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# Progesterone facilitates exploration, affective and social behaviors among wildtype, but not 5α-reductase Type 1 mutant, mice

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## Abstract

Progesterone ($P_4$) facilitates exploration, anxiety and social behaviors in estrogen ($E_2$)-primed mice. Some of these effects may be due to actions of its 5α-reduced metabolite, 5α-pregnan-3α-ol-20-one (3α,5α-THP). In order to address the role of $P_4$ and its metabolite, 3α,5α-THP, a mouse model was utilized. We hypothesized that if $P_4$'s metabolism to 3α,5α-THP is essential to facilitate exploratory, anti-anxiety and social behaviors of mice, then wildtype, but not 5α-reductase knockout (5α-RKO), mice will have greater expression of these behaviors. Experiment 1: Mice were ovariectomized (ovx), $E_2$-primed and administered $P_4$ (0, 125, 250, or 500 μg) subcutaneously and then tested 4 hours later in a battery of tasks: open field, elevated plus maze, and social interaction. Experiment 2: Ovx, $E_2$-primed mice were administered $P_4$ (4 mg/kg), 3α, 5α-THP (4 mg/kg), medroxyprogesterone acetate (MPA, which does not convert to 3α,5α-THP; 4 mg/kg), or vehicle subcutaneously and tested 4 hours later. There was a dose-dependent effect of $P_4$ to wildtype, but not 5α-RKO, mice. Neither WT, nor 5α-RKO, mice had increased exploration, anti-anxiety or pro-social behavior with MPA administration. Progesterone only exerted effects on anti-anxiety behavior, and increased 3α,5α-THP in the prefrontal cortex and hippocampus, when administered to wildtype mice. 3α,5α-THP to both WT and 5α-RKO mice increased exploration, anti-anxiety and social interaction and 3α,5α-THP levels in the hippocampus and prefrontal cortex. Thus, metabolism of $P_4$ by the 5α-reductase enzyme may be essential for enhancement of these behaviors.



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**5. Conflicts of Interest**

The authors have no conflicts of interest to report.

**Keywords**

3α; 5α-THP; medroxyprogesterone acetate; hippocampus; prefrontal cortex neurosteroid

## Introduction

Anxiety and depressive disorders are twice as common in women compared to men [1], suggesting a role of ovarian hormones [2]. In support, situations associated with changes in estradiol and progestogens (progesterone—$P_4$ and its metabolites), such as across the menstrual cycle, and during the postpartum and perimenopausal periods, are also associated with an increased risk for mood, anxiety and depression disorders [3–4]. The 5α-reduced metabolite of $P_4$, 3α-hydroxy-5α-pregnan-20-one (3α,5α-THP)is thought to play a major role in the regulation of the stress axis and has been implicated in the etiology, expression, and/or treatment of some hormone- and/or stress-sensitive neuropsychiatric disorders (anxiety, depression, schizophrenia; [5–8]). Studies in people have shown lower3α,5α-THP concentrations in plasma and cerebrospinal fluid are associated with anxiety and major depression disorders [7, 9–11]. Furthermore, pharmacological treatment with selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine and/or fluvoxamine, can increase 3α,5α-THP in CSF coincident with reductions in depression and anxiety symptoms in both men and women [7, 12–13]. Animal models support the idea that SSRIs act to alter the metabolism enzymes necessary for 3α,5α-THP production [14–16]. Thus, 3α,5α-THP may play an important role in mediating these disorders and responses to therapeutics, and therefore further investigation is important.

In considering the role of progestogens for mood, one question is the extent to which effects are due to $P_4$ or its metabolites, such as 3α,5α-THP. $P_4$ is secreted from the ovaries and metabolized in the brain to 3α,5α-THP [17]. As a neurosteroid, 3α,5α-THP can also be produced *de novo* in the brain independent of peripheral glands, such as the ovaries [18]. Formation of 3α,5α-THP involves sequential actions by the 5α-reductase and 3α-hydroxysteroid enzymes [19–20]. In addition to brain areas involved in reproduction/motivated behaviors, such as the midbrain, key regions for stress and mood, the hippocampus and prefrontal cortex, have high expression of the enzymes that are involved in progestogen metabolism and biosynthesis [21–22]. One reason it is of interest to understand the role of different sources of progestogens for affective responding is that $P_4$ and 3α,5α-THP have different mechanisms of action. Unlike $P_4$, 3α,5α-THP does not bind with progestin receptors (PRs), but can rapidly influence brain excitability as a result of its activity as a potent positive allosteric modulator of $GABA_A$ receptors [23–28], one target of anxiety treatment. Thus, it is important to further understand the respective roles of $P_4$ and/or 3α,5α-THP for anxiety and depression.

Findings from animal models demonstrate that $P_4$ and/or 3α,5α-THP can have beneficial effects for anxiety-like and depression-like behaviors. In support, across the estrous cycle, higher levels of anxiety-like and depression-like behaviors are observed during phases of low progestogens. Additionally, rodents in diestrus, compared to rodents in proestrus and estrus, show less exploration in the open field, spend less time on the open arms of the elevated plus or T-maze, spend less time interacting with a conspecific, and demonstrate less retention of emotional memories in the passive avoidance task, coincident with lower levels of $P_4$ and 3α,5α-THP [29–36]. In rodents, removal of the ovaries obviates cyclic increases of central entries in the open field as well as open arm time in the elevated plus-maze, and enhancements in these responses can be reinstated by systemic administration of $E_2$ and $P_4$, or $P_4$ alone (treatments that increase brain levels of 3α,5α-THP) [20, 37–38]. Systemic or intra-hippocampal administration of a 5α-reductase inhibitor to mice or rats in proestrus

produces similar effects as ovariectomy to increase anxiety-like responding in these tasks [39–42], suggesting a role of acute manipulations of 3α,5α-THP formation to alter anxiety behaviors. Of interest is the role of chronic alterations in the metabolism of $P_4$ to 3α5,α-THP, and whether deficits can be reversed with progestogen administration.

In order to further address whether 5α-reduction to 3α,5α-THP underlies differences in exploration and anxiety behavior, and capacity to respond to progestogen replacement, a mutant mouse model was utilized. Mice that are deficient in the 5α-reductase enzyme type 1 enzyme (5α-RKO) lack the ability to metabolize $P_4$ to 3α,5α-THP that their 5α-reductase-replete wildtype littermates [32,45]. Previous studies in our laboratory using this approach of genetic manipulation have demonstrated that proestrous, or ovariectomized and progesterone-administered, wildtype mice entered more central squares in the open field, spent more time on the open arms of the elevated plus maze, and spent more time engaging with a novel conspecific than did proestrous or ovariectomized, progesterone-administered 5α-RKO mice [45–46]. These results suggest an important role of 5α-reduction in mice for these behaviors and support the effects of pharmacological manipulations of 5α-reductase previously reported [39–42]. However, whether there are differing responses to progestogen replacement was not elucidated in these studies, which is important given potential compensatory mechanisms when utilizing a mutant mouse model. The present work is novel because the response to a progestogen that can be reduced to 3α,5α-THP (progesterone), 3α,5α-THP itself, or a progestogen that cannot be reduced to 3α,5α-THP (medroxyprogesterone acetate; MPA) were compared. As such, the present study represents a combination of genetic and pharmacological manipulations to understand the role of progestogens for exploratory, anxiety, and social responding. We investigated the effects of manipulating levels of $P_4$ and 3α,5α-THP among wildtype and 5α-RKOmice in a test of exploration (open field), anxiety (elevated plus maze), and social interaction. In Experiment 1, mice were ovariectomized (ovx), $E_2$-primed, and administered $P_4$ (0, 125, 250, or 500 μg) subcutaneously and then tested 4 hours later in the battery of tasks. In Experiment 2, ovx, $E_2$-primed mice were administered $P_4$ (4 mg/kg), 3α,5α-THP (4 mg/kg) or MPA (a synthetic progestin, which does not convert to 3α,5α-THP, and is utilized in hormone replacement therapies; 4 mg/kg) or vehicle subcutaneously and tested 4 hours later in the battery of tasks. We measured levels of $P_4$, 3α,5α-THP, and corticosterone in plasma as well as $P_4$ and 3α,5α-THP in the hippocampus and cortex. We hypothesized that progestogens that can convert to3α,5α-THP will be more effective at facilitating exploration, anti-anxiety and social behavior, and that WT mice will respond more than 5α-RKO mice to replacement of $P_4$.

## 2. Methods and Materials

These methods were pre-approved by the Institutional Animal Care and Use Committee at the University at Albany (Albany, NY).

### 2.1 Animal Housing

Adult, female (N=177) wildtype and 5α-RKO mice that were 8–10 weeks old were utilized in this study. Mice were 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 on a reversed-lighting schedule (lights off at 0800 h). All mice had access to food (Purina Rodent Chow) and tap water available at all times in their home cages.

### 2.2 Mouse Strain and Genotyping

Willdtype (+/+; n=85) or 5α-RKO (−/−; n=92) mice were offspring derived from heterozygous (+/−) breeder pairs. Typical methods of polymerase chain reaction, modified from Jackson Laboratory's protocol, were utilized to determine the genotype of mice [32]. Mice are congenic on C57Bl/6 background from a colony that had been maintained for over 3 years at the University at Albany in the Life Sciences Research Building, with random environmental exposure to stressors (e.g. construction noise, unintentional fire alarms) typical of the construction of a new building.

### 2.3 Ovariectomy and Steroid Administration

For Experiment 1 and 2, adult mice were ovx under sodium pentobarbital anesthesia (80 mg/kg, IP or to effect). Mice were allowed to recover for 7 days following surgery, with daily monitoring. Immediately after recovery, mice were $E_2$-primed (0.1 mg/kg in 0.2cc oil) 44 hours before behavioral testing and then randomly assigned to receive a regimen of $P_4$ (0, 125, 250 or 500 μg in vegetable oil SC 6 hours before testing [Steraloids, Newport, RI]; Exp 1), administered $P_4$ (the most effective $P_4$ dosage identified in Exp 1, 500 μg), MPA (4 mg/kg; Sigma, St. Louis, MO), or 3α-5α-THP (4 mg/kg, SC, Sigma, St. Louis, MO; Exp 2; [46–47].

### 2.4 Behavioral Testing

Mice were tested in a battery of tasks to determine exploratory (open field), anti-anxiety (elevated plus maze), and social behavior (social interaction). A trained observer recorded behavior using a video-tracking system (Any-Maze, Stoelting, Wood Dale, IL).

**2.4.1 Open Field—**Mice were placed in a clear Plexiglas open field (60 × 60 × 35 cm), inside an activity monitor (AccuScan Instruments, Inc., Columbus, OH), for five minutes. The number of interruptions in horizontal infrared light beams was automatically recorded, which is an index of activity. The open field has a grid floor where the number of entries total (an index of general activity), into central squares (a measure of anti-anxiety behavior), and into peripheral squares could be determined [32]

**2.4.2 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.). Mice were placed in the center of the maze, facing an open arm, and the time spent in the open arms during this five-minute test was recorded as an index of anti-anxiety behavior [32].

**2.4.3 Social Interaction—**Experimental mice were placed in the opposite corner of the plexiglas open field (described above) from a stimulus female mouse. The stimulus mouse was an intact retired breeder of the same strain that had been habituated to the chamber and trained in the task on several occasions, such that its movements during the task were minimal, compared to that of the experimental mouse. The time spent by the experimental mouse interacting with the stimulus mouse (i.e. sniffing, grooming, licking, touching, following with contact), which was only considered when the experimental mouse initiated the contact, during the five-minute task was utilized as a measure of reduced anxiety-like behavior [32].

### 2.5 Tissue collection

Immediately after testing, mice were euthanized by cervical subluxation and decapitation. Whole brain and trunk blood were collected to measure steroid hormone levels in brain. Prefrontal cortex and hippocampus were dissected on ice and stored at −80°C prior to steroid measurement.

### 2.6 Radioimmunoassay for steroid hormones

Standard steroid extraction and radioimmunoassay techniques for $P_4$ and 3α,5α-THP used by our laboratory were employed [32]. In addition, levels of corticosterone were measured in plasma to ascertain any potential differences in stress-responding. Neither genotype nor hormone condition altered corticosterone levels. All corticosterone levels were basal (i.e. less than 2 μg/dl; data not shown).

## 3. Statistical Analyses

Two-way analysis of variance (ANOVA) was used to examine effects of progestin condition and genotype (wildtype, 5α-RKO) on behavioral and endocrine measures. When the alpha level for statistical significance was reached ($p \leq 0.05$), Fisher's LSD *post-hoc* tests were used to examine group differences.

## 4. Results

### 4.1 Experiment 1: Progesterone increases exploration in the open field and anti-anxiety responding in the elevated plus maze as well as $P_4$ and 3α,5α-THPlevels among wildtype, but not 5α-RKO, mice

In the open field task, there was a main effect of genotype ($F_{1,90} = 6.8$, $p < 0.01$) on total entries made. Wildtype mice administered $P_4$ had increased total entries compared to 5α-RKO mice. In addition, there was a main effect of genotype ($F_{1,90} = 6.9$, $p < 0.009$), but not $P_4$ condition, on central entries made. There was no interaction between these variables. Wildtype mice made more central entries than did 5α-RKO mice (see Figure 1).

In the elevated plus maze, there was a main effect of $P_4$ condition ($F_{(1,90)} = 2.8$, $p < 0.04$) on time spent on the arm opens, such that $P_4$ increased time spent on the open arms compared to vehicle. Mice administered $P_4$ increased time spent on the open arms compared to other treatment groups. Although there was no significant main effect of genotype, there was a significant interaction for genotype and $P_4$ condition ($F_{3,90} = 3.3$, $p < 0.02$) on time spent on the open arms in the elevated plus maze. This interaction revealed that $P_4$ increased the time spent on the open arms among wildtype, but not 5α-RKO, mice (see Figure 1).

A different pattern of effects was observed in this experiment for the social interaction task. There were no statistically significant differences in time spent in social interaction based on genotype or $P_4$ condition, albeit a similar pattern of results was apparent (see Figure 1).

There was a main effect of genotype on $P_4$ levels in the prefrontal cortex ($F_{1,90} = 4.1$, $p < 0.04$), but not in the hippocampus. There was a main effect of $P_4$ condition on $P_4$ levels in the hippocampus ($F_{3,90} = 2.8$, $p < 0.04$), but not the prefrontal cortex. Wildtype mice had higher $P_4$ levels in the prefrontal cortex compared to 5α-RKO mice, and $P_4$ administration significantly increased $P_4$ levels in the hippocampus, compared to vehicle administration (see Figure 2).

There were significant main effects of $P_4$ condition ($F_{3,90} = 2.7$, $p < 0.04$) and genotype ($F_{1,90} = 8.2$, $p < 0.005$) on 3α,5α-THP in the prefrontal cortex. Progesterone increased 3α, 5α-THP in the prefrontal cortex compared to vehicle, and wildtype mice had higher 3α,5α-THP levels than did 5α-RKO mice. There was a significant main effect of genotype ($F_{1,90} = 8.6$, $p < 0.004$) on 3α,5α-THP levels in the hippocampus. Wildtype mice had higher 3α,5α-THP levels in the hippocampus compared to 5α-RKO mice (see Figure 2).

### 4.2 Experiment 2: 3α,5α-THP, but not $P_4$ or MPA, increases exploration in the open field, anti-anxiety behavior in the plus maze, and social interaction as well as 3α,5a-THP levels among wildtype and 5α–RKO mice

In the open field task, there were no significant differences for progestin condition or genotype to influence total entries. There were significant main effects of progestin condition ($F_{3,70}$=3.0, $p$<0.03) and genotype ($F_{1,70}$=7.9, $p$<0.006], as well as an interaction ($F_{3,70}$=17.7, $p$<0.0001), for central entries made in the open field. Compared to vehicle administration, both wildtype and 5α-RKO mice made more central entries with 3α,5α-THP administration, but only wildtype mice made more central entries with $P_4$ administration. MPA administration did not differ from vehicle for central entries made by wildtype or 5α–RKO mice (see Figure 3).

In the elevated plus maze, there was a significant main effect of genotype ($F_{1,70}$ = 4.9, $p$ < 0.03) and progestin condition ($F_{3,70}$=3.7, $p$< 0.01) on time spent on the open arms. Wildtype mice spent more time on the open arms than did the 5α-RKO mice. Moreover, 3α,5α-THP and $P_4$, but not MPA administration, increased time spent in the open arms compared to vehicle administration (see Figure 3).

There was a significant main effect of progestin condition ($F_{3,70}$ = 3.9, $p$ < 0.01) on time spent interacting in the social interaction task. $P_4$ and 3α,5α-THP increased time interacting compared to vehicle. As in the open field, there was a interaction between progestin condition and genotype ($F_{3,70}$ = 3.5, $p$ < 0.02] on time spent in social interaction with a conspecific. Wildtype and 5α-RKO mice spent more time interacting with a conspecific following 3α,5α-THP administration. Only wildtype mice spent more time interacting with a conspecific following $P_4$ administration. Neither wildtype, nor 5α-RKO mice, spent more time with a conspecific following MPA administration (see Figure 3).

There was a significant main effect of progestin condition ($F_{3,70}$ = 2.8, $p$ < 0.04) on $P_4$ levels in the prefrontal cortex. Administration of $P_4$ and/or 3α,5α-THP increased $P_4$ levels in the prefrontal cortex. There was a main effect of genotype ($F_{1,70}$ = 4.3, $p$ < 0.04) to influence $P_4$ levels in the hippocampus. Wildtype mice had increased $P_4$ levels in the hippocampus compared to 5α-RKO mice (see figure 4).

For 3α,5α-THP levels, there were significant main effects of progestin condition ($F_{1,70}$ = 4.4, $p$ < 0.04) and genotype ($F_{3,70}$ = 7.5, $p$ < 0.01) on 3α,5α-THP levels in the prefrontal cortex. Wildtype mice had increased 3α,5α-THP levels in the prefrontal cortex compared to 5α-RKO mice. Administration of 3α,5α-THP or $P_4$, but not MPA, increased 3α,5α-THP levels in the prefrontal cortex compared to vehicle. There was a significant main effect of progestin condition ($F_{1,70}$ = 7.0, $p$ < 0.009) and genotype ($F_{3,70}$ = 9.1, $p$ < 0.0001) on 3α, 5α-THP levels in the hippocampus. Wildtype mice had higher levels of hippocampal 3α, 5α-THP in the hippocampus than 5α-RKO mice. Administration of 3α,5α-THP and/or $P_4$, but not MPA, increased 3α,5α-THP levels in the hippocampus, particularly among wildtype mice (albeit the interaction was not statistically significant, see Figure 4).

## 4. Discussion

Our hypothesis that mice deficient in 5α-reductase type 1 enzyme would have decrements in exploratory, anxiety, and social behaviors, as well as the capacity to produce 3α,5α-THP, following progestin-administration was partially supported. Compared to their 5α-RKO counterparts, when wildtype mice were ovx and $E_2$-primed, $P_4$ facilitated exploration (increased central entries) and anti-anxiety (increased open arm time) behavior, and increased levels of $P_4$ and 3α,5α-THP in the prefrontal cortex and hippocampus. 3α,5α-THP enhanced exploration, anti-anxiety effects, and social interaction, coincident with 3α,

5α-THP levels of both wildtype and 5α-RKO mice. Moreover, administration of MPA was ineffective in altering behavior, or 3α,5α-THP levels, in both wildtype and 5α-RKO mice. These data demonstrate that only in situations where 3α,5α-THP was increased was there enhanced open field exploration, open arm time in the elevated plus maze, and social interaction with a conspecific.

The findings in Experiment 1, that administration of $P_4$ increased central entries in the open field and time spent on the open arms when administered to wildtype mice, extend previous work examining effects of ovarian hormone replacement for anti-anxiety behavior of rodents. Previous studies have demonstrated that ovx impairs affective behavior, such as exploration in the open field, time spent on the open arms, and retention of a passive avoidance response of rats and mice [48], and that this can be reversed with administration of $E_2$ and $P_4$ in dosing that produces levels akin to those during proestrus [34, 37]. Moreover, $P_4$ alone can have beneficial effects in rodents to increase exploration and anti-anxiety levels in the open field and elevated plus maze, respectively [45,49]. Furthermore, the levels of progestogens produced in the prefrontal cortex and hippocampus in the present study confirm previous findings in rodents [42,45,50]. In addition, other studies looking at anxiolytic effects of music and the role of 5α-reduction (with pharmacological manipulations) have shown that progesterone treatment reduced anxiety level in the open field test and marble burying task compared to other treatment groups [39]. The present study extends this work to demonstrate that lifelong knockdown of 5α-reductase increases anxiety-like behaviors, and attenuates the response to $P_4$ replacement. This extends our previous investigation on $P_4$-replacement to ovx, 5α-RKO mice (on a C57×129 background) having little effect on open field central entries, social interaction, and immobility in the forced swim test [45]. We had used mice on a congenic C57 background, in a colony that had been maintained for several years in an environment with random noise exposure. Given that the 129 strain has greater anxiety-like responding, and such noise exposures can produce a stress phenotype, it is important to note that mice with these different phenotypes demonstrated an anxiolytic response to $P_4$-replacement only when they were able to form 3α,5α-THP in the brain. Thus, these studies support that $P_4$'s beneficial effects on exploratory and anxiety behavior may be due to its conversion to its 5α-reduced metabolite, 3α,5α-THP.

The present data in Experiment 2 support a role of exogenous replacement of $P_4$ and 3α,5α-THP, but not a synthetic progestin, MPA, for affective behavior among female mice. Administration of MPA to wildtype or 5α-RKO mice did not alter 3α,5α-THP levels, exploration, anti-anxiety, or social behavior. Previous studies in rodents have shown that there are decreased 3α,5α-THP levels associated with MPA administration, and few effects on behavioral processes [46, 51–53]. Further, only a $P_4$ and 3α,5α-THP regimen that increased 3α,5α-THP levels in the prefrontal cortex and hippocampus altered exploration in the open field, open arm time in the plus maze, and time spent socially interacting with a conspecific in the present study. As such, these results support previous findings on the role of $P_4$ and 3α,5α-THP replacement for exploration in the open field, anti-anxiety effects in the EPM, and social responding with a conspecific, and extend these to suggest some brain regions involved, the prefrontal cortex and hippocampus. In support, when administered systemically, $P_4$ and or 3α,5α-THP reduced the latency to enter the mirrored chamber in the mirror task, and increased both number of entries made, total time spent in the mirrored chamber in a dose-dependent manner and increased time spent on the open arm of the elevated plus maze [54–55]. In addition, mice have increased activity in the open-field test and an increased number of light/dark transitions [56]. Moreover, when mice consume 3α, 5α-THP in drinking water, time spent on the open arms of the elevated plus maze increases compared to mice that did not consume3α,5α-THP in their drinking water [57]. Furthermore, intra-hippocampal administration of 3α,5α-THP produces similar anxiolytic

effects in the open field (increased exploration of central squares) and elevated plus maze (increased time spent on the open arms) as is observed with systemic administration, or during the proestrous phase, of female mice and rats [58–59]. These data support a role of $P_4$ and/or 3α,5α-THP to exert beneficial effects to reduce anxiety-like responding of mice and rats. In addition, these findings confirm previous reports indicating that acute inhibition of 3α,5α-THP formation disrupts enhancement anti-anxiety behavior, such exploration in the open field, time spent on the open arms, social behavior and emergence from an enclosed chamber to an open field [45, 59]. Thus, these data suggest that 3α,5α-THP formation in the prefrontal cortex and hippocampus are integral for progestogens' effects on exploration, anxiolysis, and pro-social responding.

Given that similar effects of both endogenously and exogenously -administered $E_2$ and $P_4$are observed in affective tasks, an important question is whether 3α,5α-THP is a common modulator of these effects. $P_4$ is converted by actions of the 5α-reductase enzyme to DHP, which is then metabolized to 3α,5α-THP by 3α-HSD enzymes. $E_2$ increases activity of these metabolism enzymes and, thereby, increases 3α,5α-THP concentrations and has functional effects [19–20]. Given its ability to enhance $P_4$ metabolism, some of the anti-anxiety effects of $E_2$ may be sub sequent to 3α,5α-THP formation [60]. For example, SC administration of $E_2$ to OVX rats increases 3α,5α-THP levels in the hippocampus of rodents and reduces ictal behavior following chemoconvulsant administration [61]. Throughout the life-span, women experience varied and occasionally dramatic changes in their hormonal and reproductive cycles, which may underlie some changes in mood [62–64]. Among some women, hormonal and/or reproductive events may influence the onset or expression of depression and/or anxiety disorders, such as premenstrual syndrome, premenstrual dysphoric disorder, and post-partum depression syndromes which occur when endogenous progestogen levels are low [65–67] when compared to men. Men do not experience profound changes in progestogens. As another example, in a study among women diagnosed with panic disorder, perimenstrual, but not midluteal, 3α,5α-THP levels were significantly higher than controls, and correlated with their panic-phobic symptoms [10]. Interestingly, levels of 3α,5α-THP are decreased by infusions of agents that can induce panic attacks, such as sodium lactate or cholecystokinintetrapeptide (CCK4) [68], among individuals with panic disorder, but not among those without a history of panic attacks[ 69–71]. These data suggest differences in the formation of 3α,5α-THP based upon experiential factors. Among some women at menopause, reduced levels of 3α,5α-THP and other neurosteroids have been associated with depression and other mood disorders [72–73]. Hormone replacement therapy has been an important factor for women going through menopause. However, many of the drugs used in hormone replacement therapy have profound negative effects on behavior, steroid hormone levels and peripheral tissues. This may be the case with MPA. Although $P_4$ and MPA may have equal impacts on reducing the uterotrophic effects of unopposed estrogen treatment, their effects on the brain are different [74–75]. Animal and *in vitro* work clearly shows that $P_4$ can have neuroprotective and beneficial effects on anxiety, cognition, and depression, while MPA does not [46, 52–53, 76–77]. One reason that MPA may not exert similar effects is its in ability to form 3α,5α-THP, or perhaps it even inhibit s3α,5α-THP formation [46,78–79]. Thus, further investigation of 3α,5α-THP's effects, sources, and mechanisms is clinically-relevant.

In conclusion, $P_4$'s 5α-reduced metabolite, 3α,5α-THP, is important for exploration, anxiety and social behaviors of mice, perhaps through actions in the hippocampus and prefrontal cortex. These data may be clinically relevant for neuropsychiatric and aging/ neurodegenerative disorders. Women have higher levels of $E_2$ and progestogens and are more susceptible to certain disorders than men. One question is: what is the extent to which this sex differences may be due to these differences in hormonal milieu and capacity to produce 3α,5α-THP? Indeed, 3α,5α-THP may play a crucial role in mediating sex

differences in neuropsychiatric and neurodegenerative disorders; therefore, understanding its mechanisms in this mouse model for these effects are of continued interest.

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- Progestins enhanced behavior only in mice that had increased brain 3α,5α-THP
- 3α,5α-THP to WT and 5αRKO mice enhanced exploration, anti-anxiety, social behavior
- MPA did not alter behavior or brain 3α,5α-THP levels of WT or 5α RKO mice
- Progestins' 5α-reduction is required for exploration, affect, prosocial behavior
- Brain targets of 3α,5α-THP may be the hippocampus and prefrontal cortex.

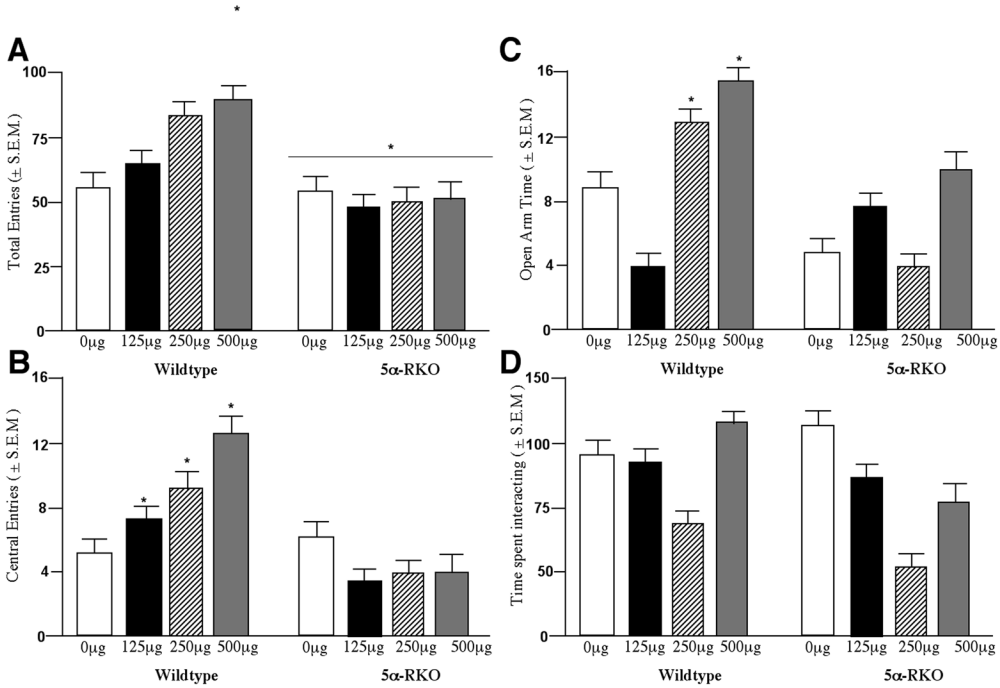


**Figure 1.**

Mean total entries (±S.E.M.; top left Panel A), mean central entries (±S.E.M.; bottom left Panel B), mean open arm time (±S.E.M.; top right Panel C), and social interaction (±S.E.M.; bottom right Panel D) of wildtype (WT) mice (left side) or 5α-RKO mice (right side), which were administered different concentrations of progesterone ($P_4$); 0 μg (white bars), 125 μg (black bars), 250 μg (striped bars), or 500 μg (gray bars). A significant difference of progesterone condition is indicated by a (*) above bar and a significant difference from genotype is indicated by a (*) above a line ($p < 0.05$).

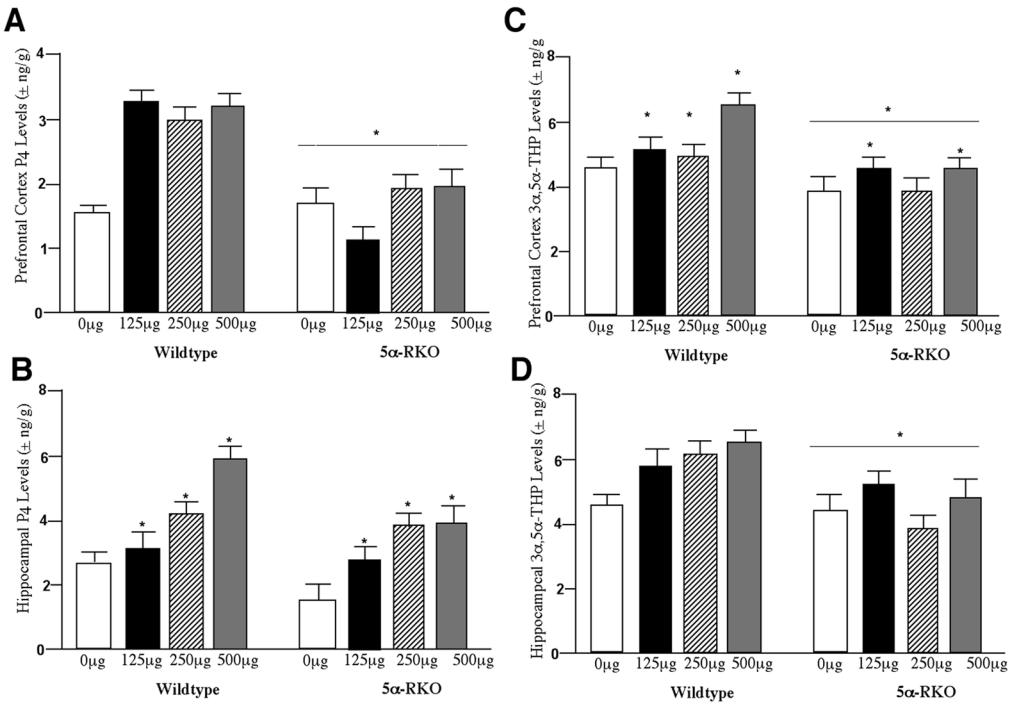


**Figure 2.**

Mean progesterone ($P_4$) levels in the prefrontal cortex levels (± ng/g.; top left Panel A), mean hippocampal $P_4$ levels (± ng/g.; bottom left Panel B), mean 3α,5α-THP levels in the prefrontal cortex (± ng/g.; top right Panel C), and mean 3α,5α-THP levels in the hippocampus (± ng/g.; bottom right Panel D) of wildtype (WT) mice (left side) or 5α-RKO mice (right side), which were administered different concentrations of $P_4$; 0 μg (white bars), 125 μg (black bars), 250 μg (striped bars), or 500 μg (gray bars). A significant difference of progesterone condition is indicated by a (*) above bar ($p < 0.05$) and a significant difference from genotype is indicated by a (*) above a line ($p < 0.05$).

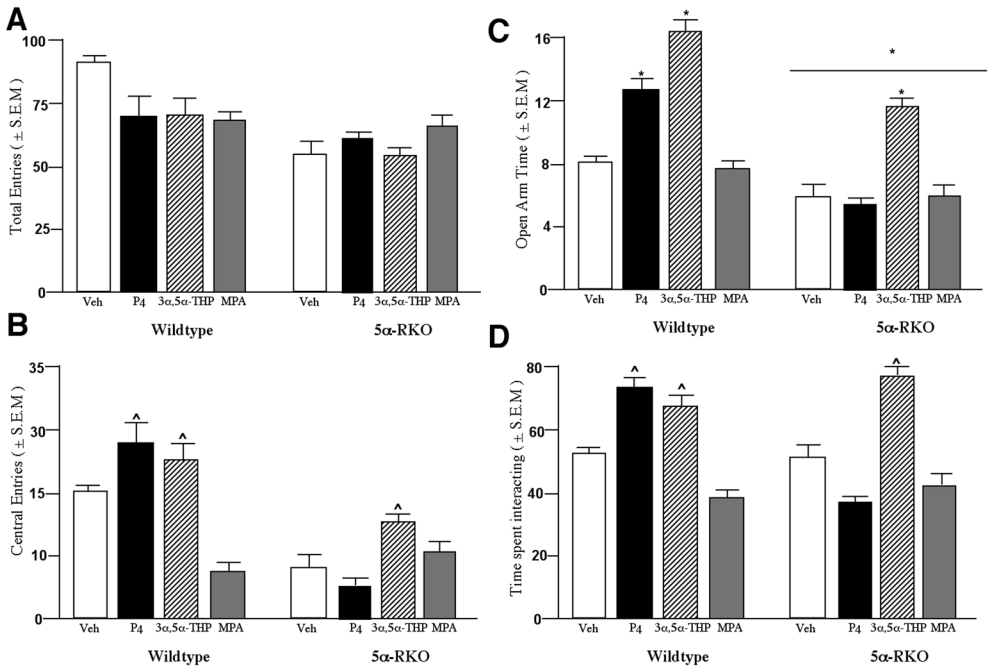


**Figure 3.**

Mean total entries (±S.E.M.; top left Panel A), mean central entries (±S.E.M.; bottom left Panel B), open arm time (±S.E.M.; bottom left Panel C), and social interaction (±S.E.M.; top right Panel D) of wildtype mice (left side) or 5α-RKO mice (right side), administered vehicle (veh, white bars), progesterone ($P_4$, 4 mg/kg, black bars), 3α,5α-THP (4 mg/kg, striped bars), or medroxyprogesterone acetate (MPA 4 mg/kg, gray bars). A significant interaction of genotype and condition is indicated by (^) above bar ($p < 0.05$). A significant effect of genotype is indicated by (*) above a line ($p < 0.05$) and a significant effect of condition is indicated by (*) above bar ($p < 0.05$).

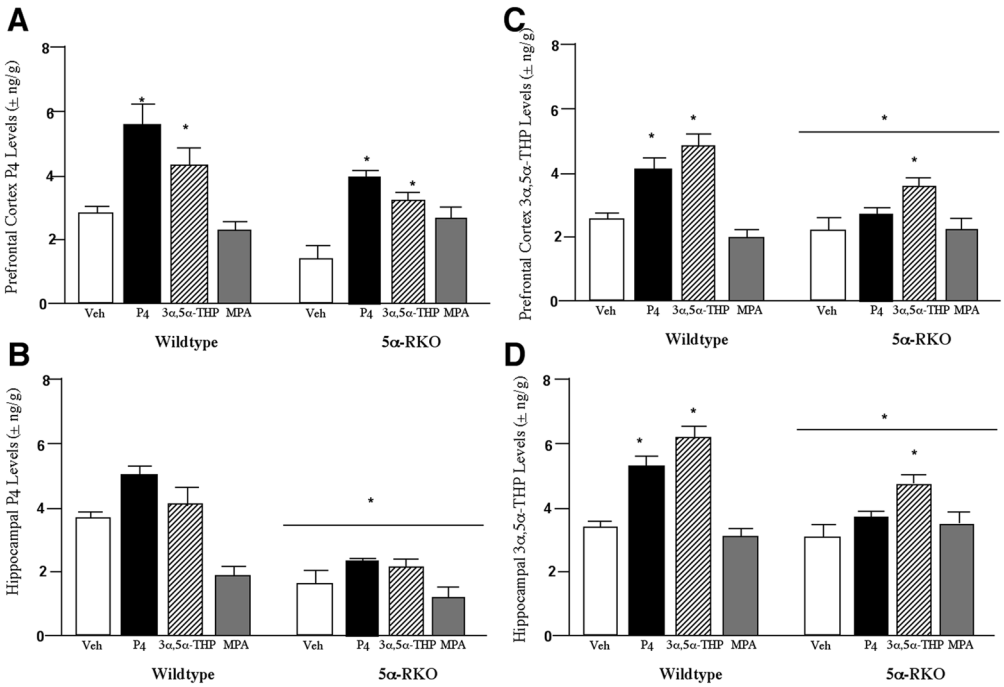


**Figure 4.**

Mean progesterone ($P_4$) levels in the prefrontal cortex (±ng/g.; top left Panel A), mean $P_4$ levels in the hippocampus (±ng/g; bottom left Panel B), mean 3α,5α-THP levels in the prefrontal cortex (±ng/g.; bottom left Panel C), mean 3α,5α-THP levels in the hippocampus (±ng/g.; top right Panel D) of wildtype mice (left side) or 5α-RKO mice (right side), administered vehicle (veh, white bars), $P_4$, (4 mg/kg, black bars), 3α,5α-THP (4 mg/kg, striped bars), or medroxyprogesterone acetate (MPA 4 mg/kg, gray bars). A significant effect of genotype is indicated by (*) above a line ($p < 0.05$) and a significant effect of condition is indicated by (*) above bar ($p < 0.05$).