

Effects of paroxetine treatment and its withdrawal on neurosteroidogenesis

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ABSTRACT

Selective serotonin reuptake inhibitors (SSRI) show high efficacy in treating depression, however during treatment side effects, like for instance sexual dysfunction, may appear, decreasing compliance. In some cases, this condition will last after drug discontinuation, leading to the so-called post-SSRI sexual dysfunction (PSSD). The etiology of PSSD is still unknown, however a role for neuroactive steroids may be hypothesized. Indeed, these molecules are key physiological regulators of the nervous system, and their alteration has been associated with several neuropathological conditions, including depression. Additionally, neuroactive steroids are also involved in the control of sexual function. Interestingly, sexual dysfunction induced by SSRI treatment has been also observed in animal models. On this basis, we have here evaluated whether a subchronic treatment with paroxetine for two weeks and/or its withdrawal (i.e., a month) may affect the levels of neuroactive steroids in brain areas (i.e., hippocampus, hypothalamus, and cerebral cortex) and/or in plasma and cerebrospinal fluid of male rats. Data obtained indicate that the SSRI treatment alters neuroactive steroid levels and the expression of key enzymes of the steroidogenesis in a brain tissue- and time-dependent manner. Indeed, these observations with the finding that plasma levels of neuroactive steroids are not affected suggest that the effect of paroxetine treatment is directly on neurosteroidogenesis. In particular, a negative impact on the expression of steroidogenic enzymes was observed at the withdrawal. Therefore, it is possible to hypothesize that altered neurosteroidogenesis may also occur in PSSD and consequently it may represent a possible pharmacological target for this disorder.

1. Introduction

Depression is a chronic and serious psychiatric illness, affecting more than 264 million people worldwide (Disease et al., 2018). This condition has major socioeconomic consequences (Greenberg et al., 2003), including social and occupational problems, and is a risk factor for lifetime suicide attempts (Birnbaum et al., 2010; Miret et al., 2013).

Many medicaments, like for instance monoamine oxidase inhibitors, tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRI), serotonin-norepinephrine reuptake inhibitors (SNRI) and the “multi-modal” drug vortioxetine (i.e., a serotonin receptor modulator with considerable affinity for dopamine and norepinephrine transporters) are

now available to treat with high efficacy this condition (Pitsillou et al., 2020; Marasine et al., 2021). However, like several other medications, the treatment is associated to side-effects. One of the most prevalent is sexual dysfunction (Rosen et al., 1999; Dewan and Anand, 1999; Piazza et al., 1997; Hirschfeld, 1998). Indeed, it has been reported that the prevalence of this complication is between 20% and 45%, in relation to the drug prescribed (Clayton et al., 2002).

In this context, it is also interesting to note that a frequent off-label prescription for some SSRIs, including paroxetine, fluoxetine, sertraline and escitalopram, is premature ejaculation (Arafa and Shamloul, 2007; Balon, 1996). A recent Cochrane review supports their use to treat this condition (Sathianathan et al., 2021). In addition to the sexual

Abbreviations: 3 α -HSOR, 3 α -hydroxysteroid oxidoreductase; 3 β -HSOR, 3 β -hydroxysteroid oxidoreductase; 3 α -diol, 5 α -androstane-3 α ,17 β -diol; 5 α -R1, 5 α -reductase type 1; 5 α -R2, 5 α -reductase type 2; ACAT, Acyl CoA:cholesterol acyl transferase; CSF, Cerebrospinal fluid; p450sc, Cytochrome p450 side chain cleavage; DHEA, Dehydroepiandrosterone; DHP, Dihydroprogesterone; DHT, Dihydrotestosterone; FSH, Follicle-stimulating hormone; HSL, Hormone sensitive lipase; ISO-ALLO, Isoallopregnanolone; LC-MS/MS, Liquid chromatography tandem mass spectrometry analysis; LH, Luteinizing hormone; PSSD, Post-SSRI sexual dysfunction; PREG, Pregnenolone; PREG-S, Pregnenolone sulfate; PROG, Progesterone; SSRI, Selective serotonin reuptake inhibitors; SNRI, Serotonin-norepinephrine reuptake inhibitors; StAR, Steroidogenic acute regulatory protein; STS, Steroid sulfatase; Sult2 β 1, Sulfotransferase family 2B member 1; T, Testosterone; THP, Tetrahydroprogesterone.

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problems observed during treatment, recently, the persistence of sexual dysfunction after stopping SSRI drugs has been increasingly reported (Bala et al., 2018; Patacchini and Cosci, 2021, 2020; Rothmore, 2020; Reisman, 2017, 2020; Healy, 2019, 2020; Healy et al., 2018; Hogan et al., 2014). This condition has been termed post-SSRI sexual dysfunction (PSSD), and it has been reported after SSRI use, but it can also be present after treatment with some SNRIs (Rothmore, 2020; Patacchini and Cosci, 2021). The pathogenesis of antidepressant-induced sexual dysfunction and that of PSSD are largely unknown (Segraves and Balon, 2014), but a role for the serotonergic inhibitory activity on the mesolimbic pathway of dopamine can be proposed (Giatti et al., 2018). However, other explanations have also been reported. Among them, a role for neuroactive steroids has been hypothesized.

Neuroactive steroids are physiological modulators of brain functions (Melcangi et al., 2014; Giatti et al., 2019b, 2020a). They can be produced by peripheral steroidogenic tissues and then metabolized in the nervous system, where they exert their functions. Evidence from the literature indicates that these molecules can also be directly produced within the nervous system itself (Melcangi et al., 2014; Giatti et al., 2019b, 2020a). Thus, specific brain areas such as the hypothalamus, hippocampus, cerebral cortex, to cite some of them, express the steroidogenic machinery to produce and metabolize neuroactive steroids (Melcangi et al., 2014; Giatti et al., 2019b, 2020a). Neurosteroidogenesis is a compartmentalized sequence of reactions starting from cholesterol, that after its transport into mitochondria, by Steroidogenic Acute Regulatory protein (StAR), is converted into pregnenolone (PREG) by the enzyme p450 side chain cleavage, coded by the CYP11A1 gene. Then, PREG is converted into progesterone (PROG) or into dehydroepiandrosterone (DHEA). PROG is then converted into its metabolites, dihydroprogesterone (DHP) by enzymes 5 α -reductase, type 1 and type 2 (5 α -R1 and 5 α -R2) and subsequently by enzymes 3 α - or 3 β -hydroxysteroid oxidoreductase (3 α /3 β -HSOR) into 3 α , 5 α -tetrahydroprogesterone (THP), also known as allopregnanolone, and its 3 β , 5 α -isomer, isoallopregnanolone, respectively. DHEA is converted into androgens, such as testosterone (T), that it is then converted into dihydrotestosterone (DHT) and 5 α -androstane-3 α ,17 β -diol (3 α -diol) by the enzymatic complex 5 α -R/3 α -HSOR or into 17 β -estradiol by the aromatase (Giatti et al., 2019b).

Neuroactive steroids are related to both depression and sexual function. For example, neuroactive steroids modulate anxiety as well as depression-related behavior in preclinical models and participate in the therapeutic effect of antidepressants (Zorumski et al., 2019, 2013; Locci and Pinna, 2017; Uzunova et al., 2006). They are also involved in the response to acute and chronic stress (Crawley et al., 1986; Purdy et al., 1991; Barbaccia et al., 2001). Moreover, the alteration of their levels, for example in condition like premenstrual dysphoric disorder (Hantsoo and Epperson, 2020) or postpartum depression (Maguire, 2019), suggests their involvement in depressive pathological mechanisms. In agreement, Brexanolone, a synthetic analog of THP, has been recently approved for the clinical management of post-partum depression (Meltzer-Brody et al., 2018; Zheng et al., 2019; Morrison et al., 2019; Leader et al., 2019). In an experimental model of depression, the treatment with fluoxetine, a SSRI drug, is able to increase the reduced levels of THP observed in the frontal cortex of socially-isolated mice (Matsumoto et al., 1999). Moreover, Uzunova and colleagues reported that three weeks of chronic treatment with different antidepressant classes restored THP levels in olfactory-bulbectomized rat, a model of depression (Uzunova et al., 2004). Clinical observations confirmed that fluoxetine was able to increase THP levels in plasma and cerebrospinal fluid (CSF) of depressed patients (Uzunova et al., 1998; Romeo et al., 1998). Overall, a consistent literature suggests that increasing THP levels can contribute to the therapeutic effects of antidepressant drugs (Guidotti et al., 2001), although it is important to point out that not all effective therapeutic interventions can increase THP levels (Schule et al., 2003, 2007; Plewnia and Padberg, 2012; Baghai et al., 2012). However,

not only THP, but also PREG sulfate produced anti-depressant action in the forced swim test (Reddy et al., 1998). Additionally, it has been reported that drugs, such as olanzapine, or fluoxetine or their co-administration can increase hippocampal levels of THP and PREG (Marx et al., 2006).

In addition, as mentioned above, neuroactive steroids also have a role in sexual function. Indeed, it is important to recall that, in men, T is the main neuroactive steroid driving sexual desire (Santi et al., 2018). In fact, hypogonadal men experience low desire and erectile dysfunction, which can be improved by T treatment (Corona et al., 2016). In agreement, loss of libido and sexual problems are reported in subjects on androgen deprivation therapy, such as during prostate cancer treatment (Mazzola and Mulhall, 2012). Animal studies revealed that the brain regions mainly involved in male sexual behavior, such as the medial preoptic area in the hypothalamus and the bed nucleus of the stria terminalis, present high levels of estrogen and androgen receptors (Hull et al., 2006). Indeed, in these regions, T has a key role in activating male sexual behavior (Hull et al., 2006).

Interestingly, the SSRI-induced sexual problems are connected to steroid levels and reproductive functions (i.e., the hypothalamus-pituitary-gonad axis). It has been reported that patients on SSRI reporting sexual dysfunction presented plasma levels of T, luteinizing and follicle-stimulating hormone (LH and FSH, respectively) which were decreased compared to SSRI-treated patients without other symptoms (Safarinejad, 2008). It is important to highlight that also animal studies reported sexual impairments after SSRI drug administration (de Jong et al., 2005; Vega Matuszyk et al., 1998; Waldinger et al., 2002), suggesting that these mechanisms can be studied in animal models. In particular, as already reported by others (Oosting et al., 2016b, 2016a; Angulo et al., 2001) male rats treated for two weeks with paroxetine at the dose of 10 mg/kg showed impaired sexual behavior, such as impaired copulatory and ejaculatory behavior and erectile dysfunction.

Therefore, using this experimental model we here explored the possibility that this drug, known to induce sexual dysfunction in patients (Serretti and Chiesa, 2009; Jing and Straw-Wilson, 2016) and frequently associated to PSSD (Waldinger et al., 2015; Giatti et al., 2018), may alter the levels of neuroactive steroids in the central nervous system and in peripheral circulation. In particular, adult male Sprague-Dawley rats have been treated with paroxetine for two weeks and analyzed 24 h after the last treatment or after a withdrawal period of one month. Neuroactive steroid levels have been assessed by mass spectrometry analysis in relevant brain areas, such as the hypothalamus, hippocampus and cerebral cortex and in plasma and CSF. Additionally, the expression of key enzymes and molecules involved in neurosteroidogenesis has been evaluated in the abovementioned brain areas.

2. Material and methods

2.1. Animals

In these experiments, adult male Sprague-Dawley rats (200–225 g at arrival, Charles River Laboratories, Lecco, Italy) were used. The procedures were approved by the local ethics committee, and by the Italian Ministry of Health (authorization 107/2018-PR). All manipulations were performed in accordance with national (D.L. No. 26, March 4, 2014, G.U. No. 61 March 14, 2014) and international laws and policies (EEC Council Directive 2010/63, September 22, 2010: Guide for the Care and Use of Laboratory Animals, United States National Research Council, 2011). All experiments have been carried in the animal care facility of the Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB) at the Università degli Studi di Milano, Italy. The rats were acclimated to the new environment for one week before starting manipulations. To collect CSF, the animals were anesthetized, until the loss of righting reflex, with a mixture of ketamine (Lobotor; la Zootecnica Group SPA, Italy) and xylazine (Rompun, la Zootecnica Group SPA, Italy). CSF was then obtained by puncturing the cisterna magna as

previously described (Lebedev et al., 2004). After CSF collection, anesthetized animals were sacrificed. Then, trunk total blood was collected in centrifuge tube with EGTA 0.25 M, while hypothalamus, hippocampus and the cerebral cortex were dissected and stored immediately at -80°C until analysis.

2.2. Treatments

Paroxetine chlorhydrate (paroxetine: 10 mg/kg/day) was suspended in tap water. Either this solution or vehicle was administered to the animals *per os* for 14 days. Paroxetine and control rats were sacrificed at 24 h and 1 month after the last treatment to investigate the effects of the subchronic treatment and of the withdrawal. Body weight was assessed at the end of the treatment and at the end of the withdrawal period.

2.3. Liquid chromatography tandem mass spectrometry analysis

2.3.1. Reagents and chemicals

5-pregnen- 3β -ol-20-one (PREG), pregnenolone-20,21- $^{13}\text{C}_2$ -16,16 D_2 ($^{13}\text{C}_2$ D_2 - PREG), 3β -hydroxy-5-pregnen-20-one 3-sulfate (PREG-S), progesterone (PROG), progesterone-2,3,4,20,25- $^{13}\text{C}_5$ ($^{13}\text{C}_5$ - PROG), 17 β -Estradiol (17 β -E), 17 β -Estradiol-2,3,4- $^{13}\text{C}_3$ ($^{13}\text{C}_3$ -17 β -E) 5 α -pregnane-3, 20-dione (DHP), 3 α -hydroxy-5 α -pregnen-20 one (THP), 3β -hydroxy-5 α -pregnen-20 one (Isoallopregnanolone), testosterone (T), 5 α -androstane-17 β -ol-3-one (DHT), 5 α -androstane-3 α 17 β -diol (3 α -diol) and dehydroepiandrosterone (DHEA) were purchased from Merck LifeScience, Italy.

24(S)-hydroxycholesterol (24-OH-C) and 25-hydroxycholesterol (25-OH-C) were bought from Cayman Chemical, USA; 7 α -hydroxycholesterol (7 α), 7 β -hydroxycholesterol (7 β) from Research Plus, USA; 10-hydroxycholesterol (19-OH-C), 7-keto-hydroxycholesterol (7-keto) and 27-hydroxycholesterol (27-OH-C) from Steraloids, USA.

Acetonitrile, acetic acid, chloroform, formic acid, hydrochloric acid 37% methanol, 2-propanol and water were HPLC grade (Merck LifeScience, Italy).

2.3.2. Neuroactive steroids

The levels of neuroactive steroids, such as PREG, PROG, DHP, THP, isoallopregnanolone, DHEA, T, DHT, 3 α -diol, 17 β -E were evaluated in the cerebral cortex, hippocampus, hypothalamus, CSF and plasma instead, PREG-S levels were assessed only in hippocampus. Plasma was obtained after blood collection, by centrifugation at 2500 g for 15 min at 4°C .

For the quantitative analysis of neuroactive steroids, all tissue and plasma samples were extracted and purified as previously described (Pesaresi et al., 2010; Caruso et al., 2008). $^{13}\text{C}_3$ -17 β -E (2 ng/sample) $^{13}\text{C}_5$ - PROG (0.4 ng/sample) and $^{13}\text{C}_2$ D_2 - PREG (10 ng/sample) were used as internal standards.

The analysis was conducted by liquid chromatography (LC) supplied of Surveyor liquid chromatography (LC) Pump Plus and Surveyor Autosampler Plus (ThermoElectron Co., San Jose, CA, USA) with a linear ion trap - mass spectrometer (LTQ, ThermoElectron Co, San Jose, CA, USA) operated in positive atmospheric pressure chemical ionization (APCI+). The chromatographic separation was achieved with a Hypersil Gold column C18 (100 \times 2.1 mm, 3 μm ; ThermoFisher Scientific) was maintained at 40°C . The mobile phases consisted of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in methanol (mobile phase B). Gradient elution was as follows: 0–1.50 min 70% A, 30% B; 1.50–2.00 min 55% A, 45%B; 2.00–3.00 min 55% A, 45% B; 3.00–35.00 min linear gradient to 36% A, 64% B; 35.00–40.00 min 25% A, 75% B; 41.00–45.00 min 1% A, 99% B; 45.00–45.20 min 70% A, 30% B and 45.40–55.00 min equilibrate with 70% A and 30% B. 25 μl sample was injected at a flowrate of 0.250 mL/min. The divert valve was set at 0–8 min to waste, 8–45 min to source and 45–55 min to waste. The injector needle was washed with MeOH/Water 1/1 (v/v).

Quantitative analysis was performed on the basis of calibration

curves prepared and analyzed using standards. LC-MS/MS peaks were appraised using software Excalibur® release 2.0 SR2 (ThermoElectron Co, San Jose, CA, USA).

2.3.3. Oxysterols

The levels of oxysterol, such as 24(S)-hydroxycholesterol (24OH-C), 25-hydroxycholesterol (25OH-C), 27-hydroxycholesterol (27OH-C), 7 α -cholesterol (7 α), 7 β -cholesterol (7 β), and 7-keto-cholesterol (7-keto) were assessed by LC-MS/MS in hippocampus. Previously described protocol (Cermenati et al., 2012) was followed for extracting oxysterols. 19-OH-Colesterol was used as an internal standard.

The quantitative analysis was performed using liquid chromatography (LC) supplied of Surveyor liquid chromatography (LC) Pump Plus and Surveyor Autosampler Plus (ThermoElectron Co., San Jose, CA, USA) with a linear ion trap - mass spectrometer (LTQ, ThermoElectron Co, San Jose, CA, USA) operated in positive atmospheric pressure chemical ionization (APCI+). The chromatographic separation was achieved with a Venunsil XBP C18(A) column (100 \times 3 mm, 3 μm ; AgelaTechnologies) and was maintained at 30°C .

The divert valve was set at 0–3.00 min to waste, 3.00–26.07 min to source and 26.07–50.00 min to waste. The injector needle was washed with MeOH/Water 1/1 (v/v). The mobile phases are chloroform/water/methanol 76/4/20 (v/v/v) (mobile phase A) and 2-propanol (IPA, mobile phase B). The flow rate was fixed at 0250 mL/min. Gradient elution was as follows: 0–15.00 min 100% A; 15.00–15.50 min linear gradient to 50% A, 50% B; 15.50–35.00 min 50% A, 50%B; 35–35.50 min linear gradient to 100% A, 0% B and 35.50–50.00 min equilibrate with 100% A.

Quantitative analysis was performed on the basis of calibration curves prepared and analysed in the same day. LC-MS/MS peaks were appraised using software Excalibur® release 2.0 SR2 (ThermoElectron Co, San Jose, CA, USA).

2.4. Real-time polymerase chain reaction

Total RNA from tissues was extracted using Trizol (Invitrogen, San Giuliano Milanese, Italy) and Direct-zol TM RNA MiniPrep kit (Zymo Research, Irvine, Calif., USA) in accordance with the manufacturer's protocol. After quantification, RNA was analyzed using a TaqMan quantitative real-time PCR instrument (CFX96 real time system; Bio-Rad Laboratories, Segrate, Italy) using the Luna Universal One-Step RT-qPCR Kit (New England BioLabs inc., Ipswich, MA). The samples were run in 96-well formats in duplicate as multiplexed reactions with a normalizing internal control, 36B4. Specific TaqMan MGB probes and primers sequence were purchased at Eurofins MWG-Operon (Milano, Italy) and are available on request: StAR, CYP17A1, 5 α -R1, 5 α -R2, 3 α -HSOR, HSL, ACAT1, STS, SULT2 β 1. Specific TaqMan MGB probes (Applied Biosystems, Thermo Fisher Scientific) were: CYP11A1 (Rn00568733_m1), 3β -HSD (Rn00820880_g1), CYP27A1 (Rn00710297_m1).

2.5. Statistical analysis

The quantitative data obtained from the experiments were analyzed by inferential statistical analysis in accordance with the experimental protocols and the data (i.e., Student's *t*-test). $p \leq 0.05$ was considered significant. Analyses were performed using GRAPHPAD PRISM, version 4.00 (GraphPad Inc., La Jolla, Calif., USA).

3. Results

3.1. The effect of paroxetine administration on body weight

Table 1 reports the body weight of animals at sacrifice. As expected, by the finding that weight gain is a common problem during antidepressant treatment, and that paroxetine, among the other SSRIs, is more likely to cause weight gain (Fava, 2000), after subchronic SSRI

Table 1
Body weight at sacrifice.

Animal	Body weight at sacrifice (g)	
	After subchronic treatment	At withdrawal
CTRL	386.03 ± 7.07	508.53 ± 6.64
PARO	412.58 ± 4.89 **	511.95 ± 7.92

Body weight of control (CTRL) and paroxetine-treated (PARO) rats. Data are expressed as mean ± SEM, n = 12 rats for each experimental group. Student's *t*-test analysis: ** *p* < 0.01 vs. control.

treatment, male rats presented an increase in body weight (*p* = 0.0054; *t* = 3.09; *df*=22; *n* = 12). After one month of withdrawal, paroxetine-treated animals showed a similar body weight than vehicle-treated controls (*p* = 0.60) (Table 1).

3.2. Assessment of neuroactive steroid levels after paroxetine subchronic treatment

Table 2 shows neuroactive steroid levels, assessed by LC-MS/MS, in plasma, CSF, hippocampus, hypothalamus, and cerebral cortex of male rats treated for 14 days *per os* with paroxetine. Interestingly, the SSRI administration was not able to modify neuroactive steroid levels in plasma, suggesting that peripheral steroidogenesis was not affected by the treatment. In contrast, several differences may be observed in CSF and brain areas analyzed, supporting the idea that SSRI administration altered neurosteroidogenesis. Among these differences, cerebral cortex is the brain structure where the pattern of neuroactive steroid levels is more altered. Indeed, we observe a significant decrease in the levels of DHP (*p* = 0.0396, *t* = 2.365, *df*=10), isallopregnanolone (*p* = 0.0221, *t* = 2.830, *df*=8) and DHEA (*p* = 0.0035, *t* = 3.798, *df*=10), associated with an increase in the levels of T (*p* = 0.0297, *t* = 2.532, *df*=10) and 3α-diol (*p* = 0.0458, *t* = 2.289, *df*=10). In the CSF, a significant decrease in 3α-diol levels (*p* = 0.0132, *t* = 3.005, *df*=10) has been detected, while 17β-estradiol was significantly increased (*p* = 0.0011, *t* = 4.496, *df*=10). In the hippocampus, PREG levels have been doubled by paroxetine treatment (*p* = 0.0138, *t* = 2.982, *df*=10), and similarly occurred for THP levels (*p* = 0.0391, *t* = 2.373, *df*=10). In hypothalamus, the only significant alteration observed is an increase in 3α-diol

levels (*p* = 0.027, *t* = 2.59, *df*=10) (Table 2).

3.3. Assessment of neurosteroidogenesis after paroxetine subchronic treatment

With the aim of correlating the alterations of neuroactive steroid levels with the enzymatic machinery, the gene expression of molecules involved in neuroactive steroid production and metabolism has been studied in the brain areas considered. In the hippocampus, the increased levels of PREG (Table 2) were associated with increased gene expression of CYP11A1, the gene encoding for the rate limiting enzymatic step in the PREG production, the cytochrome p450 side chain cleavage (P450sc; Fig. 1A), while the expression of the Steroidogenic Acute Regulatory protein (StAR), which helps the translocation of cholesterol into the mitochondria, was not affected (Fig. 1A). Likewise, in agreement with the variation of THP levels (Table 2), the expression of the 3α-hydroxysteroid oxidoreductase (3α-HSOR) was significantly increased (Fig. 1A). Similarly, in the hypothalamus, the rise in 3α-diol levels is supported by a significant increase in the gene expression of 3α-HSOR (Fig. 1B).

In the cerebral cortex, as described before, a significant decrease of DHP levels was observed in the SSRI group (Table 2) and that was associated with a significant reduction of 5α reductase type 2 (5α-R2; Fig. 1C), but not of type 1 (5α-R1; Fig. 1C). The situation for androgen in the cerebral cortex is different. Indeed, while the levels of PROG metabolites have been decreased by pharmacological treatment, the levels of the androgen were both increased and decreased in the same experimental group. In particular, the levels of DHEA, produced from PREG by the enzyme CYP17A1, were significantly decreased, but the expression of CYP17A1 was not affected (Fig. 1C). The DHEA metabolite, T, which production is mediated by the 3β-HSD enzyme, was doubled after paroxetine treatment (Table 2), and in agreement, the expression of 3β-HSD was significantly upregulated (Fig. 1C). The 3α,5α-reduced metabolite of T, 3α-diol, presented increased levels after paroxetine treatment. However, the expression of its producing enzyme, 3α-HSOR, was not significantly affected by the treatment (Fig. 1C).

Table 2
Levels of neuroactive steroids in plasma, CSF and brain areas of control and paroxetine-treated male rats: effect after subchronic treatment.

	PLASMA		CSF		HIPPOCAMPUS		HYPOTALAMUS		CEREBRAL CORTEX	
	Control	Paroxetine	Control	Paroxetine	Control	Paroxetine	Control	Paroxetine	Control	Paroxetine
PREG	0.93 ± 0.25 (6)	0.66 ± 0.25 (6)	0.09 ± 0.01 (6)	0.07 ± 0.02 (6)	6.24 ± 1.20 (6)	12.85 ± 1.87 * (6)	7.82 ± 1.64 (6)	6.75 ± 1.30 (6)	9.66 ± 1.69 (6)	8.84 ± 1.31 (6)
PROG	1.42 ± 0.40 (6)	1.85 ± 0.78 (6)	0.16 ± 0.01 (6)	0.13 ± 0.03 (6)	4.95 ± 0.71 (6)	5.27 ± 0.98 (6)	5.63 ± 1.99 (6)	6.32 ± 2.38 (6)	7.28 ± 2.84 (6)	4.14 ± 1.48 (6)
DHP	UDL (6)	UDL (6)	UDL (6)	UDL (6)	6.32 ± 1.82 (6)	4.39 ± 1.10 (6)	3.03 ± 0.62 (6)	3.98 ± 1.20 (6)	1.43 ± 0.24 (6)	0.81 ± 0.11 * (6)
THP	UDL (6)	UDL (6)	0.10 ± 0.01 (6)	0.07 ± 0.01 (6)	1.91 ± 0.50 (6)	4.85 ± 1.14 * (6)	4.69 ± 1.39 (6)	3.77 ± 1.36 (6)	3.43 ± 1.09 (6)	2.41 ± 0.88 (6)
ISOALLO	0.79 ± 0.09 (6)	0.98 ± 0.38 (6)	0.13 ± 0.04 (6)	0.10 ± 0.00 (6)	0.78 ± 0.43 (6)	1.26 ± 0.53 (6)	4.13 ± 1.50 (5)	3.74 ± 1.39 (6)	8.02 ± 1.33 (6)	2.46 ± 1.33 * (4)
DHEA	0.12 ± 0.03 (6)	0.08 ± 0.01 (6)	0.13 ± 0.05 (6)	0.23 ± 0.06 (6)	0.95 ± 0.04 (6)	0.98 ± 0.09 (6)	0.38 ± 0.15 (6)	0.34 ± 0.14 (6)	0.15 ± 0.02 (6)	0.07 ± 0.01 * * (6)
T	2.39 ± 0.61 (6)	2.88 ± 0.48 (6)	0.20 ± 0.05 (6)	0.22 ± 0.03 (6)	1.47 ± 0.33 (6)	2.01 ± 0.57 (6)	1.61 ± 0.29 (6)	3.03 ± 0.58 (6)	2.50 ± 0.56 (6)	5.08 ± 0.85 * (6)
DHT	0.35 ± 0.02 (6)	0.31 ± 0.09 (6)	0.30 ± 0.10 (6)	0.24 ± 0.09 (6)	1.64 ± 0.06 (6)	1.62 ± 0.25 (6)	4.51 ± 0.61 (6)	6.67 ± 1.11 (6)	1.35 ± 0.31 (6)	1.32 ± 0.30 (6)
3α-DIOL	1.88 ± 0.45 (6)	1.07 ± 0.16 (6)	0.41 ± 0.04 (6)	0.30 ± 0.01 * (6)	1.68 ± 0.50 (6)	1.38 ± 0.15 (6)	2.78 ± 0.72 (6)	4.79 ± 0.30 * (6)	0.22 ± 0.09 (6)	0.72 ± 0.2 * (6)
17β-E	0.06 ± 0.01 (6)	0.05 ± 0.02 (6)	0.09 ± 0.00 (6)	0.10 ± 0.00 ** (6)	0.02 ± 0.00 (6)	0.03 ± 0.00 (6)	0.03 ± 0.01 (6)	0.05 ± 0.02 (6)	0.05 ± 0.03 (6)	0.05 ± 0.01 (6)

Data (number of rats for each group is indicated in brackets) are expressed as pg/μl ± SEM in case of plasma and CSF and pg/mg ± SEM in case of brain areas. UDL = Under detection limit. Detection limits were 0.02 pg/μl or pg/mg for testosterone (T) and 17β-estradiol (17β-E), 0.05 pg/μl or pg/mg for pregnenolone (PREG), progesterone (PROG), 3α-diol, dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT); 0.1 pg/μl or pg/mg for tetrahydroprogesterone (THP) and isallopregnanolone (ISOALLO); 0.25 pg/μl or pg/mg for dihydroprogesterone (DHP). Student's *t*-test analysis: * *p* < 0.05 and * * *p* < 0.01 vs. control.

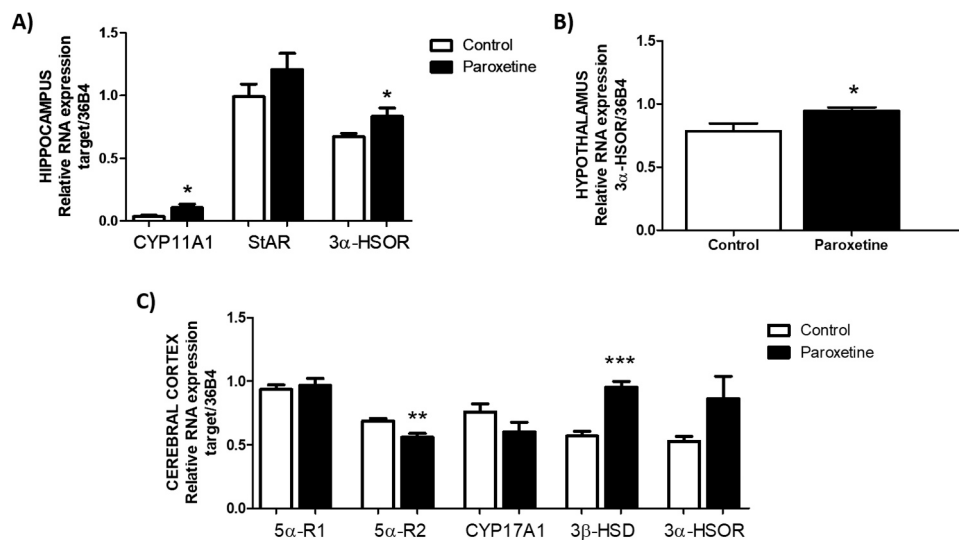


Fig. 1. Gene expression of selected targets in brain areas after subchronic paroxetine treatment. Panel A: hippocampus, panel B: hypothalamus, panel C: cerebral cortex. The columns represent the mean \pm SEM after normalization with 36B4 in control and subchronically paroxetine-treated animals. Data (n = 6, except for hypothalamus: n = 5) were analyzed by Student's *t*-test. * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ versus control.

3.4. Assessment of neuroactive steroid levels at withdrawal

After a month of drug withdrawal (Table 3), a different pattern of neuroactive steroid levels was observed compared to what reported after subchronic treatment (Table 2). Indeed, in this case a higher number of neuroactive steroids was affected and, their levels were more frequently decreased, with the only exception of isoallopregnanolone ($p = 0.0404$, $t = 2.354$, $df=10$) that was increased in the hippocampus (Table 3). Similarly to what reported after subchronic treatment, also after a month of withdrawal plasma levels of neuroactive steroids were unaffected. In the CSF, at variance to what observed after treatment (Table 2), levels of 17 β -estradiol ($p = 0.0353$, $t = 2.433$, $df=10$) were significantly decreased at withdrawal (Table 3). In the hippocampus, PREG ($p = 0.0036$, $t = 3.902$, $df=9$) and THP ($p = 0.0437$, $t = 2.307$, $df=10$) levels were significantly reduced at this time point, in contrast to the situation after subchronic treatment (Table 2). At withdrawal, in the hypothalamus, we observed altered levels for nearly all neuroactive steroids considered (Table 3), while after subchronic treatment only androgens were affected (Table 2). In particular, a significant reduction in the levels of PREG ($p = 0.0171$, $t = 2.919$, $df=9$), DHP ($p = 0.0116$, $t = 3.083$, $df=10$), THP ($p = 0.0059$, $t = 3.483$, $df=10$), isoallopregnanolone ($p = 0.0002$, $t = 5.588$, $df=10$), T ($p = 0.0429$, $t = 2.356$, $df=9$) and 3 α -diol ($p = 0.0001$, $t = 6.142$, $df=10$) has been detected in this brain area. At the same time point, in the cerebral cortex (Table 3), a significant decrease in the levels of DHEA ($p = 0.0038$, $t = 3.75$, $df=10$), which is similar to what was observed after subchronic treatment (Table 2). In this brain area, the levels of 3 α -diol, which were increased after paroxetine treatment (Table 2), at withdrawal (Table 3) showed a significant reduction ($p = 0.0332$, $t = 2.468$, $df=10$).

3.5. Assessment of neurosteroidogenesis at withdrawal: hippocampus

As reported above PREG levels in the hippocampus are decreased at the withdrawal (Table 3). Therefore, we have first evaluated whether an impairment of the enzyme P450scc and/or StAR protein occurs. As reported in Fig. 2A, the expression of these two molecules is unaffected by drug withdrawal. A possible alternative hypothesis may imply changes in the substrate availability (i.e., cholesterol). In this context, it is important to recall that cholesterol may be in a free form, ready for subsequent use in the cell, or esterified, for storage. Two enzymes are involved in these mechanisms; thus, hormone sensitive lipase (HSL) mediates the de-esterification process, while acyl CoA:cholesterol acyl

transferase (ACAT) the esterification step. Data here reported indicate that the gene expression of HSL and ACAT in the hippocampus (Fig. 2A) was unmodified at the withdrawal.

Finally, it is important to recall that PREG in the nervous system may be converted into its sulfate form (i.e., PREG-S) by the enzyme sulfotransferase family 2B member 1 (Sult2 β 1) and retro-converted to free form by the enzyme steroid sulfatase (STS). As reported in Fig. 2A, reduced expression of STS and increased expression of SULT2 β 1 was observed in the hippocampus at withdrawal, suggesting that the increased sulfonation coupled to decreased desulfonation of PREG is responsible for the observed decrease in PREG levels. Accordingly, LC-MS/MS analyses revealed at the withdrawal, an increase in PREG-S levels in the hippocampus (Control: 1.9 ± 0.61 vs paroxetine: 5.34 ± 1.13 ; $p = 0.028$; $t = 2.67$, $df=8$, $n = 5$).

As reported in Table 3, the decrease in PREG levels was associated with a decrease in its metabolite, THP. However, the decrease in THP levels at the withdrawal was not supported by changes in the gene expression (Control: 0.61 ± 0.02 vs paroxetine: 0.63 ± 0.04 , $p = 0.72$, $n = 6$) of the enzyme 3 α -HSOR, responsible for its synthesis.

3.6. Assessment of neurosteroidogenesis at withdrawal: hypothalamus

PREG levels were also reduced in the hypothalamus at drug withdrawal. However, the mechanisms leading to the PREG reduction seem different in this brain area. Indeed, at variance to what was observed in the hippocampus, the expression of STS and SULT2 β 1 (Fig. 2B) was not affected in the hypothalamus. Similarly to what was reported in the hippocampus, the expression of the machinery involved in cholesterol availability (i.e., HSL and ACAT, Fig. 2B) as well as that involved in PREG production (i.e., CYP11A1 and StAR, Fig. 2B), was not modified at withdrawal period. However, in this case the decrease in PREG levels seems related to an alteration in cholesterol metabolism in the mitochondria. In this context, it is important to recall that in this organelle, the steroid precursor cholesterol is metabolized by the enzyme CYP27A1 producing an oxysterol, such as 27-hydroxycholesterol. Indeed, as here reported, the gene expression levels of this enzyme were significantly upregulated at withdrawal (Fig. 2B). In agreement, the levels of 27-hydroxycholesterol assessed by LC-MS/MS analysis, were significantly increased at this time point (Table 4).

Interestingly, we observed that not only the levels of 27-hydroxycholesterol, but also those of other oxysterols, such as 24(S)-hydroxycholesterol, 25-hydroxycholesterol, 7 α -hydroxycholesterol and 7 β -

Table 3
Levels of neuroactive steroids in plasma, CSF and brain areas of control and paroxetine-treated male rats: effect at withdrawal.

	PLASMA		CSF		HIPPOCAMPUS		HYPOTHALAMUS		CEREBRAL CORTEX	
	Control	Paroxetine	Control	Paroxetine	Control	Paroxetine	Control	Paroxetine	Control	Paroxetine
PREG	0.72 ± 0.23 (6)	1.08 ± 0.22 (6)	0.08 ± 0.02 (6)	0.08 ± 0.01 (6)	9.73 ± 1.10 (6)	4.87 ± 0.27 * (5)	17.28 ± 2.00 (5)	10.04 ± 1.54 * (6)	11.81 ± 3.67 (5)	12.49 ± 1.98 (6)
PROG	1.61 ± 0.57 (6)	1.61 ± 0.57 (6)	0.13 ± 0.05 (6)	0.13 ± 0.04 (6)	5.08 ± 0.60 (6)	4.64 ± 0.65 (6)	6.37 ± 1.71 (6)	4.18 ± 0.31 (6)	4.58 ± 1.59 (6)	4.04 ± 1.08 (6)
DHP	UDL (6)	UDL (6)	UDL (6)	UDL (6)	6.10 ± 1.69 (6)	7.25 ± 1.06 (6)	7.47 ± 1.84 (6)	1.68 ± 0.37 * (6)	1.38 ± 0.14 (6)	1.89 ± 0.42 (6)
THP	UDL (6)	UDL (6)	0.11 ± 0.02 (6)	0.15 ± 0.06 (6)	4.66 ± 1.24 (6)	1.59 ± 0.50 * (6)	8.17 ± 1.19 (6)	3.46 ± 0.63 * * (6)	2.77 ± 0.46 (6)	2.93 ± 0.17 (6)
ISOALLO	2.74 ± 0.64 (6)	3.15 ± 0.57 (6)	0.31 ± 0.07 (6)	0.35 ± 0.02 (6)	2.14 ± 0.36 (6)	4.38 ± 0.88 * (6)	7.88 ± 1.08 (6)	1.40 ± 0.42 * * * (6)	4.91 ± 1.20 (6)	3.42 ± 0.96 (6)
DHEA	0.09 ± 0.01 (6)	0.13 ± 0.05 (6)	0.27 ± 0.05 (6)	0.35 ± 0.02 (6)	0.55 ± 0.03 (6)	0.49 ± 0.15 (6)	0.84 ± 0.19 (6)	0.66 ± 0.06 (6)	0.34 ± 0.07 (6)	0.08 ± 0.01 * * (6)
T	1.66 ± 0.37 (6)	4.40 ± 1.63 (6)	0.19 ± 0.05 (6)	0.18 ± 0.03 (6)	1.77 ± 0.54 (6)	1.43 ± 0.11 (6)	3.14 ± 0.52 (5)	1.78 ± 0.31 * (6)	1.79 ± 0.57 (6)	2.22 ± 0.44 (5)
DHT	0.30 ± 0.05 (6)	0.38 ± 0.04 (6)	0.28 ± 0.11 (6)	0.44 ± 0.09 (6)	1.31 ± 0.21 (6)	1.15 ± 0.13 (6)	5.75 ± 0.76 (5)	4.56 ± 0.75 (6)	0.87 ± 0.13 (6)	0.59 ± 0.34 (6)
3α-DIOL	1.13 ± 0.29 (6)	1.16 ± 0.25 (6)	0.36 ± 0.04 (6)	0.35 ± 0.04 (6)	0.97 ± 0.30 (6)	1.42 ± 0.20 (6)	5.40 ± 0.36 (6)	2.76 ± 0.24 * * * (6)	0.78 ± 0.24 (6)	0.17 ± 0.04 * (6)
17β-E	0.06 ± 0.02 (6)	0.06 ± 0.01 (6)	0.11 ± 0.00 (6)	0.10 ± 0.00 * (6)	0.04 ± 0.01 (6)	0.03 ± 0.01 (6)	0.07 ± 0.03 (6)	0.08 ± 0.04 (6)	0.07 ± 0.02 (5)	UDL (6)

Data (number of rats for each group is indicated in brackets) are expressed as pg/μl ± SEM in case of plasma and CSF and pg/mg ± SEM in case of brain areas. UDL = Under detection limit. Detection limits were 0.02 pg/μl or pg/mg for testosterone (T) and 17β-estradiol (17β-E), 0.05 pg/μl or pg/mg for pregnenolone (PREG), progesterone (PROG), 3α-diol, dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT); 0.1 pg/μl or pg/mg for tetrahydroprogesterone (THP) and isallopregnanolone (ISOALLO); 0.25 pg/μl or pg/mg for dihydroprogesterone (DHP). Student's *t*-test analysis: * *p* < 0.05, ** *p* < 0.01 and *** *p* < 0.001 vs. control.

hydroxycholesterol were increased, while those of 7-ketocholesterol were unaffected (Table 4). Thus, at the drug withdrawal the decreased levels of PREG in this brain area could be due to a different metabolism of cholesterol.

As reported in Table 3, the levels of the metabolites of PREG, PROG and DHEA, were not different at withdrawal. However, the levels of their further metabolites (i.e., DHP, THP and isallopregnanolone in case of PROG and T and 3α-diol in case of DHEA) were decreased (Table 3). As we reported, the pattern of the gene expression of the enzymes involved in their production (i.e., 5α-R converting PROG into DHP, 3β-HSD converting DHEA into T, 3α-HSOR converting DHP into THP and DHT into 3α-diol) was only partially in agreement. Indeed, according to the changes observed, the gene expression of the 5α-R2 was significantly decreased, but that of 5α-R1 and 3β-HSD as well as of 3α-HSOR was unmodified and increased, respectively, at the withdrawal (Fig. 2B).

3.7. Assessment of neurosteroidogenesis at withdrawal: cerebral cortex

As reported in Table 3, the levels of DHEA and 3α-diol levels at withdrawal were significantly decreased. The analysis of the gene expression of the enzymes responsible for their synthesis did not reflect the trend observed in their levels. Indeed, the gene expression of CYP17A1 (i.e., the gene coding the enzyme converting PREG into DHEA) and that of the enzyme 3α-HSOR was significantly increased and unaffected, respectively (Fig. 2C).

4. Discussion

Data here presented show for the first time that subchronic treatment with paroxetine, as well as its withdrawal is able to deeply alter the levels of several neuroactive steroids in brain areas such as hippocampus, hypothalamus and cerebral cortex. Interestingly, plasma levels do not reflect these modifications suggesting that peripheral steroidogenesis is not affected and that changes observed in brain areas are due to a specific effect on neurosteroidogenesis. Indeed, we here demonstrated that the expression of several key enzymes and molecules involved in the synthesis of neuroactive steroids is, accordingly with levels, modified in the nervous system. In particular, we observed that the levels of the first steroid synthesized by cholesterol (i.e., PREG) increased together with THP in hippocampus after paroxetine treatment. This effect seems to be specific for this brain area. Indeed, it does not occur in hypothalamus and cerebral cortex. The increase in PREG and THP levels due to hippocampal steroidogenesis is supported by the increase in the expression of the enzymes synthesizing these two neuroactive steroids (i.e., P450scc and 3α-HSOR, respectively). The increase of THP, here reported, is in agreement with the stimulatory effect exerted by different SSRIs on its levels (Uzunova et al., 2004; Guidotti and Costa, 1998; Pinna et al., 2006) and on the activity of 3α-HSOR (Griffin and Mellon, 1999) in different experimental models. In the hypothalamus the effect of treatment with paroxetine was restricted to an increase in the levels of the T metabolite, 3α-diol. Also in this case, the increase in the levels of this neuroactive steroid was due to an increase in the expression of the enzyme 3α-HSOR. In comparison to what was observed in the hippocampus and hypothalamus, a more extensive impact of paroxetine treatment was reported on the neuroactive steroid levels present in the cerebral cortex. Indeed, we observed a decrease in the levels of DHP and its metabolite isallopregnanolone as well as a decrease in DHEA associated with an increase in T and 3α-diol levels. Assessment of the gene expression of enzymes involved in the synthesis of these neuroactive steroids confirmed that the decrease in DHP levels and the increase in T is coupled with a reduction of 5α-R2 and an increase in 3β-HSD, respectively.

Therefore, this set of observations indicates that subchronic treatment with paroxetine alters differently and depending on the brain areas considered the levels of important physiological regulators of the

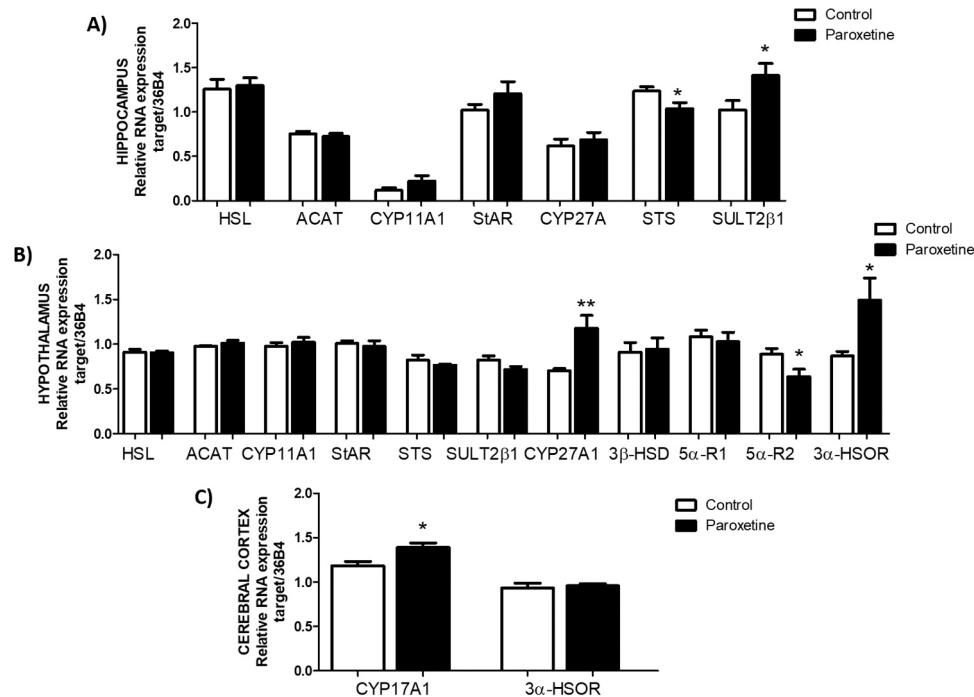


Fig. 2. Gene expression of selected targets in brain areas at withdrawal. Panel A: hippocampus, panel B: hypothalamus, panel C: cerebral cortex. The columns represent the mean \pm SEM after normalization with 36B4, in control and subchronically paroxetine-treated animals. Data (n = 6) were analyzed by Student's *t*-test. * $p \leq 0.05$ and ** $p \leq 0.01$ versus control.

Table 4

Levels of oxysterols in hypothalamus of control and paroxetine-treated male rats at withdrawal.

	Hypothalamus	
	Control	Paroxetine
24(S)-hydroxycholesterol (ng/mg tissue)	17.75 \pm 1.67	23.46 \pm 0.87 *
25-hydroxycholesterol (ng/mg tissue)	0.16 \pm 0.02	0.19 \pm 0.01 *
27-hydroxycholesterol (ng/mg tissue)	0.35 \pm 0.04	0.48 \pm 0.03 *
7α-hydroxycholesterol (ng/mg tissue)	0.11 \pm 0.01	0.17 \pm 0.02 *
7β-hydroxycholesterol (ng/mg tissue)	0.04 \pm 0.00	0.05 \pm 0.01 *
7-ketocholesterol (ng/mg tissue)	0.17 \pm 0.01	0.20 \pm 0.01

Data are expressed as mean \pm SEM, n = 6 animals for each experimental group. Student's *t*-test analysis: * $p < 0.05$ vs. control.

nervous functions, such as the neuroactive steroids (i.e., increase of PREG and THP in hippocampus, T in cerebral cortex and 3α-diol in hypothalamus and cerebral cortex, decrease of DHP and isoallopregnanolone in cerebral cortex). Paroxetine effects after subchronic treatment seem to be ascribed to a direct mechanism on the neurosteroidogenesis, as demonstrated by the findings that 1) plasma levels of neuroactive steroids are not modified, 2) CSF levels do not reflect the changes occurring in brain areas and 3) alterations in brain levels are frequently coupled with changes in brain enzymatic gene expression.

The situation seems extremely different at drug withdrawal. Indeed, except isoallopregnanolone levels that were increased in the hippocampus, the other changes observed reflect a general decrease in the levels of neuroactive steroids. Indeed, we reported decreased levels of PREG and THP in the hippocampus and hypothalamus, DHP, isoallopregnanolone, T and 3α-diol in the hypothalamus and of DHEA and 3α-diol in the cerebral cortex. Also in this case, the finding that plasma and CSF did not reflect changes occurring in the brain areas considered and that some enzymes involved in the synthesis of these neuroactive steroids are affected seems to confirm a direct effect on the neurosteroidogenesis. In this context, it is important to highlight that, even if PREG levels decrease, at the withdrawal, both in the hippocampus and

hypothalamus, the mechanisms involved in such decrease are different. Indeed, we reported that in the hippocampus the decrease is linked to an increase in its conversion into PREG-S, while in the hypothalamus to an alteration of cholesterol metabolism into oxysterols. Decreased nervous levels of PREG related to altered levels of oxysterols have already been reported in an experimental model of multiple sclerosis (Giatti et al., 2020b) and diabetic encephalopathy (Romano et al., 2018, 2017). In particular, as here reported paroxetine withdrawal induced in the hypothalamus an increase of 27-hydroxycholesterol, 24(S)-hydroxycholesterol, 25-hydroxycholesterol, 7α-hydroxycholesterol and 7β-hydroxycholesterol. Several oxysterols have cytotoxic and pro-apoptotic activities (Gold et al., 2014; Olkkonen et al., 2012), thus they have been considered to contribute to the onset and development of major chronic diseases involving inflammation, oxidative damage, and cell death (Poli et al., 2013). Altogether the observations here indicate that paroxetine treatment and its withdrawal affect neurosteroidogenesis. In particular, the finding that paroxetine withdrawal exerts an extensive decrease in the levels of many of the neuroactive steroids here considered, may suggest an interesting link with PSSD (Bala et al., 2018; Reisman, 2017, 2020; Simonsen et al., 2016; Giatti et al., 2018; Ben-Sheetrit et al., 2015; Csoka et al., 2008; Csoka and Shipko, 2006; Bahrack, 2008; Waldinger et al., 2015; Hogan et al., 2014). Indeed, even if the mechanisms causing the persistence of sexual side effects of SSRIs (i.e., genital numbing, pleasureless or absent ejaculation/orgasm, loss of libido, erectile dysfunction) are still unknown, an alteration of the serotonergic/dopaminergic pathways controlling sexual behavior has been proposed (Giatti et al., 2018; Graf et al., 2014; Abler et al., 2011, 2012). In this context, it is important to highlight neuroactive steroids acting by interaction with various classical and non-classical steroid receptors are important physiological regulators of nervous function (Giatti et al., 2020a, 2019b; Melcangi et al., 2008, 2014). In particular, in reference to the neuroactive steroids here modulated, PREG-S acts at NMDA, AMPA and GABA-A receptor (Weng and Chung, 2016; Locci and Pinna, 2017), THP as well as 3α-diol are ligands of GABA-A receptor (Belelli and Lambert, 2005; Lambert et al., 2009; Hosie et al., 2006), isoallopregnanolone antagonizes the effects of THP on the GABA-A

receptor (Wang et al., 2002; Backstrom et al., 2005), DHP and T bind to progesterone and androgen receptor, respectively (Giatti et al., 2020a). Alterations in the levels of neuroactive steroids have been related to many neurological disorders (Melcangi et al., 2016; Giatti et al., 2019a). Additionally, these molecules, among the several physiological effects exerted in the nervous system, also regulate the release of different neurotransmitters, including serotonin and dopamine (Zheng, 2009). Therefore, it is possible to hypothesize that a disturbance of their physiological levels may contribute, altering the cross talk of two neurotransmitters involved in the control of male sexual behavior, such as serotonin and dopamine (Giatti et al., 2018), to the symptomatology reported in the PSSD. This hypothesis does not exclude that peripheral actions of paroxetine may also contribute to PSSD.

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CRediT authorship contribution statement

Silvia Giatti: Conceptualization, Performed animal and gene expression experiments, Writing – original draft, Revised the text. **Silvia Diviccaro:** Performed animal experiments, Mass spectrometry analyses. **Lucia Cioffi:** Performed oxysterols analysis. **Eva Falvo:** Performed animal experiments. **Donatella Caruso:** Supervised LC-MS/MS spectrometry. **Roberto C. Melcangi:** General supervision, Conceptualization, Writing – original draft, Revised the text.

Declaration of Competing Interest

None.

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