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Allopregnanolone is required for prepulse inhibition deficits induced by D_1 dopamine receptor activation

Laura J Mosher[1,2,*], **Roberto Cadeddu**[1,*], **Sabrina Yen**[3], **Jeffrey L Staudinger**[4], **Francesco Traccis**[5], **Stephen C Fowler**[2], **Jamie L Maguire**[3], **Marco Bortolato**[1]

[1]Dept. of Pharmacology and Toxicology, College of Pharmacy, University of Utah, Salt Lake City (UT)

[2]Dept. of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, Lawrence (KS)

[3]Department of Neuroscience, School of Medicine, Tufts University, Boston (MA)

[4]Department of Basic Science, Kansas City University, School of Osteopathic Medicine, Joplin (MO)

[5]Department of Biomedical Sciences, University of Cagliari, Monserrato (CA), Italy.

Abstract

Introduction: The extraction of salient information from the environment is impaired by the activation of dopamine receptors. Using rodent models, we previously reported that this perceptual deficit, as measured by the prepulse inhibition (PPI) of startle, is effectively opposed by inhibitors of the steroidogenic enzyme 5α-reductase (5αR). The specific 5αR isoenzyme and steroids implicated in these effects, however, remain unknown.

Methods: The effects of the selective D_1 dopamine receptor agonist SKF-82958 (SKF, 0.3 mg/kg, IP) and D_2 receptor agonist quinpirole (QUIN, 0.5 mg/kg, IP) were tested in the startle reflex and PPI of knockout (KO) mice for either 5αR type 1 (5αR1) or type 2 (5αR2). Furthermore, we established whether these effects may be modified by the 5α-reduced steroids dihydroprogesterone (DHP), allopregnanolone (AP), dihydrotestosterone (DHT), 5α-androstane-3α,17β-diol (3α-diol), or androsterone. To test the mechanisms whereby 5αR products may alter the PPI-disrupting properties of D_1 agonists, we studied the involvement of GABA-A and PXR, two receptors targeted by neuroactive steroids. Specifically, we tested the effects of SKF in combination with the GABA-A antagonist bicuculline, as well as in KO mice for the GABA-A δ subunit and PXR.

Corresponding author: Marco Bortolato, MD PhD, Dept. of Pharmacology and Toxicology, College of Pharmacy, University of Utah, L.S. Skaggs Hall, 30 S 2000 E, Salt Lake City, UT 84112, USA, marco.bortolato@utah.edu.
*These authors contributed equally to the present study

Conflict of Interest
No conflicts declared.

Results: 5αR1, but not 5αR2 knockout (KO) mice, were insensitive to the PPI-disrupting effects of SKF. This sensitivity was reinstated by AP (3 mg/kg, IP), but not other 5α-reduced steroids. The PPI deficits induced by SKF were not modified by bicuculline, δ-subunit KO mice and PXR KO mice.

Conclusions: These results collectively suggest that 5αR1 enables the negative effects of D_1 dopamine receptor activation on information processing via production of AP. The contribution of AP to the PPI-disrupting mechanisms of D_1 receptor agonists, however, do not appear to be mediated by either GABA-A or PXR receptors.

Keywords

Allopregnanolone; dopamine; sensorimotor gating; D_1 receptors; 5α-reductase; behavioral studies

1. INTRODUCTION

The efficiency of information processing is contingent on the ability to filter out irrelevant stimuli. Impairments of this function – termed sensorimotor gating - have been observed across a broad range of neuropsychiatric conditions, including schizophrenia, obsessive-compulsive disorder (OCD) and Tourette syndrome (TS) (Geyer et al., 2006). The best-validated operational index to measure sensorimotor gating integrity in humans and animal models is the prepulse inhibition (PPI) of the acoustic startle reflex, a cross-species phenotype corresponding to the reduction of the startle response following a weak pre-stimulus (Hoffman and Ison, 1980).

Due to its high cross-species translational validity, PPI has been extensively used in rodent models to screen putative antipsychotic agents and investigate the molecular and neurobiological underpinnings of schizophrenia and other gating disorders. A widely used rodent model to screen antipsychotic efficacy is based on PPI deficits induced by dopaminergic agonists (Geyer et al., 2001). In most mouse strains, D_1-like receptor agonists disrupt PPI in an antipsychotic-sensitive fashion (Ralph-Williams et al., 2003); conversely, the effects of selective D_2 receptor agonists are generally poor in this species, with the only exception of a few strains (Ralph and Caine, 2005).

Our group has shown that the PPI deficits induced by dopaminergic agonists is blocked by inhibitors of 5α-reductase (5αR) (Bortolato et al., 2008). This family of steroidogenic enzymes catalyze the saturation of the 4,5-double bond of the A ring of Δ^4-3-ketosteroid substrates, including progesterone, androstenedione, and testosterone (Paba et al., 2011). This reaction leads to the synthesis of 5α-dihydrosteroids, including dihydroprogesterone (DHP), androstanedione (ASD), and dihydrotestosterone (DHT). In turn, these steroids are metabolized by 3α-hydroxysteroid oxidoreductase (3α HSOR) into 5α-pregnan-3α-ol-20-one (allopregnanolone; AP), 5α-androstan-3α-ol-17-one (androsterone), and 5α-androstane-3α,17β-diol (3α-diol) (Paba et al., 2011). Although the two main 5αR isoenzymes, termed 1 (5αR1) and 2 (5αR2), serve overlapping functional roles, they diverge with respect to several key characteristics, including localization throughout the organism, substrate affinity and pH optima (Paba et al., 2011).

We recently documented that, in mice and rats, the antipsychotic-like effects of the prototypical 5αR inhibitor finasteride is based on the opposition of the PPI-disrupting effects of D_1 receptor agonists (Frau et al., 2013; 2016). However, as finasteride is not isoenzyme-specific in rodents (Thigpen and Russell, 1992), the specific implication of 5αR1 and 5αR2 with respect to the dopaminergic modulation of PPI remains elusive. Capitalizing on these premises, we aimed the present study at studying which 5αR isoenzyme is directly implicated in the modulation of dopaminergic responses in PPI, by testing the effects of D_1- and D_2-like receptor agonists in 5αR1 and 5αR2 knockout (KO) mice. Furthermore, we examined the specific steroid-based mechanisms involved in the observed mechanisms, by testing whether the effects of 5αR inactivation on PPI were reversed by the implementation of different 5αR products, including 5α-dihydro- and 3α,5α-tetrahydro steroids.

2. MATERIALS AND METHODS

2.1. Animals.

The experiments included in this study were performed on adult (3-month old) male mice. The following mutant lines were used: 5αR1, 5αR2, GABA-A subunit δ, and pregnane X receptor (PXR) knockout (KO). These mice and their wild-type (WT) littermates were generated by mating heterozygous sires and dams. Both 5αR1 and 5αR2 KO mouse progenitors were a kind donation from Dr. Mala Mahendroo (Southwestern University). Unless stated otherwise for specific experimental purposes, all mice were housed in groups of 4–5/cage, with at least 1 mouse/genotype, and had ad libitum access to food and water. Housing facilities were maintained at 22°C with a 12 h light/dark cycle (lights on at 06:00 AM hours and on at 06:00 PM). Experimental manipulations were carried out in the animals' light cycle between 8:00 AM and 4:00 PM. All handling and experimental procedures were performed in compliance with the National Institute of Health guidelines and approved by the local Institutional Animal Care and Use Committees. To avoid potential confounds due to carry-over effects, each mouse was only used once throughout the study, with the only exception of the experiments aimed at assessing the behavioral effects of 3α-diol and androsterone in combination with SKF, for which animals were tested in counterbalanced order. Group sizes were defined based on power analyses run on preliminary studies.

2.2. Genotyping.

Mouse genotyping was performed by PCR. Samples of genomic DNA were extracted from tail biopsies acquired from mice at weaning (postnatal day 21). The following primers were used to identify 5αR1 KO mice: 1) GAT TGG GAA GAC AAT AGC AGG CAT GC 2) CCA GAC ACG AAC TTC CAC GCT TCT G 3) ATG GAG TTG GAT GAG TTG TGC. Reaction conditions were: 94°C × 1.5 min, 94°C × 20s, 55°C × 30s, 72°C × 2 min, 4°C × ∞, as previously described (Mahendroo et al., 2001). The following primers were used to identify 5αR2 KO mice: 1) GAT GAC CTC TCC GGG CTT CC 2) GAA TGT TCC AAG TCA CAG GC 3) CGC TTC TGA GGA GAG AAC TGA CTG A. Reaction conditions were: 94°C × 2 min, 94°C × 40s, 55°C × 40s, 72°C × 5 min, 4°C × ∞, as previously described (Mahendroo *et al.*, 2001). The following primers were used to identify δ KO mice: 1) GCA GGC TGT CCT ACA ACC AT; 2) ATG CCA CTC CTG ATG CCT AC; 3) GGG

GTT TGC TCG ACA TTG. Reaction conditions were: 94°C × 2 min, 94°C × 20s, 65°C × 15s, 68°C × 10s. The following primers were used to identify PXR KO: 1) CTG GTC ATC ACT GTT GCT GTA CCA; 2) GCA GCA TAG GAC AAG TTA TTC TAG AG; 3) CTA AAG CGC ATG CTC CAG ACT GC. Reaction conditions were: 95°C × 3 min, 95°C × 45s, 60°C × 30s, 72°C × 60s.

2.3. Drugs.

The following drugs were used in this study: the D_1 receptor agonist SKF-82958 (SKF; Sigma Aldrich, St. Louis, MO, USA; 0.3 mg/kg, IP; 10 min before behavioral testing); the D_2 receptor agonist quinpirole (QUIN) (Sigma Aldrich; 0.5 mg/kg, IP; 15 min before testing); the GABA-A receptor bicuculline (Sigma Aldrich; 1 mg/kg, IP; 15 min before testing); DHP (Sigma Aldrich; 3–10 mg/kg, IP; 15 min before testing); AP (Tocris Bioscience, Bristol, UK; 1–3 mg/kg, IP; 30 min before testing); DHT (Sigma; 3 mg/kg, IP; 15 min before testing); 3α-diol (Tocris; 3 mg/kg, IP; 15 min before testing); and androsterone (Tocris; 3 mg/kg, IP; 15 min before testing). SKF and QUIN were dissolved in saline. Bicuculline was dissolved in saline with few drops of concentrated acetic acid. DHP, AP, DHT, androsterone, and 3α-diol were dissolved in 2% DMSO, 3% Tween 80 and saline. Doses were based on previous studies on the effects of these compounds in PPI in mice (Yeomans et al., 2010; Frau et al., 2016) as well as pilot studies on the behavioral effects of steroids.

2.4. Acoustic startle reflex and PPI.

Startle testing was conducted as previously described (Godar et al., 2016). Briefly, the apparatus used for detection of startle reflexes (SR-LAB; San Diego Instruments, San Diego, CA, USA) consisted of five Plexiglas cages (diameter: 5 cm) in sound attenuated chambers with fan ventilation. Each cage was mounted on a piezoelectric accelerometric platform connected to an analogue digital converter. The response to each stimulus was recorded as 65 consecutive 1 ms readings. A dynamic calibration system was used to ensure comparable sensitivities across chambers. The startle testing protocol featured a 70-dB background white noise, and consisted of a 5 min acclimatization period, followed by three consecutive blocks of pulse, prepulse + pulse and ‘no stimulus’ trials. During the first and the third block, mice received only five pulse-alone trials of 115 dB. Conversely, in the second block mice were exposed to a pseudorandom sequence of 50 trials, consisting of 12 pulse-alone trials, 30 trials of pulse preceded by 73, 76 or 82 dB pre-pulses intensities (10 for each level of prepulse loudness) and eight no stimulus trials, where only the background noise was delivered. Intertrial intervals were selected randomly between 10 and 15 s. Sound levels were assessed using an A-scale setting. Percent PPI was calculated with the following formula:

$$100 - \frac{mean\ startle\ amplitude\ for\ prepulse\ pulse\ trials}{mean\ startle\ amplitude\ for\ pulse\ alone\ trials} \times 100$$

The first five pulse-alone bursts were excluded from the calculation. As no interactions between prepulse levels and treatment were found in the statistical analysis, %PPI values were collapsed across prepulse intensity to represent average %PPI.

2.5. Locomotor Activity.

Locomotor behaviors were measured in a square force-plate actometer (28cm × 28cm) as previously described (Fowler et al., 2001). In brief, mice (n=8/genotype) were placed in the center and their behavior was monitored for 20 min for the baseline locomotor activity studies. In a subsequent study 5αR1 KO, HZ, and WT mice (n=8/genotype) were placed in the center and their behavior was monitored for 60 min before they were removed, treated with SKF, QUIN or vehicle and placed back into the actometer for an additional 120 min. Each force plate actometer consisted of four force transducers placed at the corners of each load plate. Transducers were sampled 100 times s−1, yielding a 0.01 s temporal resolution, a 0.2 g force resolution and a 2-mm spatial resolution. Custom software directed the timing and data-logging processes via a LabMaster interface (Scientific Solutions Inc., Mentor, OH, USA). Distance travelled was calculated as the sum of the distances between coordinates of the location of center of force recorded every 0.50 s over the recording session.

2.6. Statistical Analyses.

Data were tested for normality by the Kolmogorov-Smirnov test. Parametric statistical analyses were performed by multiway ANOVAs, followed by Tukey's test for post-hoc comparisons. Significance was set at P = 0.05.

3. RESULTS

3.1. Genetic 5αR1 deficiency renders mice insensitive to the PPI-disruptive effects of D_1 receptor activation.

We first analyzed the effects of SKF (0.3 mg/kg, SC) on startle amplitude and PPI in 5αR1 KO and HZ mice, as compared to WT littermates (n=12/group). As described in Fig. 1A, WT mice exhibited higher startle values [Main effect for genotype: F(2,66)=8.00, P=0.0008] than HZ (P=0.02) and KO (P=0.0008; Tukey's). However, SKF did not modify this parameter in either genotype. The analyses of PPI values (Fig. 1B) revealed that SKF reduced PPI in WT [genotype x treatment interaction: F(2,66) = 5.47, P=0.006; SKF vs vehicle: P=0.0003; Tukey's), but not in either HZ or KO mice. The effects of SKF were significantly greater in WT mice than their HZ (P=0.03) and KO littermates (P=0.0003).

We then analyzed the effects of the D_2 receptor agonist QUIN in 5αR1-deficient mice (n=12–14/group). Startle amplitude was reduced by both 5αR1 deficiency [Main effect of genotype: F(2,74) = 15.59, $P < 0.0001$; WT vs HZ: P=0.0009; WT vs KO: P=0.0001, Tukey's] (Fig. 1C) and QUIN [Main effect of treatment: F(1,74) = 6.65, $P = 0.01$]. However, no interaction between these two factors was found. PPI analyses (Fig. 1D) surprisingly revealed that QUIN lowered PPI levels in KO mice [Genotype x treatment interaction: F(2,74)=3.48, P=0.04; QUIN- vs saline- treated KO mice: P=0.001; Tukey's], but not in WT (QUIN-treated WT vs QUIN-treated KO: P=0.0002) or HZ (QUIN-treated HZ vs QUIN-treated KO: P=0.004) counterparts.

3.2. Genetic 5αR1 deficiency does not alter the locomotor effects of D_1 or D_2 receptor activation.

We next tested the locomotor effects of D_1 and D_2 receptor agonists on 5αR1 KO mice (Fig 2). As expected, the D_1 receptor agonist SKF increased locomotor activity in both WT and KO mice for the 90-min period following injection (Fig 2A) [treatment x time interaction: $F(12,624)=32.04$, $P<0.0001$; Ps<0.001 for all comparisons with 0'; Tukey's]. The locomotor effects of SKF, however, were comparable across genotypes. In contrast with SKF, QUIN was found to significantly reduce locomotor activity in both genotypes [treatment x time interaction: $F(12,552)=3.69$, $P<0.0001$]. In addition, unlike WT mice, KO littermates exhibited a significant hypolocomotor response of QUIN in the 10-min bin after injection [genotype x treatment x time interaction: $F(24,552)=1.91$, $P=0.006$; $P=0.0001$ for WT vs KO mice; Tukey's]

3.3. Genetic 5αR2 deficiency does not alter the sensitivity of mice to the PPI-disruptive effects of D_1 receptor activation.

Contrary to our findings on 5αR1 KO mice, SKF did not affect startle amplitude in 5αR2-deficient mice (n=8/group; Fig. 3A); furthermore, 5αR2 KO and HZ mice were equally sensitive as their WT littermates to the PPI-disrupting effects of SKF (Fig 3B) [Main effect of treatment: $F(1,41) = 38.19$, $P < 0.001$]. Conversely, QUIN elicited a statistical trend towards a significant reduction in startle amplitude in 5αR2 KO mice and their littermates [Main effect of treatment ($F(1,53) = 3.52$, $P = 0.08$) (Fig. 3C)] (n=9–10/group); however, this drug did not elicit any significant PPI alterations in any genotype (Fig. 3D).

3.4. The insensitivity of 5αR1 KO mice to the PPI-disrupting effects of the D_1 receptor agonist is restored by AP, but not by DHP.

We next tested whether the effects of 5αR1 deficiency on the PPI-disrupting properties of SKF could be reversed by administration of two of its pregnane products, namely DHP and its metabolite AP. Analyses were run by 3-way ANOVAs, with genotype, steroid treatment, and SKF as factors. In conformity with our previous results, the analysis of the effects of DHP (3 mg/kg, IP) (Fig. 4A) showed that KO mice exhibited lower startle values than those of WT [main effect of genotype: $F(1,84)=17.08$, $P= 0.0001$]. As expected, SKF significantly reduced PPI (Figs. 4B) in WT, but not KO mice [genotype x SKF interactions: DHP: $F(1,84)=29.35$, $P= 0.00001$; Ps<0.001 for comparison between SKF-treated KO and vehicle-treated KO as well as SKF-treated WT; Tukey's test]; however, DHP did not modify the effects of SKF in either WT or KO mice. The analysis of the effects of AP on startle magnitude (Fig. 4C) revealed that SKF-treated WT mice exhibited an increased startle amplitude in comparison with both vehicle-treated WT and SKF-treated KO mice [genotype x SKF interaction: $F(1,132)=13.60$, $P=0.0003$; Ps =0.0008; Tukey's test]. Furthermore, the combination of SKF and the 3 mg/kg of AP produced a significant enhancement of startle magnitude in comparison with each of all other treatment combinations [AP x SKF interaction: $F(2,132)=5.77$, $P=0.004$; Ps<0.001, Tukey's test], irrespective of genotypes. As expected, the combination of SKF and vehicle in KO mice failed to cause PPI deficits, but the dose of 3 mg/kg of AP restored the sensitivity of these animals to the PPI-disrupting

effects of SKF [genotype x AP x SKF interaction: F(2,132)=5.00, P=0.008; P=0.0002 for comparison between SKF-treated KO mice treated with AP or its vehicle; Fig. 4D].

3.5. Androgen products of 5αR1 do not reinstate the PPI-disrupting effect of D_1 receptor agonists in 5αR1 KO mice.

We next tested whether the effects of 5αR1 deficiency on the PPI-disrupting properties of SKF could be prevented by administration of 5α-reduced androgens, including DHT, androsterone, and 3α-diol. Analyses were run by 3-way ANOVAs, with genotype, steroid, and SKF treatments as factors. The analysis of the effects of DHT (3 mg/kg, IP) (Fig. 5A), 3α-diol (3 mg/kg, IP) (Fig. 5C), and androsterone (3 mg/kg, IP) (Fig. 5E) on startle magnitude showed a significant reduction in startle values in KO mice, as compared with their WT littermates [Main genotype effect: DHT: F(1,88)=57.00, P<0.0001; 3α-diol: F(1,88)=171.45, P<0.0001; androsterone: F(1,88)=63.32, P<0.0001]. As expected, SKF significantly reduced PPI (Figs. 5B, 5D, and 5F) in WT, but not KO mice [genotype x SKF interactions: DHT: F(1,88)= 159.65 P<0.0001; 3α-diol: F(1,88)=79.43, P<0.0001; Androsterone: F(1,88)=121.98, P<0.0001; Ps<0.001 for comparisons between SKF-treated KO and WT, Tukey's test]; however, the PPI disruption induced by the D_1 receptor agonist was not modified by any of these steroids.

3.6. The sensitivity to the PPI-disrupting effects of D_1 receptor activation is not moderated by either GABA-A or PXR.

Given that our results indicated that AP enables D_1-mediated PPI disruption, we next tested whether the latter may be moderated by two of the best-characterized molecular targets implicated in the function of this neurosteroid, GABA-A and PXR receptors. We first began testing the effects of the GABA-A receptor bicuculline (1 mg/kg, IP; n=8–10/group) on the startle effects of SKF in mice (Fig. 6A–B). The analysis of PPI values showed that SKF reduced this index [Main effect of treatment: F(1,32)=24.16, P=0.0001] (Fig. 6B), but this effect was not affected by bicuculline.

We then tested the effects of SKF in δ KO mice (Fig. 6C–D) (n=10–13/ group), Unlike our previous results, SKF (0.3 mg/kg, IP) significantly enhanced startle [Main effect of treatment: F(1,41)=5.03, P=0.03], likely reflecting differences in genetic background. PPI was reduced by both KO [Main effect of genotype: F(1,41)=5.18, P=0.03] and SKF [Main effect of treatment: F(1,41)=9.50, P=0.003]; however, no interactions between these two factors were found.

The analysis of the effects of SKF (0.3 mg/kg, IP) in PXR KO mice (Fig. 6E–F) (n=13–15/ group) showed that this drug increased startle amplitude in both genotypes [F(1,51)=4.09, P=0.04]. The analysis of PPI showed that SKF significantly disrupted PP [F(1,51)=33.46, P<0.0001], irrespective of genotypes, suggesting no involvement of PXR in this effect.

4. DISCUSSION

The results of this study document that 5αR1 genetic deficiency renders mice insensitive to the sensorimotor gating deficits, but not hyperactivity, induced by the potent dopamine D_1 receptor agonist SKF. These findings complement previous data from our group on the

ability of non-selective 5αR inhibitors, such as finasteride, to counter the PPI reduction induced by D_1 receptor activation in both mice and rats, without producing specific effects on locomotor activity (Frau et al., 2013; 2016). Furthermore, we found that, in substantial agreement with our previous data on finasteride (Bortolato et al., 2008; Frau et al., 2016), 5αR1 deficiency produced a significant decrement in startle magnitude without affecting baseline PPI values, pointing to a role of this enzyme in the modulation of acoustic startle exerted by several neuroactive steroids.

Our findings also highlighted that 5αR1 KO mice are sensitive to the PPI-disrupting effects of the D_2 receptor agonist QUIN. In alignment with these results, previous data from our group showed that finasteride also renders mice sensitive to PPI deficits induced by QUIN (Frau et al., 2013). Taken together, these results suggest that changes in neuroactive steroid profiles may be critical to modulate the sensitivity to the activation of either D_1 or D_2 receptors with respect to sensorimotor gating. As previous studies have shown that the genetic background plays a critical role in defining the sensitivity of rats and mice to dopaminergic agonists (Swerdlow et al., 2000; 2002; Ralph and Caine, 2005; Mosher et al., 2016), these data raise the interesting question as to whether variations in gene expression of 5αR1 or other key neurosteroidogenic enzymes may contribute to interindividual differences in the sensitivity to D_1 and D_2 receptor activation with respect to startle modulation.

In contrast with 5αR1 KO mice, 5αR2-deficient animals were not found to exhibit alterations in startle reflex, PPI values, or their dopaminergic modulation. These data confirm previous findings on the lack of startle and PPI modifications in 5αR2 KO mice (Mosher et al., 2018). Furthermore, they point to a functional divergence between 5αR isoenzymes with respect to the modulation of dopaminergic neurotransmission, as well as perception and information processing. While both 5αR1 and 5αR2 are predicted to serve overlapping enzymatic functions, they diverge with respect to substrate affinity as well as brain distribution pattern (Paba et al., 2011; Castelli et al., 2013). The selective role of 5αR1 in the responsiveness of mice to D_1 receptor agonists in PPI may particularly reflect the predominance of this isoenzyme in the brain (Normington and Russell, 1992). From this perspective, it is worth noting that our previous results pointed to a key role of the medial prefrontal cortex and the nucleus accumbens – two regions with abundant expression of 5αR1 - in the PPI-ameliorating effects of finasteride (Devoto et al., 2012).

Another major finding of this study was that acute treatment with AP, but not other 5α-reduced steroids (including DHP, DHT, 3α-diol, and androsterone) restored the sensitivity of 5αR1 KO mice to the PPI-disrupting effects of SKF. This finding is in line with recent findings from our group, indicating that AP triggers PPI deficits in mouse models of TS (Mosher et al. 2017) and sleep-deprived rats (Frau et al., 2018). Given the well-known role of 5αR1 in the regulation of the biosynthetic pathway of AP, these data suggest that the inactivation of this enzyme may impair the synthesis of AP in the brain. The baseline AP brain and plasma levels of 5αR1 KO mice have been shown to be equivalent to that of their WT counterparts (Osborne and Frye, 2009; Koonce and Frye, 2013; Tanchuck-Nipper et al., 2015). However, specific brain-regional differences in baseline AP levels may occur between WT and KO mice; alternatively, 5αR1 KO mice may exhibit an impaired synthesis of AP in response to startle-associated stress and/or activation of D_1 receptors. Accordingly,

these mutants display a deficit in the conversion of progesterone into AP (Koonce and Frye, 2003). Unfortunately, as the limited small size of mouse brain regions represent a key limitation for steroid detection, our analyses in the present study did not include the measurement of AP levels. Future analyses, however, are warranted to verify whether brain-regional steroid profiles can be modified by either D_1 receptor activation or startle testing.

Our next experimental step focused on the identification of the molecular mechanisms whereby AP enables susceptibility to D_1 receptor-mediated PPI disruption. The best-characterized receptor of AP is GABA-A, although its mechanisms of interaction with this neurosteroid remain partially elusive. AP is a positive allosteric modulator of this receptor (Majewska et al., 1986; Paul and Purdy, 1992; Rupprecht, 2003), even though this steroid may also act as its agonist at micromolar concentrations (Belelli and Lambert, 2005). Recent evidence points to the presence of two distinct AP-binding sites within the GABA-A receptor, which are posited to be localized in the α subunit and the interface between α and β subunits, respectively (Hosie et al., 2006; 2009). However, the actions of AP on GABA-A receptors have been shown to be influenced by subunit composition (Lambert et al., 2001). In particular, AP is a powerful modulator of GABA-A receptors containing δ subunits (Adkins et al., 2001; Brown et al., 2002; Wohlfahrth et al., 2002), likely due to a primary influence of this subunit in the transduction of neurosteroid signals (Hosie et al., 2009). Accordingly, the lack of δ subunits has been shown to greatly reduce the sensitivity of GABA-A receptor to neurosteroids (Mihalek et al., 1999). Our data showed that neither the GABA-A receptor antagonist bicuculline nor the genetic deficiency of the δ subunit modified the sensitivity of mice to the PPI-disrupting effects of D_1 receptor stimulation. Although these data challenge the idea that GABA-A receptors may play a key role in the observed effects of AP, we cannot exclude that some specific families of these receptors and other neurosteroids may still contribute to the dopaminergic modulation of PPI; in particular, future studies will be needed to evaluate whether the role of AP in sensorimotor gating may be modified by fluctuations in the levels of its 3β-epimer isoallopregnanolone (3β,5α, tetrahydroprogesterone), which acts as an endogenous antagonist for the AP site within GABA-A receptors.

We also studied the potential implications of PXR in the role of AP on PPI deficits induced by D_1 receptor agonists. PXR is a promiscuous nuclear receptor involved in xenobiotic metabolism and elimination (di Masi et al., 2009). While most studies on PXR have focused on its role in the liver, this target is also present in the brain (Lamba et al., 2004) and is implicated in the metabolism of cholesterol. AP is a well-known ligand of PXR, and some of its biological actions can be attenuated by reducing the expression of this receptor (Frye et al., 2014). It should be noted that PXR is also likely involved in the biosynthesis of AP (Frye et al., 2014). However, PXR-deficient mice did not show any significant modification of the PPI-disrupting effects of D_1 receptor agonists.

These results suggest that other downstream mechanisms of AP are likely linked to the sensitivity of mice to the effects of D_1 receptor agonist on PPI. For example, the effects of AP may be mediated by other receptors, such as NMDA glutamate receptors. AP sulfate has been shown to exert a modest negative modulation of these receptors (Weaver et al., 2000). Interestingly, previous data in our lab have shown that the actions of D_1 agonists in

decreasing PPI is facilitated and potentiated by low doses of NMDA antagonists (Bortolato et al., 2005).

Several limitations to this study should be acknowledged. First, we did not test female mice. Prior testing in rats showed that the antidopaminergic effects of finasteride were preserved in both males and females, as well as castrated males (Paba et al., 2011; Devoto et al., 2012). Thus, we speculate that females may display the same effects as those observed in males; however, this conclusion will need to be confirmed by future studies. Second, while our analyses were limited to a few steroids, it is possible that other neuroactive steroids may participate to some of the observed effects. Extensive research has indicated that neuroactive steroids extensively interact with dopamine signaling to regulate a broad array of physiological functions (Sanchez et al, 2010). For example, both progesterone and testosterone alter dopamine release and turnover in rodents (Sanchez et al, 2010). Further research is warranted to address how these interactions may contribute to the pathophysiology of gating disorders.

These limitations notwithstanding, the results of these studies strongly support a role for AP in the mediation of dopamine induced PPI deficits and raise important questions for future research on the balance between D_1 and D_2 receptors in the regulation of sensorimotor gating mechanisms. As mentioned in the introduction PPI is impaired in several disorders, including schizophrenia and TS and OCD; notably, the ability of drugs to restore PPI in rodents treated with dopaminergic agonists has been shown to predict antipsychotic potency (Swerdlow et al, 1994) or validate therapeutic efficacy for TS (Godar et al., 2014). In line with this idea, it is worth noting that finasteride may have therapeutic effects for TS (Bortolato et al., 2007; Muroni et al., 2011) and schizophrenia (Koethe et al., 2008). If translationally valuable, our results may support the possibility that selective 5αR1 inhibitors could have therapeutic effects for these conditions, without some of the adverse effects of finasteride, including low libido and depression (Traish et al., 2015). A highly selective 5αR1 inhibitor, MK386, has been developed for clinical use (Schwartz et al., 1992; Ellsworth et al., 1996) and shown to be safe and well-tolerated in Phase I and II clinical trials. Future studies will be needed to evaluate whether selective 5αR1 inhibitors may have effects akin to those elicited by finasteride in animal models of schizophrenia and TS.

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Highlights

- 5α-reductases (5αRs) catalyze the rate-limiting step of neurosteroid synthesis
- In mice, D1 receptor agonists reduce the prepulse inhibition (PPI) of the startle
- 5αR1 knockout mice are insensitive to PPI deficits caused by D1 receptor activation
- The PPI-disrupting properties of D1 agonists are reinstated by allopregnanolone

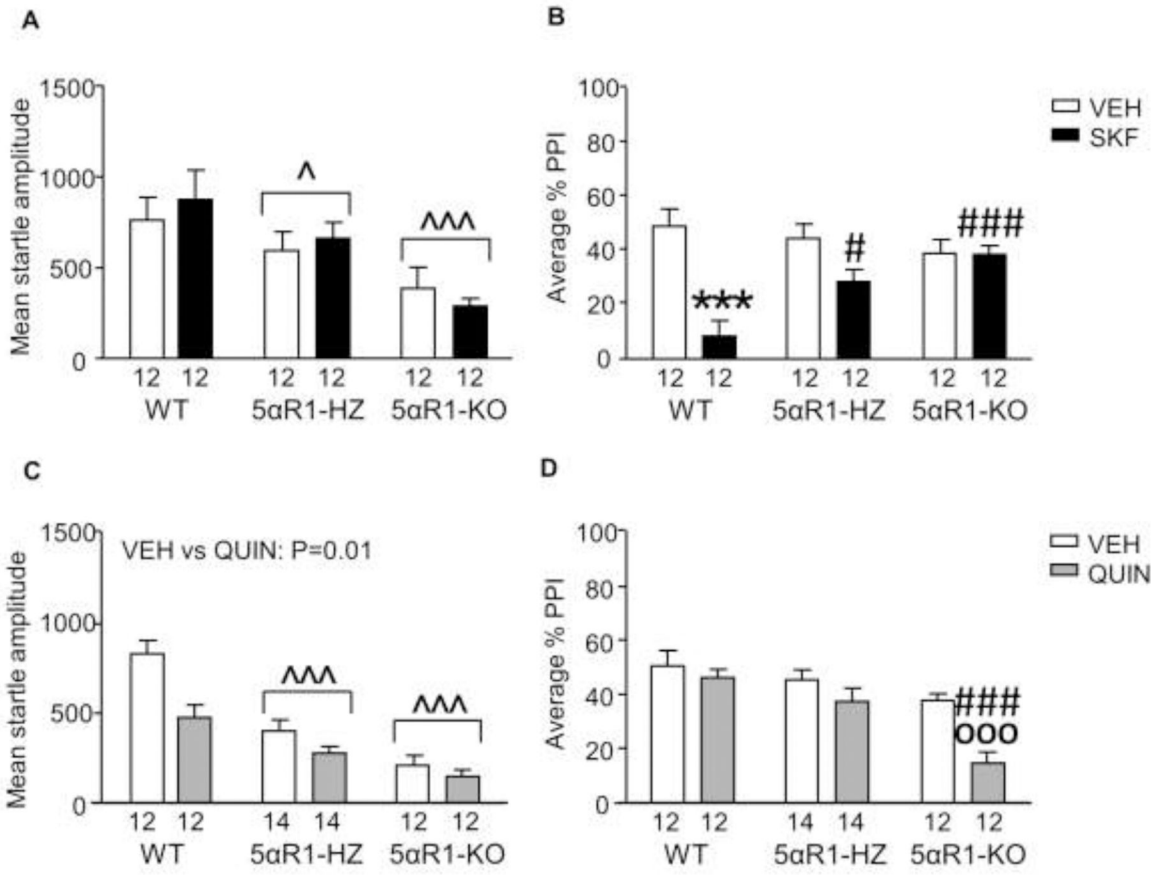

Fig. 1.

Responsiveness of 5αR1-deficient mice to the effects of D_1 and D_2 receptor agonists on startle reflex and prepulse inhibition (PPI). Data are shown as means ± SEM. ^P<0.05, ^^^P<0.001 in comparison with wildtype (WT) mice (Main effect of genotype). ***P<0.001 vs vehicle (VEH)-treated WT mice (genotype x treatment interaction). $^{\#}$,P<0.05, $^{\#\#\#}$P<0.001 vs WT mice treated with dopaminergic agonists (genotype x treatment interaction); ^{ooo},P<0.001 vs KO mice treated with VEH (genotype x treatment interaction). The number of mice in each group is indicated. Abbreviations: HZ, 5αR1 heterozygous; KO, 5αR1 knockout; SKF, SKF 82958 (0.3 mg/kg, IP); QUIN, QUIN (0.5 mg/kg, IP).

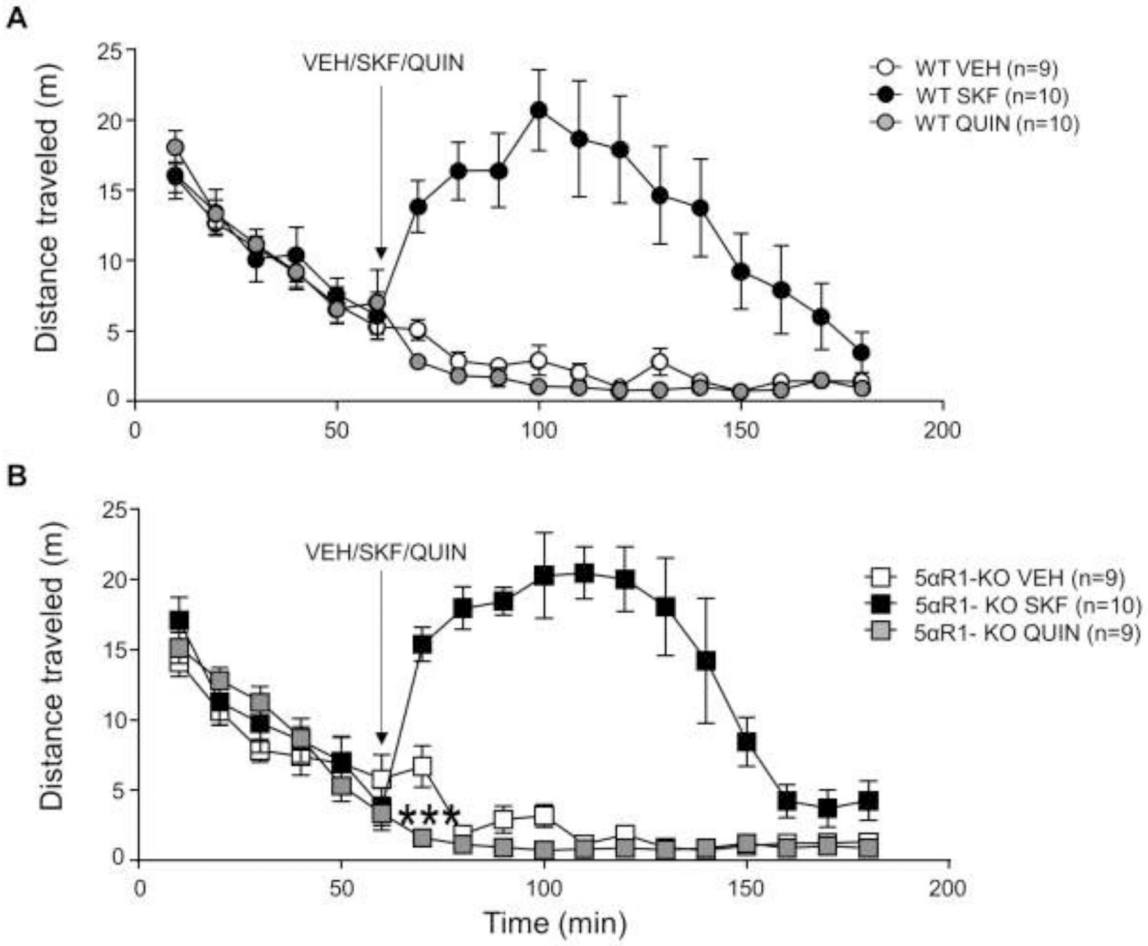

Fig. 2.

Locomotor responses to dopamine D_1 and D_2 agonists in 5αR1 knockout (KO) mice. Mice were allowed to habituate to the force plate actometer for 60 minutes. At 60 minutes the mice were briefly removed and injected with vehicle (VEH), SKF 82958 (SKF, 0.3mg/kg, IP) or QUIN (QUIN, 0.5mg/kg, IP) and returned to the actometer. The responses of wild type (WT) mice (A) and 5αR1 KO littermates (B) are described. Data are shown as mean ± SEM. ***P<0.001 for comparisons between KO mice treated with either QUIN or vehicle at 10 min after injection. The number of mice in each group is indicated.

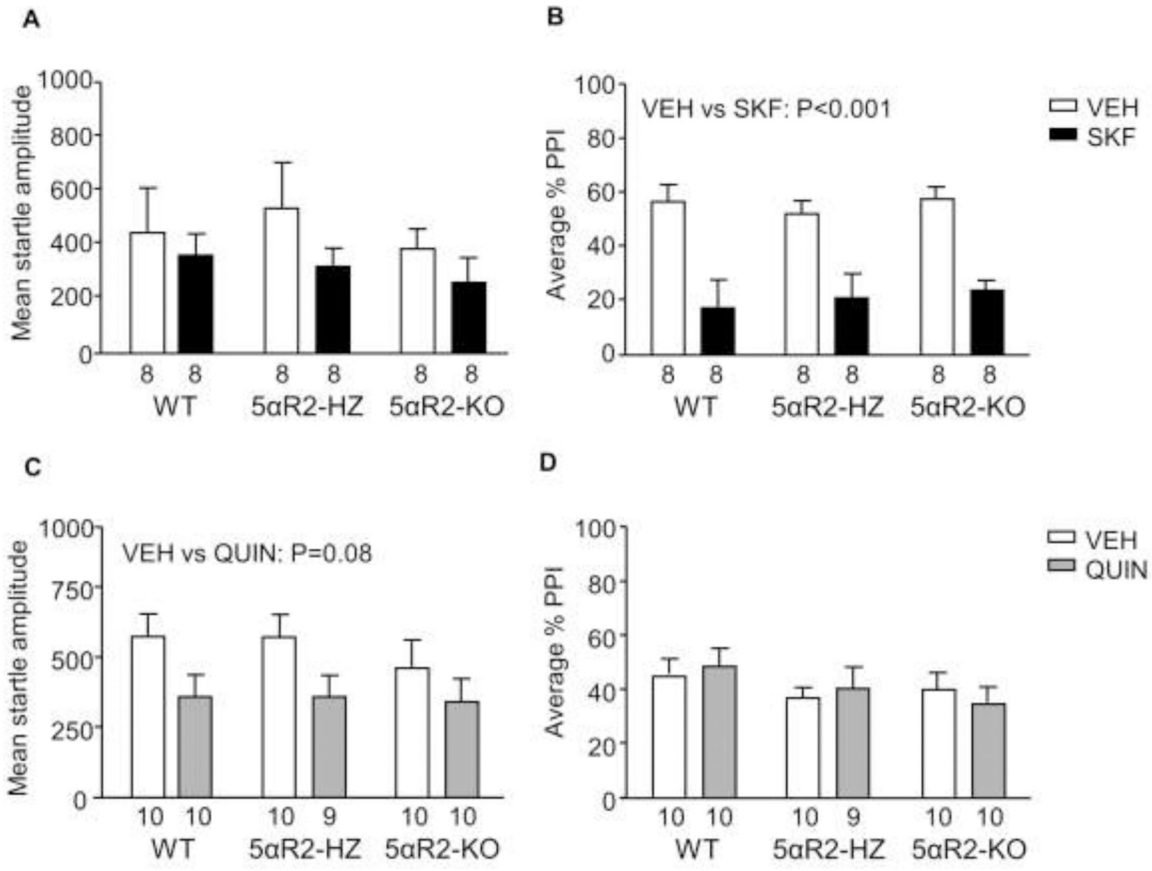

Fig. 3.

Responsiveness of 5αR2-deficient mice to the effects of D_1 and D_2 receptor agonists on startle reflex and prepulse inhibition (PPI). Data are shown as means ± SEM. The number of mice in each group is indicated. Abbreviations: WT, wildtype; HZ, 5αR1 heterozygous; KO, 5αR1 knockout; VEH, vehicle; SKF 82958 (SKF, 0.3 mg/kg, IP). QUIN, QUIN (0.5 mg/kg, IP).

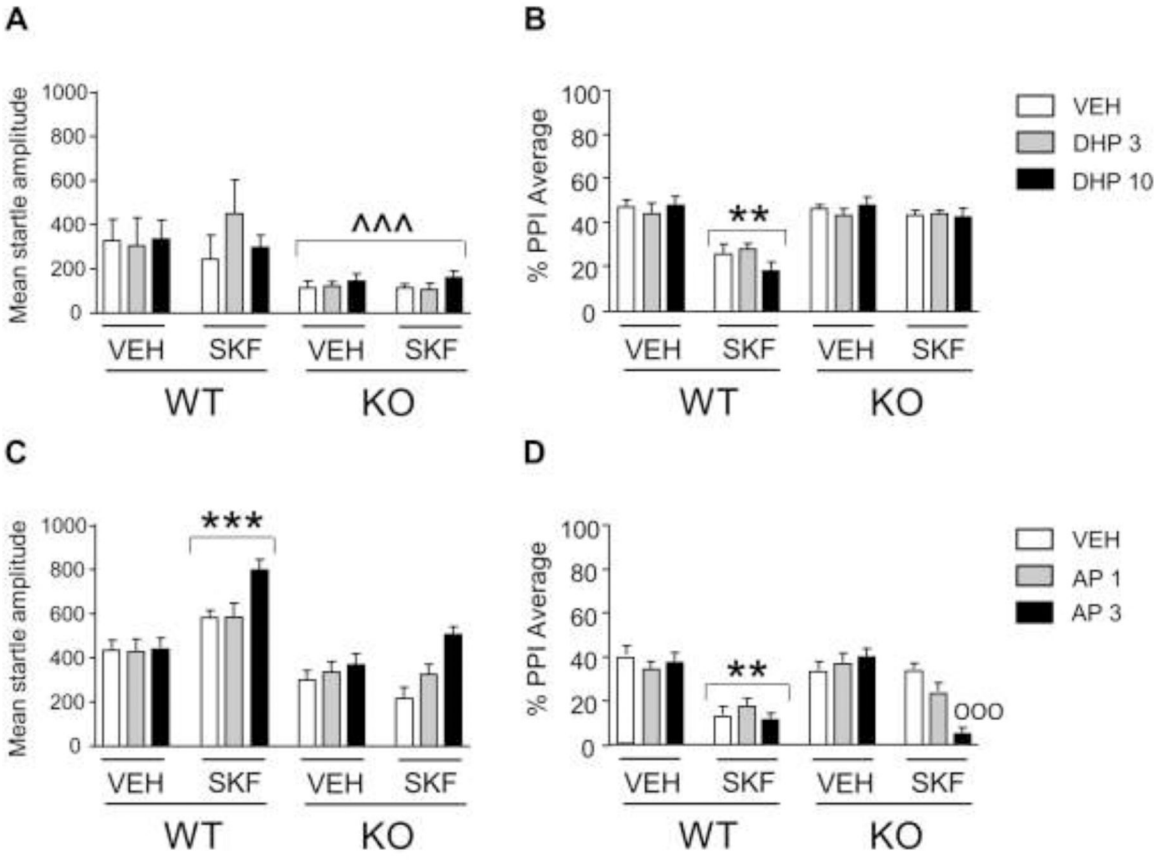

Fig. 4.

Responsiveness of 5αR1 knockout (KO) mice to the effects of the D_1 receptor agonist SKF 82958 (SKF; 0.3 mg/kg, IP) in combination with dihydroprogesterone (DHP, 3–10 mg/kg, IP; A-B; n=8/group) and allopregnanolone (AP, 1–3 mg/kg, IP; C-D; n=12/group), on startle reflex and prepulse inhibition (PPI). Data are shown as means ± SEM. ^^^, P<0.001 in comparison with wild-type (WT) mice (Main effect of genotype); **, P<0.01, ***, P<0.001 in comparison with VEH-treated WT mice (genotype x SKF interaction). ^{ooo}, P<0.001 in comparison with KO mice treated with SKF and vehicle (VEH) (genotype x SKF x AP interaction).

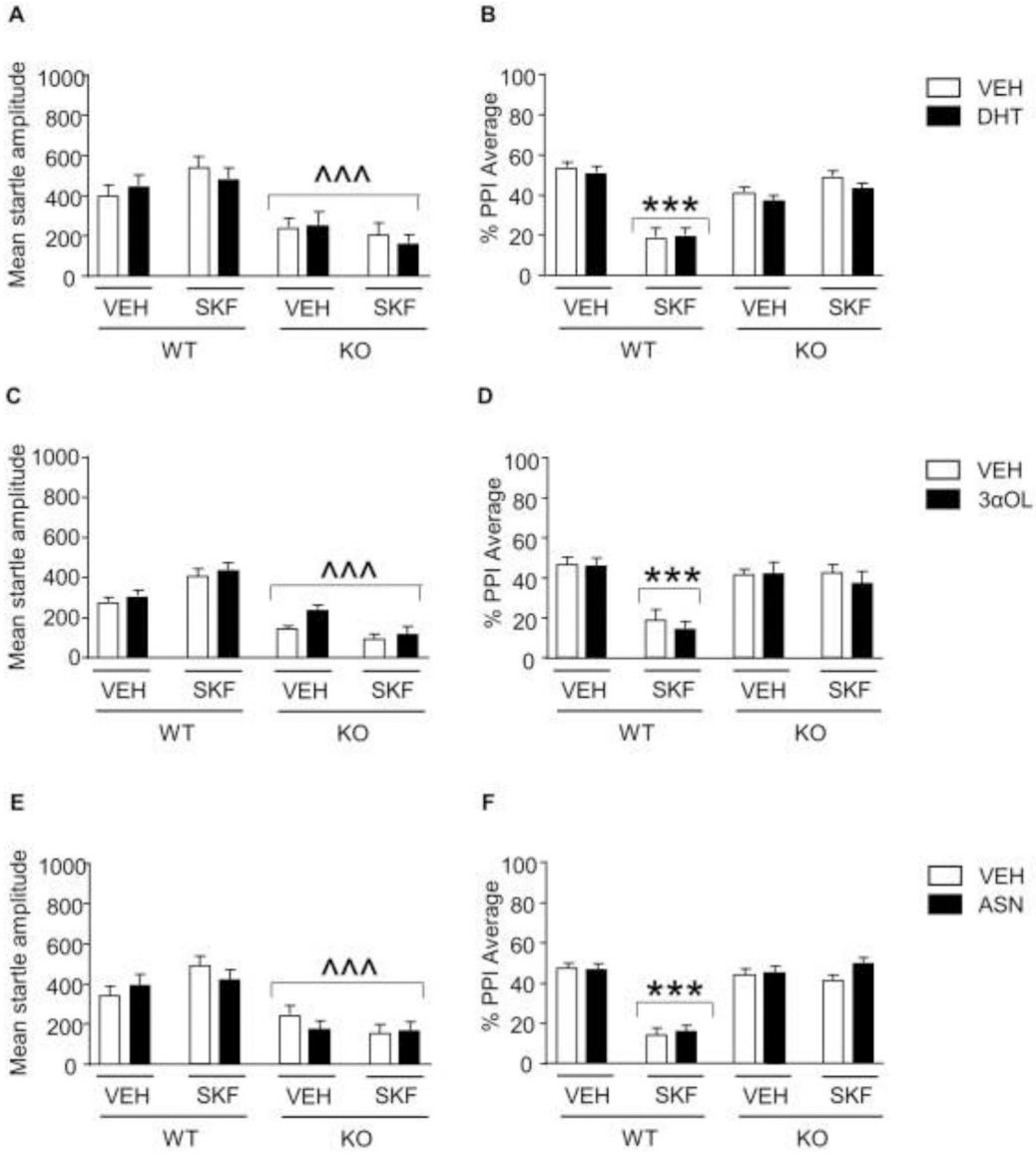

Fig. 5.

Responsiveness of 5αR1 knockout (KO) mice to the effects of the D_1 receptor agonist SKF 82958 (SKF; 0.3 mg/kg, IP) in combination with dihydrotestosterone (DHT; A-B), 5α-androstane-3α,17β-diol (3αOL; C-D), and androsterone (ASN, 3 mg/kg, IP; E-F) on startle reflex and prepulse inhibition (PPI). N=12/group for all experiments. Data are shown as means ± SEM. ^^^, P<0.001 in comparison with wild-type (WT) mice (Main effect of genotype); ***, P<0.001 in comparison with VEH-treated WT mice (genotype x SKF interaction).

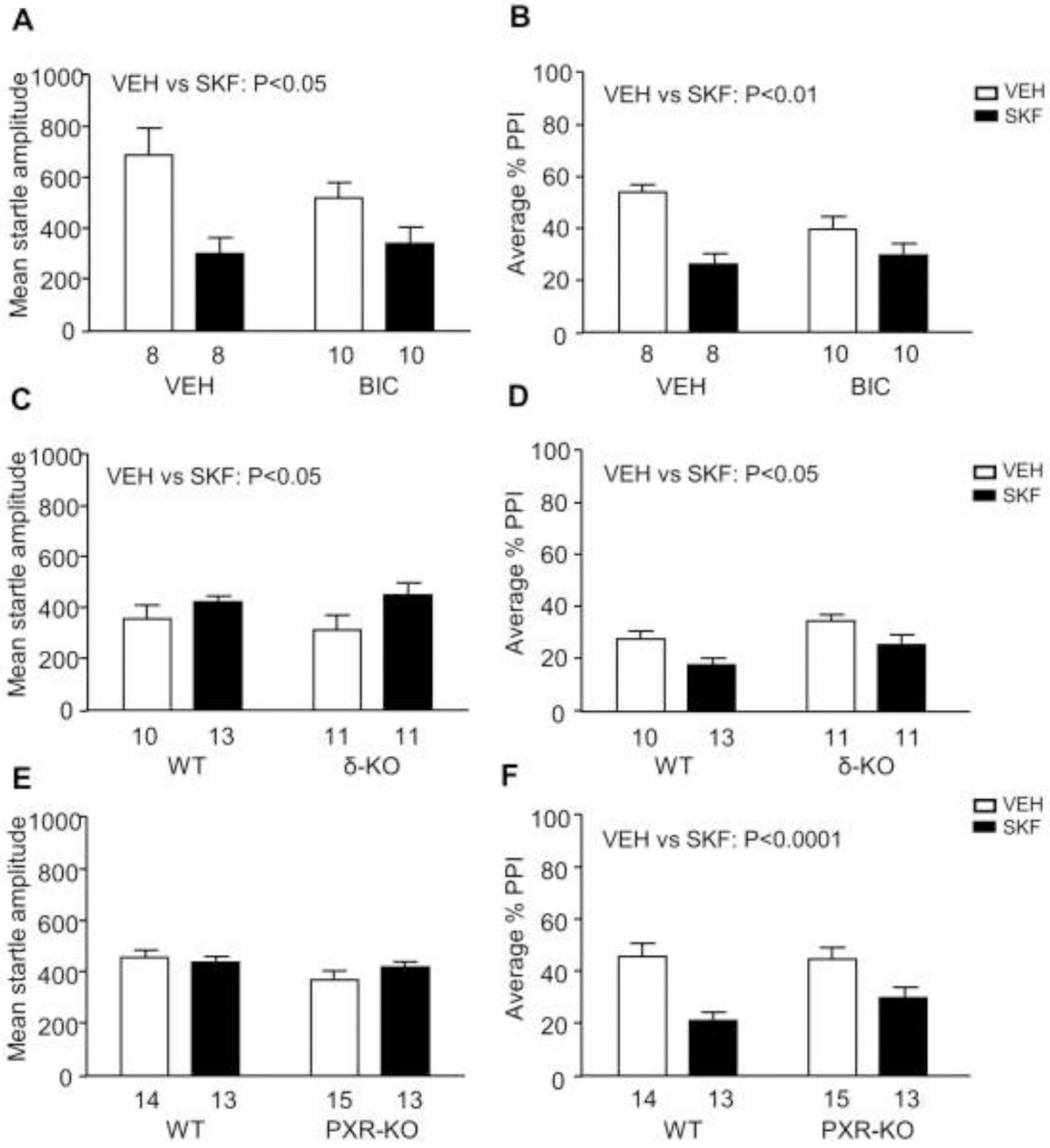

Fig. 6.

Effects of the D_1 receptor agonist SKF 82958 (SKF, 0.3 mg/kg, IP) on startle reflex and prepulse inhibition (PPI), in combination with the GABA-A receptor antagonist bicuculline (1 mg/kg, IP) (A-B), and in knockout (KO) mice for GABA-A δ-subunit (C-D) and pregnane X receptor (PXR) (E-F). Data are shown as means ± SEM. The number of mice in each group is indicated. Abbreviations: WT, wildtype; VEH, vehicle.