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5alpha-reductase inhibitors dampen L-DOPA-induced dyskinesia via normalization of dopamine D1-receptor signaling pathway and D1-D3 receptor interaction

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ABSTRACT

Although 1-3,4-dihydroxyphenylalanine (L-DOPA) is the mainstay therapy for treating Parkinson's disease (PD), its long-term administration is accompanied by the development of motor complications, particularly L-DOPA induced dyskinesia (LID), that dramatically affects patients' quality of life. LID has consistently been related to an excessive dopamine receptor transmission, particularly at the down-stream signaling of the striatal D_1 receptors (D_1R), resulting in an exaggerated stimulation of cAMP-dependent protein kinase and extracellular signal-regulated kinase (ERK) pathway. We previously reported that pharmacological blockade of 5alpha-reductase (5AR), the rate-limiting enzyme in neurosteroids synthesis, attenuates the severity of a broad set of behavioral alterations induced by D_1R and D_3R activation, without inducing extrapyramidal symptoms.

In line with this evidence, in a recent study, we found that inhibition of 5AR by finasteride (FIN) produced a significant reduction of dyskinesia induced by L-DOPA and direct dopaminergic agonists in 6-OHDA-lesioned rats.

In the attempt to further investigate the effect of 5AR inhibitors on dyskinesia and shed light on the mechanism of action, in the present study we compared the effect of FIN and dutasteride (DUTA), a potent dual 5AR inhibitor, on the development of LID, on the therapeutic efficacy of L-DOPA, on the molecular alterations downstream to the D_1R, as well as on D_1R-D_3R interaction.

The results indicated that both FIN and DUTA administration significantly reduced development and expression of LID; however, DUTA appeared more effective than FIN at a lower dose and produced its anti-dyskinetic effect without impacting the ability of L-DOPA to increase motor activation, or ameliorate forelimb use in parkinsonian rats.

Moreover, this study demonstrates for the first time that 5AR inhibitors are able to prevent key events in the appearance of dyskinesia, such as L-DOPA-induced upregulation of striatal D_1R-related cAMP/PKA/ERK signaling pathways and D_1R-D_3R coimmunoprecipitation, an index of heteromer formation.

These findings are relevant as they confirm the 5AR enzyme as a potential therapeutic target for treatment of dyskinesia in PD, suggesting the first ever evidence that neurosteroidogenesis may affect functional interaction between dopamine D_1R and D_3R.

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1. Introduction

L-DOPA-induced dyskinesias (LIDs) are extremely burdensome motor complications of long-term L-DOPA treatment in Parkinson's disease (PD) patients. The underlying mechanisms for LIDs are unclear though a number of preclinical studies indicate the importance of pulsatile stimulation of striatal postsynaptic dopamine D_1 receptors (D_1R) in their pathogenesis (Aubert et al., 2005; Bastide et al., 2015; Feyder et al., 2011; Fiorentini et al., 2016; Westin et al., 2007).

We recently showed that, in the 6-hydroxydopamine (6-OHDA) rat model of PD, LIDs were countered by finasteride (FIN) (Frau et al., 2017), a steroidogenesis inhibitor used for the therapy of benign prostatic hyperplasia and androgenic alopecia (Paba et al., 2011). FIN elicits its effects by inhibiting 5alpha-reductase (5AR), the enzyme that catalyzes the rate-limiting step in the conversion of progesterone and testosterone into their neuroactive metabolites dihydroprogesterone and dihydrotestosterone, respectively (Paba et al., 2011). Previous work showed that FIN attenuated the severity of a broad set of behavioral alterations induced by dopamine D_1R and D_3R selective and non-selective agonists, including locomotor hyperactivity, stereotyped behaviors and prepulse inhibition deficits (Frau et al., 2013, 2016). Importantly, the anti-dopaminergic effects of FIN and other 5AR inhibitors were not accompanied by motor side effects (Bortolato et al., 2008). In spite of these promising anti-dopaminergic properties, lacking extrapyramidal symptoms, the molecular underpinnings of the anti-dyskinetic effects of FIN are yet to be established.

LIDs are attributed to the abnormal signaling of D_1R located on striatal projection neurons, in response to long-term dopamine depletion (Cenci and Konradi, 2010), resulting in an exaggerated stimulation of cAMP-dependent protein kinase (PKA) and enhanced phosphorylation of downstream dopamine and c-AMP-regulated phosphoprotein, Mr32 (DARPP-32), and extracellular signal-regulated kinase (ERK) pathways (Pavón et al., 2006; Spigolon and Fisone, 2018). Of note, compelling evidence points to a functional cross-talk between striatal D_1R and D_3R in the sensitization to repeated L-DOPA administration (Bordet et al., 2000); indeed, striatal D_1R and D_3R receptors are able to form heteromeric complexes that may prevent L-DOPA-induced D_1R internalization, resulting in increased D_1R downstream signaling cascade. Accordingly, a correlation between these heteromers and the severity of LID has been found in parkinsonian rats (Farré et al., 2015; Fiorentini et al., 2015; Guitart et al., 2014; Solís and Moratalla, 2018; Solís et al., 2017).

Based on this framework, in this study we investigated the impact of a preventive treatment with either FIN or dutasteride (DUTA), a potent dual 5AR inhibitor, on the development of LID. Furthermore, in order to assess the modifications in D_1R-mediated signaling, we measured the levels of expression of G-protein α_{olf} ($G\alpha_{olf}$), the major G-protein expressed in the striatum, that mediates cAMP/PKA stimulation in response to D_1R activation, and the phosphorylation levels of ERK1/2 and DARPP-32. We next explored, in the striatum of the lesioned side, D_1R-D_3R heteromer formation which has been correlated with increased occurrence of LID.

2. Material and methods

2.1. Animals

The present study was performed on adult male Sprague-Dawley rats (275-300 g, Envigo, Italy). Animals were housed 4 per cage under standard temperature and humidity conditions, in a 12 h light/12 h dark cycle (light from 07:00 to 19:00 h) with ad libitum access to water and food. All surgical procedures and experiments were carried out in accordance with the European Union directive (EU Directive 2010/63 and Italian DLgs. 2014/26).

2.2. Drugs

FIN and DUTA (Carbosynth Limited-UK) were suspended in a vehicle (VEH) solution containing 5% Tween 80 and 95% sterile saline (SAL; 0.9% NaCl) and injected intraperitoneally (IP). L-DOPA methylester (Research Organics USA) and benserazide (Sigma-Aldrich) were dissolved in SAL and injected subcutaneously (SC). 6-OHDA (Sigma-Aldrich) was dissolved in SAL plus 0.02% ascorbic acid, and locally infused into the medial forebrain bundle (MFB). A 20:1 mixture of Fentanest (Pfizer, Italy) and Domitor® (Orion Pharma, Italy) in a volume range of 1.4–1.6 ml, was used to induce general anesthesia. Antisedan® (0.37 mg/kg, SC, Orion Pharma, Italy) was injected to reverse the sedative effect of the anesthetic. Drug doses were chosen based on our previous studies (Bortolato et al., 2008; Frau et al., 2013, 2016, 2017).

2.3. Stereotaxic surgery

According to Paxinos (2007), animals received a single unilateral injection into the right medial forebrain bundle (MFB) (AP: -2.2 mm; ML: −1.5 mm; DV: −7.9 mm from the dura, tooth bar: + 5 mm.) with 16 µg of 6-OHDA (4 µg/µl free base in SAL with 0.2% ascorbic acid) in order to achieve a complete lesion of the nigrostriatal pathway. All 6-OHDA injections were conducted using a stereotaxic frame with an attached Hamilton syringe. Injection speed was 1.0 µl/min and the syringe was kept in place for additional 3 min before removing it slowly.

2.4. Behavioral analysis

2.4.1. Stepping test

The stepping test was performed as previously described (Tronci et al., 2013). Briefly, the rat was held by the experimenter immobilizing the hindlimbs with one hand and the forelimb not to be monitored with the other, while the unrestrained forepaw was touching the table. The number of adjusting steps was counted for both paws in forehand and backhand direction while the rat was moved sideways along the table surface (90 cm in 5 s) and the average of the steps in the two directions was considered.

2.4.2. Assessment of abnormal involuntary movements (AIMs)

Abnormal Involuntary Movements (AIMs) were evaluated based on the rat dyskinesia scale according to previous procedures and methods (Cenci and Lundblad, 2007; Lundblad et al., 2002; Tronci et al., 2014). Briefly, animals were placed individually in transparent plastic single cages without bedding material and scored every 20 min after L-DOPA injection for the entire time course of dyskinesia (about 120 min). According to their topographic distribution, AIMs were classified into three subtypes as forelimb, orolingual and axial dyskinesia. A score from 0 to 4 was assessed for each AIMs subtype based on the time spent (0: absent; 1: occasional, i.e. present < 50% of the time; 2: frequent, i.e. present > 50% of the time; 3: continuous, but interrupted by strong sensory stimuli and 4: continuous, not interrupted by strong sensory stimuli). The total AIMs score was obtained by summing each involuntary movement subtype score considered during the monitoring period.

2.4.3. Activity test

The locomotor activity was assessed in motility cages (Omnitech Digiscan Animal Activity Monitor, Columbus, OH, USA), each equipped with 2 sets of 16 photocells located at right angles to each other, projecting horizontal infrared beams 2.5 cm apart above the cage floor. Animals were injected with 5AR inhibitors and placed in automated activity cages. After 40 min, animals were treated with L-DOPA and locomotor activity was recorded for additional 180 min. Horizontal activity counts were collected every 10 min.

2.5. Immunohistochemistry and biochemical studies

One hour after the last L-DOPA injection, all rats were sacrificed and the brains were collected for immunohistochemistry and biochemical analysis.

2.5.1. Immunohistochemistry study

Tyrosine Hydroxylase (TH) immunohistochemistry was performed to verify the dopaminergic lesion induced by 6-OHDA infusion into the right MFB. All animals appeared to be fully lesioned in the substantia nigra (see Fig. S1 in the supplementary material for a representative image).

2.5.2. Biochemical studies

Striatal tissue was dissected and processed for western blotting analysis in order to study the effect of drug treatments on expression of D_1R-D_3R heterodimers and molecular markers of dyskinesia. Right and left striata were excised using the rapid head-freeze dissection technique as previously described (Scheggi et al., 2009). For each animal, both lesioned and intact sides were analyzed. Frozen tissues were solubilized in cell lysis buffer (50 mM TRIS, pH 7.4, 250 mM NaCl, 5 mM EDTA, 50 mM NaF, 1 mM sodium orthovanadate, 1% Triton X-100, 0.02% NaN_3) containing 1 mM phenylmethylsulfonyl fluoride and protease inhibitor cocktail (Sigma-Aldrich). For pERK 1/2, pDARPP-32, and $G\alpha_{olf}$ analysis, lysates were centrifuged at 14,000 xg at 4 °C for 10 min and the protein concentration in the supernatant was determined by Lowry method.

2.5.3. Western blotting

Equal amounts of protein (30 μg) were separated on a 4–15% TGX Criterion precast gel (Bio-Rad Laboratories, Inc., Hercules, CA) and subsequently electro-transferred to a nitrocellulose membrane at 12 V (constant) for 1 h (Grappi et al., 2011). The membranes were incubated with primary antibodies against dopamine D_1R and D_3R (Santa Cruz Biotechnologies, Santa Cruz, CA, USA, #SC-33660, 1:1000 and #SC-9114, 1:500), phospho p44/42 MAPK (p-ERK1/2; Thr202/Tyr204, Cell Signaling Technology; #4370, 1:1000), p44/42 MAPK (ERK1/2, Cell Signaling Technology, #4695, 1:1000), phospho-Thr34 DARPP-32, DARPP-32 (Cell Signaling Technology, #12438; 1:1000 and #2306, 1:1000), and $G\alpha_{olf}$ (GNAL antibody, #PA5-50981, Thermo Fisher Scientific, 1:1000) in blocking buffer overnight at 4 °C. Specific antibody binding was detected by chemiluminescence with the ChemiDoc XRS + Imager (Bio-Rad Laboratories). Samples from each treatment group were immunoblotted and analyzed together. To control for equal loading for dopamine D_1R, D_3R, ERK 1/2, DARPP-32 and $G\alpha_{olf}$ expression, blots incubated with antibodies were stripped and reprobed using anti β-actin (Sigma-Aldrich, St.Louis, MO, USA); for phospho-ERK1/2 and phospho-Thr34 DARPP-32, blots were stripped and reprobed using anti total ERK or anti total DARPP-32 antibody, respectively. Bands were quantified in arbitrary units and normalized using the software Image Lab (Bio-Rad Laboratories) using β-actin, ERK and DARPP-32 as loading controls.

2.5.4. Immunoprecipitation

Immunoprecipitation was performed as previously described (Scheggi et al., 2017). Briefly, dopamine D_1R were immunoprecipitated from tissue lysates using monoclonal dopamine D_1R antibody against the last 123C-terminal amino acids of rat dopamine D_1R (SC-33660, Santa Cruz Biotechnology). Antibodies were coupled to protein A Dynabeads (Invitrogen) using 5 μg antibody by rotating the mixture for 10 min at room temperature. Beads were washed twice in PBS and then the antibody-conjugated beads were incubated with 200–300 μg of protein lysate for 2 h at 4 °C, followed by 3 washing steps in 0.1% Tween 20 supplemented PBS. Bound proteins were then eluted with 4 x XT Sample buffer and Reducing agent (Bio-Rad Laboratories). The immunoprecipitates were separated on a 4–15% TGX Criterion precast gel as previously described. The membranes were incubated with polyclonal anti-D_3 antibody (SC-9114, Santa Cruz Biotechnology). Chemiluminescence was detected and quantified with the ChemiDoc Imaging System (Bio-Rad Laboratories). Samples from control and treated rats were run on the same immunoblots and then analyzed together.

Band intensities of co-immunoprecipitated D_3Rs were normalized to the band intensities of immunoprecipitated D_1Rs. Band intensities were quantified using Image Lab software/Gel Doc XRS + system; values, expressed as arbitrary units, were then calculated as percentage of the Vehicle + Saline group values.

2.6. Experimental design

First, we tested the effect of a preventive treatment with the two 5AR inhibitors FIN and DUTA on LID onset, in 6-OHDA lesioned male rats. Three weeks after stereotaxic 6-OHDA lesion, rats were subjected to the stepping test in order to include in the study only rats with a severe impairment. Thus, according to stepping test score, rats were allocated in 5 balanced groups (n = 7–13 per group) and subjected to a 2-week pretreatment with FIN (30 or 60 mg/kg, IP), DUTA (15 or 30 mg/kg, IP) (Bortolato et al., 2008; Frau et al., 2017) or VEH. Thereafter, for additional 24 days, rats were daily administered with L-DOPA/benserazide (6/6 mg/kg, SC) 40 min after the administration of FIN, DUTA or VEH (FIN 30 + L-DOPA, FIN 60 + L-DOPA, DUTA 15 + L-DOPA and DUTA 30 + L-DOPA, VEH + L-DOPA groups). An additional group of 6-OHDA lesioned rats injected with VEH + SAL was used as control group for western blot and immunoprecipitation experiments. Onset and development of dyskinesia were monitored every 3–4 days by the assessment of AIMs score.

Furthermore, to investigate whether 5AR inhibitors might affect the therapeutic effect of L-DOPA in forelimb use, rats were subjected to the stepping test both at the first and last day of L-DOPA treatment. The day of the test, trained rats were first assessed for the baseline impairment; afterwards, they received FIN, DUTA or VEH 40 min before L-DOPA injection, and the number of adjusting steps was recorded: (i) 60 and 150 min after L-dopa injection at day 1; (ii) 150, 200 and 250 min after L-DOPA injection at day 23 (as the animals were more dyskinetic after chronic L-DOPA treatment, the test could not be performed at 60 min).

To further examine the effect of FIN and DUTA on the ambulatory movements, after two weeks of L-DOPA treatment, rats were tested on motility cages (Fig.1).

2.7. Statistical analysis

Behavioral data were analyzed by repeated measures two-way ANOVA, with treatment (DUTA, FIN or VEH) and time (days or minutes) as factors. Post hoc analyses were performed using Newman-Keuls or Tukey's multiple comparison test, as appropriate. Ordinary one-way ANOVA followed by Sidak's multiple comparison test was used for biochemical studies. Paired Student's t-test was used to compare data from the intact and lesioned side. Alpha was set at $p < .05$. All values are presented as mean ± SEM.

3. Results

3.1. Effect of FIN and DUTA on development of LID in 6-OHDA-lesioned rats

Since DUTA has higher efficacy in blocking both 5AR isoforms than FIN (Paba et al., 2011), the first set of experiments was aimed at comparing the ability of both inhibitors to dampen development of LID and to interfere with therapeutic effect of L-DOPA.

As shown in Fig. 2, FIN (A) and DUTA (B) treatments, in combination with L-DOPA, elicited similar extent of AIMs reduction [FIN group, main effect of treatment: $F_{(2,24)} = 9.31$, $p = .0010$; DUTA group, main effect of treatment: $F_{(2,26)} = 7.2$, $p = .0033$, two-way repeated

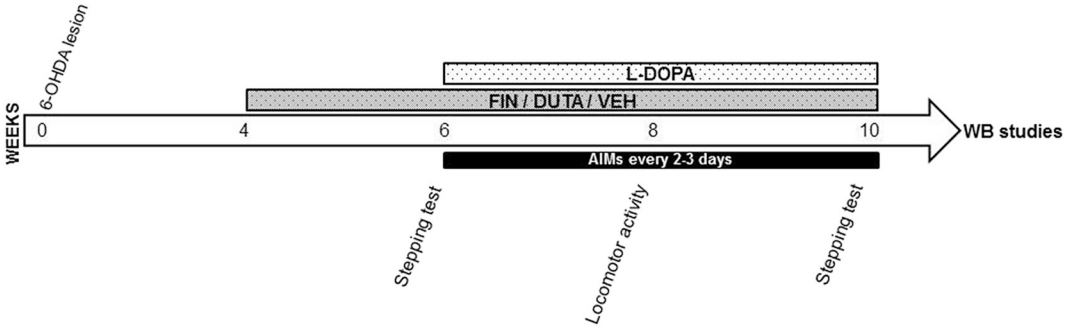


Fig. 1. Schematic representation of the experimental plan. WB (Western blotting) AIMs (Abnormal Involuntary Movements).

measures ANOVA]; however, DUTA was effective at a lower dose; moreover, the effect of the lower dose of each drug was already maximal. A main effect of time was also found [FIN group, main effect of time: $F_{(6,144)} = 17.9$, $p < .0001$; DUTA group, main effect of time: $F_{(6,156)} = 17$, $p < .0001$, two-way repeated measures ANOVA], while no treatment x time interaction was detected by the analysis for both treatment [FIN group, treatment x time interaction: $F_{(12,144)} = 1.15$, $p = .3266$, NS; DUTA group, treatment x time interaction: $F_{(12,156)} = 0.65$ $p = .7963$, NS; two-way repeated measures ANOVA]. Newman-Keuls multiple comparisons test indicated that both doses of FIN and DUTA significantly decrease the onset and development of AIMs compared to VEH group at all time points.

3.2. Effect of FIN and DUTA on L-DOPA-induced forelimb use in 6-OHDA-lesioned rats

The above experimental groups were subjected to the stepping test on day 1 and 23 of chronic L-DOPA treatment to investigate the impact of FIN or DUTA administration on L-DOPA-induced amelioration of forelimb use.

As expected, L-DOPA improved the forelimb use compared to baseline at day 1 and 23 [main effect of time at day 1: $F_{(2,48)} = 21.30$ $p < .0001$; main effect of time at day 23: $F_{(3,72)} = 30.23$ $p < .0001$]. Most importantly, DUTA (15–30 mg/kg) did not reduce forelimb use compared to L-DOPA alone, neither at day 1, nor at day 23 (Fig. 3B), while the higher dose of FIN (60 mg/kg) produced a partial reduction at day 1 (Fig. 3A). Interestingly, a trend to improved forelimb use was seen with DUTA compared to L-DOPA alone (for both doses at each time point).

3.3. Effect of FIN and DUTA on L-DOPA-induced motor activation in 6-OHDA-lesioned rats

To further investigate the impact of the 5AR inhibitor treatments on the therapeutic efficacy of L-DOPA, we tested the effect of FIN and DUTA, at the lower doses efficacious in counteracting LID, on the motor activation induced by L-DOPA, at day 16 of the L-DOPA chronic treatment period. Of note, while FIN significantly decreased the motor activation induced by L-DOPA treatment in 6-OHDA-lesioned rats, DUTA did not modify this parameter at the dose efficacious in counteracting LID (15 mg/kg, IP) [main effect of time: $F_{(18,\ 450)} = 29.95$, $p < .0001$; main effect of treatment: $F_{(2,\ 25)} = 1.96$, $p = .16$, NS; interaction time x treatment: $F_{(36,\ 450)} = 1.18$, $p = .22$, NS; two-way repeated measures ANOVA (Fig. 4).

3.4. Effect of FIN and DUTA on striatal markers of LID

To investigate whether the anti-dyskinetic effects of FIN and DUTA were accompanied by a normalization of the L-DOPA-induced alteration of dopamine D_1 receptor signaling pathway, we analyzed striatal markers of dyskinesia in 6-OHDA lesioned rats treated with L-DOPA alone or in combination with either FIN or DUTA.

In the lesioned side, the analysis of phospho-ERK 1/2 and phospho-Thr34 DARPP-32 levels by one-way ANOVA showed a significant difference between groups [FIN experiment: pERK, $F_{(3,22)} = 5.66$, $p = .0049$; pDARPP-32, $F_{(3,21)} = 3.59$, $p = .0307$; DUTA experiment: pERK, $F_{(3,21)} = 8.427$, $p = .0007$; pDARPP-32, $F_{(3,21)} = 6.99$, $p = .0029$;). As expected, in the lesioned side, chronic L-DOPA administration increased both phospho-ERK 1/2 ($p < .01$) and phospho-Thr34 DARPP-32 levels ($p < .05$). Administration of FIN prevented the

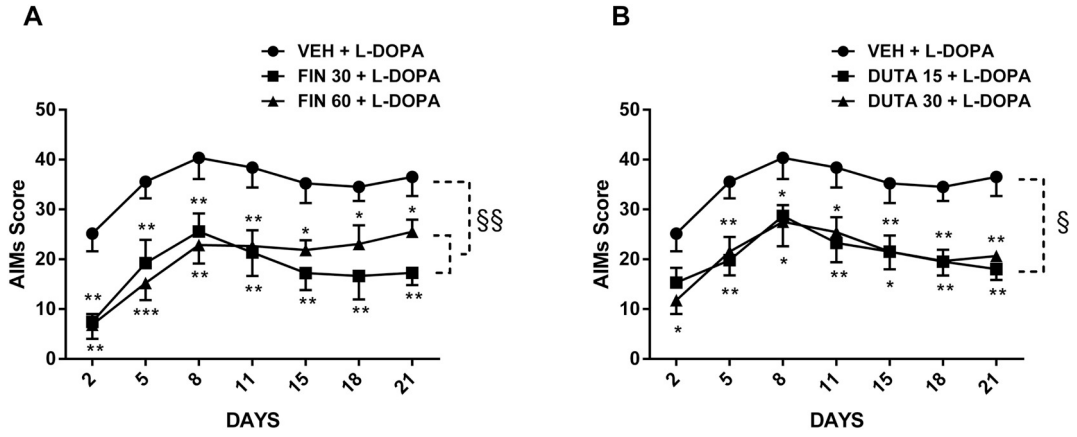


Fig. 2. Effect of preventive treatment with FIN (A) or DUTA (B) on development of LID in 6-OHDA lesioned L-DOPA naïve rats. Scores are shown as the sum of axial, limb and orolingual Abnormal Involuntary Movements (AIMs) on each testing session. Values represent mean ± SEM for each experimental group. Main effect of treatment, § $p < .05$, §§ $p < .01$ vs VEH + L-DOPA group (two way ANOVA for repeated measures). * $p < .05$, ** $p < .01$, *** $p < .001$ vs corresponding time point in VEH + L-DOPA group (Newman-Keuls multiple comparisons post hoc test).

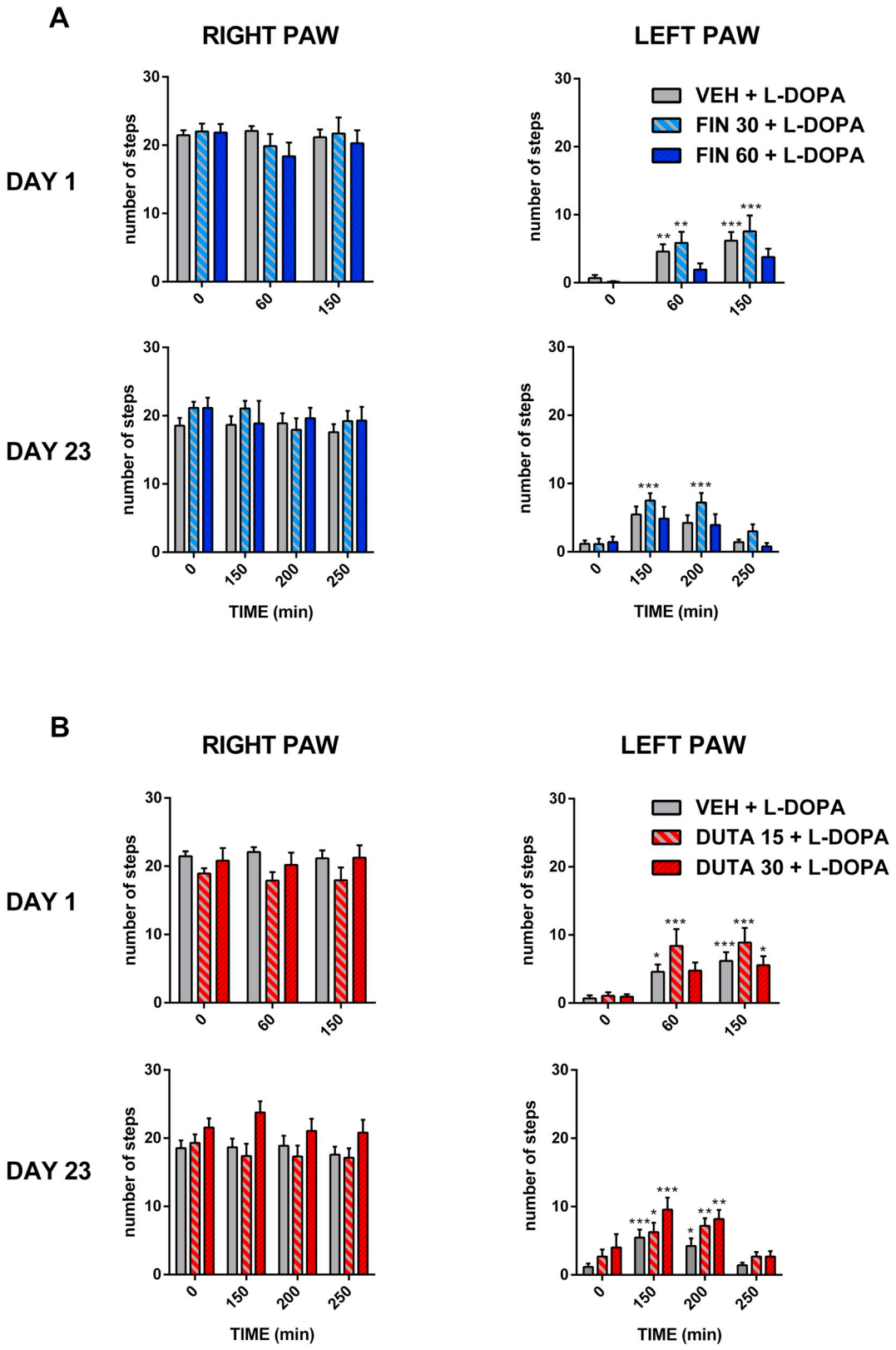


Fig. 3. Effect of preventive treatment with FIN (A) or DUTA (B) plus L-DOPA on L-DOPA-induced forelimb use in 6-OHDA lesioned rats. Stepping test was performed at day 1 and 23 of L-DOPA treatment before (time 0) and after 60, 150, 200 and 250 min following L-DOPA administration. Bar represent the mean number of adjusting steps ± SEM.* $p < .05$, ** $p < .01$, *** $p < .001$ vs respective treatment at time 0′.

increase in phospho-ERK and phospho-Thr34 DARPP-32 levels induced by L-DOPA only at the higher dose ($p < .05$). Conversely, DUTA prevented the increase in phospho-ERK and phospho-Thr34 DARPP-32 levels at both doses (pERK: $p < .001$ and $p < .05$; pDARPP-32: $p < .05$ and $p < .001$, DUTA 15 and DUTA 30 mg/kg, respectively) (Fig. 5). Levels of total ERK 1/2 and DARPP-32 were not statistically

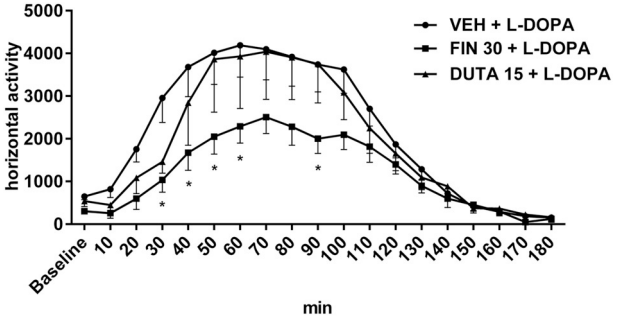


Fig. 4. Time course of the spontaneous horizontal activity of 6-OHDA lesioned rats, after preventive treatment regimen (FIN 30 or DUTA 15) and 16 days of daily L-DOPA injection. Locomotor activity was recorded for 180 min. Each point represent mean value ± SEM. * p < .05 vs corresponding time point L-DOPA group (Tukey's test).

different in the 4 experimental groups (ERK1/2: $F_{(3,21)} = 0.1283$, $p = .94$; DARPP-32: $F_{(3,21)} = 0.066$, $p = .9771$).

Next, we evaluated striatal levels of $G\alpha_{olf}$, that plays a crucial role in the intracellular effects of D_1R activation. In the lesioned side, the analysis of $G\alpha_{olf}$ levels by one-way ANOVA showed a significant difference between groups [FIN experiment: $F_{(3,16)} = 7.809$, $p = .0020$; DUTA experiment: $F_{(3,16)} = 12.530$, $p = .0002$). L-DOPA administration increased $G\alpha_{olf}$ expression ($p < .01$); administration of FIN was effective in the prevention of L-DOPA-induced $G\alpha_{olf}$ increase only at the higher dose ($p < .001$), while DUTA was effective at both doses (p < .001) (Fig. 5).

In the intact side, phospho-ERK 1/2, phospho-DARPP-32 and $G\alpha_{olf}$ levels were similar between groups (Fig. S2 A, B, C, G, H, I). Additionally, lesion with 6-OHDA per se did not affect the phosphorylation levels of ERK1/2 or DARPP-32 at Thr-34 (intact side: pERK1/2 = 100 ± 7.8, pThr34 DARPP-32 = 100 ± 6.5; lesioned side: pERK1/2 = 101.0 ± 13.8, pThr34 DARPP-32 = 105.2 ± 18.9, paired Student's *t*-test).

3.5. Effect of FIN and DUTA on L-DOPA-induced dopamine D1R-D3R heteromer formation

Next, given the suggested role of dopamine D_3R in the appearance of LID (Solís and Moratalla, 2018), we investigated the impact of 5AR inhibitors on D_1R and D_3R interaction using an immunoprecipitation assay, as indicative of receptor heteromerization.

In the lesioned side, analysis of D_3R levels in the immunoprecipitates by one-way ANOVA showed a significant difference between groups [FIN experiment: $F_{(3,22)} = 9.68$, $p = .0003$; DUTA experiment: $F_{(3,21)} = 11.68$, $p = .0001$]. In particular, post-hoc analysis showed that D_3R levels in the immunoprecipitates were significantly increased in L-DOPA-treated lesioned animals compared to vehicle-treated lesioned animals (FIN experiment: p < .001; DUTA experiment: $p < .01$) (Fig. 6A and B). Interestingly, this effect was prevented by FIN (FIN 30: $p < .05$; FIN 60: p < .001) or DUTA treatment (DUTA 15: p < .001; DUTA 30: p < .01), (Fig. 6A and B). In the intact side similar levels of D_3R in the immunoprecipitates were observed between groups (Fig. S2 D, J). Moreover, in the total lysate, dopamine D_1R and D_3R expression levels were not significantly different in the lesioned

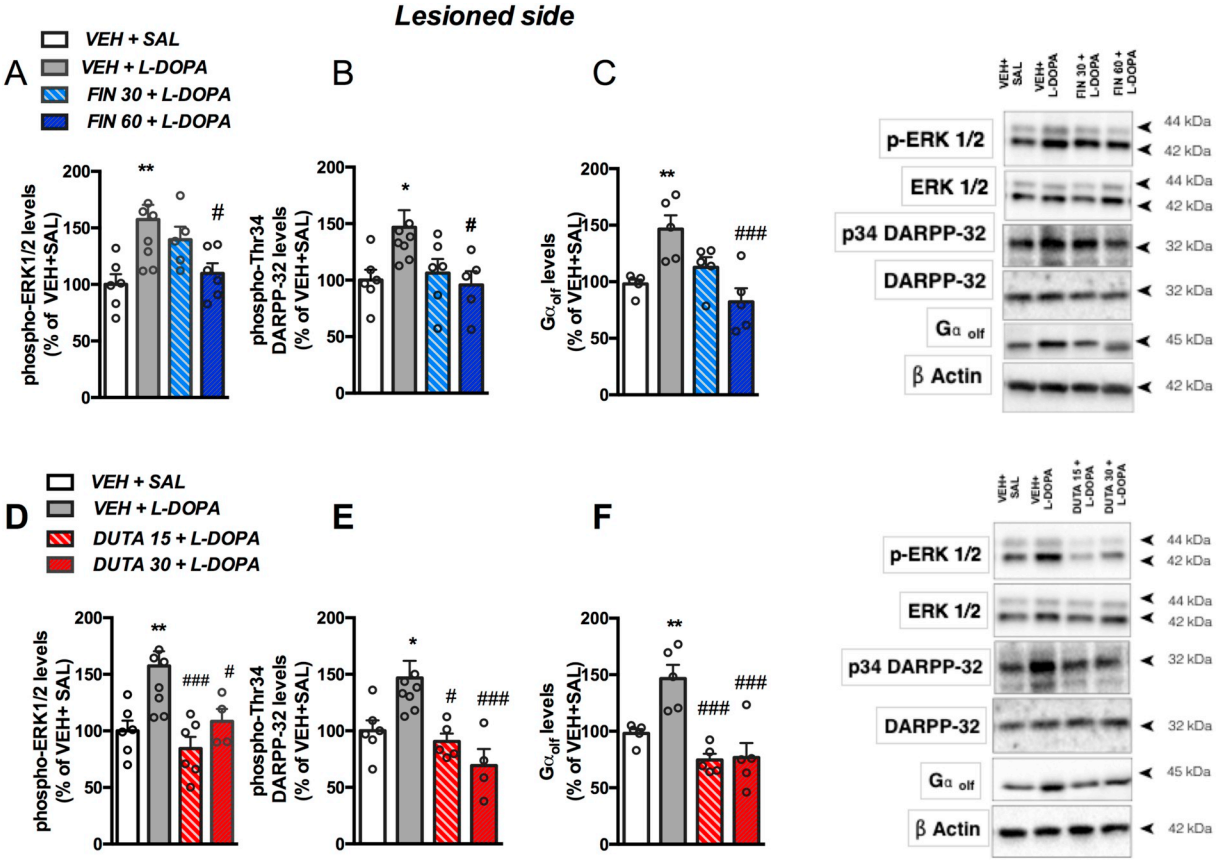


Fig. 5. Effect of FIN (A-C) and DUTA (D-F) preventive treatment on striatal markers of LIDs in the lesioned side of 6-OHDA rats treated with L-DOPA. A, D levels of phospho ERK1/2; B, E levels of phospho-Thr34 DARRP-32; C, F levels of $G\alpha_{olf}$ in lesioned striatal tissue. On the left, representative blots are shown. Phospho-ERK 1/2 and phospho-Thr34 DARPP-32 levels were normalized to those of total ERK and DARPP-32, respectively; $G\alpha_{olf}$ levels were normalized to those of β actin. Data are expressed as mean ± SEM and calculated as percentage of VEH + SAL values. (One-way ANOVA followed by Sidak's multiple comparisons test: ** p < .01, * p < .05 vs naïve group lesioned striatal tissue; ### p < .001 vs, # p < .05 vs L-DOPA group lesioned striatal tissue).

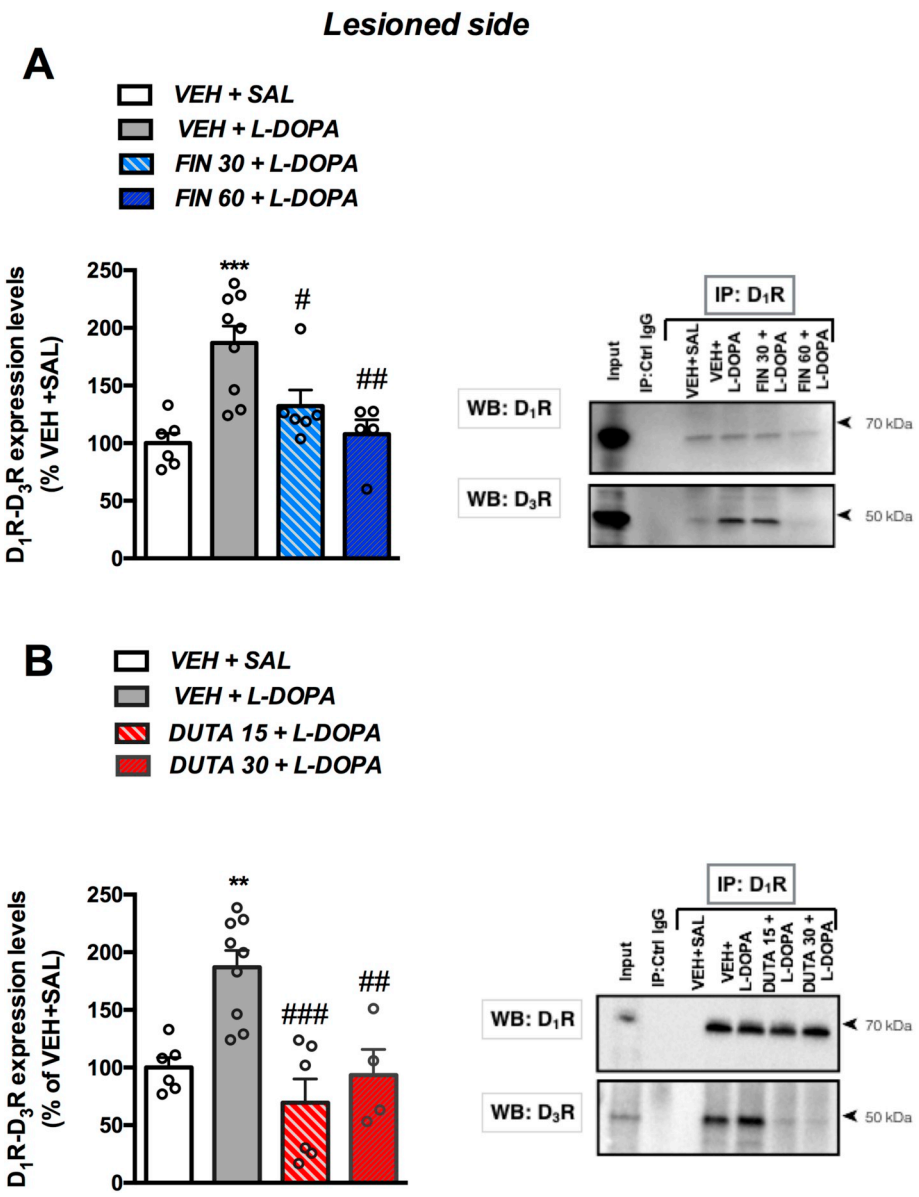


Fig. 6. Effect of FIN (A) and DUTA (B) preventive treatment on striatal dopamine D_1R-D_3R interaction in the lesioned side of 6-OHDA-lesioned rats treated with L-DOPA. Tissue sample were immunoprecipitated with anti-D_1R antibodies and immunoblotted using anti-D_3R antibodies. Band intensities of co-immunoprecipitated D_3Rs were normalized to the band intensities of immunoprecipitated D_1Rs. Normal mouse IgG was used for IP and served as a negative control, whereas total striatal lysate without Co-IP (input) served as a positive control. Data are expressed as mean ± SEM and calculated as percentage of VEH + SAL values. One-way ANOVA followed by Sidak's multiple comparisons test: *** $p < .001$, ** $p < .01$ vs VEH + SAL; ### $p < .001$, ## $p < .01$, # $p < .05$ vs VEH + L-DOPA group.

(Fig. 7 A, B, C, D) and the intact side (Fig. S2 E, F, K, L), albeit in the lesioned side a trend toward an increase in dopamine D_1R and D_3R expression was observed in VEH + L-DOPA group (Fig.7). In addition, to verify whether 6-OHDA infusion affected D_1R and D_3R levels, we compared the intact and lesioned side of 6-OHDA-lesioned rats (VEH + SAL). Analysis by paired Student's *t*-test showed no significant differences in D_1R and D_3R expression between the intact and lesioned side (intact side: $D_1R = 100 \pm 9.4$, $D_3R = 100 \pm 8.5$; lesioned side: $D_1R = 85.0 \pm 11.1$, $D_3R = 116.3 \pm 10.6$).

4. Discussion

We have recently reported for the first time that FIN produces significant dampening of LID in the rat 6-OHDA-lesioned model of PD. Here, we show that this effect is not limited to FIN but extends to another 5AR inhibitor, DUTA, which is also used in clinical setting for the treatment of benign prostatic hyperplasia (BPH) (Aggarwal et al., 2010; Desgrandchamps et al., 2006). Moreover, this study demonstrates for the first time that 5AR inhibitors are able to prevent key events in the appearance of dyskinesia in the rat model of PD, such as L-DOPA-induced upregulation of striatal dopamine D_1R-related cAMP/PKA/ERK signaling pathways, and D_1R-D_3R coimmunoprecipitation, an index of heteromer formation (Feyder et al., 2011; Fiorentini et al., 2008; Solìs and Moratalla 2018). These findings are relevant as they confirm the 5AR enzyme as a potential therapeutic target for treatment of dyskinesia in PD, suggesting the first ever evidence that neurosteroidogenesis may affect functional interaction between dopamine D_1R and D_3R, which overexpression plays a role not only in dyskinesia, but also in other brain disorders (Frau et al., 2016; Mosher et al., 2016).

Importantly, DUTA did not interfere with the ability of L-DOPA to ameliorate the forelimb use in the stepping test at any day (1 and 23), test time (60, 150, 200 and 250 min after L-DOPA treatment) and dose (15 and 30 mg/kg) assessed; by contrast, FIN reduced forelimb use at the first day of administration at the higher tested dose. Moreover, opposite to FIN, DUTA did not affect the overall motor activation produced by L-DOPA in these rats. Thus, our results indicate that DUTA is able to counteract dyskinesia without impacting the therapeutic effects of L-DOPA, supporting the potential clinical feasibility of this approach to control dyskinesia.

According to the higher efficacy in 5AR inhibition, DUTA exhibited significant antidyskinetic effects at lower doses than FIN. Indeed, preliminary experiments in our lab showed that the FIN dose of 15 mg/kg

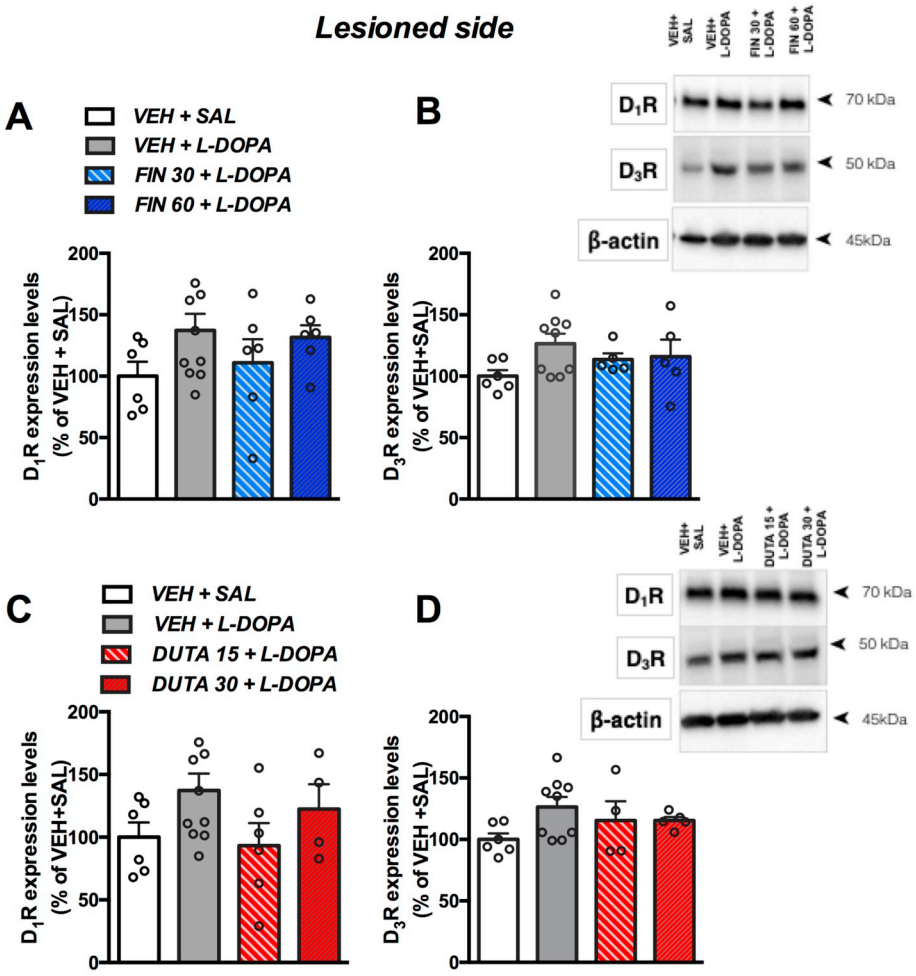
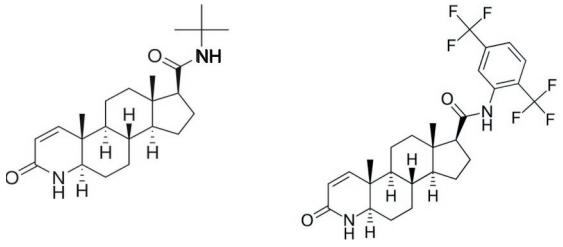


Fig. 7. Effect of FIN (A, B) and DUTA (C, D) preventive treatment on striatal dopamine D_1R and D_3R expression levels in the lesioned side of 6-OHDA-rats treated with L-DOPA. D_1R and D_3R levels were normalized to those of β-actin. Data are expressed as mean ± SEM and calculated as percentage of VEH + SAL values. In total lysate, D_1R and D_3R receptor expression levels were not significantly different, although a trend toward an increase in D_1R and D_3R receptors expression was observed in VEH + L-DOPA group.

Table 1
Comparison of serum half-life and IC50 against SRD5A1 and SRD5A1 of finasteride and dutasteride in rat and human (Xu et al., 2006).

	FINASTERIDE		DUTASTERIDE	
	rat	human	rat	human
Serum half-life	2 h	6 h	31 h	> 72 h
IC50 (nmol/L) SRD5A1	5.4 ± 0.2	360 ± 40	0.3 ± 0.02	6 ± 1
IC50 (nmol/L) SRD5A2	0.5 ± 0.1	69 ± 1	0.2 ± 0.02	7 ± 3



(a dose efficacious for DUTA) did not produce any reduction of LID. In line with these data, DUTA is used at a lower dose than FIN for the treatment of BPH. As reported by other authors in the treatment of BPH, the higher efficacy of DUTA at lower doses may possibly be ascribed to differences in their pharmacokinetic and pharmacodynamic profile (Aggarwal et al., 2010). In fact, the limited effectiveness of FIN may be due to the fact that while being a potent, time-dependent, irreversible inhibitor of the human 5AR2 (IC50, 69 nmol/l), this compound is not as potent for the human 5AR1 isoform (IC50, 360 nmol/l). Conversely, DUTA is 60-fold more potent than FIN with IC50 of 6 nM and 7 nM for the two isoforms. Furthermore, in comparison with FIN, DUTA has an extremely longer half-life (5–6 weeks vs 6–8 h), which accounts for its good efficacy even in non-daily treatment regimen. Although in humans FIN acts as a selective inhibitor of the peripheral 5AR type-2 (Paba et al., 2011), in rats it efficiently blocks both 5AR isozymes with IC 50 of 5.4 nmol/l and 0.5 nmol/l for the 5AR1 and 5AR2, respectively (Xu et al., 2006). However, similarly to humans, DUTA has lower IC50 for both rodent isoforms than FIN (IC50 of 0.3 nmol/l for 5AR1 and 0.2 nmol/l for the 5AR2; for further details see Table 1).

Although we did not identify the involvement of a specific isoform, the results of this study indirectly indicate that the better antidyskinetic profile of DUTA may rely on its higher affinity for the 5AR1. In line with this hypothesis, it is worth noting that in a recent study conducted in transgenic mice characterized by the deletion of gene expressing 5AR1 and 5AR2, mice lacking of the isoenzymes 1, but not 2, were fully protected by the psychotomimetic effects elicited by the selective dopamine D_1R agonist SKF-82958 (Mosher et al., submitted). Of note, 5AR1 KO mice become susceptible to the behavioral alterations induced by SKF-82958 if they previously received allopregnanolone injection (Mosher et al., submitted). These data strongly suggest that the negative modulation of dopamine D_1R is specifically subtended by the 5AR1, with its neurosteroidogenic pathway. In addition, it is worth noting that the irreversible blockade that characterizes both inhibitors not only decreases 5AR-related metabolites, but also significantly increases 5AR substrates. Accordingly, we previously reported that a single administration of FIN led to increased levels of 5AR1 substrates pregnenolone and dehydroepiandrosterone (DHEA) (Frau et al., 2015, 2017). Of note, a number of preclinical and clinical studies reported that pregnenolone and DHEA exhibit marked antidopaminergic activity and show beneficial properties in patients with dopamine-related disorders (Maayan et al., 2006; Romieu et al., 2006; Vallée et al., 2014; Wong et al., 2012, 2015). In addition, it has been recently reported that pregnenolone inhibited the increase in ERK 1/2 signaling in the ventral striatum of mice (Vallée et al., 2014). On the other hand, allopregnanolone and DHEA sulfate exhibited positive modulation on the behavioral effects elicited by dopamine D_1R activation (Dong et al., 2007; Frye et al., 2004); moreover, allopregnanolone has been shown to affect the phosphorylation of DARPP-32 (Frye and Walf, 2010; Mani et al., 2000), a key molecule in dopamine D_1R signaling cascade, which has been implicated in dyskinesia (Picconi et al., 2003; Santini et al., 2007; Svenningsson et al., 2004). Thus, our current and previous behavioral and biochemical data suggest that one of the plausible mechanisms by which 5AR blockade exerts antidyskinetic effects might be through the reinstatement of a favorable balance in the neurosteroidogenic pathway with ensuing normalization of D_1R signaling.

Unfortunately, to date, there is no inhibitor able to selectively target the type 1 isoenzyme in humans. Some 5AR1 inhibitors have been developed for clinical trials for the treatment of acne vulgaris, but with no promising results (Leyden et al., 2004). Furthermore, these compounds do not bind to rat 5AR1 and, therefore, cannot be used to discern the role of this isoform in the 6-OHDA rat model of PD. If 5AR1 was specifically responsible for the antidyskinetic effect of 5AR inhibitors and we could selectively target this isoenzyme, we may possibly avoid several adverse reactions related to 5AR2 blockade, i.e. anhedonia/apathy (already present in PD patients) and sexual dysfunctions due to decreased androgen signaling (Vis and Schröder, 2009).

We have previously shown that FIN completely reversed behavioral alterations induced by exaggerated dopaminergic activation in rodents. For instance, in Sprague-Dawley rats, systemic FIN injections countered the prepulse inhibition deficits and stereotyped behaviors exerted by dopaminergic agonists, through a post-synaptic negative modulation of dopaminergic receptors in the striatum (Bortolato et al., 2008; Devoto et al., 2012). In agreement with the ability of FIN to modulate dopamine transmission, FIN was also able to reduce dyskinesia produced by direct dopamine receptor agonists, either at D_1R or at D_2R/D_3R (Frau et al., 2017). In line with these data, in the present study we found that 5AR inhibitors dampened phosphorylation of DARPP-32 and ERK1/2, as well as increased $G\alpha_{olf}$ expression seen in L-DOPA-treated dyskinetic rats as compared to VEH treated animals. Moreover, FIN and DUTA reduced the levels of co-immunoprecipitation of D_1R-D_3R in striatal homogenates, as compared to the VEH + L-DOPA treated group. This latter result is of particular interest as several recent studies have suggested that dopaminergic heteromers are strictly implicated in the development of LID (Fiorentini et al., 2015; Solís and Moratalla, 2018). Among the heteromeric complexes discovered to date, the D_1R-D_3R complex formation appears particularly relevant in view of the primary role of D_1R in the onset and development of dyskinesia, and of the regulatory influence of D_3R on D_1R trafficking and signaling (Fiorentini et al., 2008). In heteromeric configuration, this complex displays functional properties remarkably different from homomeric entities. Indeed, the mutual D_1R-D_3R interaction results in increased affinity of D_1R to dopamine as well as in a stronger D_1R stimulation through the lack of its typical internalization (Marcellino et al., 2008). Previous evidence indicates that D_3R are key players in D_1R internalization and that LID are underpinned by increased D_3R expression, which in turn, potentiated dopamine stimulation of D_1R cAMP/PKA signaling (Aubert et al., 2005; Bézard et al., 2003; Bordet et al., 2000; Guillin et al., 2001). In line with these findings, here we demonstrated that L-DOPA treatment induced a significant increase in expression levels of striatal D_3R; by contrast, this condition was prevented in animals that were chronically treated with FIN or DUTA. Of note, the ability of 5AR inhibitors to interfere with D_3R expression and therefore with D_1R-D_3R heteromer formation was restricted to the lesioned striatum. These results suggest that these compounds exert their effect only under a condition of dopaminergic denervation, while they do not interfere with the physiological role/expression of D_3R.

Interestingly, FIN was recently shown to produce therapeutic effects in adult male patients affected by Tourette Syndrome (Bortolato et al., 2007; Muroni et al., 2011). This is intriguing as Tourette syndrome is a psychiatric condition characterized by motor fluctuations and phonic tics, which pathophysiology has been suggested to be due to striatal dopaminergic dysfunction (Denys et al., 2013; Felling and Singer, 2011; Jeffries et al., 2002). Furthermore, FIN was also able to attenuate motor and non-motor side-effects elicited by dopamine replacement therapies in PD patients, such as blepharospasm and pathological gambling (Bortolato et al., 2010, 2012). 5AR inhibitors have been on the market for many years for the treatment of male pattern hair loss and BPH. Thus, if beneficial effects of 5AR inhibitors will be demonstrated in PD patients, this approach may rapidly be introduced in the clinical practice, at least in male subjects.

In conclusion, this study confirms the 5AR enzyme as an intriguing target for the treatment of dyskinesia in PD, and suggests that prevention of D_1R-D_3R heteromer formation may play a key role in the mechanism of protection. However, further studies are warranted to unveil the mechanism by which neurosteroids are able to affect D_1R-D_3R interaction.

Finally, our data indicate that DUTA may be the preferred compound to be used in clinical trials for its higher efficacy and fewer complications.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2018.09.018.

Conflict of interest

All the authors included in this manuscript declare no conflict of interest.

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References

Aggarwal, S., Thareja, S., Verma, A., Bhardwaj, T.R., Kumar, M., 2010. An overview on 5α-reductase inhibitors. Steroids 75, 109–153.
Aubert, I., Guigoni, C., Håkansson, K., Li, Q., Dovero, S., Barthe, N., Bioulac, B.H., Gross, C.E., Fisone, G., Bloch, B., Bezard, E., 2005. Increased D1 dopamine receptor signaling in levodopa-induced dyskinesia. Ann. Neurol. 57, 17–26.
Bastide, M.F., Meissner, W.G., Picconi, B., Fasano, S., Fernagut, P.-O., Feyder, M., Francardo, V., Alcacer, C., Ding, Y., Brambilla, R., Fisone, G., Jon Stoessl, A., Bourdenx, M., Engeln, M., Navailles, S., De Deurwaerdère, P., Ko, W.K.D., Simola, N., Morelli, M., Groc, L., Rodriguez, M.-C., Gurevich, E.V., Quik, M., Morari, M., Mellone, M., Gardoni, F., Tronci, E., Guehl, D., Tison, F., Crossman, A.R., Kang, U.J., Steece-Collier, K., Fox, S., Carta, M., Angela Cenci, M., Bézard, E., 2015. Pathophysiology of L-dopa-induced motor and non-motor complications in Parkinson's disease. Prog. Neurobiol. 132, 96–168.
Bézard, E., Ferry, S., Mach, U., Stark, H., Leriche, L., Boraud, T., Gross, C., Sokoloff, P., 2003. Attenuation of levodopa-induced dyskinesia by normalizing dopamine D3 receptor function. Nat. Med. 9, 762–767.
Bordet, R., Ridray, S., Schwartz, J.C., Sokoloff, P., 2000. Involvement of the direct striatonigral pathway in levodopa-induced sensitization in 6-hydroxydopamine-lesioned rats. Eur. J. Neurosci. 12, 2117–2123.
Bortolato, M., Muroni, A., Marrosu, F., 2007. Treatment of Tourette's syndrome with finasteride. Am. J. Psychiatry 164, 1914–1915.
Bortolato, M., Frau, R., Orrù, M., Bourov, Y., Marrosu, F., Mereu, G., Devoto, P., Gessa, G.L., 2008. Antipsychotic-like properties of 5-alpha-reductase inhibitors. Neuropsychopharmacology 33, 3146–3156.
Bortolato, M., Cannas, A., Solla, P., Puligheddu, M., Muroni, A., Marrosu, F., 2010. Treatment of benign essential blepharospasm with finasteride: a case report. Clin. Neuropharmacol. 33, 207–208.
Bortolato, M., Cannas, A., Solla, P., Bini, V., Puligheddu, M., Marrosu, F., 2012. Finasteride attenuates pathological gambling in patients with Parkinson disease. J. Clin. Psychopharmacol. 32, 424–425.
Cenci, M.A., Konradi, C., 2010. Maladaptive striatal plasticity in L-DOPA-induced dyskinesia. Prog. Brain Res. 183, 209–233.
Cenci, M.A., Lundblad, M., 2007. Ratings of L-DOPA-induced dyskinesia in the unilateral 6-OHDA lesion model of Parkinson's disease in rats and mice. Curr. Protoc. Neurosci. 9, 25.
Denys, D., de Vries, F., Cath, D., Figee, M., Vulink, N., Veltman, D.J., van der Doef, T.F., Boellaard, R., Westenberg, H., van Balkom, A., Lammertsma, A.A., van Berckel, B.N.M., 2013. Dopaminergic activity in Tourette syndrome and obsessive-compulsive disorder. Eur. Neuropsychopharmacol. 23, 1423–1431.
Desgrandchamps, F., Droupy, S., Irani, J., Saussine, C., Comenducci, A., 2006. Effect of dutasteride on the symptoms of benign prostatic hyperplasia, and patient quality of life and discomfort, in clinical practice. BJU Int. 98, 83–88.
Devoto, P., Frau, R., Bini, V., Pillolla, G., Saba, P., Flore, G., Corona, M., Marrosu, F., Bortolato, M., 2012. Inhibition of 5α-reductase in the nucleus accumbens counters sensorimotor gating deficits induced by dopaminergic activation. Psychoneuroendocrinology 37, 1630–1645.
Dong, L.-Y., Cheng, Z.-X., Fu, Y.-M., Wang, Z.-M., Zhu, Y.-H., Sun, J.-L., Dong, Y., Zheng, P., 2007. Neurosteroid dehydroepiandrosterone sulfate enhances spontaneous glutamate release in rat prelimbic cortex through activation of dopamine D1 and sigma-1 receptor. Neuropharmacology 52, 966–974.
Farré, D., Muñoz, A., Moreno, E., Reyes-Resina, I., Canet-Pons, J., Dopeso-Reyes, I.G., Rico, A.J., Lluís, C., Mallol, J., Navarro, G., Canela, E.I., Cortés, A., Labandeira-García, J.L., Casadó, V., Lanciego, J.L., Franco, R., 2015. Stronger dopamine D1 receptor-mediated neurotransmission in dyskinesia. Mol. Neurobiol. 52, 1408–1420.
Felling, R.J., Singer, H.S., 2011. Neurobiology of tourette syndrome: current status and need for further investigation. J. Neurosci. 31, 12387–12395.
Feyder, M., Bonito-Oliva, A., Fisone, G., 2011. L-DOPA-induced dyskinesia and abnormal signaling in striatal medium spiny neurons: focus on dopamine D1 receptor-mediated transmission. Front. Behav. Neurosci. 5, 71.
Fiorentini, C., Busi, C., Gorruso, E., Gotti, C., Spano, P., Missale, C., 2008. Reciprocal regulation of dopamine D1 and D3 receptor function and trafficking by heterodimerization. Mol. Pharmacol. 74, 59–69.
Fiorentini, C., Savoia, P., Bono, F., Tallarico, P., Missale, C., 2015. The D3 dopamine receptor: from structural interactions to function. Eur. Neuropsychopharmacol. 25, 1462–1469.
Fiorentini, C., Savoia, P., Savoldi, D., Bono, F., Busi, C., Barbon, A., Missale, C., 2016. Shp-2 knockdown prevents l-dopa-induced dyskinesia in a rat model of Parkinson's disease. Mov. Disord. 31, 512–520.
Frau, R., Pillolla, G., Bini, V., Tambaro, S., Devoto, P., Bortolato, M., 2013. Inhibition of 5α-reductase attenuates behavioral effects of D1-, but not D2-like receptor agonists in C57BL/6 mice. Psychoneuroendocrinology 38, 542–551.
Frau, R., Abbiati, F., Bini, V., Casti, A., Caruso, D., Devoto, P., Bortolato, M., 2015. Targeting neurosteroid synthesis as a therapy for schizophrenia-related alterations induced by early psychosocial stress. Schizophr. Res. 168, 640–648.
Frau, R., Mosher, L.J., Bini, V., Pillolla, G., Pes, R., Saba, P., Fanni, S., Devoto, P., Bortolato, M., 2016. The neurosteroidogenic enzyme 5α-reductase modulates the role of D1 dopamine receptors in rat sensorimotor gating. Psychoneuroendocrinology 63, 59–67.
Frau, R., Savoia, P., Fanni, S., Fiorentini, C., Fidalgo, C., Tronci, E., Stancampiano, R., Meloni, M., Cannas, A., Marrosu, F., Bortolato, M., Devoto, P., Missale, C., Carta, M., 2017. The 5-alpha reductase inhibitor finasteride reduces dyskinesia in a rat model of Parkinson's disease. Exp. Neurol. 291, 1–7.
Frye, C.A., Walf, A.A., 2010. Infusions of anti-sense oligonucleotides for DARPP-32 to the ventral tegmental area reduce effects of progesterone- and a dopamine type 1-like receptor agonist to facilitate lordosis. Behav. Brain Res. 206, 286–292.
Frye, C.A., Edinger, K.L., Seliga, A.M., Wawrzycki, J.M., 2004. 5alpha-reduced androgens may have actions in the hippocampus to enhance cognitive performance of male rats. Psychoneuroendocrinology 29, 1019–1027.
Grappi, S., Marchese, G., Secci, M.E., De Montis, M.G., Gambarana, C., Scheggi, S., 2011. Morphine sensitization as a model of mania: Comparative study of the effects of repeated lithium or carbamazepine administration. Pharmacol. Biochem. Behav. 99, 749–758.
Guillin, O., Diaz, J., Carroll, P., Griffon, N., Schwartz, J.C., Sokoloff, P., 2001. BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization. Nature 411, 86–89.
Guitart, X., Navarro, G., Moreno, E., Yano, H., Cai, N.S., Sánchez-Soto, M., Kumar-Barodia, S., Naidu, Y.T., Mallol, J., Cortés, A., Lluís, C., Canela, E.I., Casadó, V., McCormick, P.J., Ferré, S., 2014. Functional selectivity of allosteric interactions within G protein-coupled receptor oligomers: the dopamine D1-D3 receptor heterotetramer. Mol. Pharmacol. 86, 417–429.
Jeffries, K.J., Schooler, C., Schoenbach, C., Herscovitch, P., Chase, T.N., Braun, A.R., 2002. The functional neuroanatomy of Tourette's syndrome: an FDG PET study III: functional coupling of regional cerebral metabolic rates. Neuropsychopharmacology 27, 92–104.
Leyden, J., Bergfeld, W., Drake, L., Dunlap, F., Goldman, M.P., Gottlieb, A.B., Heffernan, M.P., Hickman, J.G., Hordinsky, M., Jarrett, M., Kang, S., Lucky, A., Peck, G., Phillips, T., Rapaport, M., Roberts, J., Savin, R., Sawaya, M.E., Shalita, A., Shavin, J., Shaw, J.C., Stein, L., Stewart, D., Strauss, J., Swinehart, J., Swinyer, L., Thiboutot, D., Washenik, K., Weinstein, G., Whiting, D., Pappas, F., Sanchez, M., Terranella, L., Waldstreicher, J., 2004. A systemic type I 5 alpha-reductase inhibitor is ineffective in the treatment of acne vulgaris. J. Am. Acad. Dermatol. 50, 443–447.
Lundblad, M., Andersson, M., Winkler, C., Kirik, D., Wierup, N., Cenci, M.A., 2002. Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. Eur. J. Neurosci. 15, 120–132.
Maayan, R., Lotan, S., Doron, R., Shabat-Simon, M., Gispan-Herman, I., Weizman, A., Yadid, G., 2006. Dehydroepiandrosterone (DHEA) attenuates cocaine-seeking behavior in the self-administration model in rats. Eur. Neuropsychopharmacol. 16, 329–339.
Mani, S.K., Fienberg, A.A., O'Callaghan, J.P., Snyder, G.L., Allen, P.B., Dash, P.K., Moore, A.N., Mitchell, A.J., Bibb, J., Greengard, P., O'Malley, B.W., 2000. Requirement for DARPP-32 in progesterone-facilitated sexual receptivity in female rats and mice. Science 287, 1053–1056.
Marcellino, D., Ferré, S., Casadó, V., Cortés, A., Le Foll, B., Mazzola, C., Drago, F., Saur, O., Stark, H., Soriano, A., Barnes, C., Goldberg, S.R., Lluis, C., Fuxe, K., Franco, R., 2008. Identification of dopamine D1-D3 receptor heteromers. Indications for a role of synergistic D1-D3 receptor interactions in the striatum. J. Biol. Chem. 283, 26016–26025.
Mosher, L.J., Frau, R., Pardu, A., Pes, R., Devoto, P., Bortolato, M., 2016. Selective activation of D1 dopamine receptors impairs sensorimotor gating in Long-Evans rats. Br. J. Pharmacol. 173, 2122–2134.
Muroni, A., Paba, S., Puligheddu, M., Marrosu, F., Bortolato, M., 2011. A preliminary study of finasteride in Tourette syndrome. Mov. Disord. 26, 2146–2147.
Paba, S., Frau, R., Godar, S.C., Devoto, P., Marrosu, F., Bortolato, M., 2011. Steroid 5α-reductase as a novel therapeutic target for schizophrenia and other neuropsychiatric disorders. Curr. Pharm. Des. 17, 151–167.
Pavón, N., Martín, A.B., Mendialdua, A., Moratalla, R., 2006. ERK phosphorylation and FosB expression are associated with L-DOPA-induced dyskinesia in hemiparkinsonian mice. Biol. Psychiatry 59, 64–74.
Paxinos, G., 2007. The Rat Brain in Stereotaxic Coordinates. Academic Press.
Picconi, B., Centonze, D., Håkansson, K., Bernardi, G., Greengard, P., Fisone, G., Cenci, M.A., Calabresi, P., 2003. Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. Nat. Neurosci. 6, 501–506.
Romieu, P., Lucas, M., Maurice, T., 2006. Sigma1 receptor ligands and related neuroactive steroids interfere with the cocaine-induced state of memory. Neuropsychopharmacology 31, 1431–1443.
Santini, E., Valjent, E., Usiello, A., Carta, M., Borgkvist, A., Girault, J.-A., Hervé, D., Greengard, P., Fisone, G., 2007. Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. J. Neurosci. 27, 6995–7005.
Scheggi, S., Crociani, A., De Montis, M.G., Tagliamonte, A., Gambarana, C., 2009. Dopamine D1 receptor-dependent modifications in the dopamine and cAMP-

regulated phosphoprotein of Mr 32 kDa phosphorylation pattern in striatal areas of morphine-sensitized rats. Neuroscience 163, 627–639.
Scheggi, S., Ferrari, A., Pelliccia, T., Devoto, P., De Montis, M.G., Gambarana, C., 2017. Fasting biases μ-opioid receptors toward b-arrestin2-dependent signaling in the accumbens shell. Neuroscience 352, 19–29.
Solís, O., Moratalla, R., 2018. Dopamine receptors: homomeric and heteromeric complexes in L-DOPA-induced dyskinesia. J. Neural. Transm. (Vienna) 125 (8), 1187–1194.
Solís, O., Garcia-Montes, J.R., González-Granillo, A., Xu, M., Moratalla, R., 2017. Dopamine D3 receptor modulates l-DOPA-induced dyskinesia by targeting D1 receptor-mediated striatal signaling. Cereb. Cortex. 27, 435–446.
Spigolon, G., Fisone, G., 2018. Signal transduction in L-DOPA-induced dyskinesia: from receptor sensitization to abnormal gene expression. J. Neural. Transm. (Vienna) 125 (8), 1171–1186.
Svenningsson, P., Nishi, A., Fisone, G., Girault, J.-A., Nairn, A.C., Greengard, P., 2004. DARPP-32: an integrator of neurotransmission. Annu. Rev. Pharmacol. Toxicol. 44, 269–296.
Tronci, E., Lisci, C., Stancampiano, R., Fidalgo, C., Collu, M., Devoto, P., Carta, M., 2013. 5-Hydroxy-tryptophan for the treatment of L-DOPA-induced dyskinesia in the rat Parkinson's disease model. Neurobiol. Dis. 60, 108–114.
Tronci, E., Fidalgo, C., Zianni, E., Collu, M., Stancampiano, R., Morelli, M., Gardoni, F., Carta, M., 2014. Effect of memantine on L-DOPA-induced dyskinesia in the 6-OHDA-lesioned rat model of Parkinson's disease. Neuroscience 265, 245–252.
Vallée, M., Vitiello, S., Bellocchio, L., Hébert-Chatelain, E., Monlezun, S., Martin-Garcia, E., Kasanetz, F., Baillie, G.L., Panin, F., Cathala, A., Roullot-Lacarrière, V., Fabre, S., Hurst, D.P., Lynch, D.L., Shore, D.M., Deroche-Gamonet, V., Spampinato, U., Revest, J.-M., Maldonado, R., Reggio, P.H., Ross, R.A., Marsicano, G., Piazza, P.V., 2014. Pregnenolone can protect the brain from cannabis intoxication. Science 343, 94–98.
Vis, A.N., Schröder, F.H., 2009. Key targets of hormonal treatment of prostate cancer. Part 2: the androgen receptor and 5alpha-reductase. BJU Int. 104, 1191–1197.
Westin, J.E., Vercammen, L., Strome, E.M., Konradi, C., Cenci, M.A., 2007. Spatiotemporal pattern of striatal ERK1/2 phosphorylation in a rat model of L-DOPA-induced dyskinesia and the role of dopamine D1 receptors. Biol. Psychiatry 62, 800–810.
Wong, P., Chang, C.C.R., Marx, C.E., Caron, M.G., Wetsel, W.C., Zhang, X., 2012. Pregnenolone rescues schizophrenia-like behavior in dopamine transporter knockout mice. PLoS One 7, e51455.
Wong, P., Sze, Y., Chang, C.C.R., Lee, J., Zhang, X., 2015. Pregnenolone sulfate normalizes schizophrenia-like behaviors in dopamine transporter knockout mice through the AKT/GSK3β pathway. Transl. Psychiatry 5, e528.
Xu, Y., Dalrymple, S.L., Becker, R.E., Denmeade, S.R., Isaacs, J.T., 2006. Pharmacologic basis for the enhanced efficacy of dutasteride against prostatic cancers. Clin. Cancer Res. 12, 4072–4079. https://doi.org/10.1158/1078-0432.CCR-06-0184.