

The Neurosteroidogenic Enzyme 5 α -Reductase Mediates Psychotic-Like Complications of Sleep Deprivation

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Acute sleep deprivation (SD) can trigger or exacerbate psychosis- and mania-related symptoms; the neurobiological basis of these complications, however, remains elusive. Given the extensive involvement of neuroactive steroids in psychopathology, we hypothesized that the behavioral complications of SD may be contributed by 5 α -reductase (5 α R), the rate-limiting enzyme in the conversion of progesterone into the neurosteroid allopregnanolone. We first tested whether rats exposed to SD may exhibit brain-regional alterations in 5 α R isoenzymes and neuroactive steroid levels; then, we assessed whether the behavioral and neuroendocrine alterations induced by SD may be differentially modulated by the administration of the 5 α R inhibitor finasteride, as well as progesterone and allopregnanolone. SD selectively enhanced 5 α R expression and activity, as well as AP levels, in the prefrontal cortex; furthermore, finasteride (10–100 mg/kg, IP) dose-dependently ameliorated PPI deficits, hyperactivity, and risk-taking behaviors, in a fashion akin to the antipsychotic haloperidol and the mood stabilizer lithium carbonate. Finally, PPI deficits were exacerbated by allopregnanolone (10 mg/kg, IP) and attenuated by progesterone (30 mg/kg, IP) in SD-subjected, but not control rats. Collectively, these results provide the first-ever evidence that 5 α R mediates a number of psychosis- and mania-like complications of SD through imbalances in cortical levels of neuroactive steroids.

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INTRODUCTION

Alterations of sleep architecture and quality are prominent correlates of symptom severity in a wide array of neuropsychiatric disorders (Benca *et al*, 1992; Cohrs *et al*, 2001; Monti and Monti, 2004; Bauer *et al*, 2006; Parhami *et al*, 2013); the neurobiological underpinnings of these phenomena, however, remain largely elusive.

One of the best experimental paradigms to study the behavioral complications of hypsomnia is afforded by sleep deprivation (SD). This condition leads to several perceptual and cognitive abnormalities (Killgore, 2010; Daviaux *et al*, 2014) and has been shown to trigger and/or exacerbate psychotic and manic symptoms, as well as risk-taking behaviors (West *et al*, 1962; Wehr *et al*, 1987; Killgore *et al*, 2006; Kahn-Greene *et al*, 2007; McKenna *et al*, 2007);

accordingly, SD-subjected rats have been shown to exhibit a broad array of manic-like behavioral manifestations (Gessa *et al*, 1995). Along these lines, we and others have shown that sleep-deprived rats and humans develop deficits in the prepulse inhibition (PPI) of the acoustic startle reflex (Frau *et al*, 2008; Petrovsky *et al*, 2014), a well-characterized cross-species index for the measurement of sensorimotor gating. PPI deficits are observed in schizophrenia and mania (Braff *et al*, 2001; Perry *et al*, 2001), underscoring the high-translational value of this paradigm in the study of SD-induced behavioral complications. In line with this premise, we found that the PPI deficits caused by SD are reversed by antipsychotic drugs, but not by either the antidepressant citalopram or the anxiolytic diazepam (Frau *et al*, 2008).

Ample evidence has shown that neuroactive steroids have broad psychopathological implications (Dubrovsky, 2005) and may be involved in the pathophysiology of schizophrenia and bipolar disorder (Marx *et al*, 2006). Building on these premises, we recently studied the psychopathological implications of the enzymes 5 α -reductase (5 α R) type 1 and 2, which catalyze the rate-limiting step in the conversion of progesterone (PROG) and testosterone into the

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neurosteroids 5 α -dihydroprogesterone (DHP) and 5 α -dihydrotestosterone (DHT). These compounds are further converted by 3 α -hydroxysteroid oxidoreductase (3 α -HSOR) into the potent neurosteroids 3 α ,5 α -tetrahydroprogesterone (allopregnanolone; AP) and 3 α -androstenediol (Paba *et al*, 2011). Systemic administrations of the 5 α R inhibitor finasteride (FIN) countered PPI deficits in a fashion akin to antipsychotics (Bortolato *et al*, 2008; Frau *et al*, 2015). Notably, these effects were replicated by FIN infusions into the nucleus accumbens (NAc) and prefrontal cortex (PFC) (Devoto *et al*, 2012). In line with these ideas, preliminary evidence has documented that FIN may have therapeutic potential for schizophrenia and other neuropsychiatric disorders (Koethe *et al*, 2008; Muroi *et al*, 2011; Paba *et al*, 2011; Bortolato *et al*, 2012).

This background led us to hypothesize that some of the behavioral complications of SD, including PPI deficits, may be mediated by corticolimbic alterations in 5 α R. To test this possibility, we measured the impact of SD on the expression and function of 5 α R in corticolimbic regions and assessed the effects of FIN on PPI deficits and other psychosis- and mania-related behavioral consequences of SD.

MATERIALS AND METHODS

Animals

We used adult male Sprague–Dawley rats (Harlan, S. Pietro al Natisone, Italy) weighing between 280 and 320 g. Animals were housed four per cage, with *ad libitum* access to food and water and maintained on a 12/12-h light/dark cycle (lights on at 19 h). Rats were gently handled daily for three days before experimental manipulations. All experimental procedures were in compliance with the National Institute of Health guidelines and approved by the local Institutional Animal Use Committees. All possible efforts were made to minimize animal pain and discomfort and to reduce the number of experimental subjects.

Drugs

FIN, lithium carbonate, indomethacin, DHT (Sigma-Aldrich, St Louis, MO), PROG, and AP (Tocris Bioscience, Bristol, UK) (Sigma-Aldrich) were suspended in 5% Tween 80, diluted with 0.9% saline, and administered by IP injection in a volume of 2 ml/kg. Haloperidol (Sigma-Aldrich) was dissolved in 10% acetic acid buffered with NaOH and diluted with saline. FIN, haloperidol and lithium carbonate were administered 40 min before behavioral testing; conversely, AP, PROG and DHT were injected 15 min before testing.

SD

Throughout the study, SD was produced using the small-platform method (Frau *et al*, 2008), a well-validated experimental paradigm that has been shown to produce near-complete REM sleep reduction and a marked reduction in overall sleep in rodents (Grahnstedt and Ursin, 1985; Machado *et al*, 2006). Non-sleep deprived (NSD) control rats were placed in the experimental room used for SD procedures with the same temperature and humidity, but

kept individually in their home cage, after removal of cage mates. Our prior studies showed that these animals exhibited the same behavioral phenotypes as those of rats kept on large platforms (Frau *et al*, 2008). The duration of SD was kept at 72 h, based on our prior evidence that this timing is necessary to produce marked and stable PPI deficits in rats (Frau *et al*, 2008), as well as other mania-related behavioral responses (Gessa *et al*, 1995). Both SD-subjected and NSD rats had free access to food and water throughout the procedure. The following sections provide a brief description of the methods for behavioral and neurochemical analyses. Additional details are described in Supplementary Information.

Behavioral Analyses

Immediately following SD, a phenotypic battery was conducted to examine sleep-deprived behavioral sequelae. Startle reflex and PPI were tested in a Plexiglas cylinder mounted on a piezoelectric accelerometric platform (Med Associates, St Albans, VT, USA) as previously detailed (Bortolato *et al*, 2004). Locomotor activity was measured during the last 20 min of a 30 min session in Omnitech Digiscan cages (Columbus, OH, USA) as indicated (Bortolato *et al*, 2008). Risk propensity was evaluated using a variant of the wire-beam bridge task that was modified for rats, as detailed elsewhere (Bortolato *et al*, 2009a). Object exploration and social interactions were assessed for 5 and 10 min, respectively, with variants of the protocols described elsewhere (Bortolato *et al*, 2009a, b).

Neurochemical Analyses

Immediately following behavioral testing, animals were killed, brain regions were harvested, snap-frozen and stored at -80°C until processed. 5 α R1, 5 α R2 and 3 α -HSOR protein levels were examined by western blot as previously reported (Bortolato *et al*, 2011). Steroid levels were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS), as described elsewhere (Caruso *et al*, 2008). Analyses targeted the following steroids: pregnenolone, PROG, DHP, AP, DHEA, testosterone DHT and 3 α ,5 α -androstenediol. Given the low size of the NAc, analyses were performed on a broader area of the brain that included the ventral and mediodorsal striatum, in order to achieve a critical content of neurosteroids that may be detected by LC-MS/MS analysis.

Statistical Analyses

Normality and homogeneity of variance of data distribution were verified by using the Kolmogorov-Smirnov and Bartlett's tests. Statistical analyses were performed with one-way or multiway ANOVAs or linear regression and ANCOVA, as appropriate. *Post hoc* comparisons were performed by Tukey's test. Non-parametric comparisons were performed with Mann–Whitney tests. The significance threshold was set at 0.05.

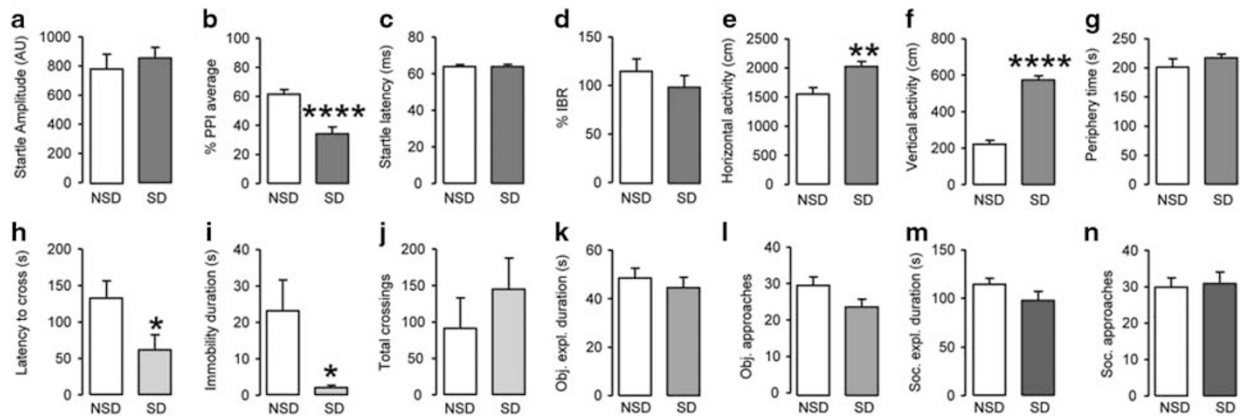


Figure 1 Sleep deprivation (SD) induces behavioral phenotypes related to manic and psychotic alterations. In comparison with non-sleep deprived (NSD) controls, SD-subjected rats displayed no significant changes in (a) startle amplitude [$F(1,19) = 0.30$, NS], but a marked reduction in (b) prepulse inhibition (PPI) [$F(1,20) = 24.39$, $P < 0.0001$]. Conversely, no differences were found in either (c) startle latency or (d) startle habituation (tested as interblock ratio; IBR). The analysis of locomotor activity (e–g) revealed that SD led to increased (e) horizontal [$F(1,13) = 11.01$, $P < 0.01$] and (f) vertical activity [$F(1,13) = 120.18$, $P < 0.0001$], but did not affect the duration of time spent in (g) the peripheral zone of the arena. In the wire-beam bridge experiment (h–j), SD-subjected rats exhibited a significant decrease in (i) latency to cross the bridge [$F(1,13) = 5.08$, $P < 0.05$] and (j) immobility duration [$F(1,13) = 7.02$, $P < 0.05$], but did not affect (k) the total distance traveled on the bridge. Notably, SD did not affect either total duration or frequency of exploratory activity directed towards either (k and l) novel objects or (m and n) social counterparts. Values represent mean \pm SEM for each group ($n = 7–11$). * $P < 0.05$; ** $P < 0.01$; **** $P < 0.0001$, compared to NSD group.

RESULTS

SD Results in Psychosis- and Mania-Related Behavioral Alterations

We first confirmed that 72 h of SD produced robust PPI deficits (Frau *et al*, 2008) (Figure 1b), but no alterations in startle amplitude (Figure 1a), startle latency (Figure 1c) or startle habituation, as tested by an inter-block ratio (IBR) index (Figure 1d). We then assessed whether the information-processing alterations in rats subjected to this SD regimen also displayed other behavioral changes related to psychosis and mania endophenotypes. In particular, we verified that SD-subjected rats exhibited significant enhancements in horizontal (Figure 1e) and vertical (Figure 1f) activity, but did not exhibit any alterations in the tendency to travel in the periphery of an open arena (Figure 1g), a well-established index of anxiety-like behavior in rats.

We then hypothesized that the hyperlocomotion in SD-subjected rats may reflect disinhibition, a core behavioral domain of mania; to test this possibility, we verified the impact of SD on the proclivity to cross a suspended wire-beam bridge task (Bortolato *et al*, 2009a). In line with clinical evidence (Killgore *et al*, 2006; McKenna *et al*, 2007; Womack *et al*, 2013), SD impaired risk assessment, as signified by a reduced the latency to cross the bridge (Figure 1h) and lower immobility duration (Figure 1i), but not overall distance traveled (Figure 1j) on the bridge. Conversely, sleep-deprived rats did not exhibit significant changes in their exploration of either novel objects (Figure 1k and l) or social counterparts (Figure 1m and n), indicating that the behavioral effects of SD were only specific to select domains of response inhibition.

SD-Induced PPI Alterations are Correlated with Increased Cortico-Accumbal Levels of 5 α R Types 1 and 2

To test whether these behavioral complications of SD were underpinned by changes in corticolimbic 5 α R expression, we

first measured the levels of the two main 5 α R isoenzymes (1 and 2) (Paba *et al*, 2011) in PFC, NAc, hippocampus and amygdala of SD-subjected rats. SD produced significant enhancements in 5 α R1 in the PFC and NAc ($P_s < 0.05$) (Figure 2a and b), but not in the hippocampus or amygdala (Figure 2c and d). Notably, mean %PPI levels were negatively correlated with the content of 5 α R1 in the NAc ($R^2 = 0.44$; $P < 0.05$; ANCOVA), but not in any of the other brain areas (Figure 2e–h). Furthermore, SD-subjected rats showed a significant enhancement of 5 α R2 in the PFC ($P < 0.05$), but not in any of the other areas (Figure 2i–l). A robust negative correlation was found between average %PPI levels and 5 α R2 levels in the PFC ($R^2 = 0.66$; $P = 0.001$; ANCOVA) (Figure 2m), but not in any of the other structures (Figure 2n–p). No significant correlations were found between startle amplitudes and 5 α R levels in any brain area. In contrast with 5 α R, SD failed to affect the expression of 3 α -HSOR in either the PFC or NAcc. (Supplementary Figure S1A and B).

SD Results in Enhanced AP Levels in the PFC, But Not Striatum

To verify whether the changes in 5 α R expression were associated with alterations in brain-regional profiles of neuroactive steroids, we measured the levels of pregnenolone, PROG, DHP, AP (see Figure 3a for a schematization of their metabolic pathway), DHEA, testosterone, DHT and 3 $\alpha,5\alpha$ -androstane diol in the PFC and striatum. Our results revealed that, in the PFC, 72-h SD resulted in a selective, significant increase in the levels of AP, but none of the other steroids (Figure 3b–d; Supplementary Table S1). A significant increase was also found in AP/PROG ratio in the PFC (Figure 3g). No differences, however, were found in the PROG/PREG ratio (Figure 3f). In contrast with the data in the PFC, no significant differences in neurosteroid levels or their ratios were detected in the striatum (including caudate-putamen and NAc) (Figure 3h–m and Supplementary

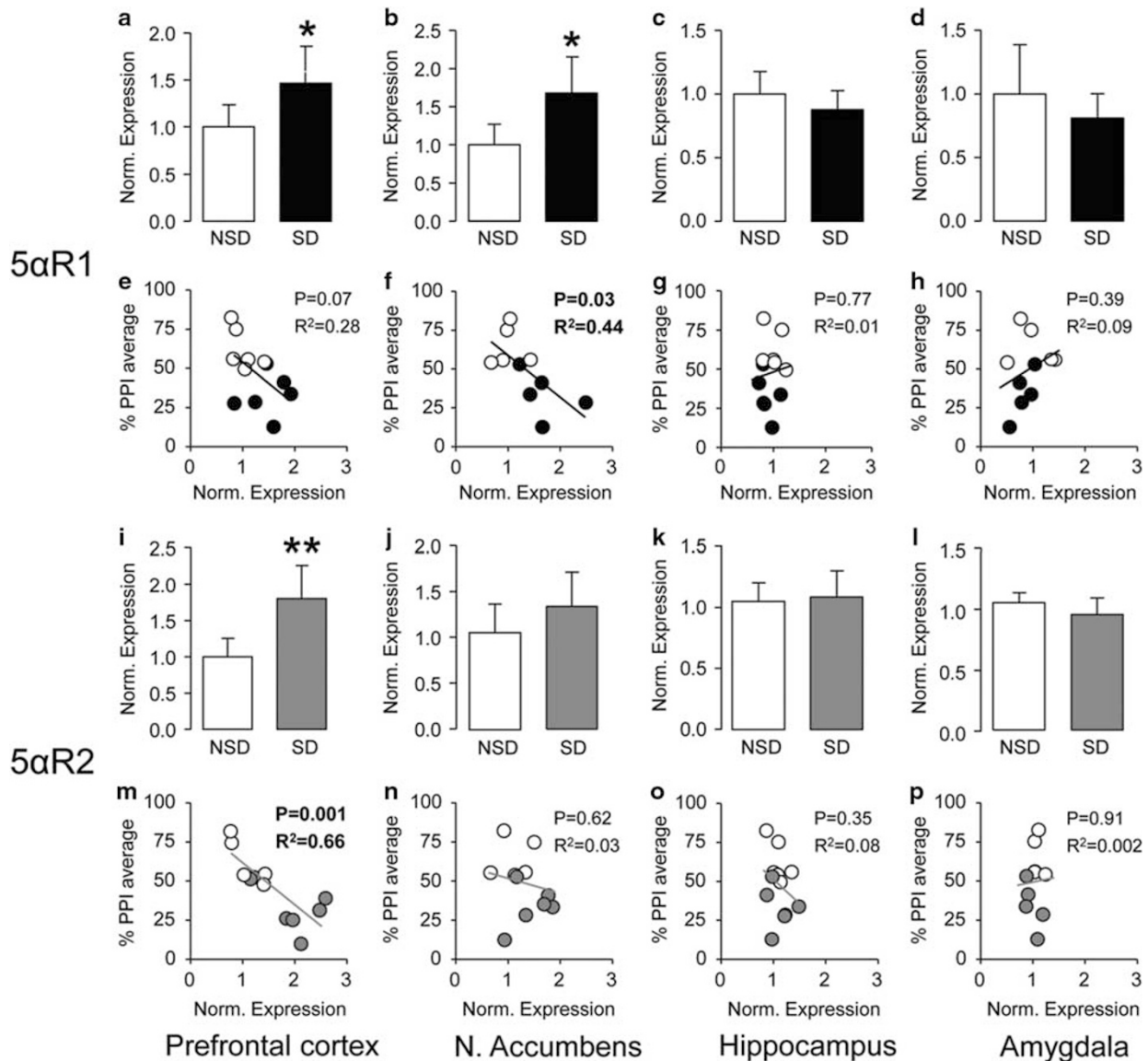


Figure 2 Sleep deprivation (SD) increases protein expressions of both 5 α -reductase isoforms (5 α R1 and 5 α R2) in different brain regions. SD-subjected rats exhibited increased 5 α R1 expression in (a) the prefrontal cortex [$F(1,10) = 6.24, P < 0.05$] and (b) nucleus accumbens [$F(1,8) = 7.59, P < 0.05$], but not in (c) hippocampus or (d) amygdala. PPI average levels were negatively correlated with 5 α R1 in the nucleus accumbens (f), but not in any other region (e, g, and h). SD led to a selective increase of 5 α R2 expression in (i) the PFC [$F(1,10) = 14.39, P < 0.01$], but not in any other brain areas (j–l). Furthermore, the expression of 5 α R2 in the PFC (m), but not in any other region (n–p) was negatively correlated with PPI levels. Bars represent means \pm SEM for each group ($n = 5$ –6). * $P < 0.05$; ** $P < 0.01$, compared to non-sleep deprived (NSD) rats.

Table S1). The levels of DHT and 3 α -diol were consistently below detection levels in either region.

FIN Reverses Behavioral and Neuroendocrine Effects of SD

To ascertain whether the behavioral impairments induced by SD were underpinned by the enhancement in 5 α R and AP levels, we tested the effects of the 5 α R inhibitor finasteride (FIN, 10–100 mg/kg, IP) on the behavioral changes induced by SD, in comparison with the benchmark mood stabilizer lithium carbonate (50 mg/kg, IP) and the antipsychotic haloperidol (0.1 mg/kg, IP) (Figure 4a–f). As expected, FIN dose-dependently reduced startle reflex in both NSD and SD

rats ($P_s < 0.0001$ for 25 and 100 mg/kg doses) (Figure 4a). Similar effects were induced by both haloperidol (Main effect: $P < 0.0001$) and lithium (Main effect: $P < 0.05$). Notably, 25 mg/kg and 100 mg/kg FIN countered SD-induced PPI deficits ($P_s < 0.05$ for both doses), in a fashion similar to lithium carbonate ($P < 0.05$) and haloperidol ($P < 0.05$); however, none of the drugs affected PPI in NSD controls (Figure 4b). The effects of FIN on the other behavioral abnormalities were only tested at the dose of 25 mg/kg, given that, in previous studies and pilot experiments, we found that higher doses of this 5 α R inhibitor significantly reduce spontaneous locomotor activity in control Sprague–Dawley rats (Bortolato *et al*, 2008). In contrast with the effects of FIN, the inhibitor of 3 α -HSOR

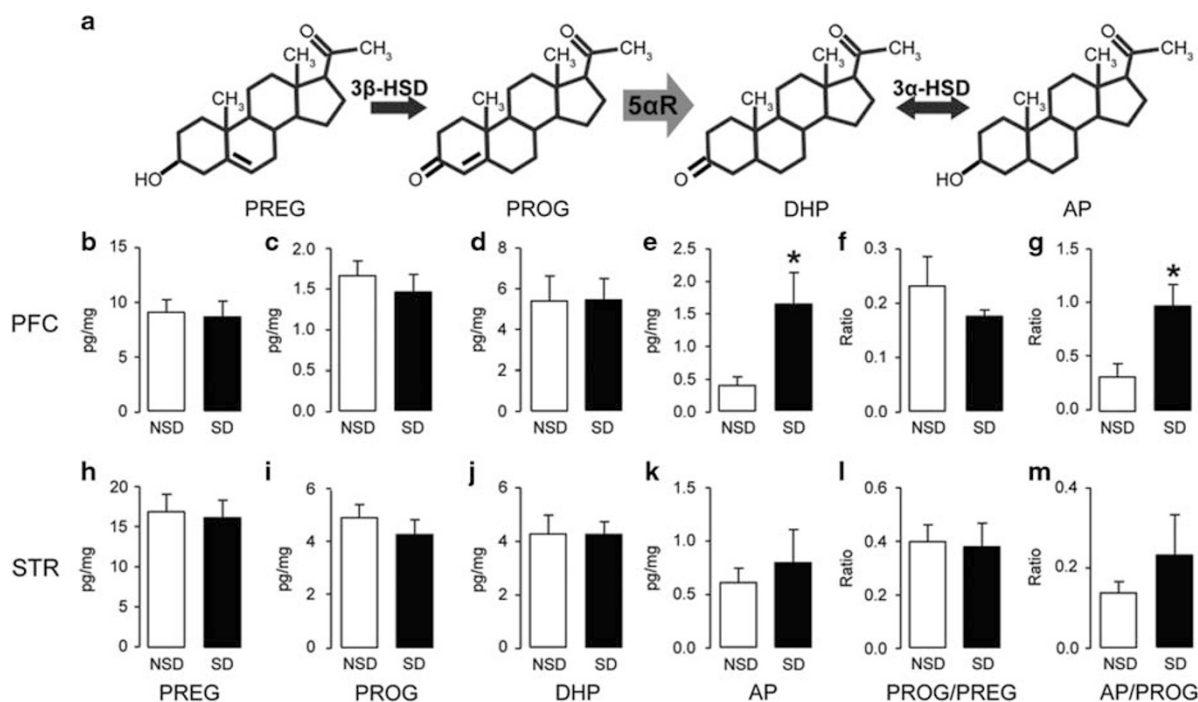


Figure 3 Effects of sleep deprivation (SD) on neurosteroid levels in the prefrontal cortex (PFC) and striatum (STR). (a) shows a schematic representation of the metabolic pathway converting pregnenolone (PREG) into progesterone (PROG), dihydroprogesterone (DHP) and allopregnanolone (AP). SD induced an enhancement in the PFC levels of AP (e) [$F(1,18) = 5.92$; $P < 0.05$], as well as its ratio with PROG (g) [$F(1,18) = 7.67$; $P < 0.05$]. Conversely, no significant differences were found in STR. 3 β -HSD: 3 β -hydroxysteroid dehydrogenase; 5 α R: 5 α -reductase; 3 α -HSD: 3 α -hydroxysteroid dehydrogenase. Values represent mean \pm SEM for each group ($n = 10$). * $P < 0.05$, compared to non-sleep deprived (NSD) rats.

indomethacin (5 mg/kg, IP) reduced startle magnitude in NSD rats ($P < 0.05$ vs vehicle-treated controls), but not in SD-subjected animals (Supplementary Figure S1C). No effects of this drug were detected on PPI (Supplementary Figure S1D) in either group.

FIN effectively countered SD-induced enhancements in horizontal (Figure 4c; $P < 0.001$) and vertical (Figure 4d; $P < 0.001$) activity, but had no significant effects on either response in NSD controls; in contrast, lithium reduced horizontal activity in both SD and NSD rats (Main effect: $P < 0.001$), but selectively reduced vertical activity in SD rats ($P < 0.01$). Finally, haloperidol reduced both horizontal and vertical activity irrespective of the experimental condition (Main effects: $P_s < 0.0001$ for both analyses).

We next explored whether FIN and lithium could restore the impairment in risk appraisal in the wire-beam bridge task. Given that haloperidol elicited profound locomotor impairments in both SD and NSD rats, this compound was not tested. Pretreatment with FIN or lithium selectively increased the latency to cross the bridge in SD-subjected animals ($P < 0.05$ for both; Figure 4e). Conversely, FIN increased the immobility time in the bridge in NSD animals ($P < 0.001$), but did not affect this index in SD-subjected rats (Figure 4f). Furthermore, FIN-treated, SD-subjected rats showed a marked reduction in immobility in comparison with NSD counterparts ($P < 0.001$). Lithium failed to significantly affect immobility (Figure 4f).

We then verified whether FIN modified the levels of neuroactive steroids in the PFC and striatum of SD and NSD rats (Supplementary Figure S2). In line with previous results (Frau et al, 2015), FIN produced a significant increase in

PREG and PROG levels ($P_s < 0.0001$; Supplementary Figure S2A, B, G and H) and reduced the concentrations of DHP ($P_s < 0.01$; Supplementary Figure S2C and I) in both regions, irrespective of the experimental group. AP levels were increased in the PFC of SD-exposed rats, irrespective of FIN treatment ($P < 0.01$ Supplementary Figure S3D). This drug, however, selectively opposed the enhancement in the AP/PROG ratio in this region ($P < 0.001$; Supplementary Figure S2F), indicating the normalization of 5 α R activity. In the striatum, FIN produced a generalized reduction of AP/PROG ratio (Main effect: $P < 0.05$), in both NSD and SD rats (Supplementary Figure S2L).

SD-Induced PPI Deficits are Exacerbated by AP and Attenuated by PROG

To verify the role of 5 α R substrates and products on the behavioral complications of SD, we tested the effects of AP and PROG. PPI deficits in SD-subjected animals were exacerbated by AP ($P < 0.01$; Figure 5b) and countered by PROG ($P < 0.01$; Figure 5d). These effects, however, were not observed in NSD rats. Furthermore, FIN significantly enhanced PPI in SD-subjected rats treated with AP, but not PROG. Startle analyses revealed that both AP and FIN reduced startle magnitude (Main effects: $P_s < 0.01$) (Figure 5a), while PROG had no such effect (Figure 5c). In additional studies, we tested the impact of DHT on startle and PPI. DHT increased startle magnitude (Main effect: $P < 0.01$; Supplementary Figure S3A) in both SD and NSD rats, but had no effect on PPI (Supplementary Figure S3B).

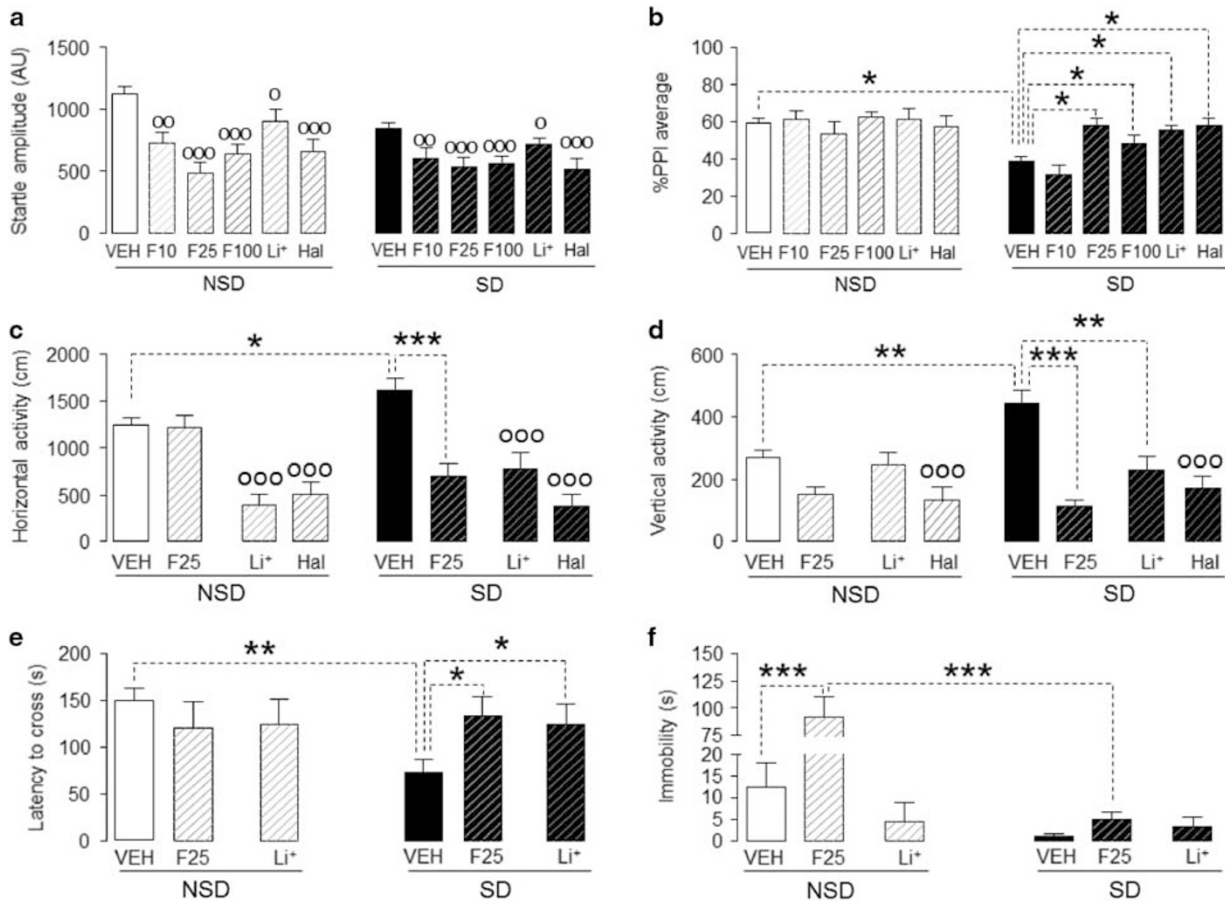


Figure 4 Effects of finasteride (F), lithium (Li^+) and haloperidol (Hal) on the behavioral changes induced by sleep deprivation (SD). (a) The startle response was significantly reduced by F(10–100 mg/kg) in both SD and non-sleep deprived (NSD) rats [Main effect for F: $F(3,63) = 12.04$; $P < 0.0001$; $P < 0.01$ for the comparison between 10 mg/kg F and vehicle; $P_s < 0.001$ for the comparisons between 25 and 100 mg/kg and vehicle]. Similarly, both Li^+ and Hal reduced this index in SD and NSD rats [Main effect for Li^+ : $F(1,39) = 4.33$; $P < 0.05$; Main effect for Hal: $F(1,39) = 37.44$; $P < 0.0001$]. (b) The analysis of PPI determined that, while SD reduced PPI in rats treated with vehicle (VEH), this effect was reversed by both doses of F [SD \times treatment interaction: $F(3,67) = 4.04$; $P < 0.05$; $P_s < 0.05$ for comparisons between VEH-NSD vs VEH-SD; VEH-SD vs VEH-F25; and VEH-SD vs VEH-F100], as well as Li^+ [SD \times treatment interaction: $F(1,38) = 4.13$; $P < 0.05$; $P < 0.05$ for comparison between VEH-SD and Li^+ -SD] and Hal [SD \times treatment interaction: $F(1,39) = 4.34$; $P < 0.05$; $P < 0.05$ for comparison between VEH-SD and Hal-SD]. (c) The analysis of horizontal locomotor activity revealed that VEH-treated, SD-subjected rats traveled for a longer distance than their NSD counterparts, and this enhancement was prevented by F25 [SD \times treatment interaction: $F(1,41) = 6.80$; $P < 0.05$ for comparison between VEH-NSD vs VEH-SD; $P < 0.001$ for comparison between VEH-SD and F25-SD]; conversely, both Li^+ and Hal reduced activity irrespective of the condition [Main effect for Li^+ : $F(1,41) = 24.94$; $P < 0.0001$; Main effect for Hal: $F(1,37) = 41.09$; $P < 0.0001$]. (d) Similarly, SD increased vertical activity, in a fashion sensitive to F [SD \times treatment interaction: $F(1,42) = 8.79$; $P < 0.01$; $P < 0.01$ for comparison between VEH-NSD vs VEH-SD; $P < 0.001$ for comparison between VEH-SD and F25-SD] and Li^+ [SD \times treatment interaction: $F(1,43) = 6.00$; $P < 0.05$; $P < 0.01$ for comparison between VEH-SD and Li^+ -SD]; conversely, Hal reduced vertical movements in both SD and NSD rats [Main effect for Hal: $F(1,38) = 35.74$; $P < 0.0001$]. (e) SD also reduced the latency of rats to cross a suspended wire-beam bridge, and this effect was significantly reversed by F [SD \times treatment interaction: $F(1,44) = 5.94$; $P < 0.05$; $P < 0.01$ for comparison between VEH-NSD vs VEH-SD; $P < 0.001$ for comparison between VEH-SD and F25-SD] and Li^+ [SD \times treatment interaction: $F(1,42) = 4.36$; $P < 0.05$; $P < 0.05$ for comparison between VEH-SD and Li^+ -SD]. (f) The immobility on the bridge was dramatically increased by F25 only in NSD, but not in SD rats F [SD \times treatment interaction: $F(1,39) = 33.38$; $P < 0.0001$; $P_s < 0.001$ for comparisons between VEH-NSD vs F-NSD and F-NSD vs F-SD]; conversely, Li^+ did not significantly affect this parameter, irrespective of the conditions. Values represent mean \pm SEM for each group ($n = 8-12$). * $P < 0.05$; ** $P > 0.01$; *** $P < 0.001$ for interaction effects (indicated by brackets). $^{\circ}P < 0.05$; $^{\circ\circ}P < 0.01$; $^{\circ\circ\circ}P < 0.0001$ for main treatment effects. The doses of F are indicated in mg/kg (IP).

Finally, we found that AP did not modify locomotor activity in either SD or NSD rats (Supplementary Figure S4).

DISCUSSION

The results of this study (summarized in Supplementary Table S2) show that SD enhanced the expression of $5\alpha\text{R1}$ and 2 , as well as AP synthesis, in the PFC of rats. These neurochemical changes were accompanied by PPI deficits, as well as other behavioral alterations, such as locomotor

hyperactivity and increased risk propensity. Notably, these alterations in SD-subjected animals were prevented by the $5\alpha\text{R}$ inhibitor FIN, in a fashion substantially analogous to the mood stabilizer lithium carbonate and the antipsychotic haloperidol; furthermore, PPI deficits were exacerbated by AP and countered by PROG in SD-subjected rats; conversely, neither neurosteroid had any effect on sensorimotor gating in NSD animals.

Taken together, these findings provide the first indication that $5\alpha\text{R}$ in the PFC mediates several of the psychotic- and

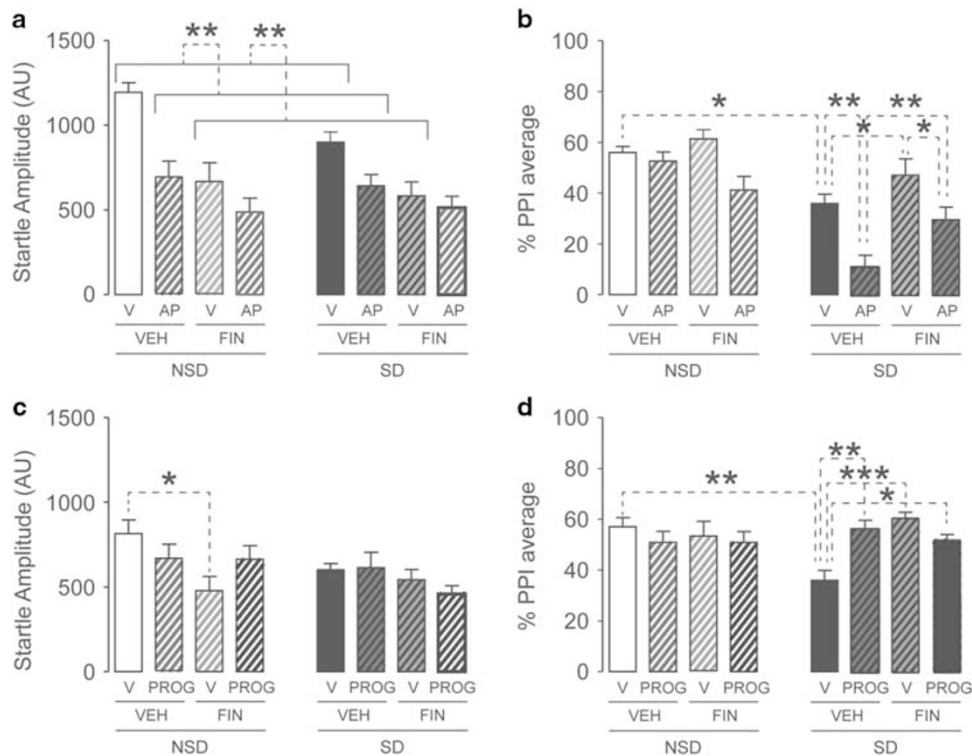


Figure 5 Effects of allopregnanolone (AP, 10 mg/kg, IP) and progesterone (PROG, 30 mg/kg, IP), in combination with finasteride (FIN, 25 mg/kg, IP) on (a and c) startle response and (b and d) prepulse inhibition (PPI) in sleep-deprived (SD) and non-sleep-deprived (NSD) rats. A 2-way interaction between AP and FIN (but not SD conditions) was found in the analysis of startle values (a) [$F(1,82) = 5.45, P < 0.05$]. This effect was shown to reflect significant differences between the rats treated with both vehicles and those treated with either AP or FIN ($P_s < 0.01$ for both comparisons). The analysis of PPI values (b) revealed a significant SD \times FIN \times AP interaction [$F(1,82) = 4.44; P < 0.05$]. Post-hoc analyses confirmed that SD reduced PPI in rats ($P < 0.05$ for comparison between SD and NSD rats treated with vehicles), and showed that this effect was significantly exacerbated by AP ($P < 0.01$), and reduced by FIN ($P < 0.05$). However, the PPI of SD rats treated with FIN and AP was significantly higher than those treated with vehicle and AP ($P < 0.01$) and lower than those treated with vehicle and FIN ($P < 0.05$). The evaluation of the effects of PROG on startle amplitude (c) revealed a significant SD \times FIN \times PROG interaction [$F(1,91) = 4.74; P < 0.05$], which was found to depend on a FIN-induced significant reduction on startle amplitude in NSD rats ($P < 0.05$). Furthermore, the analysis of PPI (d) showed a significant three-way interaction [$F(1,91) = 9.035, P < 0.05$]. Post-hoc comparisons confirmed a significant difference between NSD and SD rats, and revealed that both PROG and FIN countered the PPI deficits induced by SD ($P < 0.01$ and $P < 0.001$, respectively). Values represent mean \pm SEM for each treatment group ($n = 8-12$). V, vehicle of AP/PROG; VEH, vehicle of FIN. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Brackets indicate significant differences between experimental groups.

manic-like complications of acute SD by facilitating the conversion of PROG into AP. The translational relevance of these results is underscored by the recent demonstration that, in healthy volunteers, SD induces PPI deficits in association with phenotypic changes reminiscent of negative and cognitive symptoms of schizophrenia (Petrovsky *et al*, 2014). Based on this evidence, SD-induced PPI deficits are currently recognized as a promising surrogate model of psychosis (Petrovsky *et al*, 2014). It should also be noted that SD has been shown to increase impulsive responses and impair risk assessment (Killgore *et al*, 2006; McKenna *et al*, 2007; Venkatraman *et al*, 2007; Womack *et al*, 2013), and PPI deficits have been documented in impulse-control disorders and correlated with impulsivity levels (Stojanov *et al*, 2003; Gee *et al*, 2015); while future studies are required to verify whether SD-induced PPI deficits may serve as an operational index of impulse-control deficits, previous studies have indicated that this index may predict attentional and executive performance (Bitsios and Giakoumaki, 2005), which is typically defective in impulse-control alterations.

In contrast with $5\alpha R$, SD did not have any effect on $3\alpha HSOR$, the enzyme that converts DHP and DHT into AP

and 3α -diol, respectively. In agreement with these data, the $3\alpha HSOR$ inhibitor indomethacin did not reverse the behavioral effects of SD.

Both AP synthesis and $5\alpha R$ levels have been shown to be increased by acute stress in animal models (Purdy *et al*, 1991; Sánchez *et al*, 2008), raising the possibility that the stressful component of SD may contribute to the role of $5\alpha R$ in the neurobehavioral outcomes of SD. Indeed, the platform method used to induce SD in our study has been shown to elicit a significant stress response (Fadda and Fratta, 1997; Suchecki *et al*, 1998; Revel *et al*, 2009). Although future studies on different methods to produce SD in animal models are warranted to ascertain the influence of stress on the observed deficits, it is worth noting that we found that the PPI deficits induced by SD were not observed in rats on large platforms or rats exposed to consistent soaking, indicating that the deficits in sensorimotor gating may be indeed specific to SD (Frau *et al*, 2008). Furthermore, parsing out the outcomes of SD from those of stress response may be highly problematic, given that SD is inherently stressful and conducive to a robust response of the HPA axis (Vgontzas *et al*, 1999; McEwen, 2006; Minkel *et al*, 2014).

The association of SD-induced phenotypes with alterations in $5\alpha R$ and neurosteroids in the PFC may reflect the key role of this region in the regulation of PPI and risk-taking behaviors (Bubser and Koch, 1994; Hazlett *et al*, 1998; Knoch *et al*, 2006; St Onge and Floresco, 2010), as well as its high susceptibility to the deleterious effects of SD (Harrison *et al*, 2000; Durmer and Dinges, 2005; Yan *et al*, 2011; Acosta-Peña *et al*, 2015). Specifically, our results indicated that PPI levels were negatively correlated with the PFC concentrations of $5\alpha R2$, but not $5\alpha R1$. In adult rats, PFC $5\alpha R2$ is segregated in pyramidal cells (Castelli *et al*, 2013), the output neurons that project to other cortical areas or subcortical regions. This pattern of expression in rats is strikingly different from that of $5\alpha R1$, which has been shown to be localized also in glial cells (Melcangi *et al*, 1993). Although, $5\alpha R2$ is significantly less abundant than $5\alpha R1$ in the adult brain (Normington and Russell, 1992), it has a higher affinity for PROG than $5\alpha R1$ (Paba *et al*, 2011); these premises collectively suggest that $5\alpha R2$ may serve a distinctive role, directly related to the functional control of pyramidal cells in the PFC. Given that AP acts as a positive allosteric modulator of the γ -aminobutyric acid A (GABA-A) receptor (Belelli and Lambert, 2005), the increased synthesis of this neurosteroid in pyramidal cells may lead to a higher conductance of their GABA-A channels, ultimately resulting in reduced firing patterns of the projections of PFC towards other brain areas. Furthermore, PROG had a protective effect on SD-induced PPI, suggesting that its receptors may be implicated in the complications of SD and in the antipsychotic-like effects of FIN. Overall, these imbalances may be related to changes in signal-to-noise ratio in pyramidal neurons, leading to temporary dysfunctions of PFC activity. While GABA-A and PROG receptors likely contribute to the regulation of PPI in SD-subjected animals, we cannot exclude that these phenomena may also reflect the involvement of other receptors in the PFC with high affinity for AP and/or PROG, such as the membrane PROG receptors, PXR, NMDA and σ receptors (Cooke *et al*, 2013; Frye *et al*, 2014; Vyklícky *et al*, 2015).

The behavioral changes induced by SD may have been contributed by other regions than PFC. In particular, $5\alpha R1$ expression was significantly increased in the NAc. Due to the small size of this region, we could not assess whether these changes may lead to functional alterations of this enzyme. Pooling the region with the dorsal striatum failed to reveal any significant change; however, this result should be considered with caution, given that NAc represents only a small portion of the overall sampled tissues. Moreover, our previous results showed that the antipsychotic-like actions of FIN on the PPI deficits induced by the dopaminergic non-selective agonist apomorphine were fully replicated by intra-NAc infusions (Devoto *et al*, 2012).

In addition to rescuing the changes in AP/PROG ratio, FIN also produced a number of other changes in neurosteroid profile in the PFC and striatum. In line with previous findings (Frau *et al*, 2015), this drug increased PREG and, to a lesser extent, PROG levels. While the underpinnings of these effects are unknown, it is possible that this phenomenon may reflect an internal feedback mechanism controlling the activity of key promoters of *ex novo* neurosteroidogenesis, such as the 18-kDa translocator protein or the steroidogenic acute regulatory (StAR) protein.

On this score, it is worth noting that DHT reduces StAR expression (Houk *et al*, 2004); thus, a reduction in DHT levels induced by FIN may lead to a major enhancement in neurosteroidogenesis and PREG production. Irrespective of these issues, it is possible that the enhancement in PREG may be instrumental to enable the activity of FIN in restoring hyperactivity in SD-subjected rats. In keeping with this concept, previous reports have shown that PREG may exert antipsychotic-like actions in both patients and animal models (Marx *et al*, 2009; Wong *et al*, 2012).

A number of limitations should be acknowledged: first, our studies were only conducted in male rats; given the implication of $5\alpha R$ s in the synthesis of sex hormones, it is possible that the phenotypic effects of neurosteroids in SD-subjected rats may be different; second, although our results imply that changes in cortical steroids are conducive to alterations in PPI and other behavioral responses, this thesis should be further supported by injections of neurosteroids and FIN in the PFC. Indeed, it is possible that other regions not included in our analyses may play an important role in the phenotypic effects documented in this study; third, pharmacological treatments with FIN and other agents was generally limited to a relatively narrow dose range; in consideration of the complex dose-response profiles of neurosteroids, we cannot exclude that our analyses may have failed to identify additional effects of the drugs tested; fourth, it is possible that our assessment of the behavioral effects of FIN in SD may be limited by the intrinsic hypolocomotive effects of this drug in NSD controls. These caveats notwithstanding, the present study has provided the first-ever demonstration that the neurosteroidogenic enzyme $5\alpha R$ mediates psychosis- and mania-related complications of SD. These findings, as well as emerging evidence on the therapeutic properties of FIN in several conditions exacerbated by SD, may open up to new horizons for the development of neurosteroid-based therapies in psychiatry and as novel tools to offset the negative psychological impact of sleep disturbances. Accordingly, preliminary evidence from our group has shown that $5\alpha R$ inhibitors may exert therapeutic effects in a number of disorders characterized by sensorimotor gating deficits and exacerbated by sleep loss, such as schizophrenia, pathological gambling and Tourette syndrome (Koethe *et al*, 2008; Muroi *et al*, 2011; Bortolato *et al*, 2012).

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