Psychoneuroendocrinology

EFFECTS OF PAROXETINE TREATMENT AND ITS WITHDRAWAL ON NEUROSTEROIDOGENESIS

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Section/Category:	Preclinical studies (in animals)
Keywords:	Selective serotonin reuptake inhibitors; neuroactive steroids; sexual dysfunction; depression; hippocampus; hypothalamus
Corresponding Author:	Roberto Cosimo Melcangi Università degli Studi di Milano Milano, Italy
First Author:	Silvia Giatti
Order of Authors:	Silvia Giatti
	Silvia Diviccaro
	Lucia Cioffi
	Eva Falvo
	Donatella Caruso
	Roberto Cosimo Melcangi
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. 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 these molecules 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 synthesis of neuroactive steroids 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.
Suggested Reviewers:	Graziano Pinna gpinna@uic.edu Expert in neurosteroids
	Rainer Rupprecht rainer.rupprecht@medbo.de Expert in neurosteroids
	Luis Miguel Garcia-Segura lmgs@cajal.csic.es expert in neurosteroidogenesis
	David Healy david.healy54@googlemail.com Expert in PSSD

Powered by Editorial Manager® and ProduXion Manager® from Aries Systems Corporation

	Marco Bortolato marco.bortolato@utah.edu Expert in neurosteroids

Powered by Editorial Manager® and ProduXion Manager® from Aries Systems Corporation

UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE FARMACOLOGICHE
E BIOMOLECOLARI - DiSFeB
Direttore: Prof.ssa Monica DiLuca

Dear Prof Danzer,

Please find here enclosed the manuscript entitled:

EFFECTS OF PAROXETINE TREATMENT AND ITS WITHDRAWAL ON NEUROSTEROIDOGENESIS

by Silvia Giatti, Silvia Diviccaro, Lucia Cioffi, Eva Falvo, Donatella Caruso, Roberto Cosimo Melcangi

to be considered for publication in **Psychoneuroendocrinology**.
I look forward to receiving the Referee comments.

With cordial regards

Sincerely yours,

Roberto Cosimo Melcangi

Prof. Roberto Cosimo Melcangi
Via Balzaretti, 9 - 20133 Milano, Italia
Tel.: 02.50318238 – Fax: 02.50318204; e-mail: roberto.melcangi@unimi.it

EFFECTS OF PAROXETINE TREATMENT AND ITS WITHDRAWAL ON NEUROSTEROIDOGENESIS

Silvia Giatti, Silvia Diviccaro, Lucia Cioffi, Eva Falvo, Donatella Caruso, Roberto C. Melcangi

Highlights

- Brain neuroactive steroid levels are altered by paroxetine treatment
- Brain neuroactive steroid levels are altered after one-month withdrawal
- Paroxetine treatment and withdrawal did not affect steroid plasma levels
- Brain expression of steroidogenic enzyme is affected by paroxetine treatment
- Brain expression of steroidogenic enzyme is affected after one-month withdrawal

Manuscript File Click here to view linked References

EFFECTS OF PAROXETINE TREATMENT AND ITS WITHDRAWAL ON NEUROSTEROIDOGENESIS

Silvia Giatti, Silvia Diviccaro, Lucia Cioffi, Eva Falvo, Donatella Caruso, Roberto C. Melcangi*

Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milano, Italy

*Corresponding author:

Roberto Cosimo Melcangi, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Italy, Via Balzaretti 9, 20133 Milano, Italy. Tel. +39-02-50318238; Fax: +39-02-50318202.

Email: roberto.melcangi@unimi.it

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. 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 these molecules 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 synthesis of neuroactive steroids 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.

Keywords: Selective serotonin reuptake inhibitors**,** neuroactive steroids**,** sexual dysfunction, depression, hippocampus, hypothalamus

Abbreviations:

3α-hydroxysteroid oxidoreductase: 3α-HSOR

3β-hydroxysteroid oxidoreductase: 3β-HSOR

5α-androstane-3α,17β-diol: 3α-diol

5α-reductase type 1: 5α-R1

5α-reductase type 2: 5α-R2

Acyl coa:cholesterol acyl transferase: ACAT

Cerebrospinal fluid: CSF

Cytochrome p450 side chain cleavage: p450scc

Dehydroepiandrosterone: DHEA

Dihydroprogesterone: DHP

Dihydrotestosterone: DHT

Follicle-stimulating hormone: FSH

Hormone sensitive lipase: HSL

Isoallopregnanolone: ISOALLO

Liquid chromatography tandem mass spectrometry analysis: LC-MS/MS

Luteinizing hormone: LH

Post-SSRI sexual dysfunction: PSSD

Pregnenolone: PREG

Pregnenolone sulfate: PREG-S

Progesterone: PROG

Selective serotonin reuptake inhibitors: SSRI

Serotonin-norepinephrine reuptake inhibitors: SNRI

Steroidogenic acute regulatory protein: star

Steroid sulfatase: STS

Sulfotransferase family 2B member 1: Sult2β1

Testosterone: T

Tetrahydroprogesterone: THP

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, occupational problems, and because it 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 & 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 contest, 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 & Shamloul 2007, Balon 1996). A recent Cochrane review supports their use to treat this condition (Sathianathen *et al.* 2021). In addition to the sexual problems observed during treatment, recently, the persistence of sexual dysfunction after stopping SSRI drugs has been increasingly reported (Bala *et al.* 2018, Patacchini & Cosci 2021, Patacchini & Cosci 2020, Rothmore 2020, Reisman 2017, Reisman 2020, Healy 2019, Healy 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 & Cosci 2021). The pathogenesis of antidepressant-induced sexual dysfunction and that of PSSD are largely unknown

(Segraves & Balon 2014), but a role for the serotoninergic 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 steroid has been hypothesized.

Neuroactive steroids are physiological modulators of brain functions (Melcangi *et al.* 2014, Giatti *et al.* 2019b, Giatti *et al.* 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, Giatti et al. 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, Giatti et al. 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 alpha-reductase, type 1 and type 2 (5α-R1 and 5α-R2) and subsequently by enzymes 3α- or 3β-hydroxysteroid oxidoreductase (3α/3β-HSOR) into tetrahydroprogesterone (THP), also known as allopregnanolone, and isoallopregnanolone respectively. DHEA is converted into androgens, such as testosterone (T), that it is then converted into dihydrotestosterone (DHT) and 5α-androstane-3α,17β-diol (3α-diol) by the enzymatic complex 5α-R/3α-HSOR or into 17β-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, Zorumski *et al.* 2013,

Locci & 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 & 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). 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.* 2017). 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 & 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 Matuszczyk *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, Oosting *et al.* 2016a, Angulo *et al.* 2001) male rats treated for two weeks with paroxetine at the dose of 10mg/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 & Chiesa 2009, Jing & 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 hours 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 chloroidrate (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-$^{13}C_2$-16,16 D_2 ($^{13}C_2$ D_2– PREG), 3β-hydroxy-5-pregnen-20-one 3-sulfate (PREG-S), progesterone (PROG), progesterone-2,3,4,20,25-$^{13}C_5$ ($^{13}C_5$ – PROG), 17β-Estradiol (17β-E), 17β-Estradiol-2,3,4-^{13}C3 ($^{13}C_3$-17β-E) 5α-pregnane-3, 20-dione (DHP), 3α-hydroxy-5α-pregnen-20 one (THP), 3β-hydroxy-5α-pregnen-20 one (Isoallopregnanolone), testosterone (T), 5α-androstane-17β-ol-3-one (DHT), 5α-androstane-3α17b-diol (3α-diol) and dehydroepiandrosterone (DHEA) were purchased from Merck LifeScience, Italy.

24(S)-hydroxycolesterol (24-OH-C) and 25-hydroxycolesterol (25-OH-C) were bought from Cayman Chemical, USA; 7α-hydroxycolesterol (7α),7β-hydroxycholesterol (7β) from Research Plus, USA; 10-hydroxycholesterol (19-OH-C), 7-keto-hydroxycolesterol (7-keto) and 27-hydroxycolesterol (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, 3α-diol, 17β-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 2,500 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). $^{13}C_3$-17β-E (2 ng/sample) $^{13}C_5$ – PROG (0.4 ng/sample) and $^{13}C_2$ 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 × 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-hydroxycholesterol (24OH-C), 25(S)-hydroxycholesterol (25OH-C), 27-hydroxycholesterol (27OH-C), 7α-cholesterol (7α), 7β-cholesterol (7β), 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 × 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 0,250 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, 5α-R1, 5α-R2, 3α-HSOR, HSL, ACAT1,

STS, SULT2β1. Specific TaqMan MGB probes (Applied Biosystems, Thermo Fisher Schientific) 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 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 ($p=0.0396$, $t=2.365$, $df=10$), isoallopregnanolone ($p=0.0221$, $t=2.830$, $df=8$) 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 (P450scc; Figure 1A), while the expression of the Steroidogenic Acute Regulatory protein (StAR), which helps the translocation of cholesterol into the mitochondria, was not affected (Figure 1A). Likewise, in agreement with the variation of THP levels (table 2), the expression of the 3α-hydroxysteroid oxidoreductase (3α-HSOR) was significantly increased (Figure 1A). Similarly, in the hypothalamus, the rise in 3α-diol levels is supported by a significant increase in the gene expression of 3α-HSOR (Figure 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; Figure 1C), but not of type 1 (5α-R1; Figure 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 (Figure 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

(Figure 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 (Figure 1C).

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 17β-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 3α-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 observed after subchronic treatment (table 2) was reported. In this brain area, the levels of 3α-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 Figure 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 (Figure 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 (Sult2β1) and retro-converted to free form by the enzyme steroid sulfatase (STS). As reported in Figure 2A, reduced expression of STS and increased expression of SULT2β1 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 3α-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 SULT2β1 (figure 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, figure 2B) as well as that involved in PREG production (i.e., CYP11A1 and StAR, figure 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 (figure 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, 7α-hydroxycholesterol and 7β-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 isoallopregnanolone 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 (figure 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 (figure 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 both 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 & Costa 1998, Pinna *et al.* 2006) and on the activity of 3α-HSOR (Griffin & 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 isoallopregnanolone 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 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, Romano *et al.* 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, Reisman 2020, Simonsen *et al.* 2016, Giatti et al. 2018, Ben-Sheetrit *et al.* 2015, Csoka *et al.* 2008, Csoka & Shipko 2006, Bahrick 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 serotoninergic/dopaminergic pathways controlling sexual behavior has been proposed (Giatti et al. 2018, Graf *et al.* 2014, Abler *et al.* 2011, Abler *et al.* 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, Giatti et al. 2019b, Melcangi *et al.* 2008, Melcangi et al. 2014). In particular, in reference to the neuroactive steroids here modulated, PREG-S acts at NMDA, AMPA and GABA-A receptor (Weng & Chung 2016, Locci & Pinna 2017), THP as well as 3α-diol are ligands of GABA-A receptor (Belelli & 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 release (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.

Author contributions

S.G.: conceptualized the experiments, performed animal and gene expression experiments, wrote the original draft and revised the text; S.D.: performed animal experiments and mass spectrometry analyses; L.C.: performed oxysterols analysis; E.F.: performed animal experiments; D.C.: supervised LC-MS/MS spectrometry; R.C.M.: general supervision; conceptualized the experiments, wrote the original draft and revised the text.

Declaration of competing interest

None

Acknowledgements

The authors acknowledge sig. F. Giavarini for his technical support.

Funding sources

This work was supported by the grants from MIUR Progetto Eccellenza, PON "Ricerca e Innovazione" PerMedNet -project ARS01_01226; we also thank the Italian Group of PSSD patients for the support.

Legends to figures

Figure 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 ± 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.

Figure 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 ± 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.

Reference

Abler, B., Gron, G., Hartmann, A., Metzger, C. and Walter, M., 2012. Modulation of frontostriatal interaction aligns with reduced primary reward processing under serotonergic drugs. *J Neurosci,* **32,** 1329-1335. DOI: 10.1523/JNEUROSCI.5826-11.2012

Abler, B., Seeringer, A., Hartmann, A., Gron, G., Metzger, C., Walter, M. and Stingl, J., 2011. Neural correlates of antidepressant-related sexual dysfunction: a placebo-controlled fMRI study on healthy males under subchronic paroxetine and bupropion. *Neuropsychopharmacology,* **36,** 1837-1847. DOI: 10.1038/npp.2011.66

Angulo, J., Peiro, C., Sanchez-Ferrer, C. F., Gabancho, S., Cuevas, P., Gupta, S. and Saenz de Tejada, I., 2001. Differential effects of serotonin reuptake inhibitors on erectile responses, NO-production, and neuronal NO synthase expression in rat corpus cavernosum tissue. *Br J Pharmacol,* **134,** 1190-1194. DOI: 10.1038/sj.bjp.0704351

Arafa, M. and Shamloul, R., 2007. A randomized study examining the effect of 3 SSRI on premature ejaculation using a validated questionnaire. *Ther Clin Risk Manag,* **3,** 527-531.

Backstrom, T., Wahlstrom, G., Wahlstrom, K., Zhu, D. and Wang, M. D., 2005. Isoallopregnanolone; an antagonist to the anaesthetic effect of allopregnanolone in male rats. *Eur J Pharmacol,* **512,** 15-21. DOI: 10.1016/j.ejphar.2005.01.049

Bahrick, A., 2008. Persistence of sexual dysfunction side effects after discontinuation of antidepressant medications: emerging evidence. *The open psychology journal,* **1,** 9.

Bala, A., Nguyen, H. M. T. and Hellstrom, W. J. G., 2018. Post-SSRI Sexual Dysfunction: A Literature Review. *Sexual medicine reviews,* **6,** 29-34. DOI: 10.1016/j.sxmr.2017.07.002

Balon, R., 1996. Antidepressants in the treatment of premature ejaculation. *Journal of sex & marital therapy,* **22,** 85-96. DOI: 10.1080/00926239608404912

Barbaccia, M. L., Serra, M., Purdy, R. H. and Biggio, G., 2001. Stress and neuroactive steroids. *Int Rev Neurobiol,* **46,** 243-272. DOI: 10.1016/s0074-7742(01)46065-x

Belelli, D. and Lambert, J. J., 2005. Neurosteroids: endogenous regulators of the GABA(A) receptor. *Nat Rev Neurosci,* **6,** 565-575. DOI: 10.1038/nrn1703

Ben-Sheetrit, J., Aizenberg, D., Csoka, A. B., Weizman, A. and Hermesh, H., 2015. Post-SSRI Sexual Dysfunction: Clinical Characterization and Preliminary Assessment of Contributory Factors and Dose-Response Relationship. *Journal of clinical psychopharmacology,* **35,** 273-278. DOI: 10.1097/JCP.0000000000000300

Birnbaum, H. G., Kessler, R. C., Kelley, D., Ben-Hamadi, R., Joish, V. N. and Greenberg, P. E., 2010. Employer burden of mild, moderate, and severe major depressive disorder: mental health services utilization and costs, and work performance. *Depress Anxiety,* **27,** 78-89. DOI: 10.1002/da.20580

Caruso, D., Scurati, S., Maschi, O., De Angelis, L., Roglio, I., Giatti, S., Garcia-Segura, L. M. and Melcangi, R. C., 2008. Evaluation of neuroactive steroid levels by liquid chromatography-tandem mass spectrometry in central and peripheral nervous system: effect of diabetes. *Neurochem Int,* **52,** 560-568. DOI: 10.1016/j.neuint.2007.06.004

Cermenati, G., Abbiati, F., Cermenati, S. et al., 2012. Diabetes-induced myelin abnormalities are associated with an altered lipid pattern: protective effects of LXR activation. *J Lipid Res,* **53,** 300-310. DOI: 10.1194/jlr.M021188

Clayton, A. H., Pradko, J. F., Croft, H. A. et al., 2002. Prevalence of sexual dysfunction among newer antidepressants. *J Clin Psychiatry,* **63,** 357-366. DOI: 10.4088/jcp.v63n0414

Corona, G., Isidori, A. M., Aversa, A., Burnett, A. L. and Maggi, M., 2016. Endocrinologic Control of Men's Sexual Desire and Arousal/Erection. *J Sex Med,* **13,** 317-337. DOI: 10.1016/j.jsxm.2016.01.007

Crawley, J. N., Glowa, J. R., Majewska, M. D. and Paul, S. M., 1986. Anxiolytic activity of an endogenous adrenal steroid. *Brain Res,* **398,** 382-385. DOI: 10.1016/0006-8993(86)91500-3

Csoka, A. B., Bahrick, A. and Mehtonen, O. P., 2008. Persistent sexual dysfunction after discontinuation of selective serotonin reuptake inhibitors. *J Sex Med,* **5,** 227-233. DOI: 10.1111/j.1743-6109.2007.00630.x

Csoka, A. B. and Shipko, S., 2006. Persistent sexual side effects after SSRI discontinuation. In: *Psychother Psychosom*, Vol. 75, pp. 187-188. Switzerland. DOI: 10.1159/000091777

de Jong, T. R., Pattij, T., Veening, J. G., Waldinger, M. D., Cools, A. R. and Olivier, B., 2005. Effects of chronic selective serotonin reuptake inhibitors on 8-OH-DPAT-induced facilitation of ejaculation in rats: comparison of fluvoxamine and paroxetine. *Psychopharmacology (Berl),* **179,** 509-515. DOI: 10.1007/s00213-005-2186-6

Dewan, M. J. and Anand, V. S., 1999. Evaluating the tolerability of the newer antidepressants. *J Nerv Ment Dis,* **187,** 96-101. DOI: 10.1097/00005053-199902000-00005

Disease, G. B. D., Injury, I. and Prevalence, C., 2018. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet,* **392,** 1789-1858. DOI: 10.1016/S0140-6736(18)32279-7

Fava, M., 2000. Weight gain and antidepressants. *J Clin Psychiatry,* **61 Suppl 11,** 37-41.

Giatti, S., Diviccaro, S., Falvo, E., Garcia-Segura, L. M. and Melcangi, R. C., 2020a. Physiopathological role of the enzymatic complex 5alpha-reductase and 3alpha/beta-hydroxysteroid oxidoreductase in the generation of progesterone and testosterone neuroactive metabolites. *Front Neuroendocrinol,* **57,** 100836. DOI: 10.1016/j.yfrne.2020.100836

Giatti, S., Diviccaro, S., Panzica, G. and Melcangi, R. C., 2018. Post-finasteride syndrome and post-SSRI sexual dysfunction: two sides of the same coin? *Endocrine,* **2,** 180-193. DOI: 10.1007/s12020-018-1593-5

Giatti, S., Diviccaro, S., Serafini, M. M., Caruso, D., Garcia-Segura, L. M., Viviani, B. and Melcangi, R. C., 2019a. Sex differences in steroid levels and steroidogenesis in the nervous system: Physiopathological role. *Front Neuroendocrinol***,** 100804. DOI: 10.1016/j.yfrne.2019.100804

Giatti, S., Garcia-Segura, L. M., Barreto, G. E. and Melcangi, R. C., 2019b. Neuroactive steroids, neurosteroidogenesis and sex. *Prog Neurobiol,* **176,** 1-17. DOI: 10.1016/j.pneurobio.2018.06.007

Giatti, S., Rigolio, R., Diviccaro, S., Falvo, E., Caruso, D., Garcia-Segura, L. M., Cavaletti, G. and Melcangi, R. C., 2020b. Sex dimorphism in an animal model of multiple sclerosis: Focus on pregnenolone synthesis. *J Steroid Biochem Mol Biol,* **199,** 105596. DOI: 10.1016/j.jsbmb.2020.105596

Gold, E. S., Diercks, A. H., Podolsky, I., Podyminogin, R. L., Askovich, P. S., Treuting, P. M. and Aderem, A., 2014. 25-Hydroxycholesterol acts as an amplifier of inflammatory signaling. *Proc Natl Acad Sci U S A,* **111,** 10666-10671. DOI: 10.1073/pnas.1404271111

Graf, H., Walter, M., Metzger, C. D. and Abler, B., 2014. Antidepressant-related sexual dysfunction - perspectives from neuroimaging. *Pharmacol Biochem Behav,* **121,** 138-145. DOI: 10.1016/j.pbb.2013.12.003

Greenberg, P. E., Kessler, R. C., Birnbaum, H. G., Leong, S. A., Lowe, S. W., Berglund, P. A. and Corey-Lisle, P. K., 2003. The economic burden of depression in the United States: how did it change between 1990 and 2000? *J Clin Psychiatry,* **64,** 1465-1475. DOI: 10.4088/jcp.v64n1211

Griffin, L. D. and Mellon, S. H., 1999. Selective serotonin reuptake inhibitors directly alter activity of neurosteroidogenic enzymes. *Proc Natl Acad Sci U S A,* **96,** 13512-13517.

Guidotti, A. and Costa, E., 1998. Can the antidysphoric and anxiolytic profiles of selective serotonin reuptake inhibitors be related to their ability to increase brain 3 alpha, 5 alpha-tetrahydroprogesterone (allopregnanolone) availability? *Biol Psychiatry,* **44,** 865-873. DOI: 10.1016/s0006-3223(98)00070-5

Guidotti, A., Dong, E., Matsumoto, K., Pinna, G., Rasmusson, A. M. and Costa, E., 2001. The socially-isolated mouse: a model to study the putative role of allopregnanolone and 5alpha-dihydroprogesterone in psychiatric disorders. *Brain Res Brain Res Rev,* **37,** 110-115. DOI: 10.1016/s0165-0173(01)00129-1

Hantsoo, L. and Epperson, C. N., 2020. Allopregnanolone in premenstrual dysphoric disorder (PMDD): Evidence for dysregulated sensitivity to GABA-A receptor modulating neuroactive steroids across the menstrual cycle. *Neurobiol Stress,* **12,** 100213. DOI: 10.1016/j.ynstr.2020.100213

Healy, D., 2019. Post-SSRI sexual dysfunction & other enduring sexual dysfunctions. *Epidemiol Psychiatr Sci,* **29,** e55. DOI: 10.1017/S2045796019000519

Healy, D., 2020. Antidepressants and sexual dysfunction: a history. *J R Soc Med,* **113,** 133-135. DOI: 10.1177/0141076819899299

Healy, D., Le Noury, J. and Mangin, D., 2018. Enduring sexual dysfunction after treatment with antidepressants, 5alpha-reductase inhibitors and isotretinoin: 300 cases. *The International journal of risk & safety in medicine,* **29,** 125-134. DOI: 10.3233/JRS-180744

Hirschfeld, R. M., 1998. Sexual dysfunction in depression: disease- or drug-related? *Depress Anxiety,* **7 Suppl 1,** 21-23. DOI: 10.1002/(sici)1520-6394(1998)7:1+<21::aid-da6>3.0.co;2-i

Hogan, C., Le Noury, J., Healy, D. and Mangin, D., 2014. One hundred and twenty cases of enduring sexual dysfunction following treatment. *The International journal of risk & safety in medicine,* **26,** 109-116. DOI: 10.3233/jrs-140617

Hosie, A. M., Wilkins, M. E., da Silva, H. M. and Smart, T. G., 2006. Endogenous neurosteroids regulate GABAA receptors through two discrete transmembrane sites. *Nature,* **444,** 486-489. DOI: 10.1038/nature05324

Hull, E. M., Wood, R. I. and McKenna, K. E., 2006. The neurobiology of male sexual behavior. In: *The Physiology of Reproduction*, (J. Neill and D. Pfaff eds.), pp. 1729-1824. Elsevier, Amsterdam.

Jing, E. and Straw-Wilson, K., 2016. Sexual dysfunction in selective serotonin reuptake inhibitors (SSRIs) and potential solutions: A narrative literature review. *Ment Health Clin,* **6,** 191-196. DOI: 10.9740/mhc.2016.07.191

Lambert, J. J., Cooper, M. A., Simmons, R. D., Weir, C. J. and Belelli, D., 2009. Neurosteroids: endogenous allosteric modulators of GABA(A) receptors. *Psychoneuroendocrinology,* **34 Suppl 1,** S48-58. DOI: 10.1016/j.psyneuen.2009.08.009

Leader, L. D., O'Connell, M. and VandenBerg, A., 2019. Brexanolone for Postpartum Depression: Clinical Evidence and Practical Considerations. *Pharmacotherapy,* **39,** 1105-1112. DOI: 10.1002/phar.2331

Lebedev, S. V., Blinov, D. V. and Petrov, S. V., 2004. Spatial characteristics of cisterna magna in rats and novel technique for puncture with a stereotactic manipulator. *Bull Exp Biol Med,* **137,** 635-638. DOI: 10.1023/b:bebm.0000042732.00810.01

Locci, A. and Pinna, G., 2017. Neurosteroid biosynthesis down-regulation and changes in GABAA receptor subunit composition: a biomarker axis in stress-induced cognitive and emotional impairment. *Br J Pharmacol,* **174,** 3226-3241. DOI: 10.1111/bph.13843

Maguire, J., 2019. Neuroactive Steroids and GABAergic Involvement in the Neuroendocrine Dysfunction Associated With Major Depressive Disorder and Postpartum Depression. *Front Cell Neurosci,* **13,** 83. DOI: 10.3389/fncel.2019.00083

Marasine, N. R., Sankhi, S., Lamichhane, R., Marasini, N. R. and Dangi, N. B., 2021. Use of Antidepressants among Patients Diagnosed with Depression: A Scoping Review. *Biomed Res Int,* **2021,** 6699028. DOI: 10.1155/2021/6699028

Marx, C. E., Shampine, L. J., Khisti, R. T. et al., 2006. Olanzapine and fluoxetine administration and coadministration increase rat hippocampal pregnenolone, allopregnanolone and peripheral deoxycorticosterone: implications for therapeutic actions. *Pharmacol Biochem Behav,* **84,** 609-617. DOI: 10.1016/j.pbb.2006.07.032

Mazzola, C. R. and Mulhall, J. P., 2012. Impact of androgen deprivation therapy on sexual function. *Asian J Androl,* **14,** 198-203. DOI: 10.1038/aja.2011.106

Melcangi, R. C., Garcia-Segura, L. M. and Mensah-Nyagan, A. G., 2008. Neuroactive steroids: state of the art and new perspectives. *Cell Mol Life Sci,* **65,** 777-797. DOI: 10.1007/s00018-007-7403-5

Melcangi, R. C., Giatti, S., Calabrese, D., Pesaresi, M., Cermenati, G., Mitro, N., Viviani, B., Garcia-Segura, L. M. and Caruso, D., 2014. Levels and actions of progesterone and its metabolites in the nervous system during physiological and pathological conditions. *Prog Neurobiol,* **113,** 56-69. DOI: 10.1016/j.pneurobio.2013.07.006

Melcangi, R. C., Giatti, S. and Garcia-Segura, L. M., 2016. Levels and actions of neuroactive steroids in the nervous system under physiological and pathological conditions: Sex-specific features. *Neurosci Biobehav Rev,* **67,** 25-40. DOI: 10.1016/j.neubiorev.2015.09.023

Meltzer-Brody, S., Colquhoun, H., Riesenberg, R. et al., 2018. Brexanolone injection in post-partum depression: two multicentre, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet,* **392,** 1058-1070. DOI: 10.1016/S0140-6736(18)31551-4

Miret, M., Ayuso-Mateos, J. L., Sanchez-Moreno, J. and Vieta, E., 2013. Depressive disorders and suicide: Epidemiology, risk factors, and burden. *Neurosci Biobehav Rev,* **37,** 2372-2374. DOI: 10.1016/j.neubiorev.2013.01.008

Morrison, K. E., Cole, A. B., Thompson, S. M. and Bale, T. L., 2019. Brexanolone for the treatment of patients with postpartum depression. *Drugs Today (Barc),* **55,** 537-544. DOI: 10.1358/dot.2019.55.9.3040864

Olkkonen, V. M., Beaslas, O. and Nissila, E., 2012. Oxysterols and their cellular effectors. *Biomolecules,* **2,** 76-103. DOI: 10.3390/biom2010076

Oosting, R. S., Chan, J. S., Olivier, B., Banerjee, P., Choi, Y. K. and Tarazi, F., 2016a. Differential effects of vilazodone versus citalopram and paroxetine on sexual behaviors and serotonin transporter and receptors in male rats. *Psychopharmacology (Berl),* **233,** 1025-1034. DOI: 10.1007/s00213-015-4198-1

Oosting, R. S., Chan, J. S. W., Olivier, B. and Banerjee, P., 2016b. Vilazodone does not inhibit sexual behavior in male rats in contrast to paroxetine: A role for 5-HT1A receptors? *Neuropharmacology,* **107,** 271-277. DOI: 10.1016/j.neuropharm.2016.03.045

Patacchini, A. and Cosci, F., 2020. A Paradigmatic Case of Postselective Serotonin Reuptake Inhibitors Sexual Dysfunction or Withdrawal After Discontinuation of Selective Serotonin Reuptake Inhibitors? *Journal of clinical psychopharmacology,* **40,** 93-95. DOI: 10.1097/JCP.0000000000001154

Patacchini, A. and Cosci, F., 2021. Exposure to serotonin selective reuptake inhibitors or serotonin noradrenaline reuptake inhibitors and sexual dysfunction: Results from an online survey. *The International journal of risk & safety in medicine*. DOI: 10.3233/JRS-200074

Pesaresi, M., Maschi, O., Giatti, S., Garcia-Segura, L. M., Caruso, D. and Melcangi, R. C., 2010. Sex differences in neuroactive steroid levels in the nervous system of diabetic and non-diabetic rats. *Horm Behav,* **57,** 46-55. DOI: 10.1016/j.yhbeh.2009.04.008

Piazza, L. A., Markowitz, J. C., Kocsis, J. H., Leon, A. C., Portera, L., Miller, N. L. and Adler, D., 1997. Sexual functioning in chronically depressed patients treated with SSRI antidepressants: a pilot study. *Am J Psychiatry,* **154,** 1757-1759. DOI: 10.1176/ajp.154.12.1757

Pinna, G., Costa, E. and Guidotti, A., 2006. Fluoxetine and norfluoxetine stereospecifically and selectively increase brain neurosteroid content at doses that are inactive on 5-HT reuptake. *Psychopharmacology (Berl),* **186,** 362-372. DOI: 10.1007/s00213-005-0213-2

Pitsillou, E., Bresnehan, S. M., Kagarakis, E. A., Wijoyo, S. J., Liang, J., Hung, A. and Karagiannis, T. C., 2020. The cellular and molecular basis of major depressive disorder: towards a unified model

for understanding clinical depression. *Mol Biol Rep,* **47,** 753-770. DOI: 10.1007/s11033-019-05129-3

Poli, G., Biasi, F. and Leonarduzzi, G., 2013. Oxysterols in the pathogenesis of major chronic diseases. *Redox Biol,* **1,** 125-130. DOI: 10.1016/j.redox.2012.12.001

Purdy, R. H., Morrow, A. L., Moore, P. H., Jr. and Paul, S. M., 1991. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. *Proc Natl Acad Sci U S A,* **88,** 4553-4557.

Reddy, D. S., Kaur, G. and Kulkarni, S. K., 1998. Sigma (sigma1) receptor mediated anti-depressant-like effects of neurosteroids in the Porsolt forced swim test. *Neuroreport,* **9,** 3069-3073. DOI: 10.1097/00001756-199809140-00028

Reisman, Y., 2017. Sexual Consequences of Post-SSRI Syndrome. *Sexual medicine reviews*. DOI: 10.1016/j.sxmr.2017.05.002

Reisman, Y., 2020. Post-SSRI sexual dysfunction. *BMJ,* **368,** m754. DOI: 10.1136/bmj.m754

Romano, S., Mitro, N., Diviccaro, S., Spezzano, R., Audano, M., Garcia-Segura, L. M., Caruso, D. and Melcangi, R. C., 2017. Short-term effects of diabetes on neurosteroidogenesis in the rat hippocampus. *J Steroid Biochem Mol Biol,* **167,** 135-143. DOI: 10.1016/j.jsbmb.2016.11.019

Romano, S., Mitro, N., Giatti, S. et al., 2018. Diabetes induces mitochondrial dysfunction and alters cholesterol homeostasis and neurosteroidogenesis in the rat cerebral cortex. *J Steroid Biochem Mol Biol,* **178,** 108-116. DOI: 10.1016/j.jsbmb.2017.11.009

Romeo, E., Strohle, A., Spalletta, G., di Michele, F., Hermann, B., Holsboer, F., Pasini, A. and Rupprecht, R., 1998. Effects of antidepressant treatment on neuroactive steroids in major depression. *Am J Psychiatry,* **155,** 910-913.

Rosen, R. C., Lane, R. M. and Menza, M., 1999. Effects of SSRIs on sexual function: a critical review. *Journal of clinical psychopharmacology,* **19,** 67-85.

Rothmore, J., 2020. Antidepressant-induced sexual dysfunction. *Med J Aust,* **212,** 329-334. DOI: 10.5694/mja2.50522

Safarinejad, M. R., 2008. Evaluation of endocrine profile and hypothalamic-pituitary-testis axis in selective serotonin reuptake inhibitor-induced male sexual dysfunction. *Journal of clinical psychopharmacology,* **28,** 418-423. DOI: 10.1097/JCP.0b013e31817e6f80

Santi, D., Spaggiari, G., Gilioli, L., Poti, F., Simoni, M. and Casarini, L., 2018. Molecular basis of androgen action on human sexual desire. *Mol Cell Endocrinol*, **467**, 31-41. DOI: 10.1016/j.mce.2017.09.007

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Sathianathen, N. J., Hwang, E. C., Mian, R., Bodie, J. A., Soubra, A., Lyon, J. A., Sultan, S. and Dahm, P., 2021. Selective serotonin re-uptake inhibitors for premature ejaculation in adult men. *Cochrane Database Syst Rev,* **3,** CD012799. DOI: 10.1002/14651858.CD012799.pub2

Segraves, R. T. and Balon, R., 2014. Antidepressant-induced sexual dysfunction in men. *Pharmacol Biochem Behav,* **121,** 132-137. DOI: 10.1016/j.pbb.2013.11.003

Serretti, A. and Chiesa, A., 2009. Treatment-emergent sexual dysfunction related to antidepressants: a meta-analysis. *Journal of clinical psychopharmacology,* **29,** 259-266. DOI: 10.1097/JCP.0b013e3181a5233f

Simonsen, A. L., Danborg, P. B. and Gotzsche, P. C., 2016. Persistent sexual dysfunction after early exposure to SSRIs: Systematic review of animal studies. *The International journal of risk & safety in medicine,* **28,** 1-12. DOI: 10.3233/JRS-160668

Uzunova, V., Sampson, L. and Uzunov, D. P., 2006. Relevance of endogenous 3alpha-reduced neurosteroids to depression and antidepressant action. *Psychopharmacology (Berl),* **186,** 351-361. DOI: 10.1007/s00213-005-0201-6

Uzunova, V., Sheline, Y., Davis, J. M., Rasmusson, A., Uzunov, D. P., Costa, E. and Guidotti, A., 1998. Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. *Proc Natl Acad Sci U S A,* **95,** 3239-3244.

Uzunova, V., Wrynn, A. S., Kinnunen, A., Ceci, M., Kohler, C. and Uzunov, D. P., 2004. Chronic antidepressants reverse cerebrocortical allopregnanolone decline in the olfactory-bulbectomized rat. *Eur J Pharmacol,* **486,** 31-34. DOI: 10.1016/j.ejphar.2003.12.002

Vega Matuszcyk, J., Larsson, K. and Eriksson, E., 1998. The selective serotonin reuptake inhibitor fluoxetine reduces sexual motivation in male rats. *Pharmacol Biochem Behav,* **60,** 527-532. DOI: 10.1016/s0091-3057(98)00010-0

Waldinger, M. D., van Coevorden, R. S., Schweitzer, D. H. and Georgiadis, J., 2015. Penile anesthesia in Post SSRI Sexual Dysfunction (PSSD) responds to low-power laser irradiation: a case study and hypothesis about the role of transient receptor potential (TRP) ion channels. *Eur J Pharmacol,* **753,** 263-268. DOI: 10.1016/j.ejphar.2014.11.031

Waldinger, M. D., van De Plas, A., Pattij, T., van Oorschot, R., Coolen, L. M., Veening, J. G. and Olivier, B., 2002. The selective serotonin re-uptake inhibitors fluvoxamine and paroxetine differ in sexual inhibitory effects after chronic treatment. *Psychopharmacology (Berl),* **160,** 283-289. DOI: 10.1007/s00213-001-0980-3

Wang, M., He, Y., Eisenman, L. N. et al., 2002. 3beta -hydroxypregnane steroids are pregnenolone sulfate-like GABA(A) receptor antagonists. *J Neurosci,* **22,** 3366-3375. DOI: 10.1523/JNEUROSCI.22-09-03366.2002

Weng, J. H. and Chung, B. C., 2016. Nongenomic actions of neurosteroid pregnenolone and its metabolites. *Steroids,* **111,** 54-59. DOI: 10.1016/j.steroids.2016.01.017

Zheng, P., 2009. Neuroactive steroid regulation of neurotransmitter release in the CNS: action, mechanism and possible significance. *Prog Neurobiol,* **89,** 134-152. DOI: 10.1016/j.pneurobio.2009.07.001

Zheng, W., Cai, D. B., Zheng, W., Sim, K., Ungvari, G. S., Peng, X. J., Ning, Y. P., Wang, G. and Xiang, Y. T., 2019. Brexanolone for postpartum depression: A meta-analysis of randomized controlled studies. *Psychiatry Res,* **279,** 83-89. DOI: 10.1016/j.psychres.2019.07.006

Zorumski, C. F., Paul, S. M., Covey, D. F. and Mennerick, S., 2019. Neurosteroids as novel antidepressants and anxiolytics: GABA-A receptors and beyond. *Neurobiol Stress,* **11,** 100196. DOI: 10.1016/j.ynstr.2019.100196

Zorumski, C. F., Paul, S. M., Izumi, Y., Covey, D. F. and Mennerick, S., 2013. Neurosteroids, stress and depression: Potential therapeutic opportunities. *Neurosci Biobehav Rev,* **37,** 109-122. DOI: 10.1016/j.neubiorev.2012.10.005

Click here to access/download;Figure;Figure 1 bis.tif

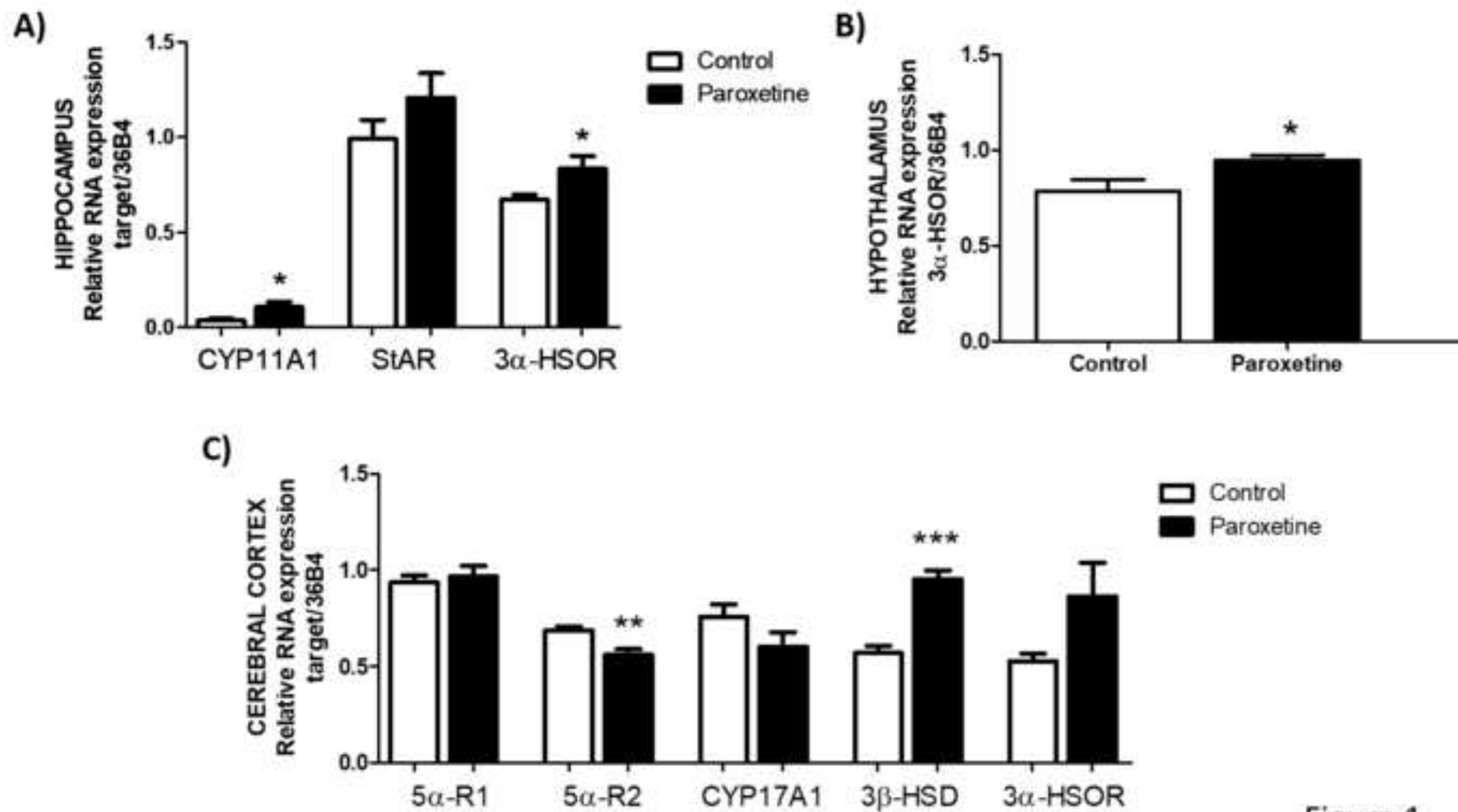

Figure 2

Click here to access/download;Figure;Figure 2 bis.tif

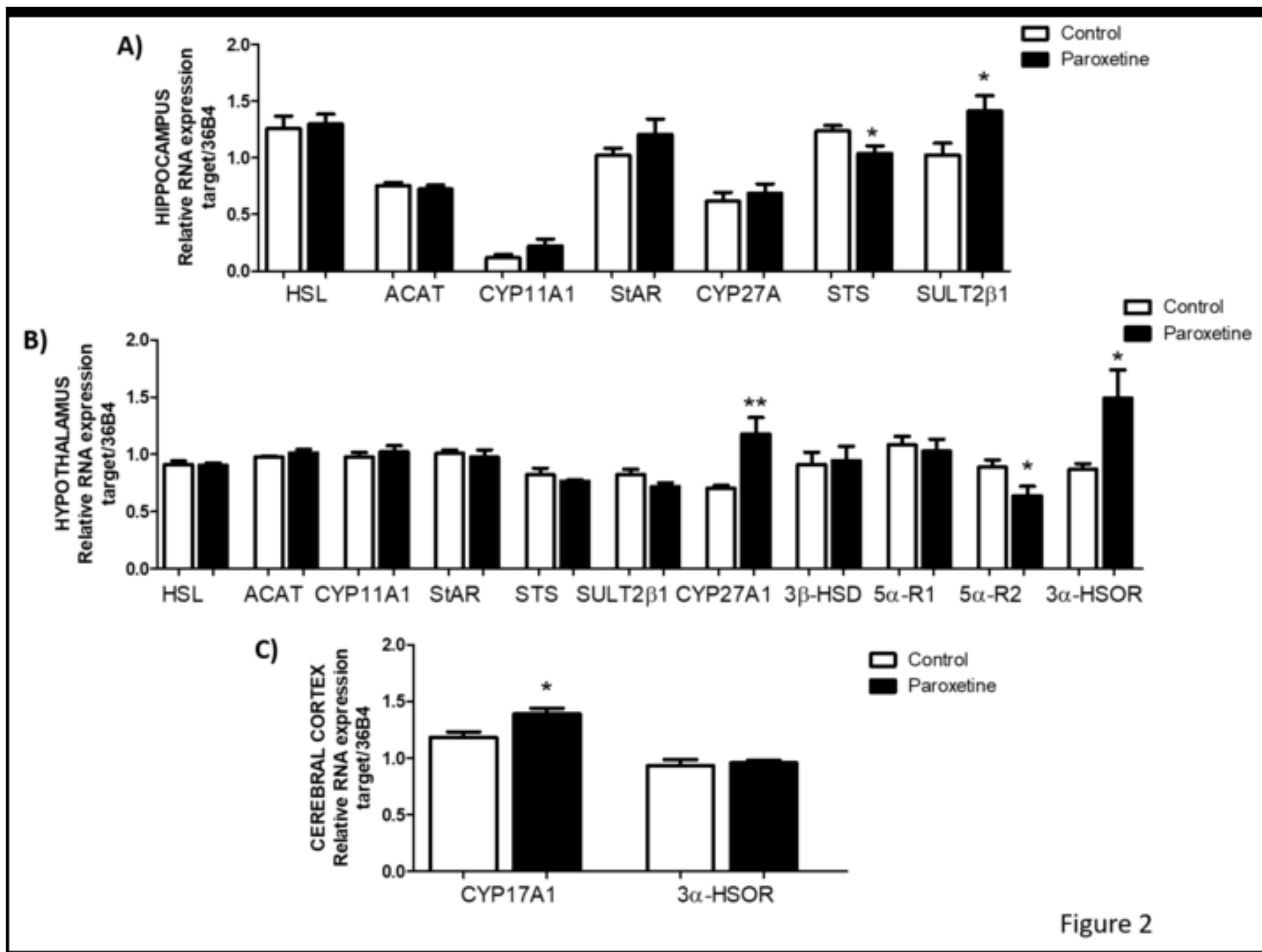

Figure 2

 Click here to access/download;Table;Table 1.docx

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.

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)	0.24±0.01 (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)
3A-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)
17B-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 isoallopregnanolone (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.

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		HYPOTALAMUS		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)	0.09±0.01 (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)	0.15±0.06 (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)
3A-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)

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 isoallopregnanolone (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.

 Click here to access/download;Table;Table 4.docx

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±1.67	23.46±0.87 *
25-hydroxycholesterol (ng/mg tissue)	0.16±0.02	0.19±0.01 *
27-hydroxycholesterol (ng/mg tissue)	0.35±0.04	0.48±0.03 *
7α-hydroxycholesterol (ng/mg tissue)	0.11±0.01	0.17±0.02 *
7β-hydroxycholesterol (ng/mg tissue)	0.04±0.00	0.05±0.01 *
7-ketocholesterol (ng/mg tissue)	0.17±0.01	0.20±0.01

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

Declaration of competing interest

None

Psychoneuroendocrinology Submission Checklist

By submitting the enclosed manuscript to Psychoneuroendocrinology the authors attest the following:

- The authors have read through the guidelines described in the Guide for Authors for the preparation of the manuscript and the report of their results

- The standard reporting guidelines appropriate to the studies reported to the manuscript have been followed

- All precautions have been taken to ensure that the studies described in the manuscript are not underpowered and appropriate power analyses have been conducted

- Guidelines for specific types of studies have been followed

- Care has been taken to ensure the manuscript is written in clear scientific English

Done in Milano on June 19, 2021

Signature of all authors (electronic signature is permitted):

All the authors belong to the Dipartimento di Science Farmacologiche e Biomolecolari, Università degli studi di Milano, Italy.

Silvia Giatti (silvia.giatti@unimi.it), Silvia Diviccaro (silvia.diviccaro@unimi.it), Lucia Cioffi (lucia.Cioffi@unimi.it), Eva Falvo (eva.falvo@unimi.it), Donatella Caruso (donatella.caruso@unimi.it)

ON THE BEHALF OF ALL THE AUTHORS
Roberto Cosimo Melcangi (corresponding authors)
roberto.melcangi@unimi.it

MELCANGI
COSIMO
Universita'
degli Studi
di Milano
19.06.2021
07:47:26
GMT+00:00

EFFECTS OF PAROXETINE TREATMENT AND ITS WITHDRAWAL ON NEUROSTEROIDOGENESIS

Silvia Giatti, Silvia Diviccaro, Lucia Cioffi, Eva Falvo, Donatella Caruso, Roberto C. Melcangi*

Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milano, Italy

***Corresponding author:**

Roberto Cosimo Melcangi, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Italy, Via Balzaretti 9, 20133 Milano, Italy. Tel. +39-02-50318238; Fax: +39-02-50318202.

Email: roberto.melcangi@unimi.it