye CA, Walf AA. Changes in progesterone metabolites in the hippocampus can modulate open field and forced swim test behavior of proestrous rats. *Horm Behav.* 2002; 41:306–15. [PubMed: 11971664]
41. Rhodes ME, Frye CA. Inhibiting progesterone metabolism in the hippocampus of rats in behavioral estrus decreases anxiolytic behaviors and enhances exploratory and antinociceptive behaviors. *Cogn Affect Behav Neurosci.* 2001; 1:287–96. [PubMed: 12467128]
42. Walf AA, Sumida K, Frye CA. Inhibiting 5 α -reductase in the amygdala attenuates antianxiety and antidepressive behavior of naturally receptive and hormone-primed ovariectomized rats. *Psychopharmacology.* 2006; 186:302–11. [PubMed: 16220340]
43. Chikahisa S, Sano A, Kitaoka K, Miyamoto K, Sei H. Anxiolytic effect of music depends on ovarian steroid in female mice. *Behav Brain Res.* 2007; 179:50–9. [PubMed: 17280725]
44. Frye CA, Rhodes ME, Walf A, Harney J. Progesterone reduces pentylentetrazol-induced ictal activity of wild-type mice but not those deficient in type I 5 α -reductase. *Epilepsia.* 2002; 43 (Suppl 5):14–7. [PubMed: 12121288]
45. Edinger KL, Frye CA. Testosterone's analgesic, anxiolytic, and cognitive-enhancing effects may be due in part to actions of its 5 α -reduced metabolites in the hippocampus. *Behav Neurosci.* 2004; 118:1352–64. [PubMed: 15598144]
46. Edinger KL, Frye CA. Testosterone's anti-anxiety and analgesic effects may be due in part to actions of its 5 α -reduced metabolites in the hippocampus. *Psychoneuroendocrinology.* 2005; 30:418–30. [PubMed: 15721054]
47. Frye CA, Edinger KL. Testosterone's metabolism in the hippocampus may mediate its anti-anxiety effects in male rats. *Pharmacol Biochem Behav.* 2004; 78:473–81. [PubMed: 15251256]
48. Frye CA, Koonce CJ, Edinger KL, Osborne DM, Walf AA. Androgens with activity at estrogen receptor β have anxiolytic and cognitive-enhancing effects in male rats and mice. *Horm Behav.* 2008; 54:726–34. [PubMed: 18775724]
49. Frye CA, Rhodes ME, Walf AA, Harney JP. Testosterone reduces pentylentetrazole-induced ictal activity of wildtype mice but not those deficient in type I 5 α -reductase. *Brain Res.* 2001; 918:182–6. [PubMed: 11684057]
50. Frye CA, Rhodes ME, Walf A, Harney JP. Testosterone enhances aggression of wild-type mice but not those deficient in type I 5 α -reductase. *Brain Res.* 2002; 948:165–70. [PubMed: 12383970]
51. Seeman MV. Psychopathology in women and men: focus on female hormones. *Am J Psychiatry.* 1997; 154:1641–7. [PubMed: 9396940]
52. Brambilla F, Biggio G, Pisu MG, Bellodi L, Perna G, Bogdanovich-Djukic V, Purdy RH, Serra M. Neurosteroid secretion in panic disorder. *Psychiatry Res.* 2003; 118:107–16. [PubMed: 12798975]
53. Rasmusson AM, Pinna G, Paliwal P, Weisman D, Gottschalk C, Charney D, Krystal J, Guidotti A. Decreased cerebrospinal fluid allopregnanolone levels in women with posttraumatic stress disorder. *Biol Psychiatry.* 2006; 60:704–13. [PubMed: 16934764]
54. Romeo E, Ströhle A, Spalletta G, di Michele F, Hermann B, Holsboer F, Pasini A, Rupprecht R. Effects of antidepressant treatment on neuroactive steroids in major depression. *Am J Psychiatry.* 1998; 155:910–3. [PubMed: 9659856]
55. Uzunova V, Sheline Y, Davis JM, Rasmusson A, Uzunov DP, Costa E, Guidotti A. Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. *Proc Natl Acad Sci U S A.* 1998; 95:3239–44. [PubMed: 9501247]

56. Carter RN, Paterson JM, Tworowska U, Stenvers DJ, Mullins JJ, Seckl JR, Holmes MC. Hypothalamic-pituitary-adrenal axis abnormalities in response to deletion of 11 β -HSD1 is strain-dependent. *J Neuroendocrinol.* 2009; 21:879–87. [PubMed: 19602102]
57. Yehuda R, Bierer LM, Sarapas C, Makotkine I, Andrew R, Seckl JR. Cortisol metabolic predictors of response to psychotherapy for symptoms of PTSD in survivors of the World Trade Center attacks on September 11, 2001. *Psychoneuroendocrinology.* 2009; 34:1304–13. [PubMed: 19411143]

Highlights

- There are estrous cycle changes in exploratory and anxiety behavior of adult female mice
- 5 α -reductase may be required for proestrous increases in exploration and anti-anxiety behavior
- 5 α -reduction in the hippocampus and cortex is important for exploration and anti-anxiety effects

Figure 1.

The mean time spent (± S.E.M.) in the central entries of the open field (bars) and plasma 3α, 5α-THP levels (indicated by gray circles). * indicates a significant effect of cycle + over a bar indicates a significant effect of genotype ^ indicates a significant interaction

Figure 2.

The mean time spent (± S.E.M.) on the open arms of the plus maze (bars) and hippocampus 3α,5α-THP levels (indicated by gray circles). * indicates a significant effect of cycle + over a bar indicates a significant effect of genotype ^ indicates a significant interaction

Table 1

Levels of corticosterone, progesterone (P), and estradiol (E₂) and total entries made in the open field of wildtype and 5 α -reductase knockout (5 α RKO) mice in proestrus or diestrus.

Genotype	wildtype		5 α -reductase KO	
Condition	Diestrous	Proestrous	Diestrous	Proestrous
E ₂				
Plasma (pg/ml)	13.1 $\pm$ 2.2	23.4 $\pm$ 1.0 *	23.7 $\pm$ 3.0 ⁺	32.7 $\pm$ 0.9 * ⁺
Hippocampus (ng/g)	3.9 $\pm$ 1.5	5.1 $\pm$ 0.3 *	10.4 $\pm$ 0.5 ⁺ ^	4.8 $\pm$ 0.2 * ⁺
Cortex (ng/g)	2.3 $\pm$ 0.7	11.3 $\pm$ 1.0 *	1.0 $\pm$ 0.1	11.7 $\pm$ 1.4 *
P				
Plasma (ng/ml)	10.6 $\pm$ 0.2	11.3 $\pm$ 0.1 *	10.8 $\pm$ 0.3	11.3 $\pm$ 0.1 *
Hippocampus (ng/g)	5.1 $\pm$ 0.1	5.4 $\pm$ 0.2	4.9 $\pm$ 0.1 ⁺	4.9 $\pm$ 0.1 ⁺
Cortex (ng/g)	4.8 $\pm$ 0.1	4.8 $\pm$ 0.1	4.5 $\pm$ 0.1 ⁺	3.9 $\pm$ 0.2 ⁺
3 α ,5 α -THP				
Cortex (ng/g)	2.0 $\pm$ 0.4	3.8 $\pm$ 0.3 *^	2.2 $\pm$ 0.4	2.6 $\pm$ 0.3 *
Corticosterone				
Plasma (ng/dl)	0.7 $\pm$ 0.2	1.7 $\pm$ 0.3	1.2 $\pm$ 0.3	1.5 $\pm$ 0.4
Open Field				
Total entries (mean $\pm$ SEM)	75.6 $\pm$ 7.1	100.1 $\pm$ 10.4	88.8 $\pm$ 9.0	90.9 $\pm$ 6.6

* indicates a significant effect of cycle

⁺ indicates a significant effect of genotype

^ indicates a significant interaction