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Antidepressant-like effect induced by Cannabidiol is dependent on brain serotonin levels

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ABSTRACT

Cannabidiol (CBD) is a compound of *Cannabis sativa* with relevant therapeutic potential in several neuropsychiatric disorders including depression. CBD treatment has shown significant antidepressant-like effects in different rodent preclinical models. However, the mechanisms involved in CBD-induced antidepressant effects are still poorly understood. Therefore, this work aimed at investigating the participation of serotonin (5-HT) and/or noradrenaline (NA) in CBD-induced antidepressant-like effects in the forced swimming test (FST) by: 1) testing if CBD co-administration with serotonergic (fluoxetine, FLX) or noradrenergic (desipramine, DES) antidepressants would have synergistic effects; and 2) investigating if 5-HT or NA depletion would impair CBD-induced behavioral effects. Results showed that CBD (10 mg/kg), FLX (10 mg/kg) and DES (5 mg/kg) induced antidepressant-like effects in mice submitted to FST. Ineffective doses of CBD (7 mg/kg), when co-administered with ineffective doses of FLX (5 mg/kg) or DES (2.5 mg/kg) resulted in significant antidepressant-like effects, thus implicating synergistic and/or additive mechanisms. Pretreatment with PCPA (an inhibitor of serotonin synthesis: 150 mg/kg, i.p., once per day for 4 days), but not DSP-4 (a noradrenergic neurotoxin: 1 µg/µl, i.c.v., 24 h before the test), reduced monoamine levels in the brain. However, only PCPA treatment abolished CBD-induced behavioral effects in FST, indicating the participation of serotonergic mechanisms. None of the treatments induced locomotor effects. Our results suggest that the antidepressant-like effect induced by CBD in the FST is dependent on serotonin levels in the central nervous system (CNS).

1. Introduction

Cannabidiol (CBD) is a major component of *Cannabis sativa* with a broad spectrum of beneficial pharmacological actions in psychiatric disorders, including anxiolytic, antipsychotic, neuroprotective and antidepressant properties (Campos et al., 2016; Ligresti et al., 2016). Accordingly, preclinical studies have demonstrated that administration of CBD induces antidepressant-like effects in several animal models (Zanelati et al., 2010; El-alfy et al., 2010; Réus et al., 2011; Shoval et al., 2016; Linge et al., 2016; Sartim et al., 2016; Schiavon et al., 2016). However, the pharmacological mechanisms responsible for CBD effects remain unclear.

In the central nervous system (CNS), noradrenaline (NA) and serotonin (5-HT) systems play an important role in emotional regulation, stress response and depression neurobiology (Ressler and Nemeroff, 2000). Currently available antidepressant treatments, such as selective serotonin reuptake inhibitors (SSRI) and tricyclic drugs, have their therapeutic effect related to the increase in monoaminergic neurotransmission in limbic brain regions (Ressler and Nemeroff, 2000). Accordingly, the antidepressant action of CBD seems to be mediated by serotonin $5HT_{1A}$ receptors, since systemic administration of WAY100635, a $5\text{-}HT_{1A}$ receptor antagonist, prevented the antidepressant-like effects induced by CBD in both forced swimming test (FST; Zanelati et al., 2010) and olfactory bulbectomy mouse model of depression (OBX, Linge et al., 2016).

Important brain structures involved in depression and antidepressant response, such as the prefrontal cortex (PFC) and hippocampus (HPC), have been associated with the effects induced by CBD. In rats, CBD administration into the ventral medial prefrontal cortex (vmPFC) produces antidepressant-like effects in the forced swimming test (FST), an effect blocked by systemic pretreatment with the $5\text{-}HT_{1A}$ receptor antagonist WAY100635 (Sartim et al., 2016). A similar

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antidepressant-like effect was observed after intra-vmPFC injection of 8-OH-DPAT, a 5-HT_{1A} receptor agonist (Sartim et al., 2016). However, it is not clear if 5-HT_{1A} participation in CBD effects is a consequence of increased serotonin availability or direct receptor activation/facilitation by CBD itself. Therefore, it becomes relevant to investigate CBD effects under conditions of decreased serotonin availability in the CNS.

Additionally, several studies have shown important interactions between cannabinoids and the noradrenergic system (Oropeza et al., 2005, 2007; Page et al., 2007; Fox et al., 2009), but there is no study evaluating the participation of NA in CBD-induced antidepressant-like effects.

The present study, therefore, tested the hypothesis that the antidepressant-like effects induced by CBD involve the facilitation of noradrenergic and/or serotonergic neurotransmitter systems in the brain. To this aim, we investigated if: 1. the co-administration of CBD with serotonergic (fluoxetine, FLX) or noradrenergic (desipramine, DES) reuptake blockers would promote significant antidepressant-like effects in the FST; 2. the depletion of serotonin or noradrenaline levels in the brain would abrogate CBD effects.

2. Materials and methods

2.1. Animals

Male *Swiss* mice (8 weeks) were purchased from the University of São Paulo (USP) breeding facility. The animals were housed in plexiglass cages (200 × 120 × 300 mm), 4 animals per cage, under controlled conditions of temperature (24 ± 1 °C) and lighting (lights on from 06:30 to 18:30 h), with food and water *ad libitum*. The bedding was changed 3 times per week. The experimental protocol was approved by the local Committee of Ethics of the School of Medicine of Ribeirão Preto – USP (protocol n° 072/2014) and carried out in accordance to the guidelines of the Brazilian Council for Control of Animal Experimentation (COBEA) for care and use of laboratory animals, which are in compliance with international laws and politics. All efforts were made to minimize suffering and the number of animals used.

2.2. Drugs and treatment

In this study, we used the following drugs: Cannabidiol (**CBD**, THC Pharma, Frankfurt, Germany): 3, 7 and 10 mg/kg (Zanelati et al., 2010); Fluoxetine (**FLX**, selective inhibitor of serotonin reuptake; Sigma-Aldrich, St. Louis, MO, USA): 1, 5 and 10 mg/kg (Sales and Joca, 2016); Desipramine hydrochloride (**DES**, tricyclic antidepressant; Sigma-Aldrich): 2.5 and 5 mg/kg (Sales and Joca, 2016); Para-Clorophenylalaninemethyl ester (**PCPA**, 5-HT synthesis inhibitor): 100 mg/kg/day during 4 days (Diniz et al., 2017); -N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (**DSP-4**, noradrenergic neurotoxin): 1 μg/μL (Diniz et al., 2017). DES was dissolved in sterile saline; CBD, FLX and PCPA were dissolved in 2% Tween 80/isotonic sterile saline. DSP-4 was dissolved in 10% DMSO/sterile isotonic saline. All drugs, except DSP-4, were administered intraperitoneally (i.p.) in a volume of 10 mL/kg. DSP-4 was administered intracerebroventricularly (i.c.v, 1 μL) to protect peripheral noradrenergic system. In case of co-administration of drugs, injections were given 30 min apart.

2.3. Behavioral experiments

After arrival from the breeding facility, mice were kept in the animal house of the Pharmacology Discipline for two weeks prior to the start of the experiments. They were brought to the experimental rooms two hours prior to the start of the experiments and allowed to habituate to the environment. Animals were randomly sorted to the different treatment groups and tested also in random order to avoid circadian influences on behavioral recordings. All procedures were carried between 14:00–18:00 h. A total of 306 animals were used.

2.3.1. Forced swimming test (FST)

FST was used to measure the immobility time (IT, characterized by floating of the animal with minor movements, only the ones necessary to keep the head above the water) of the mice. A decrease in IT is characteristic of antidepressant-like effects, since this effect is shared by all classical antidepressants (Porsolt et al., 1977; Sales and Joca, 2016). In this test, the animals were placed into a glass cylinder (height 25 cm, diameter 17 cm) with water (10 cm high; 23–25 °C) and forced to swim during the 6 min testing session (inescapable situation) (Sales and Joca, 2016). The test was videotaped and the IT was subsequently measured during the last 4 min. The water was changed after each test (Sales and Joca, 2016).

2.3.2. Open field test (OFT)

The OFT was performed to assess potential changes in locomotor activity that could interfere with mobility in the FST (Sales and Joca, 2016). In this test, the animals were placed in a circular open-field arena (50 cm high, 40 cm diameter) during 6 min. The test was videotaped and the number of crossings between the quadrants of the arena was measured. After each test, the arena was cleaned with 70% alcohol solution (Sales and Joca, 2016).

2.4. Stereotaxic surgery and intracerebroventricular (i.C.V) injection

Mice underwent stereotaxic surgery to have guide-cannulae (0.9 mm, 26 G) implanted into the lateral ventricle (coordinates: AP = +0.3 mm from bregma; L = +1.2 mm; V = −2.5 mm from the skull) (Paxinos and Franklin, 2001). The cannulae were fixed to the skull with acrylic cement. To do that, mice were anesthetized with 2,2,2 tribromoethanol (i.p., 2.5%, 0.1 mL/10 g - Sigma-Aldrich) and placed in the stereotaxic frame (Stoelting, Wood Dale, IL, USA). Immediately after the surgery procedure, the animals received the antibiotic (i.m., Pentabiotic 1.200.000UI – 0.1 mL/mice) to prevent infection in the post-surgical recovery period. After the recovery period (7 days), the animals were submitted to the experiments 3 and 4. The injection into the lateral ventricule (1 μL) was infused during 1 min with the help of a micro-syringe (Hamilton). After the behavioral tests, mice received an anaesthetic injection (i.p., chloral hydrate solution 5%, 0.1 mL/10 g, $C_2H_3Cl_3O_2$, VETEC, Brazil) and were sacrificed by decapitation. Evan's blue dye (1 μL) was injected into the lateral ventricle as a marker of the injection site. The animals that received injections outside the lateral ventricle were excluded from statistical analysis.

2.5. High-performance liquid chromatography (HPLC)

The animals were deeply anesthetized with 5% chloral hydrate (10 mL/kg), decapitated, and had their brain structures (HPC and PFC) dissected and the tissues stored at −80 °C. The samples were homogenized in ultrasound with 0.1 M perchloric acid (400 μL), centrifuged at 13000 rpm for 10 min at 4 °C. The supernatant (40 μL) was injected into the chromatographic system (Waters® Alliance), column Symmetry® C18, 5 μm (150 × 4.6 mm), mobile phase containing 88.03% buffer (citric acid 50 mM, potassium chloride 2 mM, heptane sulfonic acid 1.2 mM, EDTA 0.1 mM) pH 3.2, 9.86% methanol and 2.11% acetonitrile. The mobile phase was vacuum filtered with a membrane (0.45 μm) and flow 1 mL/min during 2–5 min. The serotonin and noradrenaline neurotransmitters were quantified by electrochemical detection in HPLC (Waters® Alliance). The calibration curve was constructed with standard solutions of 1, 2.5, 5, 10, 25, 50 and 100 ng/mL injected in triplicate. The concentrations of the samples were expressed in ng of substance/mg of tissue (Diniz et al., 2017).

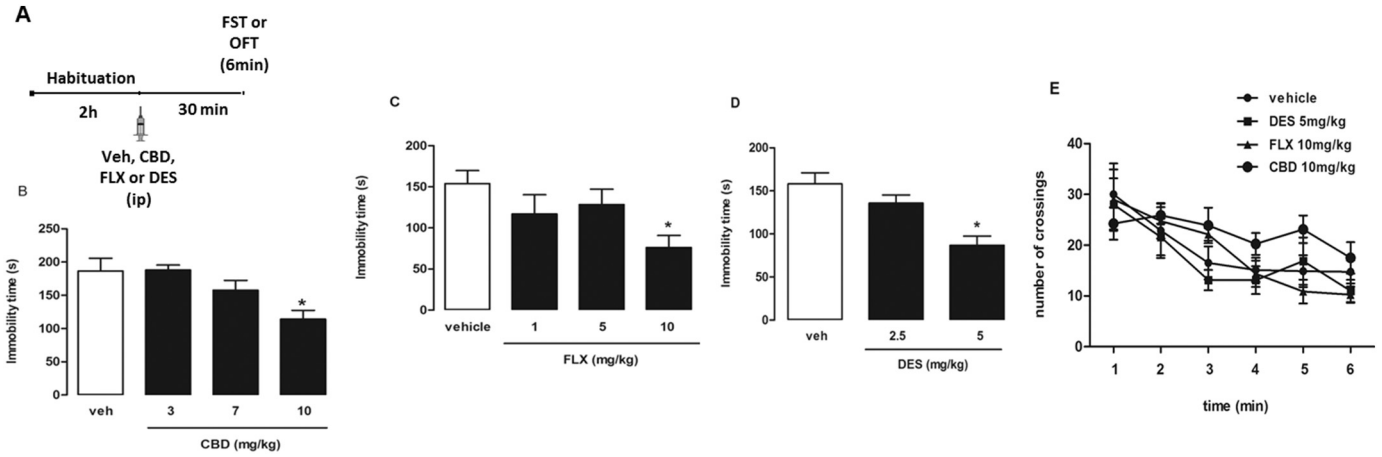

Fig. 1. Systemic administration of CBD, FLX and DES induces antidepressant-like effect in the FST and does not change the number of crossings in the OFT. (A) Representative schedule of the experimental protocol. Mice received injection of CBD (**B**; n = 7–8/group), FLX (**C**; n = 7/group), DES (**D**; n = 6/group) or vehicle (veh) and, 30 min later, their behavior were recorded in a 6 min forced swimming test (B-D) or open field test (**E**; n = 8/group). *$P < .05$, compared to vehicle-treated group.

2.6. Experimental design

Representative schedule of the experimental protocols are shown in Figs. 1A, 2A, 3A–B, 4A, and D.

2.6.1. Experiment 1 - antidepressant and CBD effects in mice submitted to the FST and OFT

The aims of this experiment were to confirm the antidepressant-like effects induced by FLX, DES and CBD; and to determine the subeffective doses of the treatments. The animals received an injection (i.p.) of FLX (1, 5 and 10 mg/kg; n = 7/group), DES (2.5 and 5 mg/kg; n = 6/group), CBD (3, 7 and 10 mg/kg; n = 7–8/group) or vehicle and, 30 min later, were submitted to the FST (6 min), when immobility time was measured during the last four minutes. Independent groups of animals received only the effective doses of the same treatments (FLX: 10 mg/kg; DES: 5 mg/kg and CBD: 10 mg/kg) and were submitted to the OFT, where the number of crossings was measured for six minutes (n = 8/group).

2.6.2. Experiment 2 - behavioral effects of the co-administration of CBD with FLX or DES in mice submitted to the FST and OFT

The aim of this experiment was to assess the behavioral effects induced by combining ineffective doses of CBD with ineffective doses of the two pharmacologically distinct monoaminergic antidepressants (FLX or DES). To do that, animals received injections (i.p.) of vehicle or CBD (7 mg/kg) followed, 5 min later, by vehicle, FLX (5 mg/kg; n = 8/group) or DES (2.5 mg/kg; n = 7–8/group). Thirty minutes later, mice were submitted to the FST (6 min), where immobility time was measured during the last 4 min. Independent groups of animals received the same treatments and were submitted to the OFT, where the number of crossings was measured for six minutes (n = 7–8/group).

2.6.3. Experiment 3 - effects of PCPA and DSP-4 treatments on CBD effects in the FST and OFT

This experiment investigated if CBD-induced behavioral effects would be dependent on intact serotonergic and/or noradrenergic (5-HT or NA) brain levels. To assess serotonergic participation, animals received an injection of vehicle or PCPA (i.p., 100 mg/kg/day, n = 7/group) for four days and, one hour after the last injection, mice received an injection of CBD (i.p., 10 mg/kg). Thirty minutes after that, mice

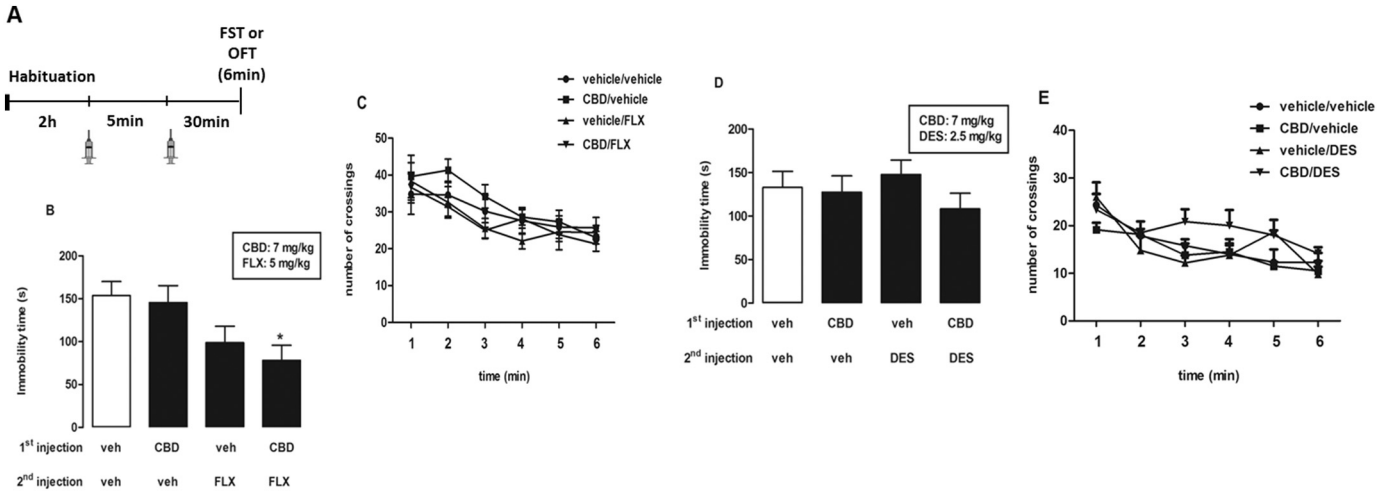

Fig. 2. Systemic administration of CBD and FLX, but not DES, induces antidepressant-like effect in the FST and does not change number of crossings in the OFT. (A) Representative schedule of the experimental protocol. Mice received injection (subeffective doses) of vehicle or CBD and, 5 min later, received injection (subeffective doses) of vehicle or FLX **(B**, n = 8/group; **C**, n = 10/group**)** or DES **(D**, n = 7–8/group; **E**, n = 6/group**).** 30 min later, their behavior were recorded in a 6 min FST **(B and D)** or OFT **(C and E)**. *P < .05, compared to vehicle-treated group.

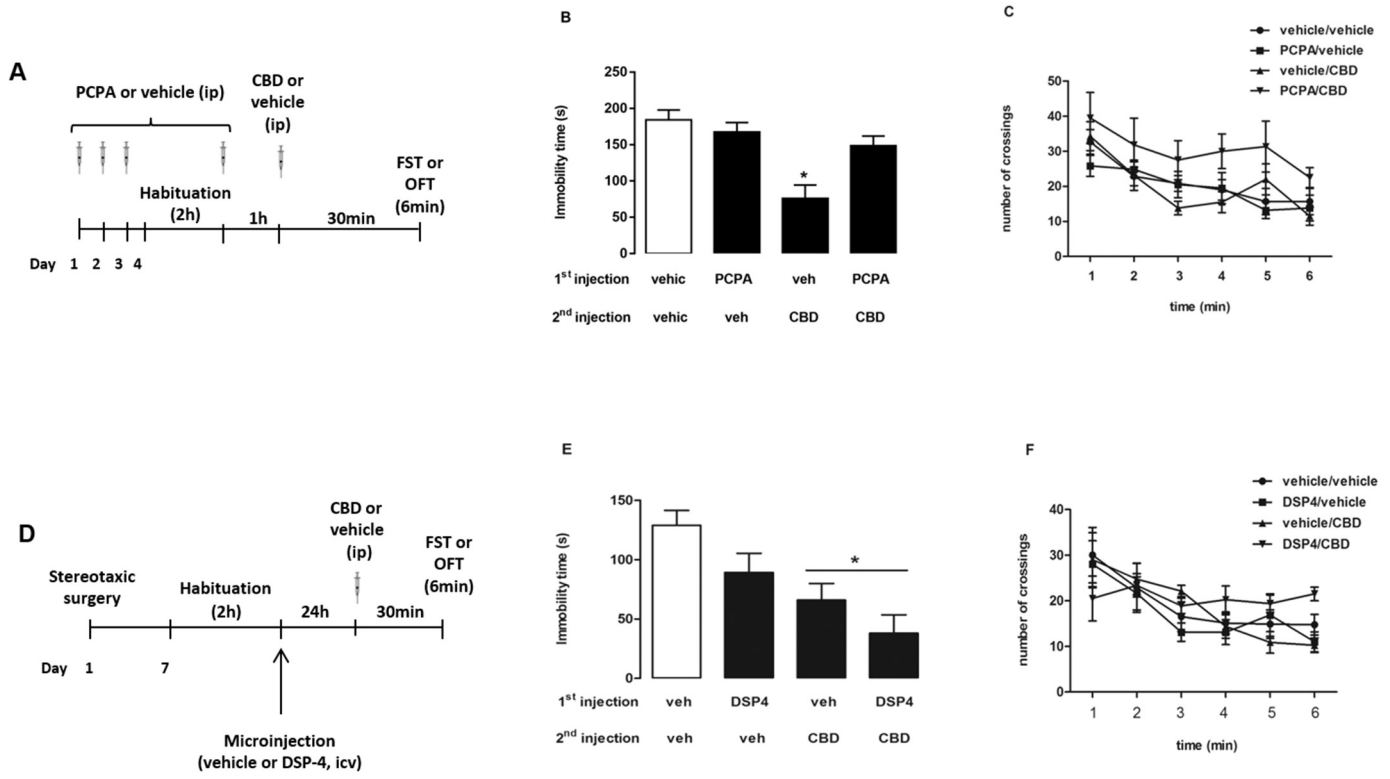

Fig. 3. The pretreatment with PCPA, but not DSP-4, blocks the antidepressant-like effect induced by CBD in the FST. (A) Representative schedule of the experimental protocol with PCPA and CBD administration: Mice received injection of PCPA or vehicle (i.p., 100 mg/kg/day, n = 6/group) every 24 h for 4 day) and 1 h after the last injection, the animals received injection of CBD (10 mg/kg) or vehicle. 30 min after, it was submitted to the FST **(B)** or OFT **(C)**. **(D)** Representative schedule of the experimental protocol with DSP-4 and CBD administration: Mice were submitted to the stereotaxic surgery and after 7 days received i.c.v. injection of the DSP-4 (1 μg/μL, n = 6/group) or vehicle. 24 h later, the animals received a systemic injection of CBD (10 mg/kg) or vehicle and it was submitted to the FST **(E)** or OFT **(F).** *P < .05, compared to vehicle/vehicle-vehicle/CBD group **(B)** and vehicle/vehicle-vehicle/CBD and DSP-4/CBD group **(E).**

were submitted to FST or OFT.

To evaluate noradrenergic participation on CBD effects, independent groups of animals were submitted to the surgery stereotaxic and, after the recovery period (7 days), they received an injection of vehicle or DSP-4 (1 μg/μL, *n* = 6/group) into lateral ventricle. Twenty four hours later, the animals received a systemic injection of CBD (i.p., 10 mg/kg) and were submitted to the FST or OFT, 30 min after that.

2.6.4. Experiment 4 - determination of 5-HT and NA levels in PFC and in mice treated with PCPA or DSP-4

To confirm if PCPA and DSP-4 treatments were able to reduce brain serotonin and noradrenaline levels, respectively, independent groups of animals received vehicle or PCPA (i.p., 100 mg/kg/day, *n* = 6/group) every 24 h for four days. They were killed 1 h after the last injection to have their brain dissected for HPLC analysis. Another group of mice

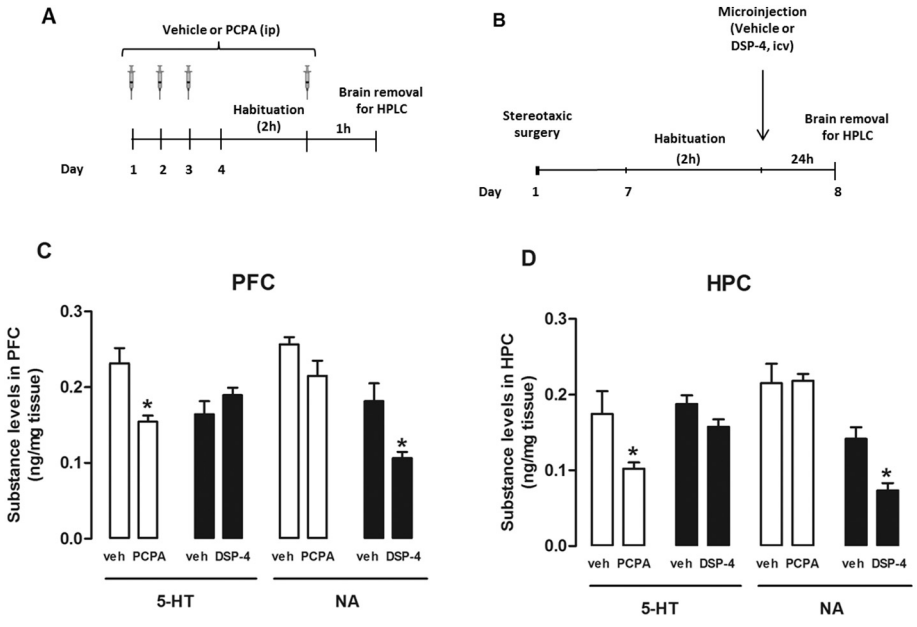

Fig. 4. The treatment with PCPA and DSP-4 selectively reduces 5-HT and NA levels, respectively, in the PFC and HPC. (A-B) Representative schedule of the experimental protocol with PCPA and DSP-4 administration; **(A)** Mice received injection of PCPA or vehicle (i.p., 100 mg/kg/day, n = 6/group) every 24 h for 4 day with the last injection 1 h before brain dissection for HPLC. **(B)** Mice was submitted to the stereotaxic surgery and after 7 days received i.c.v. injection of the DSP-4 (1 μg/μL, n = 6/group). 24 h later, the brain were dissected for HPLC. PCPA and DSP-4 selectively reduce 5-HT and NA levels, respectively, in PFC **(C)** and HPC **(D).**

were submitted to stereotaxic surgery and, 7 days later, received an injection of vehicle or DSP-4 (i.c.v., 1 μg/μL, n = 6/group). Twenty-four h later they were killed and had their brains dissected for posterior HPCL analysis.

2.7. Statistical analysis

Treatment effects on immobility time were compared using one-way ANOVA followed by Dunnett's test for *post hoc* comparisons. In case of treatment combinations, a Two-way ANOVA followed by Bonferroni's test was employed with the factors being treatment and injection. Data from OFT were analyzed by a two-way repeated measures ANOVA with the factors being treatment and time. The comparison was performed for each minute test. The Student's *t*-test was used to compare neurotransmitter levels in HPLC measurements. Probability < .05 was accepted as significant. GraphPad Prism 7.0 software was used for statistical analysis.

3. Results

3.1. Experiment 1 - behavioral effects of the administration of CBD, FLX and DES in mice submitted to FST and OFT

CBD significantly reduced the immobility time at the higher dose tested only (10 mg/kg; $F_{3,25} = 6.104$, $p < .05$; Fig. 1B). Similar effects were observed in response to FLX (10 mg/kg; $F_{3,24} = 3.077$, $p < .05$; Fig. 1C) and DES (5 mg/kg; $F_{3,15} = 10.95$, $p < .05$; Fig. 1D). Therefore, the intermediate doses of CBD (7 mg/kg), FLX (5 mg/kg) and DES (2.5 mg/kg) were considered subeffective. Regarding the OFT, the exploration reduced over time (time factor: $F_{5,140} = 13.89$, $p < .05$), but no effect of treatment ($F_{3,140} = 1.044$, $p > .05$) or interaction between factors was observed (treatment *vs* time: $F_{15,140} = 1.224$, $p > .05$; treatment: Fig. 1E).

3.2. Experiment 2 - behavioral effects of the co-administration of CBD with FLX or DES in mice submitted to the FST and OFT

CBD (7 mg/kg) and FLX (7 mg/kg) were ineffective when administered alone, but significantly decreased the immobility time in the FST when administered in combination (interaction: $F_{1,28} = 0.1174$, $p > .05$; injection 1: $F_{1,28} = 0.6211$, $p > .05$; injection 2: $F_{1,28} = 11.28$; $p < .05$; Fig. 2B). In contrast, the association of CBD (7 mg/kg) and DES (2.5 mg/kg) in subeffective doses did not result in significant antidepressant-like effect (interaction: $F_{1,26} = 0.8855$, $p > .05$; injection 1: $F_{1,26} = 1.549$, $p > .05$; injection 2: $F_{1,26} = 0.0135$; $p > .05$; Fig. 2C). No locomotor changes were induced by any of the treatments in the OFT (CBD/FLX: interaction: $F_{15,180} = 0.7974$, $p > .05$; time: $F_{5,180} = 16.11$, $p < .05$; treatment: $F_{3,180} = 0.846$, $p > .05$; Fig. 2C; CBD/DES: interaction: $F_{15,90} = 1.277$, $p > .05$; time: $F_{5,90} = 10.68$, $p < .05$; treatment: $F_{3,90} = 4.727$, $p < .05$; Fig. 2E).

3.3. Experiment 3 - effects of PCPA and DSP-4 treatments on CBD effects in the FST and OFT

PCPA attenuated the behavioral effects induced by CBD in FST (interaction: $F_{1,12} = 6.98$, $p < .05$; injection 1: $F_{1,12} = 5.24$, $p < .05$; injection 2: $F_{1,12} = 13.83$, $p < .05$; Fig. 3B), without inducing locomotor changes in OFT (interaction: $F_{15,100} = 1.026$, $p > .05$; time: $F_{5,100} = 14.40$, $p < .05$; treatment: $F_{3,100} = 2.698$, $p > .05$; Fig. 3C). On the other hand, DSP-4 pretreatment failed to block CBD effects in the (FST: interaction: $F_{1,20} = 0.1652$, $p > .05$; injection 1: $F_{1,20} = 5.376$, $p < .05$; injection 2: $F_{1,20} = 15.38$, $p < .05$; Fig. 3E). Also, no effect was observed in the OFT (interaction: $F_{15,140} = 1.738$, $p > .05$; time: $F_{5,140} = 12.04$, $p < .05$; treatment: $F_{3,140} = 0.3852$, $p > .05$; Fig. 3F).

3.4. Experiment 4 - determination of 5-HT and NA levels in the PFC of mice treated with PCPA or DSP-4

PCPA and DSP-4 selectively reduced 5-HT and NA levels, respectively, in the PFC [**PCPA** (5-HT: $t_{10} = 3.559$, $p < .05$; NA: $t_{10} = 1.871$, $p > .05$); **DSP-4** (5-HT: $t_{10} = 1.281$, $p > .05$; NA: $t_{10} = 3.027$, $p < .05$); Fig. 4C] and HPC [**PCPA** (5-HT: $t_8 = 2.324$, $p < .05$; NA: $t_{10} = 0.1214$, $p > .05$); **DSP-4** (5-HT: $t_{10} = 1.976$, $p > .05$; NA: $t_{10} = 3.778$, $p < .05$); Fig. 4D]. Still, the statistical analysis indicated significant difference between vehicle treated animals of DSP-4 compared with vehicle treated animals of PCPA group [$p < .05$; $n = 6$/group; **PFC** (5-HT: $t_{10} = 2.544$ and NA: $t_{10} = 2.963$); **HPC** (NA: $t_{10} = 2.443$)].

4. Discussion

The main purpose of the present work was to investigate the participation of serotonin and/or noradrenaline in CBD-induced antidepressant-like effects in the FST. The results showed that the combined administration of subeffective doses of CBD with subeffective doses of FLX, but not DES, reduced immobility time in the FST. Moreover, serotonin reduction abrogated the antidepressant-like effects of CBD in the FST. Noradrenaline reduction produced no effect in this test. Together, the results suggest that CBD antidepressant-like effect in the FST depends on serotonin, but not noradrenaline, levels in the CNS.

Monoamines have a well-established key role in mood regulation and behavioral and emotional responses to stress. It has long been suggested that dysregulation of serotonin and noradrenaline levels, or their target receptors, participate in the pathophysiology of depression (revised in Yohn et al., 2017 and Montoya et al., 2016). Accordingly, the central serotoninergic and noradrenergic systems are the main targets of classical antidepressants, including serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs). These drugs increase monoaminergic neurotransmitters levels in the synaptic cleft through reuptake inhibition (Frazer and Morilak, 2005; Pariante, 2003). Conversely, depletion of serotonin or noradrenaline levels dampens the mood-improving effects of serotonergic (FLX) and noradrenergic (DES) antidepressants, respectively, in remitted depressive patients (Lucki and O'Leary, 2004). This clearly indicates that monoamines are necessary for the therapeutic effect of conventional serotonergic and noradrenergic antidepressant drugs.

In animals, monoaminergic antidepressant drugs decrease immobility time in the FST. This animal model is the most widely used test to detect antidepressant-like effects, showing acceptable predictive validity when used with this aim (Porsolt et al., 1977; Borsini and Meli, 1988; Chourbaji et al., 2005; Frazer and Morilak, 2005). As in humans, the antidepressant-like effect of these drugs is sensitive to monoamine levels. Serotonin depletion blocks the behavioral effects of serotonergic drugs in the FST, whereas noradrenaline depletion abrogates those of noradrenergic drugs (Page et al., 1999).

In mice, acute CBD administration (i.p. and intra-vmPFC) exhibited a dose-dependent antidepressant-like effect in the FST and the tail suspension test (Zanelati et al., 2010; El-alfy et al., 2010; Réus et al., 2011; Schiavon et al., 2016; Sartim et al., 2016). It also reversed the hyperactivity observed in the olfactory bulbectomy mouse model of depression (OBX) (Linge et al., 2016). In Wistar Kyoto rats, a genetic animal model of depression (Overstreet, 2012), CBD oral administration (30 mg/kg) showed a prohedonic effect in the saccharin preference test (SPT) (Shoval et al., 2016). Corroborating these results, the current study found that acute injection of CBD (10 mg/kg) induces antidepressant-like effect in the FST without changing locomotor activity in the OFT.

Evidence suggests that, despite a somewhat complex pharmacology (Bisogno et al., 2001; De Petrocellis et al., 2012; Giacoppo et al., 2017; Carrier et al., 2006; Campos and Guimarães, 2009; Roser et al., 2010; Castillo et al., 2010; Campos et al., 2012; Ligresti et al., 2016), the

antidepressant-like effect of CBD is mediated by activation of 5-HT_{1A} receptors (Zanelati et al., 2010; Sartim et al., 2016). In fact, pretreatment with WAY100635, a 5-HT_{1A} antagonist (i.p.: 0.1 mg/kg, intra-vmPFC: 30 nmol/side), blocked antidepressant-like effects induced by systemic administration of CBD in mice FST (Zanelati et al., 2010; Sartim et al., 2016). However, when injected into the prelimbic cortex, CBD effects in the FST were blocked by both local CB1 and 5-HT_{1A} antagonism (Sartim et al., 2016). Considering that the effects of anandamide in this brain region were also blocked by previous local 5-HT_{1A} blockade, it was suggested that CBD effects on 5-HT_{1A} was indirect to an endocannabinoid-mediated facilitation of local serotonin levels (Sartim et al., 2016). In agreement with that, a microdialysis study demonstrated that acute systemic administration of CBD increased extracellular 5-HT levels in the ventromedial prefrontal cortex (vmPFC) in OBX mice and WAY100635 (0.3 mg/kg) prevented both the antidepressant-like effect and increased cortical 5-HT neurotransmission induced by CBD treatment (Linge et al., 2016). Altogether, this data suggests that CBD effects would mainly involve the activation of post-synaptic 5-HT_{1A} receptors in limbic brain regions, such as the vmPFC. However, it cannot be discarded that somatodendritic 5-HT_{1A} autoreceptors could also play an important role, especially upon repeated treatment, as it is the case for monoaminergic antidepressants (Albert and François, 2010).In summary, CBD induces antidepressant-like effects possibility by enhancing 5-HT neurotransmission followed by subsequent activation of post-synaptic 5-HT_{1A} receptors. In addition to this mechanism, however, there is also evidence that CBD can facilitate, probably by an allosteric mechanism, serotonin action on 5-HT_{1A} receptors (Rock et al., 2012).

To further examine the participation of monoamines in the antidepressant action of CBD, we performed in mice submitted to the FST, the association of CBD and antidepressant drugs of different pharmacological classes (noradrenergic and serotonergic), both in subeffective doses. The results of this study showed that the combination of CBD with FLX, a SSRI, induce antidepressant-like effect in FST. On the other hand, the association of CBD with the tricyclic noradrenergic antidepressant, DES, did not show synergistic/additive effects. These results indicated that a facilitation of serotonin, but not noradrenalin, levels would be more likely involved in CBD-induced behavioral effects in the FST. In addition to providing important mechanistic information regarding mechanism of action, drug combination studies may also facilitate some favorable perspectives to the therapeutic point of view, such as 1) increasing the efficacy of the final therapeutic effect; and/or 2) decreasing the dosage of each individual drug can potentially decrease side effects while maintaining the same overall efficacy (Chou, 2006). For these therapeutic benefits, drug combinations strategies have been widely used and became important choice in the treatment for several diseases. In the present study, we primarily aimed at providing evidence regarding monoamine involvement on CBD effects. However, our data also allow us to argue that CBD could facilitate the antidepressant effect induced by serotoninergic antidepressant drugs, thus allowing SSRIs to be administered at lower doses, which could result in fewer side-effects. This, hypothesis remains to be tested.

The results of the drug combination studies were further supported by neurochemical experiments which revealed that the antidepressant-like effect induced by CBD was prevented by PCPA, a drug that depletes serotonin in the CNS by inhibiting tryptophan hydroxylase (TH) (Koe and Weissman, 1966); but not by DSP-4, a selective neurotoxin for the noradrenergic system (Ross and Stenfors, 2015). In this study, a limited reduction of serotonin levels was able to prevent behavioral effects induced by CBD suggesting that the full functional serotonin nerve endings in PFC and HPC are needed for the antidepressant-like effects of CBD. These effects were independent of locomotor activity changes since no effect were observed in the OFT.

PCPA and DSP-4 selectively reduced 5-HT and NA levels, respectively in the PFC and HPC, two brain areas implicated in depression neurobiology and antidepressant effects. In fact, several reports described similar results in these regions after treatment with PCPA or DSP-4, and demonstrated that they can interfere with the behavioral effects of antidepressant drugs (Shutoh et al., 2000; Fletcher et al., 2001; Page et al., 1999; Dailly et al., 2006). Interestingly, animals submitted to the stereotaxic surgery and treated with vehicle (i.c.v.) showed reduced monoamines levels in PFC (5-HT and NA) and HPC (NA) in comparison with vehicle-treated animals of the PCPA group (i.p. injections), thus suggesting that the stress of the surgery and/or i.c.v. injection could have affected the basal levels of 5-HT and NA.

In conclusion, our results are in line with previous studies suggesting that the antidepressant-like effect induced by CBD is dependent on serotonin levels. In addition, we show for the first time that noradrenaline does not appear to be involved in CBD effects in the FST. However, the mechanisms through which CBD may facilitate 5-HT neurotransmission are not clear and require further investigation.

Conflicts of interest

The authors declare no conflict of interest.

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