Neuropharmacology 103 (2016) 16–26

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Cannabidiol induces rapid-acting antidepressant-like effects and enhances cortical 5-HT/glutamate neurotransmission: role of 5-HT_{1A} receptors

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ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form
15 December 2015
Accepted 16 December 2015
Available online 19 December 2015

Chemical compounds:
(−)Cannabidiol (PubChem CID: 12302390)
WAY-100635 maleate salt (PubChem CID: 11957721)
AM251 (PubChem CID: 2125)

Keywords:
Cannabidiol
Antidepressant
Glutamate
Serotonin
Olfactory bulbectomy
5-HT_{1A} receptor

ABSTRACT

Cannabidiol (CBD), the main non-psychotomimetic component of marihuana, exhibits anxiolytic-like properties in many behavioural tests, although its potential for treating major depression has been poorly explored. Moreover, the mechanism of action of CBD remains unclear. Herein, we have evaluated the effects of CBD following acute and chronic administration in the olfactory bulbectomy mouse model of depression (OBX), and investigated the underlying mechanism. For this purpose, we conducted behavioural (open field and sucrose preference tests) and neurochemical (microdialysis and autoradiography of 5-HT_{1A} receptor functionality) studies following treatment with CBD. We also assayed the pharmacological antagonism of the effects of CBD to dissect out the mechanism of action. Our results demonstrate that CBD exerts fast and maintained antidepressant-like effects as evidenced by the reversal of the OBX-induced hyperactivity and anhedonia. *In vivo* microdialysis revealed that the administration of CBD significantly enhanced serotonin and glutamate levels in vmPFCx in a different manner depending on the emotional state and the duration of the treatment. The potentiating effect upon neurotransmitters levels occurring immediately after the first injection of CBD might underlie the fast antidepressant-like actions in OBX mice. Both antidepressant-like effect and enhanced cortical 5-HT/glutamate neurotransmission induced by CBD were prevented by 5-HT_{1A} receptor blockade. Moreover, adaptive changes in pre- and post-synaptic 5-HT_{1A} receptor functionality were also found after chronic CBD. In conclusion, our findings indicate that CBD could represent a novel fast antidepressant drug, via enhancing both serotonergic and glutamate cortical signalling through a 5-HT_{1A} receptor-dependent mechanism.

1. Introduction

Cannabinoid compounds have been used by different cultures to improve mood since ancient times. For this reason, the study of the endocannabinoid system and cannabinoid derivatives has gained a great interest in anxiety/depression research (Bambico et al., 2007; Hill and Gorzalka, 2005; McLaughlin et al., 2007; Shearman et al., 2003).

In this regard, cannabidiol (CBD), the main non-psychotomimetic component of marijuana (Mechoulam et al., 2002), has shown anxiolytic properties both in humans and rodents (Bergamaschi et al., 2011; Guimaraes et al., 1990) after acute

Abbreviations: Cannabidiol, CBD; ventromedial prefrontal cortex, vmPFCx; olfactory bulbectomy, OBX; selective serotonin reuptake inhibitors, SSRIs.

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http://dx.doi.org/10.1016/j.neuropharm.2015.12.017
0028-3908/

or chronic administration (Campos and Guimaraes, 2008; Campos et al., 2013b; Resstel et al., 2009). Nevertheless, little is known about its potential for treating depression. It was proposed as a putative novel antidepressant as it displayed positive responses in the forced swimming test (FST) (El-Alfy et al., 2010; Zanelati et al., 2010) and also in the novelty suppressed feeding test (NSF) under chronic stress conditions (Campos et al., 2013b). Furthermore, CBD exerts a positive impact on some neuroplasticity markers of antidepressant effects, such as increased brain-derived neurotrophic factor levels (Magen et al., 2010). It also restores the impaired neuroproliferation of chronically stressed animals (Campos et al., 2013b), and presents anti-inflammatory and immunomodulatory effects (Esposito et al., 2011; Malfait et al., 2000).

The mechanism of action of CBD has been extensively scrutinized (McPartland et al., 2015). This multifaceted drug produces different pharmacological actions modulating several receptors in the central nervous system (CNS) (CB_1, CB_2, 5-HT_{1A}, TRPV1 and PPARγ receptors, among others) (Campos et al., 2013a, 2013b; Casarotto et al., 2010; Costa et al., 2004; Do Monte et al., 2013; Esposito et al., 2011; Pazos et al., 2013; Soares Vde et al., 2010; Thomas et al., 2007). Given the crosstalk among systems involved in mood control, the ability of CBD to modulate some of them, could result advantageous for the treatment of complex diseases like depression. Among all the above highlighted mechanisms, the CB_1 and 5-HT_{1A} receptors seem to be the most strongly implicated in the regulatory effects of CBD upon mood. CBD has been reported to act as an antagonist/inverse agonist of CB_1 receptors (Thomas et al., 2007) and also to increase anandamide (AEA) levels (Bisogno et al., 2001; Leweke et al., 2012). It also exerts a positive allosteric modulation of 5-HT_{1A} receptors rather than a direct agonism (Rock et al., 2012), a fact that could explain the unexpected key role of these serotonergic receptors in many of the effects of CBD. Pharmacological approaches with selective receptor antagonists showed that the acute anxiolytic-like and panicolytic-like properties of CBD are predominantly mediated by 5-HT_{1A} receptors (Campos et al., 2013a; Campos and Guimaraes, 2008; Resstel et al., 2009; Soares Vde et al., 2010; Zanelati et al., 2010), whereas the anxiolytic-like effects induced by its chronic administration involving neurogenic actions, seem to be CB_1-receptor dependent (Campos et al., 2013b).

Classical antidepressants act through serotonergic potentiation whereas the effects of fast-acting agents seem to be mediated by glutamatergic signalling (Du et al., 2006). However, there is scarce knowledge about the impact of CBD's administration on serotonergic and glutamatergic pathways. In this regard, 5-HT_{1A} receptor is expressed within dorsal raphe nucleus (DRN) on 5-HTergic neurons and local GABAergic interneurons, where it controls 5-HT neuronal firing (Celada et al., 2001). They are also located in cortical interneurons and pyramidal cells modulating neurotransmitters efflux (Santana et al., 2004). In addition, endocannabinoid system is also strongly implicated in the control of 5-HT and glutamate release at different locations (Bambico et al., 2007; Bisogno et al., 2001; Brown et al., 2003; McLaughlin et al., 2012; Mendiguren and Pineda, 2009; Navarrete and Araque, 2008). Thus, the study of CBD's effects upon these pattern of neurotransmitter release would shed light on the mechanistic basis of the behavioural actions of CBD.

Herein, we have evaluated the behavioural and neurochemical actions of CBD in the olfactory bulbectomy mouse model of depression (OBX) (Linge et al., 2013), since its antidepressant efficacy under pathological conditions has not been investigated yet. Firstly, we assayed the behavioural effects induced by acute and chronic administration of CBD, and in parallel we performed microdialysis studies to assess the effects of CBD on the serotonin (5-HT) and glutamate dialysate output in the ventromedial prefrontal cortex (vmPFCx), a pivotal area for the behavioural outcome depending on the emotional status. In addition, the 5-HT_{1A} receptor functionality ([^{35}S]GTPγS autoradiography) following chronic administration of CBD was analysed in different brain areas, given their role in the mechanism of action of antidepressants. Finally, pharmacological antagonism studies were performed to determine the receptor implicated in the behavioural and neurochemical actions of CBD.

2. Material and methods

2.1. Animals and OBX surgery

Experiments were conducted with 3 month old male C57BL6 mice weighing 25–30 g. All procedures were carried out with the previous approval of the Animal Care Committee of the University of Cantabria and according to the Spanish legislation (RD 53/2013) and the European Communities Council Directive (2010/63/UE) on "Protection of Animals Used in Experimental and Other Scientific Purposes. All efforts were made to minimise animal suffering, to reduce the number of animals used, and to utilise alternatives to *in vivo* techniques, if available. Animals were individually caged all throughout the study, housed in climate controlled rooms with 12 h light–12 h dark cycle, and provided with food and water *ad libitum*.

The OBX and sham-operation (SHAM) procedure was performed as previously described (Linge et al., 2013). The following experimental groups were designed: vehicle-treated sham (SHAM VEH), CBD-treated sham (SHAM CBD), vehicle-treated OBX (OBX VEH), and CBD-treated OBX (OBX CBD). The initial group size was n = 10 for behavioural studies, n = 8 for microdialysis studies, and n = 8 for antagonist studies. After a 4-week recovery period, treatments and experiments were performed using different sets of animals for each procedure (see Experimental schedule in Supplementary experimental procedures). The final animal number used in each experiment is indicated in Section 3 (see Figure legends).

2.2. Drugs and treatments

(−)Cannabidiol, WAY100635 (WAY) and AM251 (AM) were dissolved in vehicle (VEH) (2% Tween 80®: 5% Propilenglycol®: saline). All the drugs were purchased from TOCRIS (Bristol, United Kingdom).

Acute and chronic administration studies were conducted to investigate the behavioural (open field and sucrose preference tests) and neurochemical (microdialysis studies and 5-HT_{1A} receptor autoradiography) effects induced by CBD (see Experimental schedule in Supplementary experimental procedures). In the acute studies, the effect of CBD alone (50 mg/kg) or in combination with antagonists was assessed in the open field test and microdialysis studies (30 min post-administration; i.p.). Antagonists doses were those normally reported in the literature and devoid of any effect by themselves on the parameters analysed in the open field (WAY 0.3 mg/kg, AM251 0.3 mg/kg). In the chronic treatment, CBD was administered for 14 days following a drug regime (50 mg/kg/day for 3 days + 10 mg/kg/day until the end of treatment; i.p.) that was selected after preliminary assays. After 2 weeks of treatment with CBD, animals were sacrificed and brain samples collected and stored at −80 °C for the autoradiographic studies.

2.3. Behavioural testing (supplementary experimental procedures)

Open field test (OFT): we evaluated the effects of CBD on the OBX-induced hyperactivity (to assess antidepressant-like effects) and central ambulation (to assess anxiolytic-like effects) (Linge et al., 2013). Both the acute effects (30 min post-injection) and

persistent actions (24 h post-injection after 1, 3, 7 and 14 days of treatment in the same animal) of CBD were evaluated.

Sucrose preference test: OBX-induced anhedonia was assessed in the sucrose preference test to check depressive-like behaviour and antidepressant-like actions of CBD. A choice of sucrose (1%) and water solutions were provided in the home cage and the consumption was quantified (Linge et al., 2013).

2.4. Microdialysis studies

Concentric dialysis probes were implanted in the vmPFCx. Microdialysis experiments were conducted 24 h after surgery in freely moving mice by continuously perfusing probes. After a 180 min stabilization period, six 20-min fractions were collected to obtain basal values and another six samples after the i.p. administration of drugs. 5-HT and glutamate were determined by high-performance liquid chromatography (HPLC) (Supplementary experimental procedures).

At the completion of the experiments, mice were sacrificed and brain tissue was processed according to standard histological procedures (cresyl violet staining) to verify the correct placement of dialysis probe. Mice with incorrect probe placement were discarded (<10%) (see Supplementary experimental procedures; Fig. S1).

2.5. [^{35}S]GTPγS autoradiography of 5-HT$_{1A}$ receptor functionality

[^{35}S]GTPγS autoradiography in coronal brain sections was carried out as previously described (Sim et al., 1995), using 10 μM (±)8-OH-DPAT for stimulated condition. Autoradiographic values of net agonist-stimulated [^{35}S]GTPγS binding were calculated by subtracting basal binding from agonist-stimulated binding. Data are expressed as percentage of agonist-stimulated binding over basal activity (100%) (Supplementary experimental procedures).

2.6. Data analysis

All the values are expressed as mean ± standard error of mean (S.E.M). For the behavioural and autoradiographic studies, the data were statistically analysed by one/two-way ANOVA (surgery and treatment as main factors), and for the microdialysis studies by three-way repeated measures ANOVA with surgery, treatment and time as main factors. A *Student-Newman-Keuls* test was applied for the *post-hoc* analysis. GraphPad Prism 5.01 (San Diego, CA, USA) and Statistica 8 (Statsoft, Inc., Tulsa, USA) were used for the statistical analysis. A p value < 0.05 was considered significant.

3. Results

Different regimes of administration were initially assayed to choose the most appropriate. The first dose of CBD evaluated (10 mg/kg/day, i.p.) produced a significant reversal of OBX-induced hyperactivity after 2 weeks of treatment (Supplementary Fig. S1). As shown later (Section 3.1.2; Fig. 2A), a higher dose of CBD (50 mg/kg/day, i.p.) induced a sustained hyperactivity reversal from the first injection but it significantly decreased sucrose consumption in half of sham animals, and a trend was observed in OBX mice (Supplementary Fig. S2a). This behavioural outcome was accompanied by a reduction in food intake (Supplementary Fig. S2b) and body weight (Supplementary Fig. S2c), reflecting an anorectic effect of CBD that would explain the decrease in sucrose preference. Therefore, a regime combining both doses (50 mg/kg for 3 days + 10 mg/kg until the end of treatment) was chosen as appropriate for achieving fast onset of antidepressant-like actions, avoiding the interference of the anorectic actions.

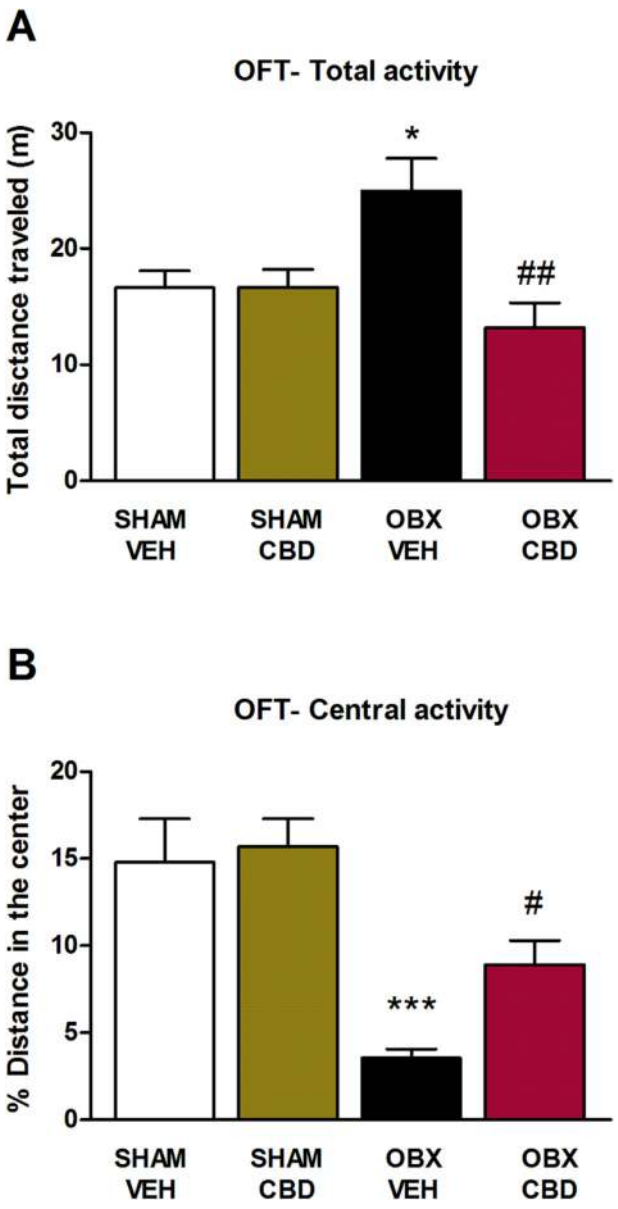

Fig. 1. Acute effects of CBD in the open field test. Acute CBD (50 mg/k; i.p.) significantly reversed both OBX-induced hyperactivity (A) and decreased central ambulation (B) 30 min *post*-injection and it was devoid of any behavioural effect in sham counterparts. Data represented as mean ± SEM, n = 6–7 mice per experimental group ($^{*}p < 0.05$ and $^{***}p < 0.001$ *vs.* SHAM VEH; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* OBX VEH).

3.1. CBD induces immediate and maintained antidepressant-like effects in OBX mice

3.1.1. Acute antidepressant-like and anxiolytic-like effects of CBD in the open-field test

The acute effect of CBD (50 mg/kg; 30 min post-injection) was evaluated in the open field test. Regarding total distance travelled, a two-way ANOVA analysis revealed a significant interaction between surgery and treatment [$F(1,22) = 6.80$, $p < 0.05$]. *Post-hoc* comparison indicated that CBD significantly reversed OBX-induced hyperactivity ($p < 0.01$; Fig. 1A). Acute CBD also increased central ambulation of OBX mice, as reflected by the higher percentage of distance travelled in the central zone ($p < 0.05$; Fig. 1B). CBD did not alter locomotor activity or central ambulation in sham animals.

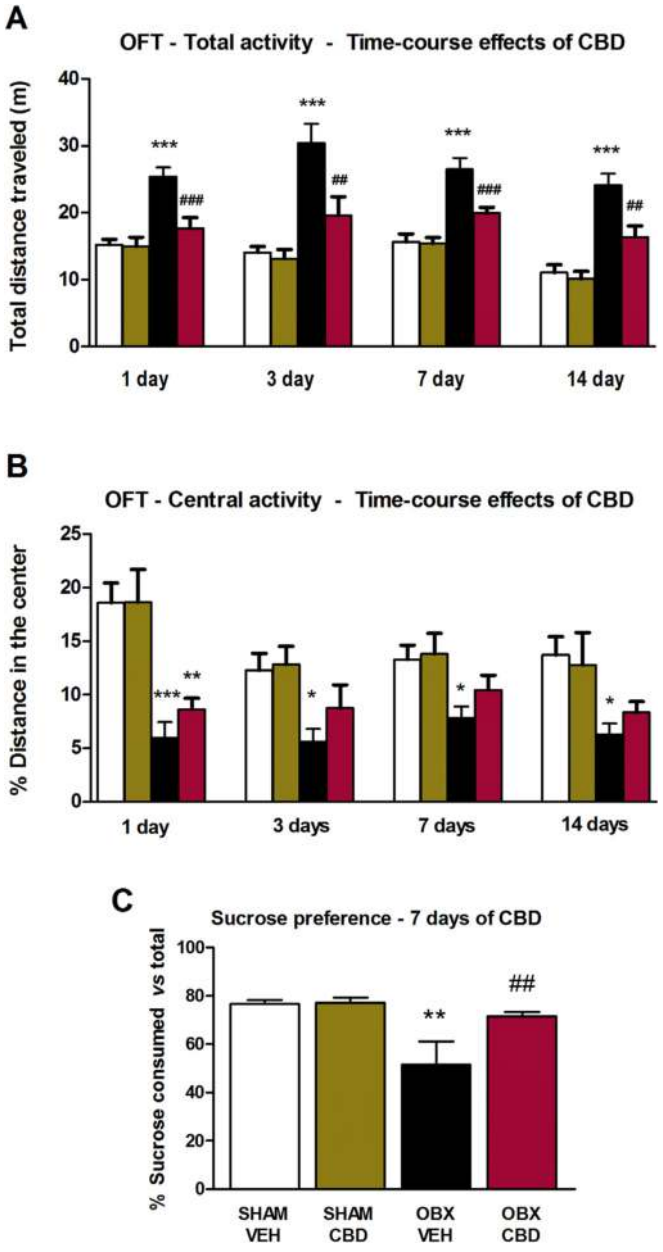

Fig. 2. Time-course effects of CBD administration in the *open field* and *sucrose preference tests*. The effect of CBD upon the OBX-induced hyperactivity (A) and decreased central activity (B) was evidenced throughout the treatment assessed 24 h post 1, 3, 7 and 14 days of drug administration. Additionally, chronic CBD reversed OBX-induced anhedonia following 7 days of administration (C). Data represented as mean ± SEM of n = 7–9 mice per experimental group ($^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs.* SHAM VEH; $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ *vs.* OBX VEH).

3.1.2. Time-course antidepressant-like effects of CBD administration

3.1.2.1. Open field test. As shown in Fig. 2A, the reversal of OBX-hyperactivity by CBD was still present 24 h after the first injection (OBX VEH *vs.* OBX CBD, $p < 0.001$). Moreover, this hyperactivity attenuation was still measured throughout the treatment with CBD when assessed 24 h post 3 ($p < 0.01$), 7 ($p < 0.001$) and 14 ($p < 0.01$) days of drug administration. Two-way ANOVA analysis revealed a significant interaction between surgery and treatment ([$F(1,29) = 7.48$, $p < 0.05$] for day 1; [$F(1,25) = 5.20$, $p < 0.05$] for day 3; [$F(1,25) = 7.29$, $p < 0.05$] for day 7; and [$F(1,25) = 5.68$, $p < 0.05$] for day 14). Interestingly, CBD did not alter sham animals locomotor activity.

Regarding central activity, the anxiolytic-like effect of CBD observed immediately after the first injection was not significantly preserved 24 h later. However, as shown in Fig. 2B, central activity in OBX mice was increased by chronic CBD. This result was not statistically different from that obtained in OBX VEH group, but there was also no difference with SHAM VEH group. Therefore, CBD partially attenuated the OBX-induced anxiety-like behaviour.

3.1.2.2. Sucrose preference test. Two-way ANOVA analysis revealed a significant interaction between surgery and treatment [$F(1,27) = 4.30$, $p < 0.05$] after 7 days of CBD treatment. As shown in Fig. 2C, OBX animals exhibited anhedonia, as reflected by a lower preference for sucrose solutions than their sham counterparts ($p < 0.01$). The sucrose intake of OBX-mice was totally restored after one week ($p < 0.01$) and two weeks (data not shown) of treatment with CBD. At the same time points sham mice did not exhibit any alteration in the sucrose preference test.

3.2. Differential effects of acute and chronic CBD upon 5-HT and glutamate output in ventro-medial prefrontal cortex

In order to explore the underpinning mechanism of the fast antidepressant-like actions of CBD, we firstly evaluated the acute effect of a single dose of CBD (50 mg/kg; i.p.) upon the dialysate levels of 5-HT and glutamate in the ventromedial prefrontal cortex (vmPFCx) of sham and OBX-mice. Then, the effect of a challenge dose of CBD after a 14 days treatment was also assessed in sham and OBX mice, in order to analyse the possible adaptive changes induced by its chronic administration.

3.2.1. Acute effects of CBD

In vivo microdialysis studies showed that acute administration of 50 mg/kg CBD prompted a significant increase in extracellular 5-HT contents in the vmPFCx of OBX-mice ($p < 0.05$ *vs.* OBX VEH), but not in sham counterparts (Fig. 3A). In addition, CBD increased extracellular glutamate levels in both sham ($p < 0.01$) and OBX ($p < 0.01$) animals (Fig. 3B).

3.2.2. Chronic effects of CBD

Microdialysis studies after chronic administration of CBD demonstrated alterations in the pattern of neurotransmitters levels, compared to the effects of a single injection of CBD. After chronic administration, a challenge dose of CBD induced a significant augmentation of extracellular 5-HT in all CBD-treated animals (SHAM CBD $p < 0.01$; OBX CBD $p < 0.05$) *vs.* vehicle-treated groups (Fig. 3C). However, the glutamate content was only increased in OBX-mice ($p < 0.01$; Fig 3D) since the sham counterparts treated with chronic CBD did not retain the response observed after acute administration of CBD.

The analysis of the absolute basal neurotransmitter levels after chronic administration of CBD revealed a significant elevation in glutamate content in all CBD treated mice (Fig. 3F), more pronounced in the sham group (OBX CBD *vs.* OBX VEH $p < 0.05$, SHAM CBD *vs.* SHAM VEH $p < 0.01$). No significant differences were obtained in the analysis of the 5-HT absolute basal levels (Fig. 3E).

3.3. Chronic CBD induces differential adaptive changes on 5-HT$_{1A}$ receptor functionality in OBX and sham mice

As shown in Fig. 4, densitometric analysis revealed a decrease in (±)8-(OH)-DPAT-induced stimulation of [^{35}S]GTPγS binding in the DRN of OBX mice (−22 ± 4% *vs.* SHAM VEH, $p < 0.05$), that was normalized after chronic administration of CBD (OBX CBD *vs.* OBX VEH, $p < 0.05$). A significant interaction between surgery and treatment was found in DRN [$F(1,23) = 5.55$, $p < 0.05$].

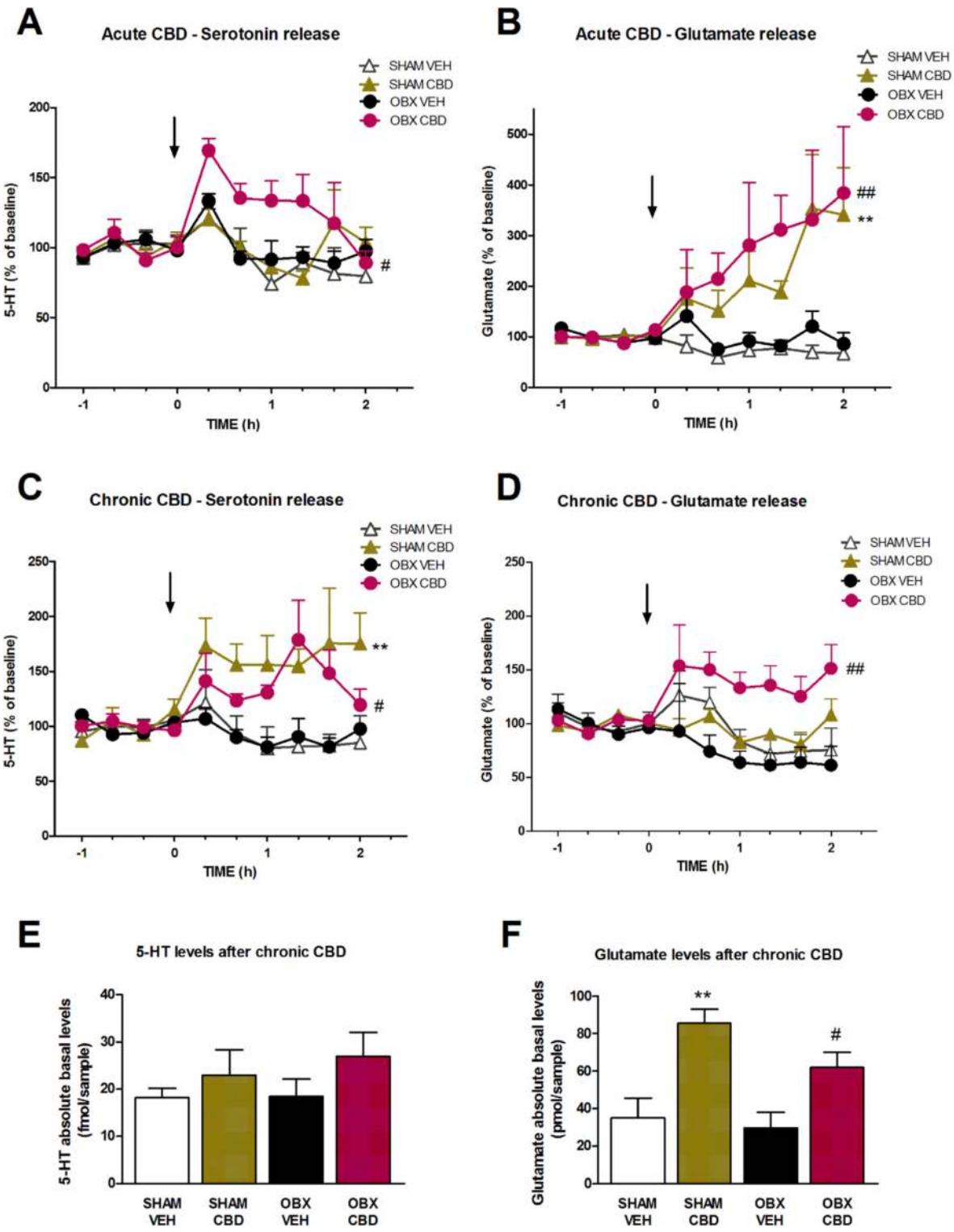

Fig. 3. Differential effects of acute and chronic CBD upon 5-HT and glutamate levels in ventro-medial prefrontal cortex of OBX and sham mice. Acute CBD (50 mg/kg; i.p.) increased extracellular 5-HT in the vmPFCx of OBX-mice but not in sham animals (A), and increased glutamate levels in both groups (B). Following chronic administration, a challenge dose of CBD increased extracellular 5-HT in sham and OBX mice (C), whereas it induced an increase of glutamate efflux only in OBX mice (D). Chronic CBD did not produce any significant change in 5-HT absolute basal levels (E) though it did increase glutamate absolute basal levels in both sham- and OBX-treated mice (F). Data represented as mean ± SEM, n = 5–7 animals per experimental group ($^{**}p < 0.01$ *vs*. SHAM VEH; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs*. OBX VEH).

A decrease in the stimulated [^{35}S]GTPγS binding in OBX mice was also observed in postsynaptic areas such as amygdala (AMY: -30 ± 2%, $p < 0.01$) and CA1-CA2 fields of the hippocampus (CA1-CA2: -89 ± 14%, $p < 0.05$) compared to SHAM VEH group. Chronic administration of CBD significantly reversed (±)8-(OH)-DPAT-induced stimulation of [^{35}S]GTPγS binding in both structures in OBX mice (OBX CBD *vs*. OBX VEH, $p < 0.05$ for both areas). Two-way ANOVA analysis revealed a significant interaction between surgery

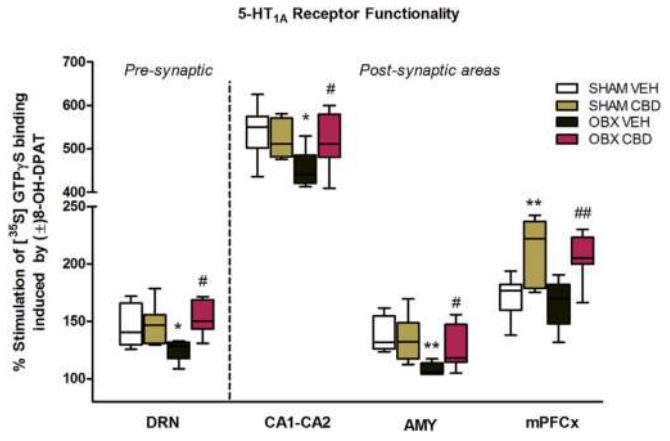

Fig. 4. Box and whiskers plot of 5-HT$_{1A}$ receptors functionality in different brain areas after chronic administration of CBD. A decreased (±)8-(OH)-DPAT stimulated [^{35}S]GTPγS binding was measured in DRN, CA1-CA2, and AMY in OBX mice compared with sham animals. This impaired 5-HT$_{1A}$ receptor functionality in OBX-mice was restored after chronic administration of CBD. A higher (±)8-(OH)-DPAT stimulated [^{35}S] GTPγS binding was detected in the mPFCx in all CBD-treated mice. Results are expressed as percentage of [^{35}S]GTPγS binding stimulation over basal values, as mean ± minimum/maximum of n = 6–9 mice per experimental group ($^{*}p < 0.05$ and $^{**}p < 0.01$ *vs.* SHAM VEH; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* OBX VEH).

and treatment in CA1-CA2 [F(1,29) = 5.37, $p < 0.05$].

In mPFCx, no changes were detected between sham and OBX mice. However, chronic administration of CBD induced an increase in the stimulated [^{35}S]GTPγS binding in both sham (SHAM CBD: + 42 ± 12%, $p < 0.01$ *vs.* SHAM VEH) and OBX (OBX CBD: + 40 ± 7, $p < 0.01$ *vs.* OBX VEH) mice.

3.4. 5-HT$_{1A}$ receptor plays a key role in the behavioural and neurochemical effects of CBD

3.4.1. Fast effects of CBD in the OFT are mediated by 5-HT$_{1A}$ receptors

Selective antagonists of either 5-HT$_{1A}$ or CB$_1$-receptors were used to investigate the neurochemical mechanisms underlying acute effects of CBD in the OBX-induced behaviour. Antagonists of 5-HT$_{1A}$ (WAY100635, 0.3 mg/kg; i.p.) and CB$_1$ (AM251, 0.3 mg/kg; i.p.) receptors were coadministered with CBD (50 mg/kg; i.p.) 30 min before the OFT session. Antagonists doses were those normally reported in the literature and devoid of any effect by themselves on the parameters analysed in the open field. Interestingly, WAY100635 was able to prevent the CBD-induced reversal of OBX-hyperactivity (OBX CBD *vs.* OBX CBD + WAY, $p < 0.01$) (Fig. 5A). It also inhibited the beneficial effect of CBD on central ambulation scores in these subjects (OBX CBD *vs.* OBX CBD + WAY, $p < 0.05$) (Fig. 5B), not altering sham mice activity (data not shown). By contrast, AM251 did not counteract nor mimic the behavioural effects of CBD in OBX animals (Fig. 5A and B).

3.4.2. 5-HT$_{1A}$ receptor blockade prevented the increase in 5-HT and glutamate cortical output induced by CBD

Since the 5-HT$_{1A}$ receptor blockade abolished the behavioural actions of acute CBD in the open field, we studied the effect of WAY100635 upon the CBD-induced changes on 5-HT and glutamate dialysate levels.

Administration of WAY100635 (0.3 mg/kg) prevented the 5-HT outflow induced by acute administration of CBD in OBX animals ($p < 0.05$ *vs.* OBX CBD) (Fig. 6A). In sham animals, where CBD alone did not induce any change in 5-HT output, the blockade of 5-HT$_{1A}$ receptors resulted in a significant increase of 5-HT efflux ($p < 0.01$ *vs.* SHAM CBD) (Fig. 6B). Additionally, WAY100635 blocked the increase of glutamate dialysate levels induced by acute CBD in both OBX (Fig. 6C; $p < 0.05$) and sham (Fig. 6D; $p < 0.01$) mice. No significant alterations were measured upon 5-HT and glutamate levels when WAY100635 was administered alone to sham and OBX mice (Supplementary Fig. S4).

4. Discussion

In this paper we demonstrate for the first time that CBD exerts rapid antidepressant-like effects as evidenced by the reversal of OBX-induced hyperactivity immediately after the first injection. Moreover, its efficacy is maintained and improved with the repeated administration, as the anhedonia was completely relieved after one week of treatment. The dose adjustment appears to be particularly important for the fast antidepressant effects of CBD. Hence, we found that 10 mg/kg of CBD exerts antidepressant-like actions after two weeks of treatment. Nevertheless, when a higher dose is administered at the beginning of the treatment (50 mg/kg), the reversal of OBX-hyperactivity is evident from the first injection and the anti-anhedonic effect appears after just one week administration.

Regarding anxiety-related behaviours, CBD also exhibited an anxiolytic-like effect in OBX mice. Our results are in good agreement with the anxiolytic actions observed after acute (Guimaraes

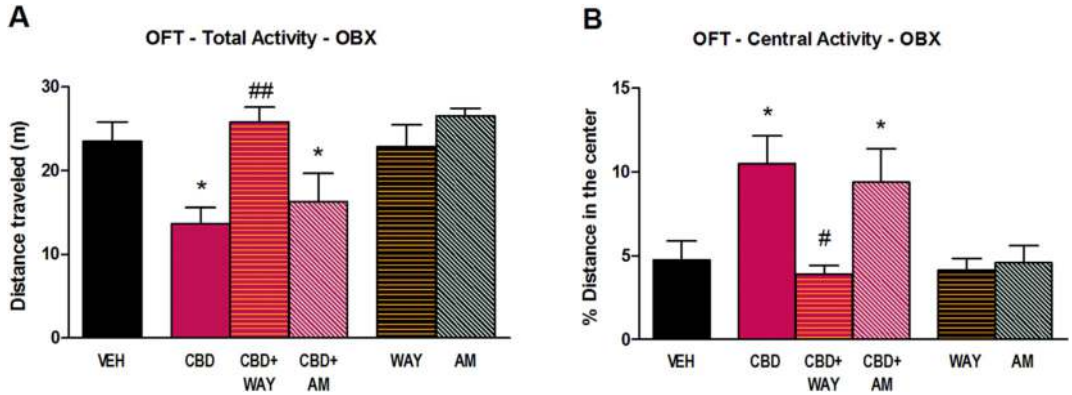

Fig. 5. Behavioural effects of acute CBD in OBX mice were prevented by 5-HT$_{1A}$ receptor blockade. In the *open field test*, WAY100635 (0.3 mg/kg; i.p) prevented both the reversal of OBX-hyperactivity (A) and the increase of central activity (B) induced by CBD (50 mg/kg). By contrast the selective CB$_1$ receptor antagonist AM251 (0.3 mg/kg; i.p.) did not counteract any of these effects. Data represented as mean ± SEM of n = 5–7 animals per experimental group ($^{*}p < 0.05$ *vs.* vehicle-treated group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* CBD-treated group).

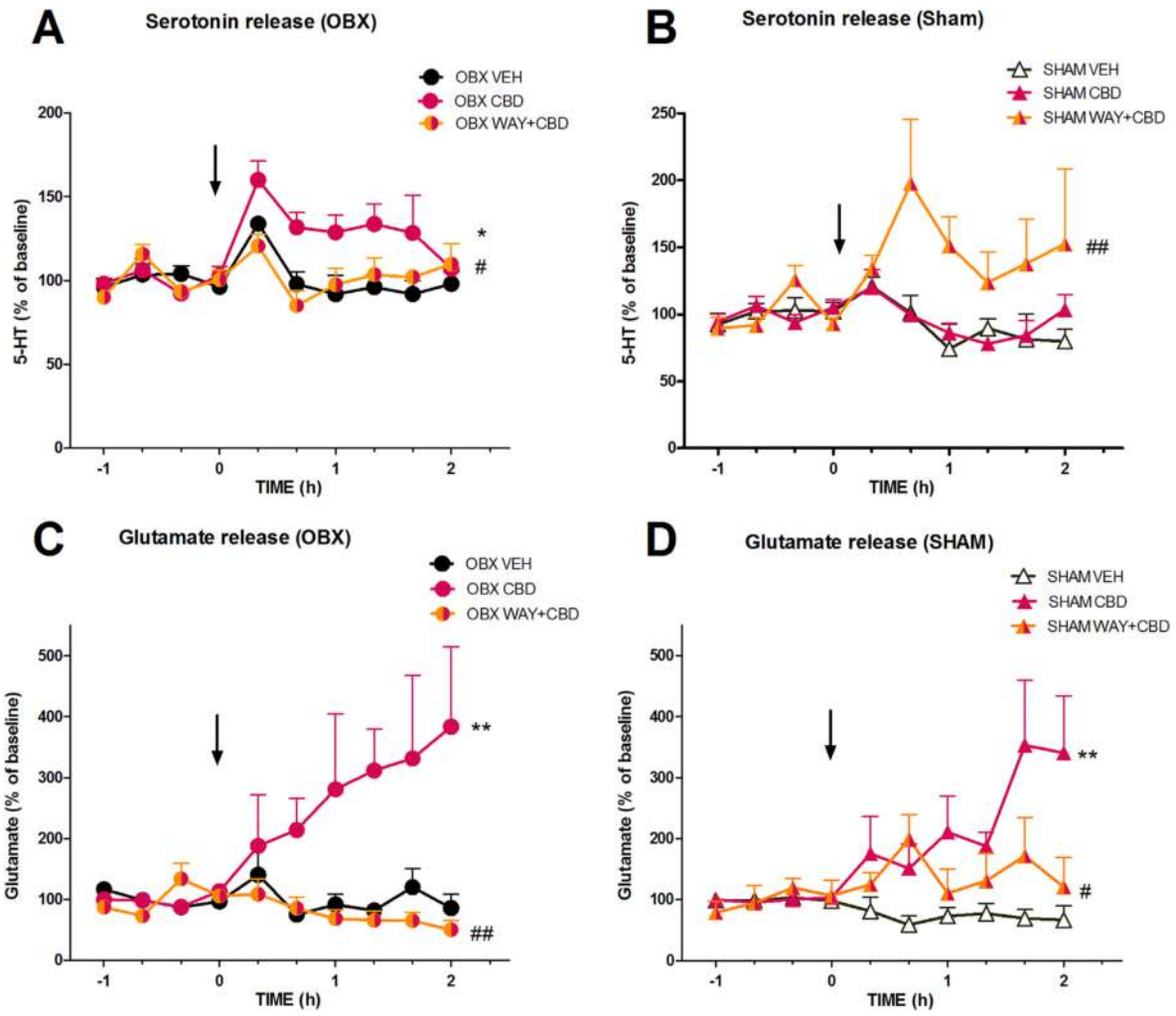

Fig. 6. Neurochemical effects of acute CBD were prevented by 5-HT$_{1A}$ receptor blockade. In microdialysis studies, cortical 5-HT outflow induced by CBD in OBX animals was prevented by WAY100635 coadministration (A). In sham mice, the coadministration of WAY100635 and CBD resulted in increased 5-HT levels in vmPFCx (B). Increased cortical glutamate levels induced by CBD were also abolished by WAY100635 in both OBX (C) and sham mice (D). Data represented as mean ± SEM of n = 5–7 animals per experimental group ($^{*}p < 0.05$ and $^{**}p < 0.01$ *vs.* respective vehicle-treated group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* respective CBD-treated group).

et al., 1990; Moreira et al., 2006; Resstel et al., 2009; Casarotto et al., 2010), or chronic administration (Campos et al., 2013a, 2013b) of CBD in rodents. Moreover, chronic administration of CBD in rats induce anxiolytic-like effects per se (Campos et al., 2013a), while in mice this effect was only observed in chronic stress models (Campos et al., 2013b). We did not detect an anxiolytic-like effect of CBD in our sham animals, and neither anxiogenic-like effects as reported by other authors (ElBatsh et al., 2012; Fogaca et al., 2014). This discrepancy could be due to methodological differences related to the use of different animal species and/or strains, behavioural tests to assess anxiety (Ohl, 2003), as well as housing conditions (Linge et al., 2013). Collectively, our findings demonstrate the antidepressant efficacy of CBD in OBX mice, an animal model of depression with comorbid anxiety –*face validity*– and extensively used for the preclinical research of antidepressant and anxiolytic effects of drugs –*predictive validity*– (Song and Leonard, 2005). Even more, CBD does not only show an earlier onset of action compared to classic antidepressants (Rodriguez-Gaztelumendi et al., 2009), but also triggers an immediate antidepressant-like effect, similarly to that reported for ketamine (Li et al., 2010; Maeng et al., 2008).

In our study, the initial high dose of CBD (50 mg/kg) reduced sucrose preference in some sham animals in parallel with a decline in food consumption and body weight. This is in line with the food intake decrease demonstrated in rats (Farrimond et al., 2012; Ignatowska-Jankowska et al., 2011; Scopinho et al., 2011), and the anorectic effects of other CB_1 antagonists/inverse agonists as Rimonabant or AM251 (Colombo et al., 1998; Shearman et al., 2003). Therefore, we assumed that this initial decrease in sucrose consumption is likely caused by an alteration of the appetite rather than to an emotional detriment, since the parallel behavioural assessment revealed an improved emotional response of OBX mice and no decline in sham animals after the administration of CBD. All the above behavioural findings reinforce the need of adequate pharmacological strategies to optimize the benefits of the treatment with CBD (McCarberg and Barkin, 2007).

In order to analyse the concurrent neurochemical events that may account for the behavioural benefits of CBD, microdialysis studies in vmPFCx were performed after acute and chronic administration. PFCx is a key area in the maladaptive behavioural regulation (Davidson, 2002), specifically exhibited by depressed individuals and a typical feature of OBX mice (Fitzgerald et al., 2008; Song and Leonard, 2005). Interestingly, acute CBD induced an increase in cortical 5-HT levels only in OBX animals, a finding

that could explain the differential behavioural effects of CBD under physiological or pathological conditions, similar to the anxiolytic effects in animals previously subjected to acute stress conditions (Fogaca et al., 2014), or chronic stress (Campos et al., 2013b). We found a decreased functionality of somatodendritic 5-HT_{1A} receptors in the DRN of OBX animals similarly to that described for depressed suicide patients (Savitz et al., 2009). Consequently, this lower inhibitory tone onto the DRN firing would drive an increased 5-HT efflux in the projection areas (Casanovas et al., 1999; Celada et al., 2001), when CBD is administered acutely to OBX mice but not to sham counterparts. Accordingly, we detected increased cortical 5-HT levels in sham mice after CBD only when 5-HT_{1A} receptors were blocked by the administration of an antagonist. The increased 5-HT cortical levels after CBD's administration in bulbectomized animals would be the consequence of an enhanced firing rate of DRN serotonergic neurons. This could be due to a preferential action of CBD over 5-HT_{1A} receptors located in GABAergic interneurons. Indeed, a sensitization of GABAergic interneurons was reported in DRN in the social defeat animal model of depression (Challis et al., 2013). Moreover, the OBX animal model presents an increased serotonergic neuron degeneration in DRN (Saitoh et al., 2007), that would favour the action of CBD on 5-HT_{1A} receptors in GABAergic interneurons, instead of serotonergic cell bodies.

5-HT augmentation in mPFCx has been described after chronic (Gardier et al., 1996) but not acute (Beyer et al., 2002) administration of antidepressants, and it has been pointed as the main underpinning mechanism for their behavioural actions, together with adaptive changes in the serotonergic system. Following chronic administration of CBD, a challenge dose induced 5-HT efflux in the vmPFCx, although in this case in both OBX and sham animals, likely indicating the occurrence of adaptive changes in the serotonergic system. In this sense, chronic CBD treatment promoted an increase in postsynaptic 5-HT_{1A} receptors functionality in mPFCx, not only in OBX but also in sham mice, as it occurs with SSRIs (Matsuda, 2013). Concomitantly, we expected to find a decreased functionality of somatodendritic DRN 5-HT_{1A} receptors of sham animals, that justified the increased 5-HT efflux induced by chronic CBD, but we did not observe any alteration. However, it should be noted that desensitization of somatodendritic 5-HT_{1A} receptors is not always detectable by [^{35}S]GTPγS binding techniques (Gi-proteins coupling to the receptor) but with other methodological approaches (e.g. 5-HT_{1A} receptor-mediated GIRK currents electrophysiology) (Rossi et al., 2006). Anyway, we did not find behavioural changes associated to the increased 5-HT efflux induced by chronic CBD observed in sham mice, though other predictive paradigms may provide valuable information. On the other hand, OBX animals exhibited impaired functionality of 5-HT_{1A} receptors in limbic brain areas (i.e. amygdala and hippocampus) that were restored after chronic administration of CBD. This effect might be associated to the increased 5-HT efflux and improvement of behavioural deficits, suggesting a crucial role of these receptors in the pharmacodynamics of CBD.

Microdialysis in vmPFCx revealed that acute CBD promoted a marked glutamate elevation in both sham and OBX mice. This facilitated glutamatergic neurotransmission has been associated with the fast antidepressant efficacy of ketamine (Maeng et al., 2008). Thus, it could be postulated that the increased glutamate efflux triggered by CBD from the first injection could underlie the fast antidepressant-like effects of CBD in OBX mice. In contrast to the immediate increase in 5-HT levels induced by CBD, a delayed pattern on glutamate level increase was observed. A similar pattern of glutamate level changes has been reported after NMDA receptor antagonists administration (Adams and Moghaddam, 2001; López-Gil et al., 2007). This higher glutamate basal levels after chronic administration of CBD could indicate the implication of second messengers as the mitogen-activated protein kinase (MAPK) pathway, the inhibition of intracellular cAMP, or the stimulation of phospholipase C (PLC), as reported in *in vitro* studies following 5-HT_{1A} receptor activation (Banerjee et al., 2007). In the chronic approach, the increase of glutamate efflux induced by CBD was observed only in OBX mice. It is noteworthy that after chronic administration of CBD, basal levels of glutamate were elevated in both sham and OBX mice, though in a lower magnitude in the OBX group. This finding could explain the lack of relative glutamate increment in sham animals after a challenge dose of CBD. A minor basal glutamatergic tone of OBX animals after chronic administration of CBD compared with sham mice, could be related to a dysfunction in glutamatergic system previously described in OBX animals (Webster et al., 2000) and also in depressed patients (Hashimoto, 2009).

To gather more information about the mechanism of action of CBD in its behavioural responses, we analysed the implication of the two main targets of this compound, 5-HT_{1A} and CB_1 receptors (Fernandez-Ruiz et al., 2013). Our findings revealed a crucial role of 5-HT_{1A} receptors in the behavioural effects of CBD, since WAY100635 but not AM251, prevented both the reversal of OBX-hyperactivity and the anxiolytic-like effects displayed by CBD. These findings are in good agreement with studies in which CBD decreased immobility in the FST (Zanelati et al., 2010) and prevented anxiety/panic responses (Campos et al., 2013a; Campos and Guimaraes, 2008; Fogaca et al., 2014; Soares Vde et al., 2010) in a 5-HT_{1A} receptor-dependent manner. However, it has been also described that some acute and chronic anxiolytic-like effects of CBD are mediated by CB_1 receptors (Campos et al., 2013b; Casarotto et al., 2010; Do Monte et al., 2013). As anxiety is a complex syndrome affected by different brain processes (Davidson, 2002; Kheirbek et al., 2012), these two receptors could be implicated in the anxiety outcome at different levels. Nevertheless, the behavioural antidepressant-like effects of CBD were neither prevented nor mimicked by AM251, suggesting that the modulation of the endocannabinoid system is not contributing to the fast antidepressant-like effects of CBD. Accordingly, we postulated that CBD could induce an increase in 5-HT/glutamate neurotransmitter in vmPFCx through a 5-HT_{1A} receptor-dependent mechanism, responsible of its fast antidepressant-like effects. Microdialysis studies confirmed our hypothesis since not only 5-HT but also glutamate increase induced by CBD were prevented by WAY100635.

Altogether, our findings demonstrate that a 5-HT_{1A}-dependent enhancement of glutamatergic and serotonergic neurotransmission in OBX mice immediately after the first injection of CBD could lie behind its fast antidepressant-like effects. Likewise, the sustained increase in prefrontocortical glutamate contents, together with serotonergic increase and the adaptive changes in 5-HT_{1A} receptor functionality, might drive the consolidation and improvement of the antidepressant-like effects of chronic CBD (including anti-anhedonic actions).

Although further investigation is still required to fully elucidate how CBD acts on 5-HT_{1A} receptors to induce the boost of 5-HT and glutamate, here we propose a putative neurochemical mechanism (Fig. 7). Both serotonergic and glutamatergic potentiation, through the allosteric modulation of the 5-HT_{1A} receptor (Rock et al., 2012), might underlie the fast antidepressant-like effects of CBD in the OBX model of depression. In prefrontal cortex, CBD would potentiate the inhibitory function of 5-HT_{1A} receptors upon GABAergic interneurons (Santana et al., 2004), favouring glutamate signalling in postsynaptic areas (Llado-Pelfort et al., 2012). This enhanced glutamatergic transmission, through pyramidal descending projections to DRN, might stimulate the neuronal firing of serotonergic

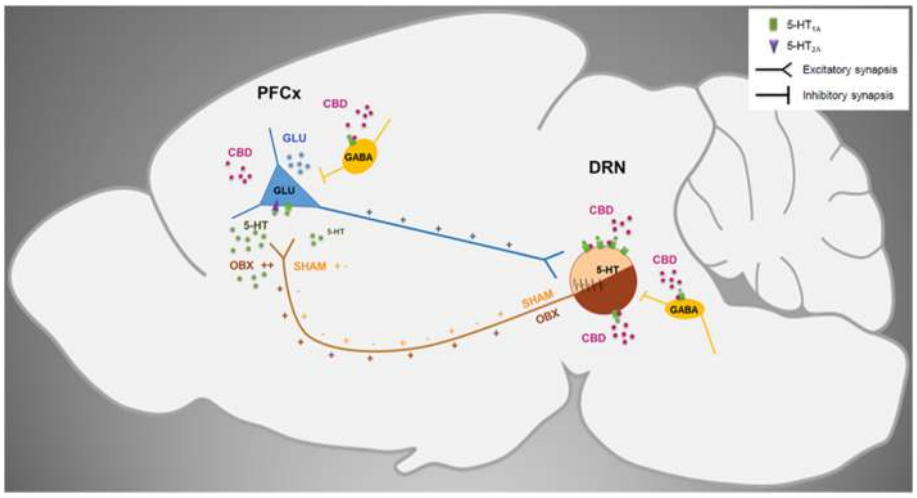

Fig. 7. Proposed neurochemical mechanism of action of CBD to induce fast antidepressant effects. In prefrontal cortex, CBD would potentiate the inhibitory function of 5-HT_{1A} receptors upon GABAergic interneurons, favouring glutamate signalling in postsynaptic areas, the stimulation of pyramidal descending projections to DRN, and therefore the neuronal firing of serotonergic neurons, and the 5-HT increase in mPFCx. In DRN, CBD would increase the firing of serotonergic neurons by reducing the inhibitory effect of GABAergic interneurons, without the detrimental effect of somatodendritic 5-HT_{1A} receptors which are desensitised in OBX mice, therefore leading to an increase in 5-HT levels in PFCx.

neurons, driving to a 5-HT increase in mPFCx (Celada et al., 2001). In DRN, CBD would reduce the inhibitory effect of GABAergic interneurons upon the discharge rate of serotonergic neurons, contributing to the increased cortical serotonergic output. The concurrent activation of somatodendritic 5-HT_{1A} receptors is known to inhibit the firing rate of DRN serotonergic neurons. The decreased functionality of these presynaptic 5-HT_{1A} receptors in OBX mice might explain the higher 5-HT levels in mPFCx after CBD due to a lower inhibitory feedback. Finally, increased serotonin efflux in mPFCx of OBX mice induced by CBD might also modulate pyramidal neurons activity through the activation of 5-HT_{1A} and 5-HT_{2A} membrane receptors.

Although the herein proposed mechanism for CBD's effects seem to be the most feasible accounting for our results, we do not discard the additional involvement of other receptors and/or the crosstalk among systems in the overall observed effects. In all, CBD is a multitarget drug that can modulate a variety of systems implicated in mood control and therefore, result in a great value from a clinical point of view.

5. Conclusions

This work evidences that CBD could represent a novel drug for treating depressive disorders in a very fast manner, acting via the enhancement of serotonergic and glutamatergic transmission through the modulation of 5-HT_{1A} receptors. The fast onset of antidepressant action of CBD and the simultaneous anxiolytic effect would solve some of the main limitations of the current antidepressant therapies. Furthermore, the broad range for therapeutic dosage and the lack of psychotomimetic effects confers a fundamental advantage for its use in clinical practice compared to other fast-acting antidepressant alternatives. Finally, this novel strategy consisting in the dual potentiation of serotonergic and glutamatergic transmission could bring new light to the discovery of new fast and effective antidepressant therapies.

Author contributors

The bulk of the experimental work, data analysis and interpretation, and the draft of the paper was carried out by Raquel Linge. Laura Jiménez-Sánchez equally contributed to the microdyalisis experiments performance and related data analysis and interpretation. Leticia Campa analysed microdyalisis samples in the HLPC. Fuencisla Pilar-Cuéllar and Rebeca Vidal participated in autoradiography and behavioural protocols development and in data interpretation. Angel Pazos contributed to the study design and data interpretation. Albert Adell supervised microdyalisis studies participating in the experimental design, data analysis and interpretation. Alvaro Diaz supervised the experimental work, participated in the study design, data analysis and interpretation. All authors contributed to and approved the final version of the paper.

Disclosure

The authors declare no conflict of interest.

Acknowledgments

This research was supported by Spanish Ministry of Economy and Competitiveness (SAF2011-25020), Instituto de Salud Carlos III (FIS Grant PI13-00038) co-funded by the European Regional Development Fund ('A way to build Europe') and Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM). Raquel Linge Méndez is a recipient of a predoctoral research contract of the Instituto de Biomedicina y Biotecnología de Cantabria (IBBTEC) and Laura Jiménez-Sánchez a predoctoral fellowship from the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). We thank the medical student Marc Grifoll Escoda for the helpful participation in microdialysis experiments.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.neuropharm.2015.12.017.

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