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Antidepressant-like effect of cannabidiol injection into the ventral medial prefrontal cortex – possible involvement of 5-HT1A and CB1 receptors

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Highlights

- CBD administration into the rat vmPFC, either into PL or IL, reduced the immobility time in the forced swimming test, an antidepressant-like effect.
- The administration of 8-OH-DPAT, a 5-HT1A agonist, into PL or IL induced antidepressant-like effects similar to CBD.
- CBD-induced antidepressant-like effects were blocked by local pretreatment with WAY100635, a 5-HT1A receptor antagonist.

Abstract

Rationale: systemic administration of Cannabidiol (CBD), the main non-psychotomimetic constituent of *Cannabis sativa*, induces antidepressant-like effects. The mechanism of action of CBD is thought to involve the activation of 5-HT1A receptors and the modulation of endocannabinoid levels with subsequent CB1 activation. The brain regions involved in CBD-induced antidepressant-like effects remain unknown. The ventral medial prefrontal cortex (vmPFC), which includes the infralimbic (IL) and prelimbic (PL) subregions, receives dense serotonergic innervation and plays a significant role in stress responses. *Objective:* to test the hypothesis that the administration of CBD into the IL or PL would induce an antidepressant-like effect through 5-HT1A and CB1 activation. *Methods:* Rats received intra-IL or -PL microinjections of CBD (10-60 nmol/side), 8-OH-DPAT (5-HT1A agonist, 5-10nmol/side), anandamide (AEA, 0.5 pmol/side) or vehicle (0.2µl/side) and were submitted to the forced swimming (FST) or to the open field (OFT) tests. Independent CBD-treated groups were pre-treated with WAY100635 (10, 30 nmol/side, 5-HT1A antagonist) or AM251 (10 pmol/side, CB1 antagonist) and submitted to the same tests. An additional group was treated with WAY100635 followed by anandamide. *Results:* CBD (PL: 10-60 nmol; IL:45-60 nmol) and 8-OH-DPAT (10 nmol) administration significantly reduced the immobility time in the FST, without changing locomotor activity in the OFT. WAY100635 (30 nmol) did not induce effect per se but blocked CBD, 8-OH-DPAT and AEA effects. Additionally, AM251 blocked CBD-effects. *Conclusion:* administration of CBD into the vmPFC induces antidepressant-like effects possibly through indirect activation of CB1 and 5-HT1A receptors.

Keywords: Cannabidiol; vmPFC; forced swimming test; antidepressant; 5HT1A

1 Introduction

Cannabidiol (CBD) is the main non-psychotomimetic compound of *Cannabis sativa*. CBD systemic administration induces several effects in the central nervous system, resulting in anxiolytic- [1] and [2], anticonvulsant- [3], [4] and [5], anticompulsive- [6] and antipsychotic-like effects [7] and [8]. Recently, our group demonstrated that systemic treatment with CBD is also able to induce antidepressant-like effects in mice submitted to the forced swimming test [9], an effect that was later confirmed by another group [10].

Despite such an impressing potential therapeutic profile in preclinical studies, the molecular mechanisms involved in CBD-induced behavioral effects remain poorly understood. Evidence has suggested that CBD may act through a variety of mechanisms, therefore being able to promote different behavioral effects [11]. For instance, CBD has a low affinity for cannabinoid (CB1) receptors [12], but can potentiate the endocannabinoid neurotransmission by inhibiting the hydrolysis or the reuptake of anandamide [13] and [14]. Furthermore, CBD also causes direct or indirect activation of serotonin, adenosine and TRPV receptors, as well as receptors of the PPAR family and ion channels [15]. Animal studies have indicated that the contribution of each of these mechanisms for CBD-induced pharmacological profile depends on the behavioral tasks under investigation [11]. For instance, some central CBD-induced effects, such as the compulsive and anxiolytic, depend on the activation of CB1 receptors [6] and [18]. On the other hand, most of the behavioral effects induced by CBD, including the antidepressant-like, involve 5-TH1A receptor activation, since it can be blocked by pretreatment with selective antagonists [2], [9], [20], [21] and [22]. This effect could either happen through direct activation of 5HT1A by CBD due to its binding and agonist properties at this receptor [19], or indirectly through the modulation of serotonin levels in response to increased endocannabinoid levels and CB1 activation [15] and [20]. Corroborating this latter proposal, the antidepressant-like effect induced by genetic and pharmacological manipulation of the endocannabinoid system have been shown to involve the modulation of the serotonergic neurotransmission [23], [24] and [25]. For instance, genetic deletion of fatty acid amide hydrolase

causes antidepressant-like effect that is associated with increased activity of dorsal raphe nucleus serotonergic neurons [24].

Therefore, it is possible to hypothesize that CBD-induced antidepressant-like effect may involve the activation of 5-HT1A receptors, either by direct agonistic properties or indirectly through the modulation of local endocannabinoid levels and CB1 activation. However, this possible interaction and the brain regions responsible for mediating this effect are not yet known.

Since the antidepressant-like effect induced by FAAH inhibitor involves the modulation of the serotonergic system in the medial prefrontal cortex (mPFC) [25], this brain region could be an important target for CBD-induced effects. The mPFC is a brain region deeply involved in several higher-order functions, such as memory, learning, decision-making and goal-directed behavior, in addition to the modulation of autonomic and endocrine responses to stress [26], [27] and [28]. The ventral portion of the medial prefrontal cortex (vmPFC) is divided into infralimbic (IL) and prelimbic (PL) subregions [29], which are cytoarchitectonically different and able to play distinct or complementary roles in the modulation of physiological and behavioral functions [30], [31] and [32]. Scopinho and colleagues (2010) [33] investigated a possible differential participation of the PL and IL in the modulation of behavioral immobility of rats submitted to the FST and found that acute reversible inactivation of these regions produced similar antidepressant-like effects, depending on the moment at which the pharmacological manipulation was performed. In addition, the antidepressant-like effect induced by inactivation of the vmPFC depended on the integrity of the serotonergic system [34]. This evidence supports the hypothesis that signaling within the vmPFC mediates stress adaptation and antidepressant-like effects.

Based on the aforementioned evidence, we hypothesized that CBD injection into the PL or the IL would induce antidepressant-like effects in rats submitted to the FST, an animal model predictive of antidepressant effects [36], with the participation of local 5HT1A and CB1 receptors.

2 Material and methods

2. 1 Animals

The experiments were performed with male Wistar rats (230-270 g). The animals were housed in pairs in acrylic boxes measuring 20X30X12 cm, under a 12 h light cycle (lights on at 7 am), with free access to food and water and in a temperature-controlled room (24±1°C). No environmental enrichment was presented. Procedures were conducted in conformity with the CONCEA for the care and use of laboratory animals, which are in compliance with international laws and politics, and were approved by the local Ethical Committee (protocol number 11.1.459.53.7).

2.2 Drugs

CBD (kindly supplied by THC-Pharma, Frankfurt, Germany): 10, 30, 45 and 60 nmol/0.2µl/side; 8-OH-DPAT (RBI): 5 and 10nmol/0.2µl/side; WAY100635 (Sigma-Aldrich): 10 and 30nmol/0.2µl/side; anandamide-AEA (Tocris): 0.5pmol/0.2µl/side. WAY100635 and 8-OH-DPAT were dissolved in sterile isotonic saline solution, AEA in Tocrisolve solution and CBD in grape seed oil [22]. Each control group received the corresponding vehicle of the drug being tested.

2.3 Stereotaxic surgery

Rats were anesthetized with 2,2,2 tribromoethanol (250mg/kg); intraperitoneally; Sigma-Aldrich, St Louis, Missouri, USA) and immobilized in a stereotaxic frame. Stainless steel guides cannula (0.11mm, 26 G) were implanted bilaterally into the vmPFC-PL [AP = +3.3 mm from bregma; L = +1.9 mm from the medial suture, V = −2.4 mm from the skull with a lateral inclination of 22◦] and vmPFC-IL [AP = +3.3 mm from bregma; L = +2.7 mm from the medial suture, V = −3.2 mm from the skull with a lateral inclination of 24◦] [37]. The cannulae were attached to the bone with stainless steel screws and acrylic cement.

Thirty-three Ga. Needles (Small Parts, Miami Lakes, Florida, USA) 1 mm longer than the guide cannulae and connected to a 10 µL syringe (7001

KH, Hamilton Co., Reno, Nevada, USA) through a PE-10 tube were used for the microinjections. The needles were carefully inserted into the guide cannulae, and the solutions were infused over a 60s period at a rate of 0.2µl/min with the help of an infusion pump (KD Scientific Inc., Holliston, Massachusetts, USA). The needles remained in place for an additional 20-30 s period to prevent reflux.

2.4 Histology

At the end of the experiments the rats were anesthetized with chloral hydrate solution 5 % (1 ml/100 g, intraperitoneally, C2H3Cl3O2, VETEC, Brazil) and the Evans blue dye was injected (0.2 µl). The chest was surgically opened and the brain perfused with 10% formalin through the left ventricle. The injection sites were identified using a rat brain atlas as reference [31]. The injection sites can be seen in Figure 1. Rats that received CBD injections outside the targeted area were pooled together in another group named “OUT”.

2.5 Forced Swimming Test

The forced swimming test was performed as described by Joca and Guimarães [38]. Rats were placed individually in plastic cylinders filled with water (24±1ºC) for 15 minutes (pre-test session). After pretest, animals were dried and returned to their home cage. Twenty-four hours later, rats were submitted to a forced swim section for 5 minutes (test) [39]. The water was changed between each section to avoid the influence of alarm substance [40]. All experiments were videotaped with Sony digital video camera model DCR-SR47 and an observer that was blind to the treatments recorded the immobility time later on.

2.6 Open Field Test

Since the forced swimming test evaluates a parameter that depends on locomotor activity, it is necessary to exclude possible confounding drug-

inducing locomotor effects. Therefore, an additional animal group was placed individually in a square open field arena, divided into 16 quadrants, where exploratory activity was videotaped during 5 minutes. During this time the number of crossings between the quadrants was counted and used as an index of locomotor activity. All experiments were videotaped and an observer that was blind to the treatments recorded the number of crossings later on.

2.7 Statistical analysis

Behavioral data from the forced swimming test were analyzed by one-way ANOVA followed by Dunnett's post-hoc test. The open field test was analyzed by two-way repeated measures ANOVA. The significance level was set at $p < 0.05$.

3 Experimental Design

Experiment 1: Effects of CBD (10, 30, 45, 60nmol) microinjection into the vmPFC of rats submitted to the forced swimming test

This experiment was designed to assess possible differences of CBD-induced effects when injected either into the PL or the IL. The animals received bilateral injections of vehicle or CBD into the PL (10, 30 or 60 nmol/0.2 µL per site) or into the IL (30, 45 or 60nmol/0.2 µL per site) 10 min before the test session.

Experiment 2: Effects of CBD (10, 30, 45, 60nmol) microinjection into the vmPFC of rats submitted to the open field test

To investigate possible unspecific locomotor effects induced by the CBD at the doses that were effective in the FST, an independent group of animals received CBD administration into the PL or the IL and were individually submitted to the open field test, 10 minutes after the injection.

Experiment 3: Effects of 8-OH-DPAT (5, 10nmol) microinjection into the vmPFC of rats submitted to the forced swimming test

This experiment was designed to investigate if the administration of a 5-HT1A agonist into the IL or the PL would induce similar effects as those induced

by CBD administration into the same cortical regions. The protocol was similar to that of experiment 1, except that animals received intra-PL or IL injections of saline or 8-OH-DPAT (5 and 10 nmol; 0.2µL per site), ten minutes before the test session.

Experiment 4: Effects of 8-OH-DPAT and WAY100635 microinjection into the vmPFC of rats submitted to the forced swimming test.

This experiment was designed to test if the effects induced by 8-OH-DAT administrations into the PL and IL could be blocked by the local administration of a 5-HT1A antagonist, WAY100635. The protocol was similar to that of experiment 1, except that animals received intra -PL or -IL injections of saline or WAY100635 (10 or 30 nmol; 0.2µL per site) followed, 5 min later, by a second injection of vehicle or 8-OH-DPAT (10 nmol; 0.2µL per site). Ten minutes after the last injection, the animals were submitted to the forced swimming test.

Experiment 5: Effects of CBD and WAY100635 microinjection into the vmPFC of rats submitted to the forced swimming test.

This experiment was designed to investigate if CBD-induced effects would be mediated by local 5-HT1A receptors. The protocol was similar to that of experiment 4, except that the second injection was of vehicle or CBD at the dose of 10 nmol into the PL and 45nmol into the IL.

Experiment 6: Effects of WAY100635 and 8-OH-DPAT microinjection into the vmPFC of rats submitted to the open field test

An independent group of animals received WAY100635 (30nmol; 0.2µl per site) or 8-OH-DPAT (10nmol; 0.2µl per site) administration and, 10 minutes after, were individually subjected to the open field test.

Experiment 7: Effects of CBD and AM251 microinjection into the vmPFC of rats submitted to the forced swimming test.

This experiment was designed to investigate if CBD-induced effects would involve local CB1 receptors. The protocol was similar to that of experiment 5, except that the first injection was of vehicle or AM251 at the dose of 10pmol per side into the PL region.

Experiment 8: Effects of AEA and WAY100635 microinjection into the vmPFC of rats submitted to the forced swimming test.

This experiment was designed to investigate if AEA-induced effects would involve local 5HT1A receptors. The protocol was similar to the experiment 4, except that the second injection was of AEA at the dose of 0.5 pmol per side into the PL region.

4 Results

Experiment 1: Effects of CBD (10, 30, 45, 60nmol) microinjection into the vmPFC of rats submitted to the forced swimming test

When administered into the PL, CBD (10, 30, 60 nmol) significantly reduced the immobility time of rats submitted to the FST, when compared to vehicle treated group ($F_{4,50}$ =6.142, p<0,05). Furthermore, the "out" group and the control group were not different from each other (n=7-16, Dunnett's test, p<0,05, Figure.2-a).

CBD administration into the IL at the doses of 45 and 60nmol significantly reduced the immobility time in the FST ($F_{4,38}$ =8.605, p<0.05) compared to the vehicle treated group (n=7-10, Dunnett's test, p<0.001, figure 2-b).

Experiment 2: Effects of CBD (10, 30, 45, 60nmol) microinjection into the vmPFC of rats submitted to the open field test

No difference was observed in the number of crossings performed by animals treated with vehicle or CBD in any of the doses tested (PL: $F_{3,128}$=0.7104, p>0.05; IL: $F_{3,72}$=0.1895, p>0.05). Moreover, there was no interaction between factors (PL: $F_{12,128}$ = 0.1800, p>0.05; IL: $F_{12,72}$ =0.2527, p>0.05, figures 3-a and 3-b, respectively).

Experiment 3: Effects of 8-OH-DPAT (5, 10nmol) microinjection into the vmPFC of rats submitted to the forced swimming test

8-OH-DPAT administration reduced immobility time, at the dose of 10nmol/0.2µL, when injected into the PL ($F_{2,16}$ =3.593, p<0.05; Dunnett's test, p<0.05, n=5-8, figure 4-a) or into the IL ($F_{2,11}$ =4.674, p<0.05; Dunnett's test, p<0.05, n=4-6, figure 4-b).

Experiment 4: Effects of 8-OH-DPAT and WAY100635 microinjection into the vmPFC of rats submitted to the forced swimming test.

As previously observed (experiment 3), 8-OH-DPAT injection into the PL reduced immobility time at the dose of 10nmol/0.2µL ($F_{5,24}$ =5.806, $p<0.05$). Moreover, intra PL administration of WAY100635 at the dose of 30nmol/0.2µL reversed 8-OH-DPAT effects (Dunnett's test, $p>0.05$, compared to vehicle treated group; figure 5-a). Similarly, 8-OH-DPAT injection (10nmol/0.2 µL) into the IL reduced the immobility time ($F_{3,23}$ =3,261, $p<0.05$), an effect that was blocked by pre-treating with WAY100635 at the dose of 30nmol/0.2µl (Dunnett's, $p>0.05$, figure 5-b).

Experiment 5: Effects of CBD and WAY100635 microinjection into the vmPFC of rats submitted to the forced swimming test.

Confirming results obtained in the first experiment, CBD injection into the PL (10nmol/0,2µl) or the IL (45nmol/0,2 µl) reduced immobility time in the forced swimming test (PL: $F_{3,27}$ =8.933, $p<0.05$ and IL: $F_{3,22}$= 7.125, $p<0.05$). Moreover, pre-administration of WAY100635 at the dose of 30nmol/0,2µL, into either the PL or IL, prevented CBD effects (Dunnett's, $p>0,05$, figures 6-a and 6-b, respectively).

Experiment 6: Effects of WAY100635 and 8-OH-DPAT microinjection into the vmPFC of rats submitted to the open field test

No significant difference in the number of crossings was observed between rats that had received vehicle, 8-OH-DPAT or WAY100635 in any of the doses tested into the PL ($F_{3,80}$ =0.7873, $p>0.05$, figure 6-A) or the IL ($F_{4,48}$ =0.1925, figure 6-B). Moreover, there was no interaction between factors (PL: $F_{12,80}$ =0.0608, $p>0.05$; IL: $F_{8,48}$ =0.6844, $p>0.05$, figures 7-a and 7-b, respectively).

Experiment 7: Effects of CBD and AM251 microinjection into the vmPFC of rats submitted to the forced swimming test.

Confirming results obtained in the first experiment, CBD injection into the PL (10nmol/0,2µl) reduced immobility time in the forced swimming test ($F_{3,23}$

=4.268, p<0.05). Moreover, pre-administration of AM251 at the dose of 10nmol/0,2µL, prevented CBD effects (Dunnett's, p>0,05, figures 8).

Experiment 8: Effects of AEA and WAY100635 microinjection into the vmPFC of rats submitted to the forced swimming test.

AEA (0.5pmol/0.2µl) injection into the PL, reduced immobility time in the forced swimming test ($F_{3,36}$ =4.830, p<0.05). Moreover, pre-administration of WAY100635 at the dose of 30nmol/0.2 µl), prevented AEA- induced effects (Dunnett's, p>0,05, figures 9).

5 Discussion

The present study showed that CBD administration into the vmPFC-PL reduced immobility time of rats during the test session at the doses of 10, 30 or 60 nmol. Similarly, CBD administration into the vmPFC-IL induced antidepressant-like effects at the doses of 45 and 60 nmol. Additionally, none of the CBD doses employed changed the number of crossings in the open field test, thus indicating that the reduction in the immobility time in the FST was not secondary to changes in locomotor activity.

The FST is a widely used animal model to predict antidepressant activity [36]. It is based on the exposure of rodents to the stress of being enclosed in a cylinder filled with water, where the animal initially assumes an escape-oriented behavior and soon become immobile. Although the interpretation of immobility in this test is still a matter of debate in the literature, antidepressant drugs of different classes decrease immobility time in the test session in rats and mice [36] and [39]. Therefore, despite its weak face validity, the FST has been the most used animal model to investigate potential antidepressant effects induced by drugs and better understand the mood disorders. According to Slattery and Cryan, 2012, the FST is still able to identify pro-depressant effects in response to different stressors. This fact shows that the FST could be safely used for pre-clinical study of mood disorders [40].

The results obtained herein are in accordance with previous data from our research group showing that systemic administration of CBD induces

antidepressant-like effects in mice submitted to the FST. These effects were also dissociated from locomotor effects [9]. Although similar, the active doses of CBD were lower in the PL than the IL. Therefore, both vmPFC subregions, seems to be involved in the antidepressant-like effects of CBD after systemic administration, although with different sensitivity to the drug. While it is not possible to assess the precise extension of CBD diffusion from its injection site, it is unlikely that the results observed herein would rely in neighboring regions, since no effect was observed when the drug was injected in adjacent regions to the PL or IL.

The vmPFC is functionally connected with limbic structures such as the hippocampus, amygdala and nucleus accumbens and modulates several behavioral responses [42]. In addition, the mPFC is involved in numerous higher-order functions and in responses to stress [26], [27] and [28]. Accordingly, stressful situations activate the vmPFC [28], [43] and [44]. However, the specific role of PL and IL in stress responses is not entirely clear. Several studies have demonstrated opposite functions of the PL and the IL in autonomic, endocrine and emotional responses to stress [9], [30], [45], [46], [47], [48] and [49]. On the other hand, similar roles for PL and IL in emotional control have been described [50]. A study of our own group showed that the blockade of either PL or IL induced antidepressant-like effects, indicating a similar participation in this behavioral response [33]. On the other hand, Hamani and colleges [34] reported different results after manipulations of the PL or IL, where only blockade of the former subregion induced antidepressant-like effect by a serotonin-dependent mechanism. Therefore, we decided to investigate CBD effects when injected separately into these two cortical subregions. The present results demonstrated that administration of CBD into the PL or IL produced similar antidepressant-like effects. This result reinforces the idea that the PL and IL are similarly engaged in modulating behavioral responses to swimming stress. Although both subregions, the PL and IL, are intimately linked to brain structures involved in behavior control, they present distinctive projections throughout the brain, including limbic structures [30]. This could be a possible explanation to the higher sensitivity of the PL to the CBD effects although more studies are necessary since they lack work relating the systems modulated by the CBD and differences between PL and IL.

The mechanisms of CBD-induced behavioral effects are not yet completely understood [11]. Initially, it was shown that some CBD-induced effects, such us anxiolytic [2] and [51], neuroprotective [21] and [52] and antidepressant [9], depended on 5HT1A receptors activation, although it is not yet clear if it is a direct or indirect effect. Russo and colleagues, 2005, showed that CBD (32 μM) *in vitro* can displace the binding of a known agonist, 8-OH-DPAT, from 5-HT1A in a ligand-binding assay, and to activate this receptor. This result demonstrated that CBD may act as a 5-HT1A receptor agonist [19]. On the other hand, CBD could also indirectly facilitate 5-HT1A receptor activation by 8-OH-DPAT at lower concentrations, as showed by Rock and colleagues, 2012 [20]. Corroborating these results, the facilitation of 5HT1A-mediated neurotransmission has been shown to mediate CBD effects in various behavioral tasks [2], [20], [21], [22] and [51]. The present data add additional evidence for the involvement of 5HT1A receptors in CBD-induced behavioral effects, since the antidepressant-like effect of CBD injected into the vmPFC was also blocked by pre-treatment with WAY100635, a 5HT1A receptor antagonist, and mimicked by 8-OH-DPAT, a 5HT1A receptor agonist. They also agree with previous data observed after systemic pre-treatment with WAY100635 [9].

Although some in vitro results have suggested that CBD acts as an antagonist or inverse agonist at cannabinoid CB1 receptors [52], it can also block anandamide reuptake or inhibit its hydrolysis by fatty acid amide hydrolase [12], [13] and [53], thus facilitating endocannabinoid-mediated neurotransmission. In vivo studies have shown that this latter mechanism is probably responsible for some of the behavioral effects of CBD, such as anti-compulsive-like [6] and pro-neurogenic [18]. Accordingly, in the present study pretreatment with AM251 blocked the antidepressant-like effects of CBD. Therefore, our data indicate that the acute antidepressant-like effect induced by CBD in the vmPFC is mediated by both CB1 and 5-HT1A activation.

The literature has consistently shown that the serotonergic system of the vmPFC is under the control of local endocannabinoid levels and CB1 receptor activation [23], [24] and [25] . For instance, administration of a FAAH inhibitor or its genetic deletion increases the firing rate of serotonin neurons and elicits antidepressant-like effects, in a CB1 dependent-fashion [54] and [24]. Furthermore, the administration of a CB1 agonist into the vmPFC induced

similar effects, suggesting that CB1 agonists modulate 5-HT neuronal activity via the vmPFC [23]. FAAH genetic deletion also produced antidepressant-like effects paralleled by altered 5-HT transmission and postsynaptic 5-HT1A activation [24].

Corroborating these data, the present study showed that the antidepressant-like effect of anandamide in the PL is blocked by a 5-HT1A receptor antagonist (WAY100635). Considering that the activation of CB1 receptors located in the PFC increases local serotonin release [23] and knowing that pre-frontal cortex is intensely connected with the raphe nuclei through glutamatergic projections [55], it is possible that CBD, by indirectly activating CB1 receptors, modulates serotonin release in the vmPFC. Our results are consistent with this hypothesis, since the antidepressant-like effect induced by anandamide was blocked by local administration of a 5HT1A antagonist.

Whereas pre-synaptic 5-HT1A receptors are located in cell bodies of serotonergic neurons in the raphe nuclei of the brain stein, post-synaptic 5HT1A receptors are found predominantly in limbic structures, including the prefrontal cortex [56] and [57]. These receptors have been extensively related to the neurobiology of depression and the therapeutic effects of antidepressant drugs [35]. For example, systemic administration of 5-HT1A agonists causes antidepressant-like effects in animal models such as the forced swimming test and olfactory bulbectomy [58], [59] and [60]. In addition, activation of postsynaptic 5-HT1A receptors seems to be required for the antidepressant effects of 5-HT1A agonists and, probably, selective serotonin reuptake inhibitors [61]. Our results, therefore, corroborate the hypothesis that activation of post-synaptic 5-HT1A receptors located in the vmPFC induces antidepressant-like effects. In the PFC, 5-HT1A receptors are found in postsynaptic pyramidal receptors and their stimulation decreases neuronal excitability and glutamate release [62] and [63]. Since inactivation of either the PL or IL or the local blockade of glutamate NMDA receptors promotes antidepressant-like effects [33] and [64], it could be speculated that the antidepressant-like effects observed with the 5-HT1A agonist administration into the vmPFC would involve modulation of glutamate release in these brain regions. This hypothesis, however, warrants further investigation.

In conclusion, the present results show that the activation of 5-HT1A receptors in the PL or IL subregions of the vmPFC decreases immobility in the forced swimming test, indicating an antidepressant-like effect. A similar effect is produced by local administration of CBD into these subregions. This latter effect depends on the activation of local 5-HT1A and CB1 receptors, and could involve CB1 facilitation of the local serotonergic system.

Conflicts of Interest

The authors declare no conflict of interest.

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References

[1] Guimarães FS, Chiaretti TM, Graeff FG, Zuardi AW (1990) Antianxiety effect of cannabidiol in the elevated plus-maze. Psychopharmacology (Berl) 100: 558-9.

[2] Resstel LB, Tavares RF, Lisboa SF, Joca SR, Corrêa FM, Guimarães FS (2009) 5-HT1A receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats. Br J Pharmacol 156: 181-8.

[3] Carlini EA, Cunha JM (1981) Hypnotic and antiepileptic effects of cannabidiol. J Clin Pharmacol 21: 417S-427S.

[4] Cunha JM, Carlini EA, Pereira AE, Ramos OL, Pimentel C, Gagliardi R, Sanvito WL, Lander N, Mechoulam R (1980) Chronic administration of cannabidiol to healthy volunteers and epileptic patients. Pharmacology 21: 175-85.

[5] Jones NA, Hill AJ, Smith I, Bevan SA, Williams CM, Whalley BJ, Stephens GJ (2010) Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo. J Pharmacol Exp Ther. 332(2):569-77.

[6] Casarotto PC, Gomes FV, Resstel LB, Guimarães FS (2010) Cannabidiol inhibitory effect on marble-burying behaviour: involvement of CB1 receptors. Behav Pharmacol 21: 353-8.

[7] Zuardi AW, Rodrigues JA, Cunha JM (1991) Effects of cannabidiol in animal models predictive of antipsychotic activity. Psychopharmacology (Berl) 104: 260-4.

[8] Zuardi AW, Crippa JA, Hallak JE, Moreira FA, Guimarães FS (2006) Cannabidiol, a Cannabis sativa constituent, as an antipsychotic drug. Braz J Med Biol Res 39: 421-9.

[9] Zanelati TV, Biojone C, Moreira FA, Guimarães FS, Joca SR (2010) Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT1A receptors. Br J Pharmacol 159: 122-8.

[10] El-Alfy AT, Ivey K, Robinson K, Ahmed S, Radwan M, Slade D, Khan I, ElSohly M, Ross S (2010) Antidepressant-like effect of delta9-tetrahydrocannabinol and other cannabinoids isolated from Cannabis sativa L. Pharmacol Biochem Behav 95: 434-42.

[11] Campos AC, Moreira FA, Gomes FV, Del Bel EA, Guimarães FS (2012) Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. Philos Trans R Soc Lond B Biol Sci 367: 3364-78.

[12] Petitet F, Jeantaud B, Reibaud M, Imperato A, Dubroeucq MC (1998) Complex pharmacology of natural cannabinoids: evidence for partial agonist activity of

delta9-tetrahydrocannabinol and antagonist activity of cannabidiol on rat brain cannabinoid receptors. Life Sci 63: PL1-6.

[13] Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I, Moriello AS, Davis JB, Mechoulam R, Di Marzo V (2001) Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br J Pharmacol 134: 845-52.

[14] Watanabe K, Ogi H, Nakamura S, Kayano Y, Matsunaga T, Yoshimura H, Yamamoto I (1998) Distribution and characterization of anandamide amidohydrolase in mouse brain and liver. Life Sci 62: 1223-9.

[15] Fernández-Ruiz J, Sagredo O, Pazos MR, García C, Pertwee R, Mechoulam R, Martínez-Orgado J (2013) Cannabidiol for neurodegenerative disorders: important new clinical applications for this phytocannabinoid? Br J Clin Pharmacol 75: 323-33.

[16] Watanabe K, Kayano Y, Matsunaga T, Yamamoto I, Yoshimura H (1996) Inhibition of anandamide amidase activity in mouse brain microsomes by cannabinoids. Biol Pharm Bull 19(8):1109-11.

[17] Rakhshan F, Day TA, Blakely RD, Barker EL (2000) Carrier-mediated uptake of the endogenous cannabinoid anandamide in RBL-2H3 cells. J Pharmacol Exp Ther 292(3):960-7.

[18] Campos AC, Ortega Z, Palazuelos J, Fogaça MV, Aguiar DC, Díaz-Alonso J, Ortega-Gutiérrez S, Vázquez-Villa H, Moreira FA, Guzmán M, Galve-Roperh I, Guimarães FS (2013) The anxiolytic effect of cannabidiol on chronically stressed mice depends on hippocampal neurogenesis: involvement of the endocannabinoid system. Int J Neuropsychopharmacol 16: 1407-19.

[19] Russo EB, Burnett A, Hall B, Parker KK (2005) Agonistic properties of cannabidiol at 5-HT1a receptors. Neurochem Res 30: 1037-43.

[20] Rock EM, Bolognini D, Limebeer CL, Cascio MG, Anavi-Goffer S, Fletcher PJ, Mechoulam R, Pertwee RG, Parker LA (2012) Cannabidiol, a non-psychotropic component of cannabis, attenuates vomiting and nausea-like behaviour via indirect agonism of 5-HT(1A) somatodendritic autoreceptors in the dorsal raphe nucleus. Br J Pharmacol 165(8):2620-34.

[21] Hayakawa K, Mishima K, Nozako M, Ogata A, Hazekawa M, Liu AX, Fujioka M, Abe K, Hasebe N, Egashira N, Iwasaki K, Fujiwara M (2007) Repeated treatment with cannabidiol but not Delta9-tetrahydrocannabinol has a neuroprotective effect without the development of tolerance. Neuropharmacology 52: 1079-87.

[22] Campos AC, Guimarães FS (2008) Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. Psychopharmacology (Berl) 199: 223-30.

[23] Bambico FR, Katz N, Debonnel G, Gobbi G (2007) Cannabinoids elicit antidepressant-like behavior and activate serotonergic neurons through the medial prefrontal cortex. J Neurosci 27:11700-11.

[24] Bambico FR, Cassano T, Dominguez-Lopez S, Katz N, Walker CD, Piomelli D, Gobbi G (2010) Genetic deletion of fatty acid amide hydrolase alters emotional behavior and serotonergic transmission in the dorsal raphe, prefrontal cortex, and hippocampus. Neuropsychopharmacology 35:2083-100.

[25] Häring M, Grieb M, Monory K, Lutz B, Moreira FA (2013) Cannabinoid CB_1 receptor in the modulation of stress coping behavior in mice: the role of serotonin and different forebrain neuronal subpopulations. Neuropharmacology 65:83-9.

[26] Liston C, Miller MM, Goldwater DS, Radley JJ, Rocher AB, Hof PR, Morrison JH, McEwen BS (2006) Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. J Neurosci 26: 7870-4.

[27] Beeman CL, Bauer PS, Pierson JL, Quinn JJ (2013) Hippocampus and medial prefrontal cortex contributions to trace and contextual fear memory expression over time. Learn Mem 20: 336-43.

[28] Resstel LB, Joca SR, Guimarães FG, Corrêa FM (2006) Involvement of medial prefrontal cortex neurons in behavioral and cardiovascular responses to contextual fear conditioning. Neuroscience 143: 377-85.

[29] Kesner RP, Churchwell JC (2011) An analysis of rat prefrontal cortex in mediating executive function. Neurobiol Learn Mem 96: 417-31.

[30] Vertes RP (2004) Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse 51: 32-58.

[31] Sangha S, Robinson PD, Greba Q, Davies DA, Howland JG (2014) Alterations in Reward, Fear and Safety Cue Discrimination after Inactivation of the Rat Prelimbic and Infralimbic Cortices. Neuropsychopharmacology.

[32] Burgos-Robles A, Bravo-Rivera H, Quirk GJ (2013) Prelimbic and infralimbic neurons signal distinct aspects of appetitive instrumental behavior. PLoS One 8: e57575.

[33] Scopinho AA, Scopinho M, Lisboa SF, Correa FM, Guimarães FS, Joca SR (2010) Acute reversible inactivation of the ventral medial prefrontal cortex induces antidepressant-like effects in rats. Behav Brain Res 214: 437-42.

[34] Hamani C, Diwan M, Macedo CE, Brandão ML, Shumake J, Gonzalez-Lima F, Raymond R, Lozano AM, Fletcher PJ, Nobrega JN (2010) Antidepressant-like effects of medial prefrontal cortex deep brain stimulation in rats. Biol Psychiatry 67: 117-24.

[35] Savitz J, Lucki I, Drevets WC. (2009) 5-HT(1A) receptor function in major depressive disorder. Prog Neurobiol 88:17-31.

[36] Cryan JF, Markou A, Lucki I (2002) Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol Sci 23(5): 238-45.

[37] Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates. Academic Press, London.

[38] Joca SR, Guimarães FS (2006) Inhibition of neuronal nitric oxide synthase in the rat hippocampus induces antidepressant-like effects. Psychopharmacology (Berl) 185: 298-305.

[39] Porsolt RD, Le Pichon M, Jalfre M (1977) Depression: a new animal model sensitive to antidepressant treatments. Nature 266: 730-2.

[40] Slattery DA, Cryan JF (2012) Using the rat forced swim test to assess antidepressant-like activity in rodents. Nat Protoc 7:1009-14.

[41] Abel EL, Bilitzke PJ (1990) A possible alarm substance in the forced swimming test. Physiol Behav 48: 233-9.

[42] Gabbott PL, Warner TA, Jays PR, Bacon SJ (2003) Areal and synaptic interconnectivity of prelimbic (area 32), infralimbic (area 25) and insular cortices in the rat. Brain Res 993: 59-71.

[43] Yuen EY, Wei J, Liu W, Zhong P, Li X, Yan Z (2012) Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex. Neuron 73: 962-77.

[44] Silva M, Aguiar DC, Diniz CR, Guimarães FS, Joca SR (2012) Neuronal NOS inhibitor and conventional antidepressant drugs attenuate stress-induced fos expression in overlapping brain regions. Cell Mol Neurobiol 32: 443-53.

[45] Barbas H (1995) Anatomic basis of cognitive-emotional interactions in the primate prefrontal cortex. Neurosci Biobehav Rev 19: 499-510.

[46] Vidal-Gonzalez I, Vidal-Gonzalez B, Rauch SL, Quirk GJ (2006) Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. Learn Mem 13: 728-33.

[47] Tavares RF, Corrêa FM, Resstel LB (2009) Opposite role of infralimbic and prelimbic cortex in the tachycardiac response evoked by acute restraint stress in rats. J Neurosci Res 87:2601-7.

[48] Radley JJ, Arias CM, Sawchenko PE (2006) Regional differentiation of the medial prefrontal cortex in regulating adaptive responses to acute emotional stress. J Neurosci 26: 12967-76.

[49] Marquis JP, Killcross S, Haddon JE (2007) Inactivation of the prelimbic, but not infralimbic, prefrontal cortex impairs the contextual control of response conflict in rats. Eur J Neurosci 25: 559-66.

[50] Heidbreder CA, Groenewegen HJ (2003) The medial prefrontal cortex in the rat: evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. Neurosci Biobehav Rev 27: 555-79.

[51] Campos AC, Ferreira FR, Guimarães FS (2012) Cannabidiol blocks long-lasting behavioral consequences of predator threat stress: possible involvement of 5HT1A receptors. J Psychiatr Res 46: 1501-10.

[52] Mishima K, Hayakawa K, Abe K, Ikeda T, Egashira N, Iwasaki K, Fujiwara M (2005) Cannabidiol prevents cerebral infarction via a serotonergic 5-hydroxytryptamine1A receptor-dependent mechanism. Stroke 36: 1077-82.

[53] Thomas A, Baillie GL, Phillips AM, Razdan RK, Ross RA, Pertwee RG (2007) Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. Br J Pharmacol 150(5):613-23.

[54] Gobbi G, Bambico FR, Mangieri R, Bortolato M, Campolongo P, Solinas M, Cassano T, Morgese MG, Debonnel G, Duranti A, Tontini A, Tarzia G, Mor M, Trezza V, Goldberg SR, Cuomo V, Piomelli D (2005) Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. Proc Natl Acad Sci U S A 102:18620-5.

[55] Jankowski MP, Sesack SR (2004) Prefrontal cortical projections to the rat dorsal raphe nucleus: ultrastructural features and associations with serotonin and gamma-aminobutyric acid neurons. J Comp Neurol 468:518-29.

[56] Chalmers DT, Watson SJ (1991) Comparative anatomical distribution of 5-HT1A receptor mRNA and 5-HT1A binding in rat brain--a combined in situ hybridisation/in vitro receptor autoradiographic study. Brain Res 561: 51-60.

[57] Burnet PW, Eastwood SL, Lacey K, Harrison PJ (1995) The distribution of 5-HT1A and 5-HT2A receptor mRNA in human brain. Brain Res 676: 157-68.

[58] Wieland S, Lucki I (1990) Antidepressant-like activity of 5-HT1A agonists measured with the forced swim test. Psychopharmacology (Berl) 101: 497-504.

[59] Cryan JF, Redmond AM, Kelly JP, Leonard BE (1997) The effects of the 5-HT1A agonist flesinoxan, in three paradigms for assessing antidepressant potential in the rat. Eur Neuropsychopharmacol 7: 109-14.

[60] Carr GV, Lucki I (2011) The role of serotonin receptor subtypes in treating depression: a review of animal studies. Psychopharmacology (Berl) 213: 265-87.

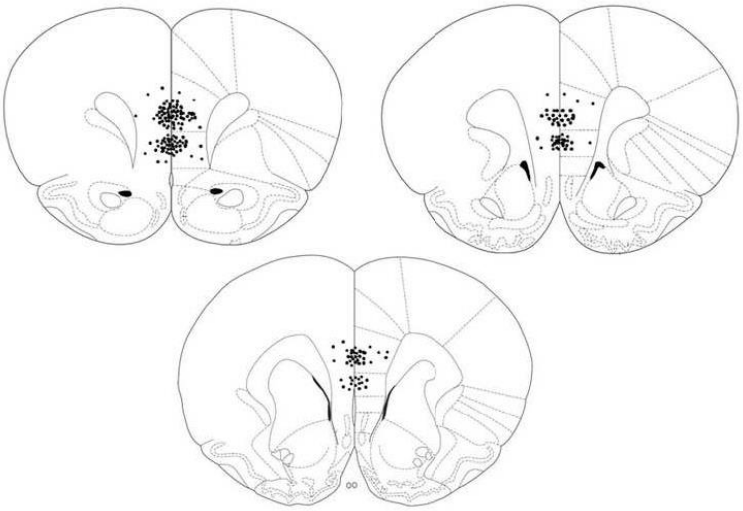
[61] Haddjeri N, Blier P, de Montigny C (1998) Long-term antidepressant treatments result in a tonic activation of forebrain 5-HT1A receptors. J Neurosci 18: 10150-6.

[62] López-Gil X, Artigas F, Adell A (2010) Unraveling monoamine receptors involved in the action of typical and atypical antipsychotics on glutamatergic and serotonergic transmission in prefrontal cortex. Curr Pharm Des 16: 502-15.

[63] Araneda R, Andrade R (1991) 5-Hydroxytryptamine2 and 5-hydroxytryptamine 1A receptors mediate opposing responses on membrane excitability in rat association cortex. Neuroscience 40: 399-412.

[64] Pereira VS, Romano A, Wegener G, Joca SR. (2015) Antidepressant-like effects induced by NMDA receptor blockade and NO synthesis inhibition in the ventral medial prefrontal cortex of rats exposed to the forced swim test. Psychopharmacology (Berl). [Epub ahead of print] PubMed PMID: 25589143.

Figure Captions



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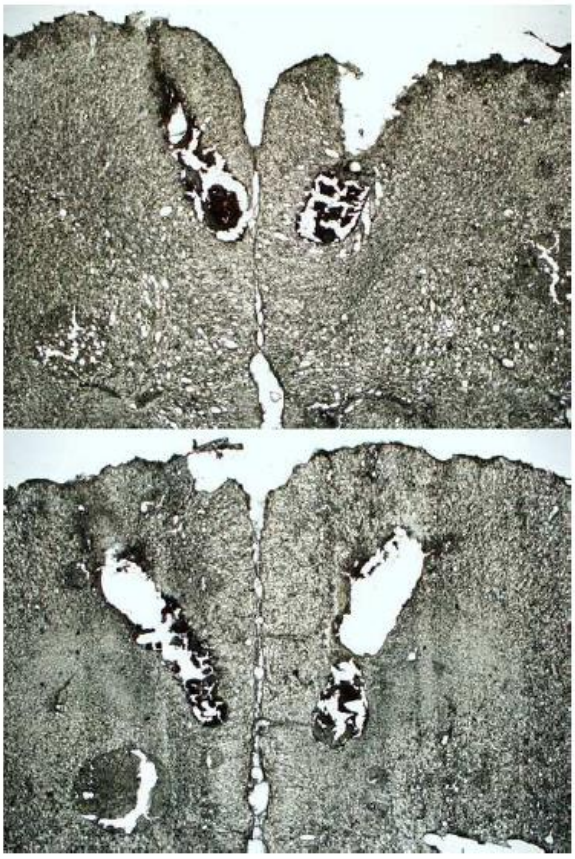
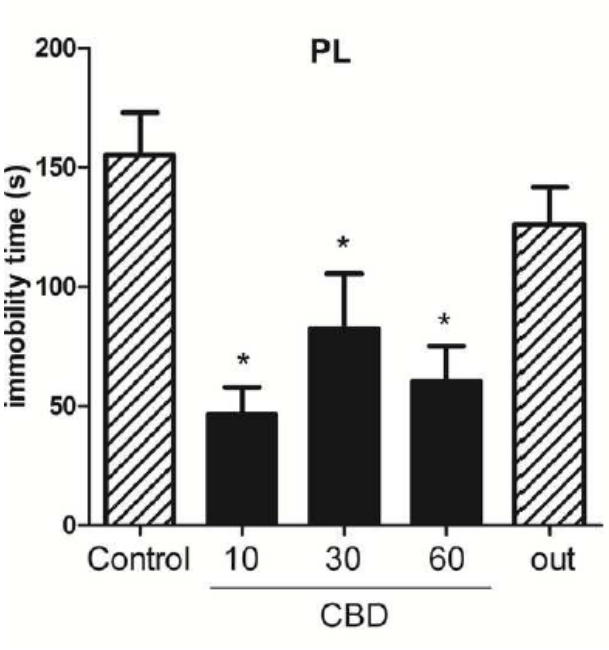


(b)

Figure 1 Histological (a) and photomicrograph (b) localization of injection sites (0.2 µL) located in the ventral medial prefrontal cortex (prelimbic and infralimbic) in diagrams (3.20, 2.70 and 2.20 mm from Bregma) based on the atlas of Paxinos and Watson (1998).



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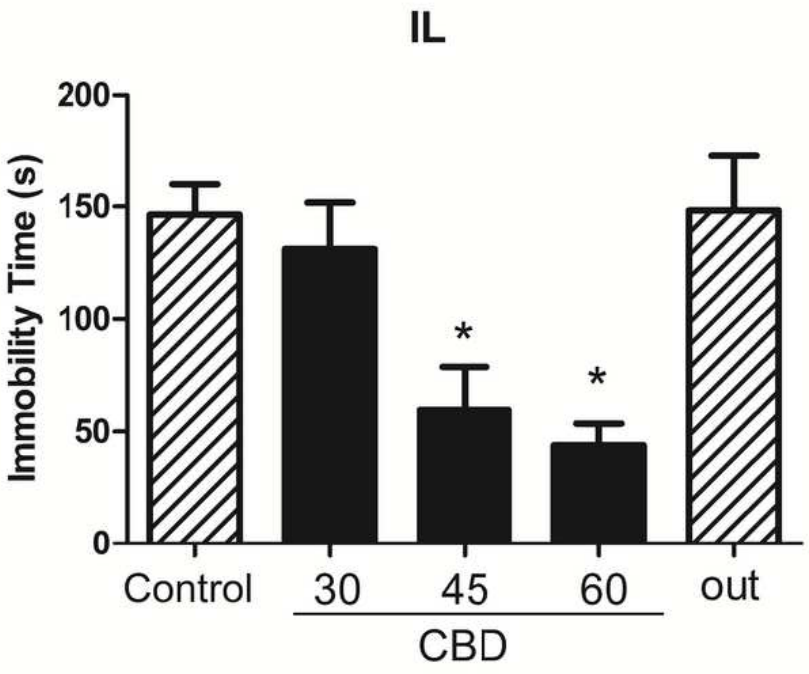
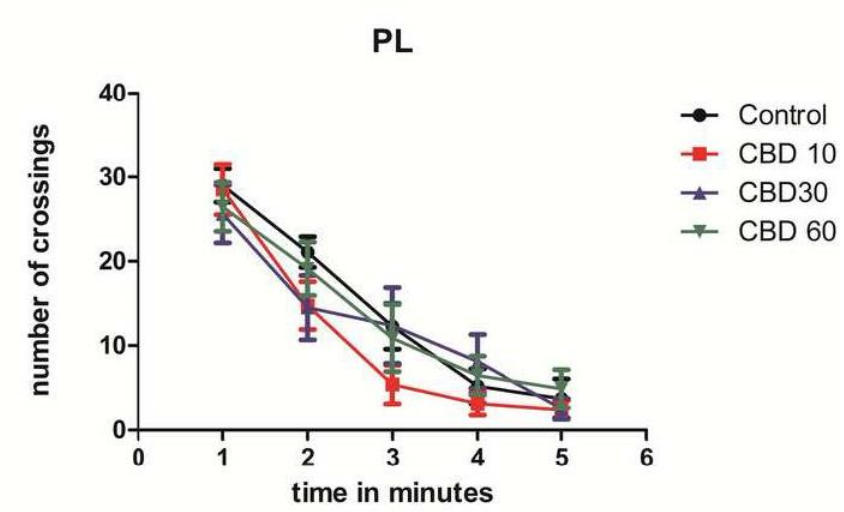


(b)

Figure 2 Effects of CBD in the forced swimming test. **a-** CBD (10, 30 and 60nmol), administered into the PL, induced antidepressant-like effects in the FST ($p < 0.05$, n 7-13/group). **b-** CBD (45 and 60 nmol), administered into the IL, induced antidepressant-like effects in the FST (*$p<0.05$, n 7-10/group). The control group represents vehicle treated animals and "out" group represents animals that received CBD outside the PL or IL. Each bar represents the mean +/- standard error (one-way ANOVA followed by Dunnett's test, $p<0,05$).



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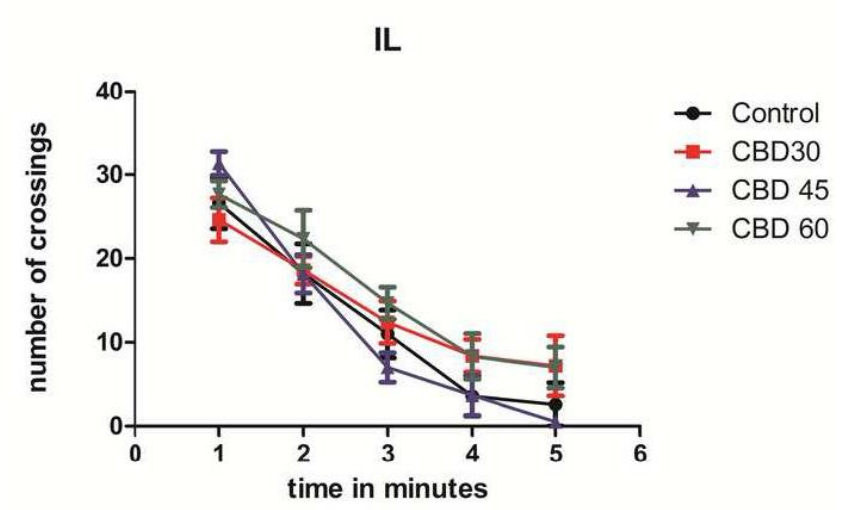
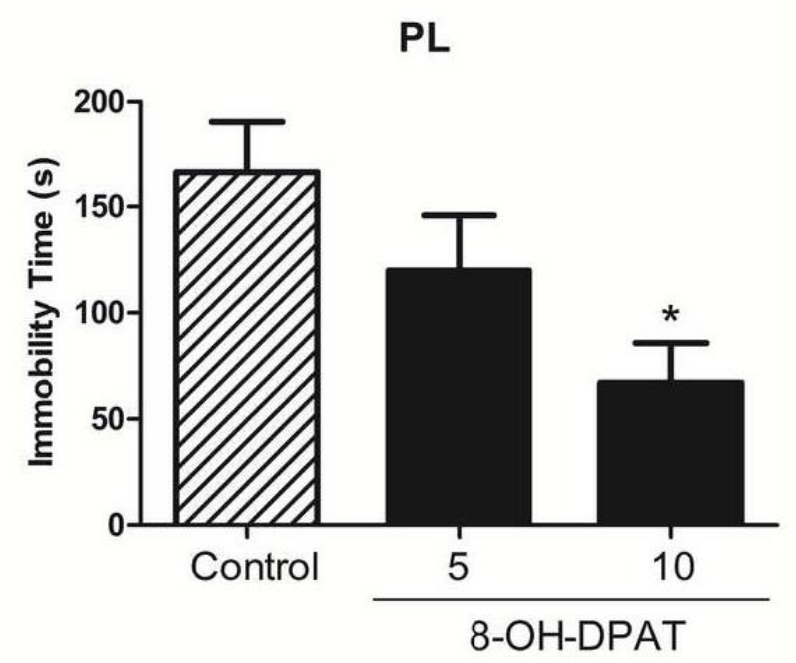


(b)

Figure 3 Effects of CBD in the Open Field Test. **a-** CBD (10, 30 and 60nmol), administered into the PL, did not change locomotor activity in the OFT ($p>0.05$, n 5-11/group). **b-** CBD (30, 45 and 60nmol), administered into the IL, did not change locomotor activity in the OFT ($p>0.05$, n 5-11/group). Points represent the mean +/- standard error (2-way ANOVA, $p>0.05$ for treatment effect).



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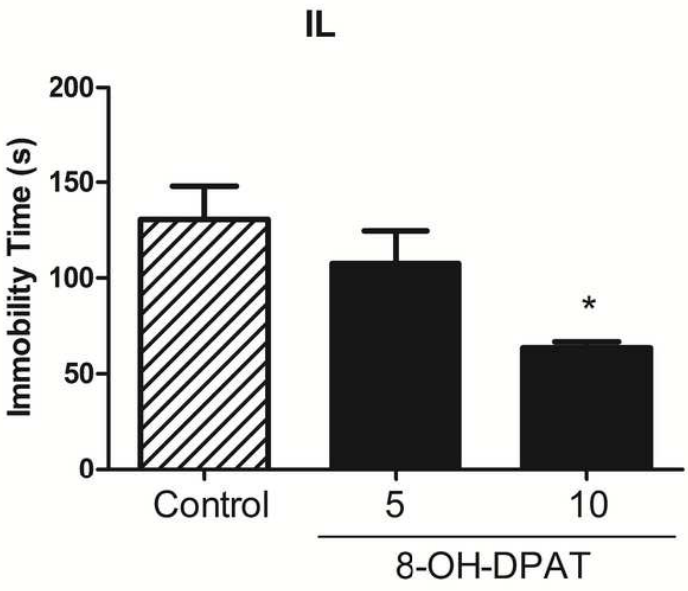
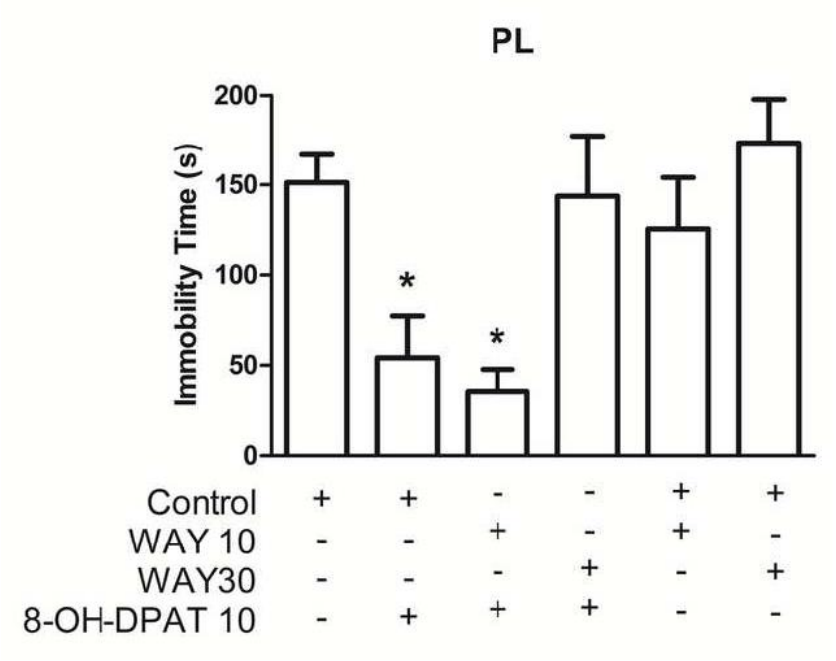


(b)

Figure 4 Effects of 8-OH-DPAT in the forced swimming test. **a-** 8-OH-DPAT (10 nmol), administered into the PL, induced antidepressant-like effects in the FST ($p<0.05$, n 5-8/group). **b-** 8-OH-DPAT (10 nmol), administered into the IL, induced antidepressant-like effects in the FST (*$p<0.05$, n 4-6/group). Control group represents vehicle treated animals. Each bar represents the mean +/- standard error (one-way ANOVA followed by Dunnett's test, $p<0,05$).



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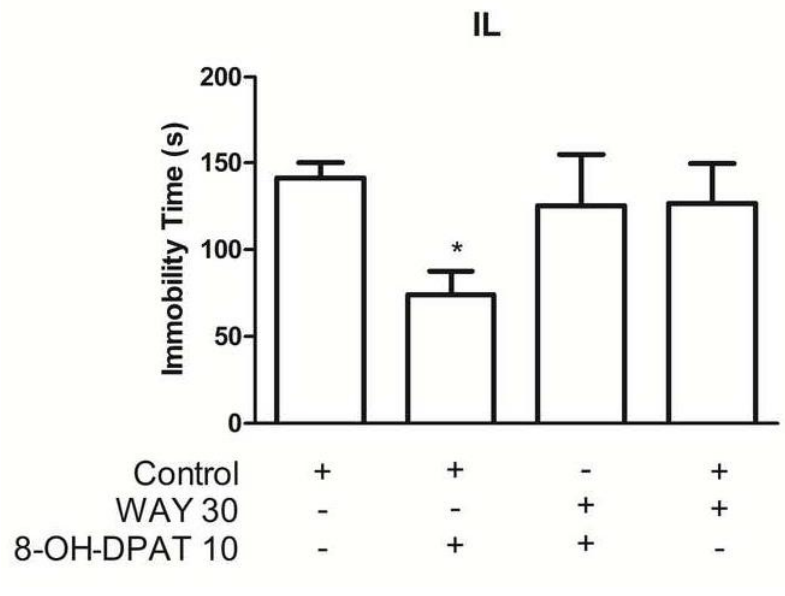
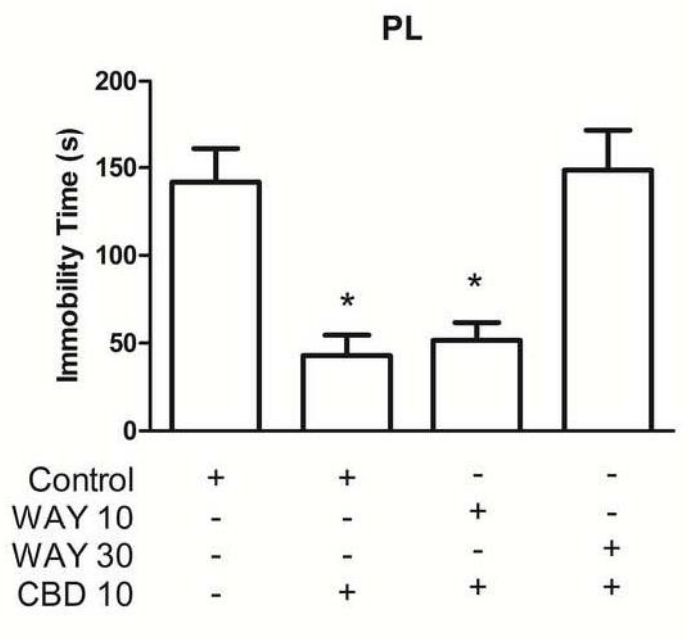


(b)

Figure 5 Effects of WAY100635 and/or 8-OH-DPAT in the forced swimming test. **a-** The 5-HT1A agonist (8-OH-DPAT, 10nmol), administered into the PL, reduced immobility time, an effect that was attenuated by previous treatment with the 5-HT1A antagonist (WAY100635, 30nmol) ($p<0.05$, n 4-8/group). **b-** The 5-HT1A agonist (8-OH-DPAT, 10nmol), administered into the IL, reduced immobility time, an effect that was attenuated by previous treatment with the 5-HT1A antagonist (WAY100635, 30nmol) (*$p<0.05$, n 5-8/group). Control group represents vehicle treated animals. Each bar represents the mean +/- standard error (one-way ANOVA followed by Dunnett's test, $p<0,05$).



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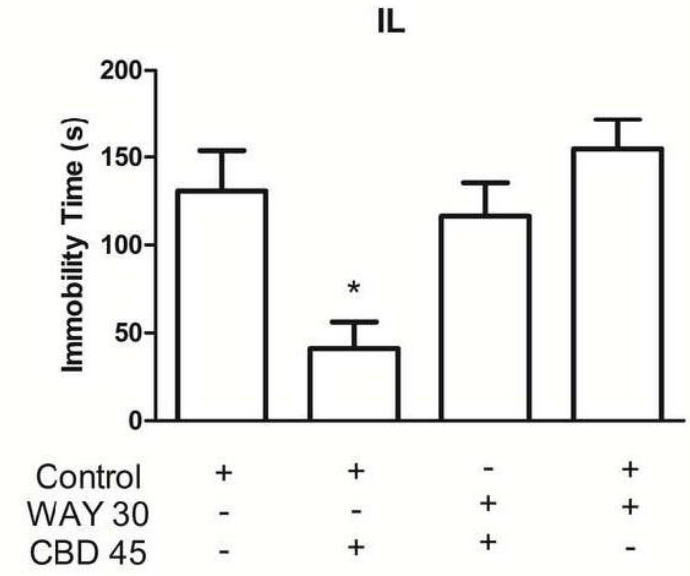
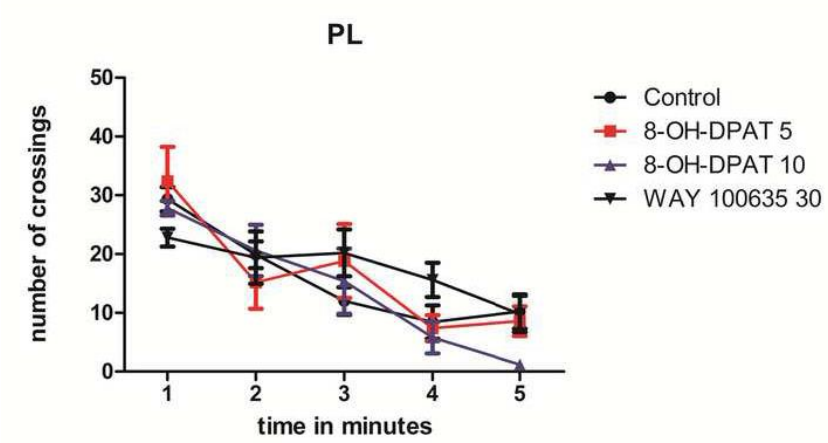


(b)

Figure 6 Effects of WAY100635 and/or CBD in the forced swimming test. **a-** CBD (10nmol), administered into the PL, reduced immobility time, an effect attenuated by previous treatment with the 5-HT1A antagonist (WAY100635, 30nmol) ($p<0.05$, n 6-10/group). **b-** The CBD (45nmol), administered into the IL, reduced the immobility time, an effect that was attenuated by previous treatment with the 5-HT1A antagonist (WAY100635, 30nmol) (*$p<0.05$, n 6-7/group). Control group represents vehicle treated animals. Each bar represents the mean +/- standard error (one-way ANOVA followed by Dunnett's test, $p<0,05$).



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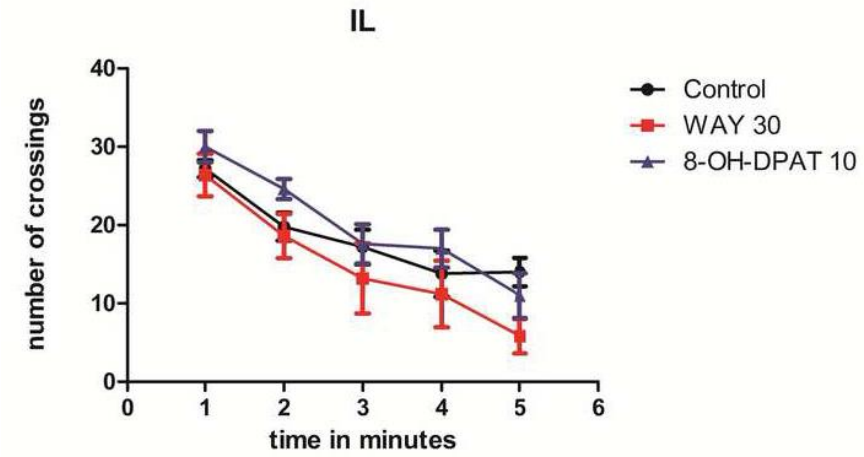

(b)

Figure 7 Effects of 8-OH-DPAT and WAY100635 in the OFT. **a-** 8-OH-DPAT (5 e10nmol) and WAY100635 (30 nmol), administered into the PL, did not change locomotor activity in the OFT ($p>0.05$, n 5-9/group). **b-** 8-OH-DPAT (10nmol) and WAY100635 (30 nmol), administered into the IL, did not change locomotor activity in the OFT ($p>0.05$, n 5/group). Points represent the mean +/- standard error (2-way ANOVA, $p>0.05$ for treatment effect).

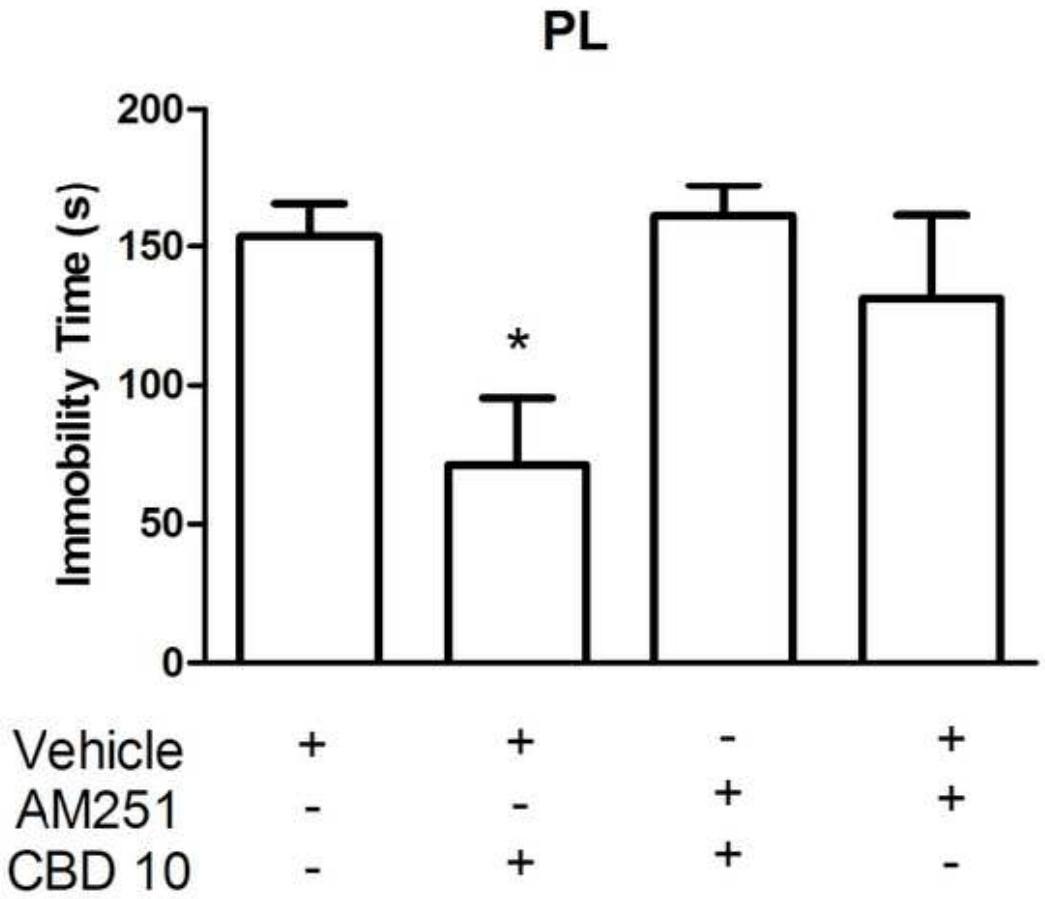

Figure 8 Effects of AM251 and/or CBD in the forced swimming test. CBD (10nmol), administered into PL, reduced immobility time, an effect blocked by previous treatment with the CB1 receptor antagonist (AM251, 10nmol) ($p<0.05$, n 6-7/group). Control group represents vehicle treated animals. Each bar represents the mean +/- standard error (one-way ANOVA followed by Dunnett's test, $p<0,05$).

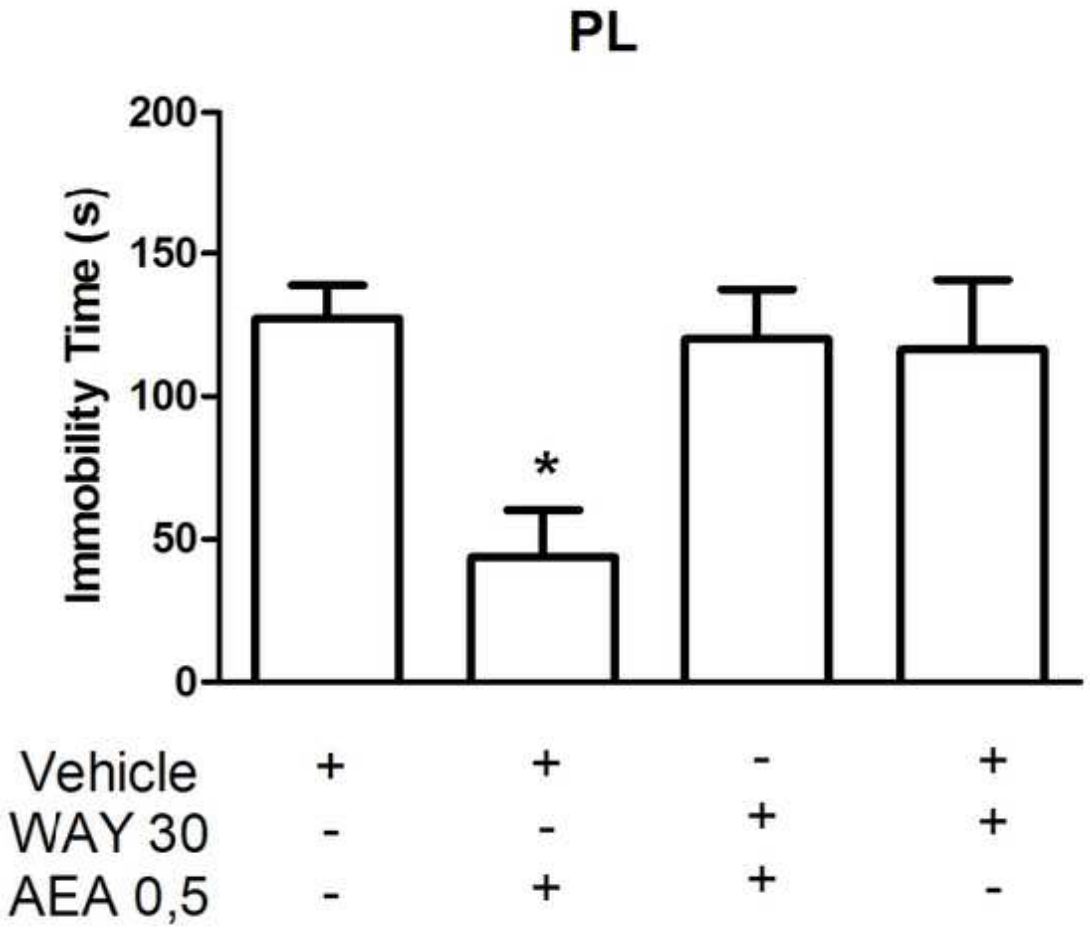

Figure 9 Effects of WAY100635 and/or AEA in the forced swimming test. AEA (0.5pmol), administered into PL, reduced immobility time, an effect blocked by previous treatment with the 5-HT1A antagonist (WAY100635, 30nmol). ($p<0.05$, n 9-12/group). Control group represents vehicle treated animals. Each bar represents the mean +/- standard error (one-way ANOVA followed by Dunnett's test, $p<0,05$).