



Cannabidiol Induces Rapid and Sustained Antidepressant-Like Effects Through Increased BDNF Signaling and Synaptogenesis in the Prefrontal Cortex

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Abstract

Currently available antidepressants have a substantial time lag to induce therapeutic response and a relatively low efficacy. The development of drugs that addresses these limitations is critical to improving public health. Cannabidiol (CBD), a non-psychotomimetic component of *Cannabis sativa*, is a promising compound since it shows large-spectrum therapeutic potential in preclinical models and humans. However, its antidepressant properties have not been completely investigated. Therefore, the aims of this study were to investigate in male rodents (i) whether CBD could induce rapid and sustained antidepressant-like effects after a single administration and (ii) whether such effects could be related to changes in synaptic proteins/function. Results showed that a single dose of CBD dose-dependently induced antidepressant-like effect (7–30 mg/kg) in *Swiss* mice submitted to the forced swim test (FST), 30 min (acute) or 7 days (sustained) following treatment. Similar effects were observed in the Flinders Sensitive and Flinders Resistant Line (FSL/FRL) rats and the learned helplessness (LH) paradigm using Wistar rats. The acute antidepressant effects (30 min) were associated with increased expression of synaptophysin and PSD95 in the medial prefrontal cortex (mPFC) and elevated BDNF levels in both mPFC and hippocampus (HPC). CBD also increased spine density in the mPFC after 30 min, but not 7 days later. Intracerebroventricular injection of the TrkB antagonist, K252a (0.05 nmol/μL), or the mTOR inhibitor, rapamycin (1 nmol/μL), abolished the behavioral effects of CBD. These results indicate that CBD induces fast and sustained antidepressant-like effect in distinct animal models relevant for depression. These effects may be related to rapid changes in synaptic plasticity in the mPFC through activation of the BDNF-TrkB signaling pathway. The data support a promising therapeutic profile for CBD as a new fast-acting antidepressant drug.

Keywords Cannabidiol · Antidepressant · BDNF · Prefrontal cortex

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Introduction

Major depressive disorder (MDD) is a recurrent condition, being among the leading contributors to social and economic burden, affecting approximately 20% of the global population [1]. According to the World Health Organization, MDD is a leading cause of disability [2]. The current pharmacological treatment approaches indicate the use of serotonin reuptake inhibitors (SSRI) as first-line medications [3, 4]. However, several weeks of treatment are needed to induce a therapeutic response, and up to 33% of the patients are considered treatment-resistant, failing to respond to two or more treatment attempts [5]. Also, the adherence of patients to these medicines is relatively low, as they cause several undesired side effects [6, 7].

In recent years, new fast-acting effective antidepressants have been proposed based on promising data from clinical and preclinical studies [8, 9]. Among them, ketamine, a mixed profile drug with high affinity for the NMDA-receptor, is the most studied [10]. Ketamine seems to disinhibit glutamate release, facilitating neuroplastic changes in several brain areas, including the prefrontal cortex (PFC), which in turn contributes to the restoration of the neuronal circuitries altered in stress and depression [11]. Importantly, a single dose of ketamine rapidly increases both intra- and extracellular brain-derived neurotrophic factor (BDNF), which could constitute part of its fast-acting antidepressant mechanism [12]. Stress and depression are associated to decreased BDNF levels and expression of its receptor tropomyosin-related kinase B (TrkB) in the hippocampus (HPC) [13, 14] and PFC [15, 16].

BDNF binds and activates TrkB receptors, triggering multiple intracellular signaling cascades (reviewed in [17, 18]), such as those regulated by the mammalian target of rapamycin complex 1 (mTORC1), resulting in fast protein synthesis and synaptogenesis [19–21]. Therefore, the increase in BDNF levels induced by ketamine may activate TrkB-mTOR signaling, thereby contributing to its sustained antidepressant effects [17, 22].

Although ketamine is effective in reducing depressive symptoms, it produces psychotomimetic undesired effects, even in low doses [23]. In this sense, the discovery of new drugs that could act as rapid antidepressants without inducing significant side effects is of great importance. In this scenario, cannabidiol (CBD), a non-psychotomimetic cannabinoid present in the *Cannabis sativa* plant, seems to be a promising compound. It has shown therapeutic potential in different psychiatric disorders, including anxiety, schizophrenia, and epilepsy, with significant effects in humans and rodent models [24]. Importantly, antidepressant-like effects have been described for CBD in the forced swimming test, in the tail suspension test, and in the olfactory bulbectomy model [25–30]. However, CBD antidepressant effects have not been tested in animal models with more appropriate face and construct validity. It also remains to be investigated if CBD can induce acute and sustained effects.

The mechanisms involved in CBD-induced psychotropic effects are not entirely understood. CBD activates 5-HT_{1A} and peroxisome proliferator-activated (PPAR γ) receptors [31, 32]. It can also facilitate endocannabinoid signaling through inhibition of the fatty acid amide hydrolase enzyme (FAAH) [33, 34]. Additionally, CBD increases BDNF and mTOR signaling in models of neurodegeneration [35, 36]. It is not known, however, if these mechanisms participate in CBD-induced antidepressant effects.

Therefore, the aim of the present study was, for the first time, to investigate whether (i) CBD could produce acute and sustained antidepressant-like effects in distinct animal models, and (ii) to assess whether such effects would involve facilitation of BDNF signaling and neuroplastic mechanisms.

Materials and Methods

Animals

Male *Swiss* mice (25–30 g, 8 weeks) and male *Wistar* from the FMRP-USP Facility, *Sprague-Dawley* (SD) from Taconic (Copenhagen—Denmark), and *Flinders Resistant* (FRL) or *Flinders Sensitive* (FSL, 280–350 g) line rats from Translational Neuropsychiatry Unit—Aarhus University breeding colonies—were used to conduct the experiments. Mice were housed in groups of 10 animals per cage (1147 cm²). *Wistar* rats were housed individually (570 cm²), and SD, FRL, and FSL rats were housed in pairs. All animals were housed in temperature-controlled room (23 ± 2 °C) with a 12/12-h light-dark cycle (lights on 6:30 a.m./ lights off 6:30 p.m.). Food and water were available ad libitum throughout the study period. The total number of animals used in the present study was 367.

The protocols described in the present study were approved by the respective ethical committees (Danish National Committee for Ethics in Animal Experimentation (2012-15-3934-00254) and CETEA (no. 072/2014), and all efforts were made to minimize animal suffering and to reduce the number of animals used.

Drugs and Reagents

Cannabidiol (CBD, THC Pharma, Germany, 7, 10, and 30 mg kg⁻¹) was dissolved in Tween 80 (Synth, Brazil) 2% in sterile isotonic saline for systemic administration in mice and *Wistar* rats [25], or in an aqueous solution containing 30% of 1,2-Propyleneglycol (Riedel-de Haen, Germany) and 2.5% of ethanol (CCS Healthcare AB, Sweden) for systemic administration in SD, FRL, and FSL rats (according to pilot experiments). For intracerebral administration, CBD (50, 150, and 300 nmol/μL) was dissolved in grape fruit oil, as described in [29]. K252a (Sigma-Aldrich, St. Louis, MO, USA, 0.05 nmol μL⁻¹; [37]) and S-Ketamine (Pfizer, IL, USA, 15 mg kg⁻¹; [38]) were dissolved

in sterile isotonic saline, and rapamycin (LC laboratories, MA, USA, 1 nmol μL^{-1}) in DMSO [39]. When two drugs used in the same experiment had different vehicles, half of the animals in the final control group received administration of each vehicle group.

Experimental Design

Experiment 1—Evaluation of Acute and Sustained Effects Induced by CBD in Mice Submitted to the FST

After 2 h habituation in the experimental room, mice received intraperitoneal (ip) injections of CBD (7, 10, and 30 mg kg^{-1}) or vehicle (10 mL kg^{-1}) and were submitted to the FST 30 min later (acute effect). Independent groups of mice received ip injections of CBD (10 and 30 mg kg^{-1}) or vehicle (10 mL kg^{-1}) and were submitted to the FST 7 days later (sustained effect). Mice FST consisted of 6 min swimming session when animals were individually placed in glass cylinders (25 cm height, 17 cm diameter) containing 10 cm of water (25 °C), as described in [25, 40].

To investigate possible unspecific locomotor effects induced by CBD, the drug (10 mg kg^{-1}) or its vehicle (10 mL kg^{-1}) was administered intraperitoneally to independent groups of mice, which were submitted to the open field test, OFT, 30 min (acute effect) or 7 days later (sustained effect), as described in [25].

Experiment 2—Participation of Trk and mTOR Signaling Mechanisms in CBD Effects

Since TrkB and mTOR signaling have been implicated in mediating rapid and sustained antidepressant effect [17, 22], we investigated if CBD effects in the FST could involve the same signaling pathway. To do that, intracerebroventricular (icv) drug injections were performed with the aim to address: (1) the effects of direct injection of CBD into the CNS; (2) the effects induced by blockade of TrkB or mTOR upon CBD effects.

Therefore, mice underwent stereotaxic surgery to have guide-cannulas implanted into the lateral ventricle, as described in the [supplementary material](#). The first experiment consisted of an icv injection of vehicle (1 μL) or CBD (50, 150, and 300 nmol μL^{-1}). Thirty minutes later, mice were submitted to the OFT and, immediately after, to the FST, as previously described. In the second experiment, mice received a first icv injection of vehicle, K252a (0.05 nmol μL^{-1}) or rapamycin (1 nmol μL^{-1}) followed by a second injection (ip), 5 min later, of vehicle or CBD (10 mg kg^{-1}). Thirty minutes after the systemic injection, the animals were individually submitted to the OFT (6 min) and then to the FST (6 min).

After the behavioral tests, animals were euthanized by decapitation preceded by ip administration of an anesthetic (chloral hydrate solution 5%, 0.1 mL/10 g, C2H3Cl3O2, VETEC, Brazil). Soon after, Evan's blue dye (1 μL) was

injected into the lateral ventricle as a marker. The injection sites were visually identified by dye spread over the ventricles. Animals that received injections outside the ventricle were excluded from statistical analysis.

Experiment 3—CBD Effects on BDNF and Synaptic Protein Levels

To investigate if CBD effects could be associated to rapid and/or sustained changes in BDNF and synaptic protein levels in the HPC and PFC, independent groups of animals received ip injections of CBD (10 mg kg^{-1}) or vehicle (10 mL kg^{-1}) and were submitted to the FST, 30 min or 7 days later. Immediately after the FST, the animals were deeply anesthetized with 5% chloral hydrate (1 mL kg^{-1} , Sigma-Aldrich) and decapitated. The HPC and PFC were dissected for further analysis of BDNF levels by ELISA or PSD95 and SYP by WB, as detailed in the [supplementary material](#).

Experiment 4—CBD Effects on the Number of Dendritic Spines in Mice

To investigate if CBD effects could be associated with rapid and/or sustained changes in the number of dendritic spines in the PFC and HPC, independent groups of animals received ip injections of CBD (10 mg kg^{-1}) or vehicle (10 mL kg^{-1}), and 30 min or 7 days later, were submitted to the FST. Immediately after or 7 days later, the animals were sacrificed, and their brain removed for further processing with the Golgi-Cox method. The procedure was performed using the FD Rapid Golgi Stain Kit (FD Neurotechnologies, USA) according to fabricant's recommendations, with detailed protocol in [supplementary material](#). The number of spines in 10 μm of secondary and tertiary apical dendrites located into the dentate gyrus of the HPC and in the PFC (layer II/III) was analyzed using a light microscope (Zeiss, Germany). An analyzer blind to the experimental conditions measured six to eight neurons per animal distributed over defined plans according to Paxinos and Franklin Atlas [41], complying with the following criteria: the neurons were relatively isolated, displayed a defined cell body and a complete dendritic tree evidenced by well-defined endings, and presented intact primary, secondary, and tertiary branches.

Experiment 5—Acute and Sustained Effect of CBD in Rats Submitted to Different Preclinical Models

To further substantiate the acute and sustained behavioral effects induced by CBD, we used two different animal models: a stress-based animal model (learned helplessness, LH) and an animal model based on selective breeding (FRL/FRL combined with FST).

The learned helplessness paradigm was performed as previously described [42], with further details in the [supplementary](#)

material. On day 1, rats were submitted to a pretest session (PT) with 40 inescapable electric foot shocks (0.4 mA, 10 s duration; PT: stressed group) and, on the 7th day, they were submitted to the test (T, 30 escapable foot shocks, 0.4 mA, 10 s duration, 30–90 s interval) preceded by a tone (60 dB, 670 Hz) that started 5 s before each shock and lasted until its end. Animals could avoid the shock during the sound presentation or interrupt its presentation (escape) by crossing to opposite side of the chamber. The absence of this behavior was considered an escape failure. It is well-documented that PT exposure increases, whereas antidepressants decrease, the number of escape failures in this paradigm [43]. To investigate CBD effects, rats received a single injection of CBD (10 and 30 mg kg⁻¹) or vehicle (10 mL kg⁻¹) in one of the following periods: immediately after or 24 h after PT, or on day 7 (1 h before the test).

To evaluate CBD effects in FRL/FSL, we first tested CBD in Sprague-Dawley rats, the strain background from which FRL and FSL animals were developed [44]. We also included ketamine as a positive control for the acute and sustained effects [38]. The animals were exposed to the PT and T sessions of the forced swim as described earlier [38, 45]. One hour before the T, they received an ip injection of ketamine (15 mg kg⁻¹), CBD (10 and 30 mg kg⁻¹) or vehicle. Locomotor activity was assessed in the OFT immediately before the FST. After 7 days, rats were re-exposed to the test session of FST. FRL and FSL animals were exposed only to the test session of forced swim, as previously described [44]. One hour after the administration of ketamine (15 mg kg⁻¹), CBD (10 and 30 mg kg⁻¹) or

vehicle, the animals were exposed to the OFT. Immediately after the OFT, the animals were submitted to the FST (acute effect). After 7 days, the rats were re-exposed to the test session of the FST (sustained effect).

In rats, OFT was carried out in a squared arena (100 cm × 100 cm × 40 cm high), in a dimly lit (10 lx), temperature-controlled room (24 ± 1 °C). The experiment was videotaped, and the total distance traveled was analyzed using Ethovision XT 11 software (Noldus Information Technology).

Statistical Analysis

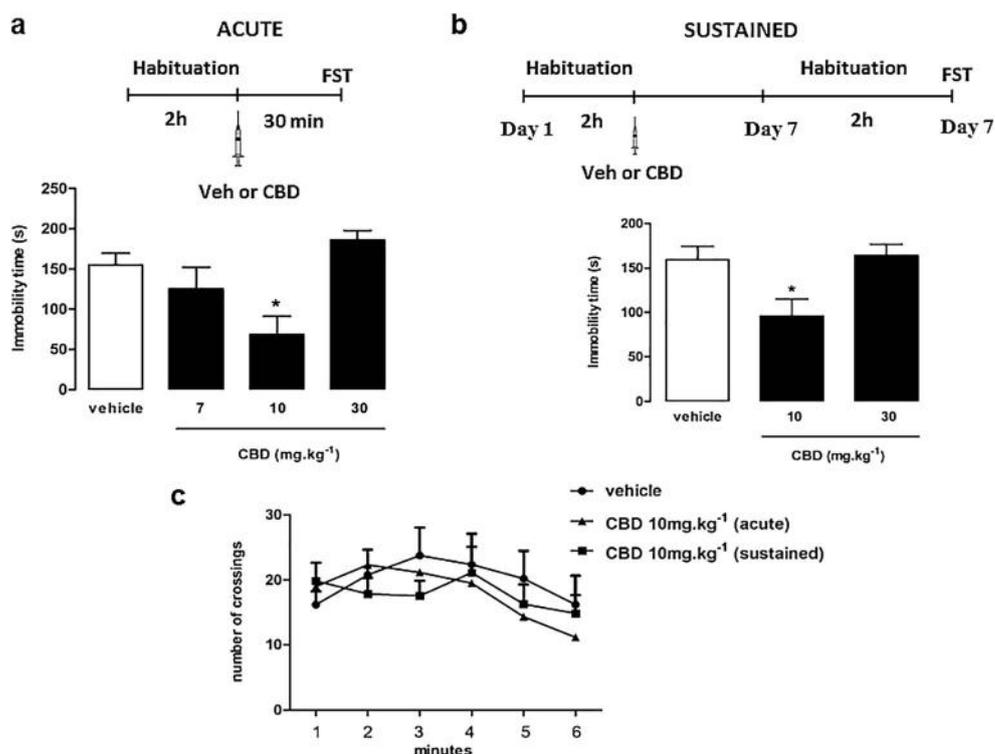
Analyses of the FST and LH data were performed using one-way ANOVA (post-test: Dunnett). Repeated measures (RM)-ANOVA was used to compare the number of crossings in OFT, and unpaired *t* test was used for the molecular data. The GraphPad Prism 5.0 software was used for statistical analyses. A 95% confidence interval and a significance level of 5% (*p* < 0.05) were considered for all analyses.

Results

CBD Induces Acute and Sustained Antidepressant-Like Effects in the Mice FST

CBD treatments significantly reduced the immobility time, 30 min ($F_{3,23} = 3.871$, *p* < 0.05, Fig. 1a) and 7 days ($F_{2,18} =$

Fig. 1 Acute and sustained effects of cannabidiol (CBD) in the mice forced swimming test (FST) and open field test (OFT). **a** Acutely, CBD treatment reduced immobility time (IT) in FST (*n* = 6–7 mice per group). **b** The IT was reduced in FST 7 days after CBD treatment (*n* = 7 mice per group). **c** The number of crossings did not differ between treatment groups (*n* = 6 mice per group). The data are presented as mean ± SEM. **p* < 0.05 compared to vehicle (VEH) mice



5.910, $p < 0.05$; Fig. 1b) after its single administration (10 mg kg^{-1}), thus revealing acute and sustained antidepressant-like effect, respectively. As shown in Fig. 1c, CBD (10 mg kg^{-1}) did not induce any significant difference in the number of crossings in the OFT when compared to vehicle (RM-ANOVA; interaction, $F_{10,75} = 5.15$, $p > 0.05$; treatment, $F_{2,75} = 0.2862$, $p > 0.05$; time, $F_{5,75} = 0.8006$, $p < 0.05$), which excluded possible unspecific effects of CBD on locomotor activity.

Participation of TrkB Receptors and mTOR Signaling in CBD-Induced Effects

CBD significantly decreased the immobility time in the FST after icv administration (Fig. 2a; $300 \text{ nmol}/\mu\text{L}$; one-way ANOVA, $F_{3,24} = 7.219$, $p < 0.05$), without changing the number of crossings in the OFT (Fig. 2b; RM-ANOVA; interaction, $F_{9,66} = 1.49$, $p > 0.05$; treatment, $F_{3,66} = 1.605$, $p > 0.05$;

time, $F_{3,66} = 4.813$, $p < 0.05$). Next, we analyzed if CBD effects could be blocked by a TrkB receptor antagonist (K252a) or by an mTOR signaling inhibitor (rapamycin). As shown in Fig. 2c, d, systemic CBD administration reduced the immobility time in the FST (one-way ANOVA, $F_{5,29} = 8.699$, $p < 0.05$), which was blocked by icv K252a or rapamycin administration (Fig. 2c; $p > 0.05$ compared to the vehicle group). In the OFT, CBD did not change the number of crossings (Fig. 2d; treatment, $F_{5,84} = 0.5770$, $p > 0.05$; interaction, $F_{15,84} = 0.3906$, $p > 0.05$). Therefore, systemic effects of CBD are dependent on intact TrkB and mTOR signaling in the CNS.

CBD Effects on BDNF and Synaptic Protein Levels

CBD administration (10 mg kg^{-1}) acutely increased BDNF levels in both the HPC and PFC (Fig. 3a; $p < 0.05$; $t_{16} = 3.535$; and $t_{17} = 2.277$, respectively), but this effect was not

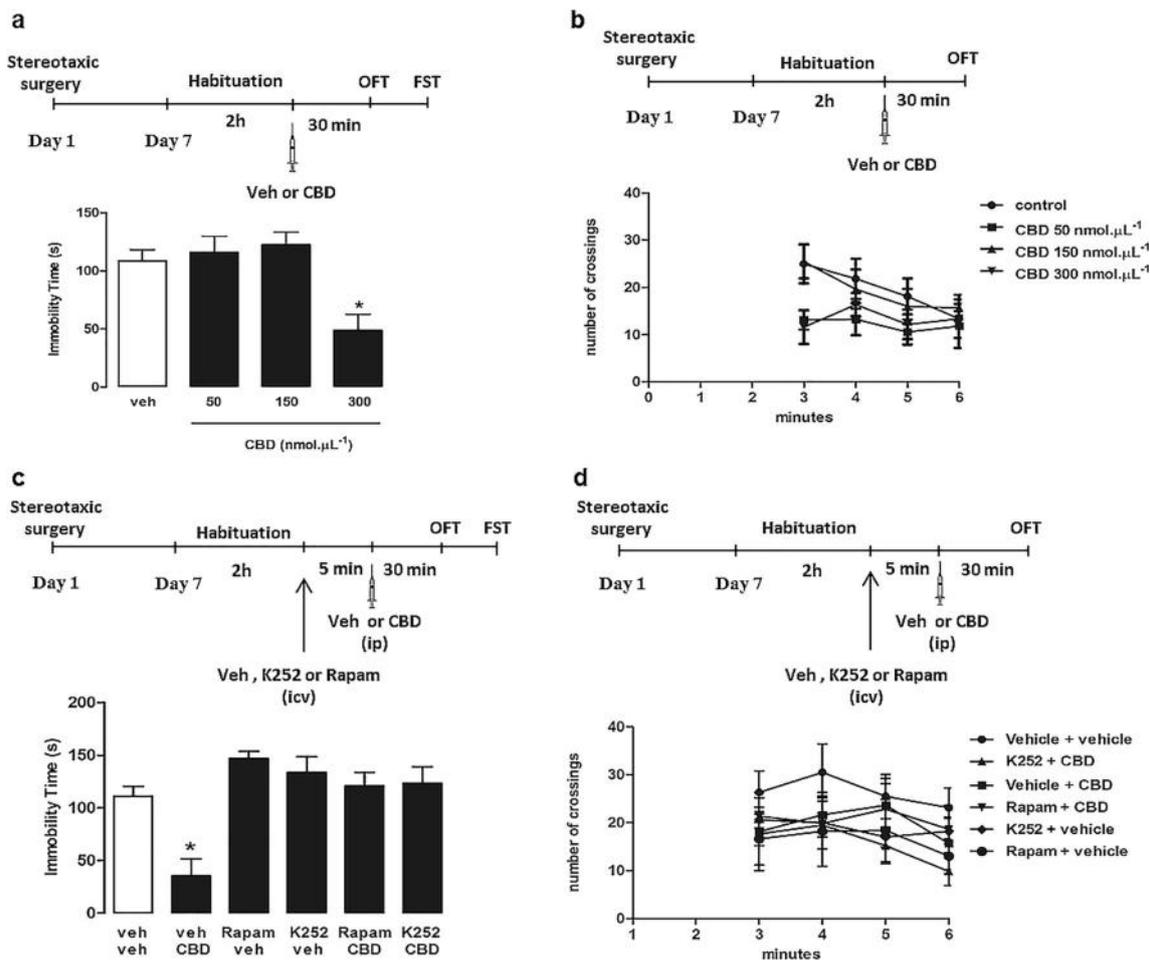


Fig. 2 Trk and mTOR blockade impair cannabidiol (CBD) effects in the forced swimming test (FST). **a** CBD treatment (icv) reduced immobility time (IT) in FST, when compared to vehicle (VEH) ($n = 6-8$ mice per group). **b** The number of crossings did not differ between treatment groups ($n = 6-8$ mice per group). **c** The inhibition of mTOR

(Rapamycin, Rapam) and blockade of Trk (K252) prevented the reduction in IT in FST induced by CBD administration ($n = 5-7$ mice per group). **d** The number of crossings did not differ between treatment groups ($n = 5-6$ mice per group). Data are presented as mean \pm SEM. * $p < 0.05$ compared to VEH mice

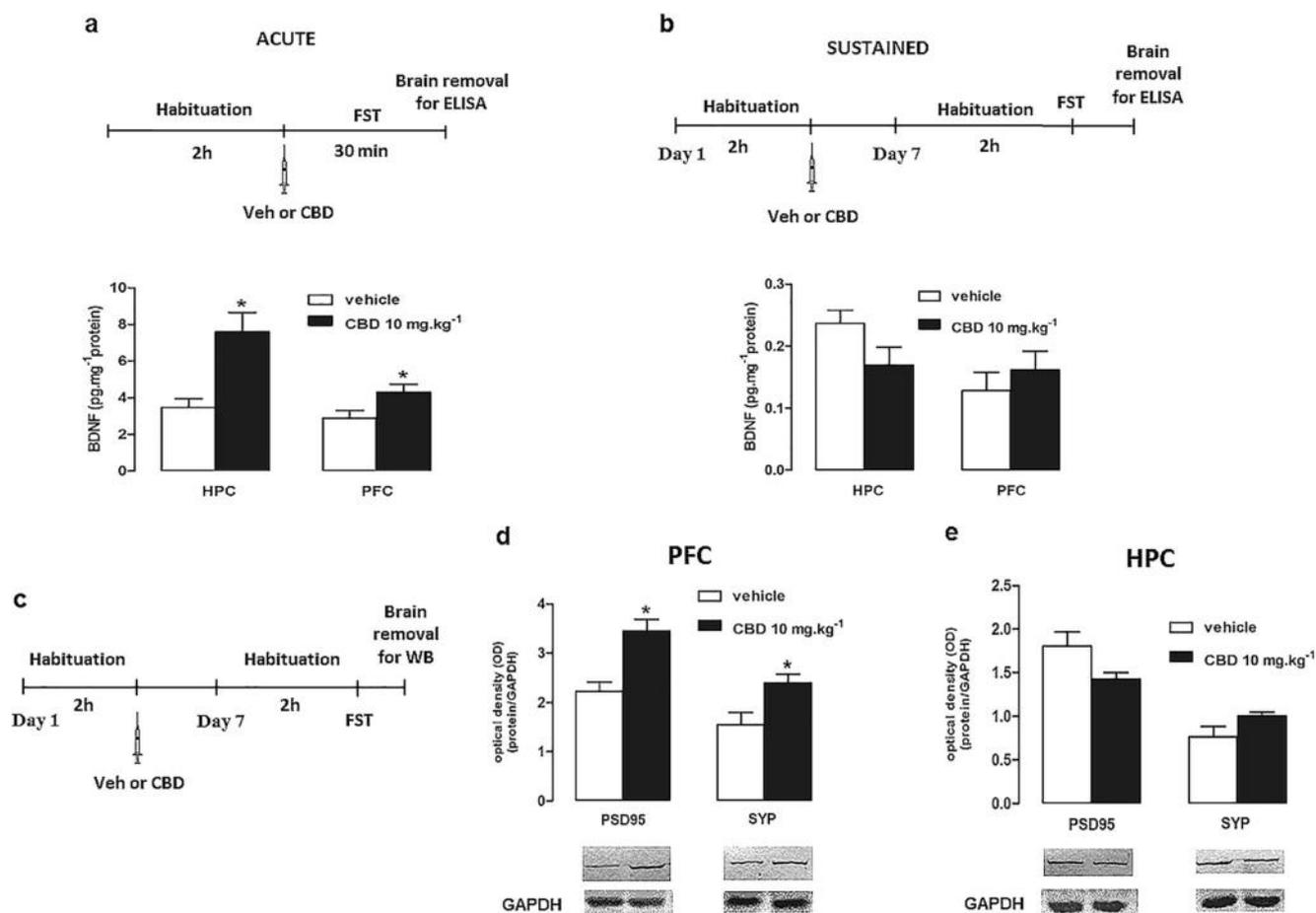


Fig. 3 Levels of BDNF, mTOR, and synaptic proteins in CBD-treated mice submitted to forced swimming test (FST). **a** Acutely, BDNF levels are increased in hippocampus (HPC) and prefrontal cortex (PFC) of mice treated with CBD ($n = 9-10$ animals per group). **b** Seven days after CBD administration, BDNF levels are not changed ($n = 9-10$ mice per group).

c Experimental scheme for WB of synaptic proteins. **d** CBD treatment increased synaptic proteins levels in PFC ($n = 8$ mice per group). **e** CBD treatment did not change synaptic proteins levels in HPC ($n = 6-8$ mice per group). The data are presented as mean \pm SEM. * $p < 0.05$ compared to VEH mice

detected 7 days later (Fig. 3b; $p > 0.05$, HPC, $t_{16} = 1.874$ and PFC, $t_{18} = 0.799$). On the other hand, 7 days after the CBD administration, the synaptic protein (PSD95 and SYP) expression was increased only in the PFC (Fig. 3d; $p < 0.05$; $t_{14} = 3.915$; and $t_{14} = 2.761$, respectively). No change was found in the HPC (Fig. 3e; $p > 0.05$; PSD95, $t_{12} = 1.876$ and SYP, $t_{11} = 1.767$). These results are consistent with a rapid increase in BDNF levels in the HPC and PFC, associated with a delayed increase in synaptic proteins in the PFC, in response to a single CBD injection.

CBD Rapidly Increases the Number of Dendritic Spines

To investigate if dendritic remodeling would be associated with the behavioral and molecular effects induced by CBD, we analyzed the number of dendritic spines in the PFC and HPC, 30 min and 7 days after drug injection. Acute administration of CBD increased the number of dendritic spines in both prelimbic and infralimbic areas of the medial PFC

(Fig. 4a, b; prelimbic PFC: secondary branch, $t_8 = 2.688$; tertiary branch, $t_8 = 2.636$; infralimbic PFC: secondary branch, $t_8 = 2.322$; tertiary branch, $t_8 = 2.814$; $p < 0.05$ for all). However, no difference was found 7 days later (Fig. 4c, d; prelimbic PFC: secondary branch, $t_6 = 0.7769$; tertiary branch, $t_6 = 0.9873$; infralimbic PFC: secondary branch, $t_6 = 1.666$; tertiary branch, $t_6 = 0.2708$; $p > 0.05$ for all). Also, CBD did not change the number of dendritic spines in the dorsal HPC (dentate gyrus) when compared to the vehicle group at any time of analysis ($p > 0.05$; Fig. 4e, 30 min after the injection: secondary branch, $t_8 = 0.1136$; tertiary branch, $t_8 = 0.4951$; Fig. 4f, 7 days after the injection: secondary branch, $t_8 = 1.668$; tertiary branch, $t_8 = 0.01073$).

Acute and Sustained Effects of CBD in Rats Submitted to Different Preclinical Models

To better support our findings regarding the rapid and sustained effects induced by CBD in the FST, we analyzed

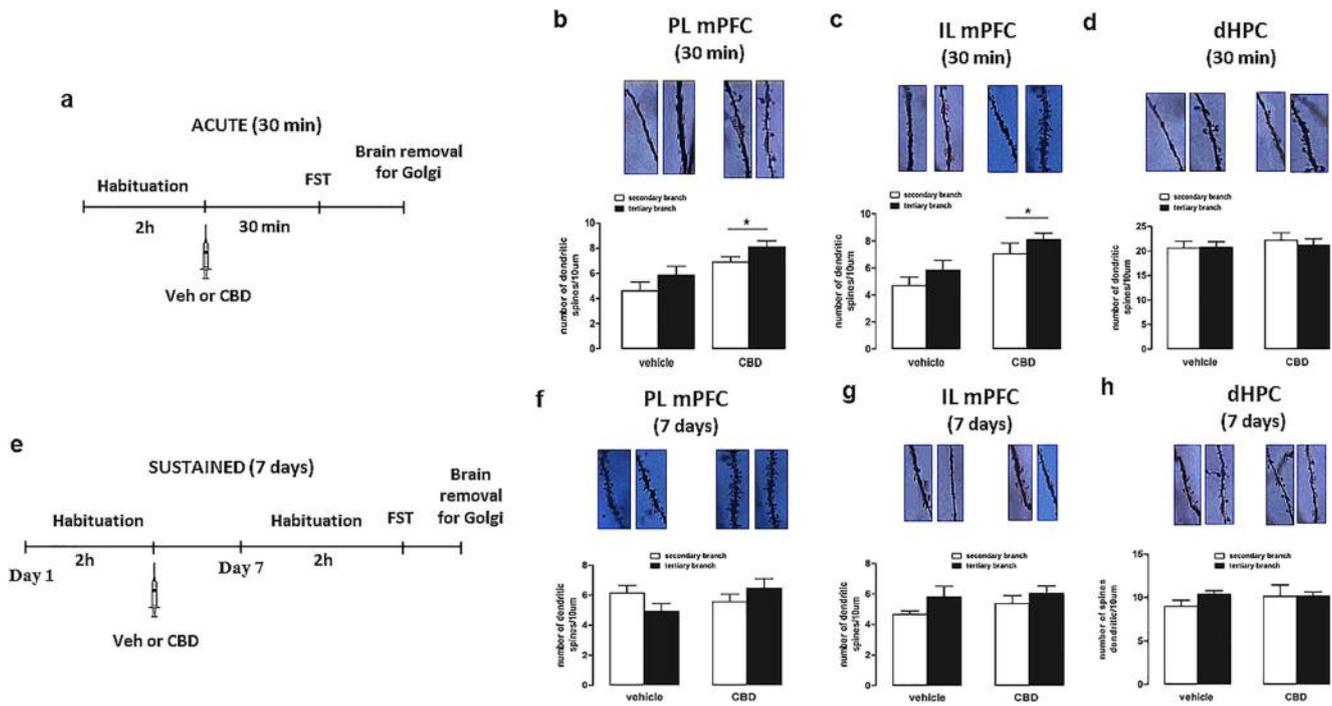


Fig. 4 Effect of cannabidiol (CBD) or vehicle (VEH) administration in dendritic spines number. **a** Experimental scheme for acute treatment. In prefrontal cortex (PFC), acute CBD treatment (ip) increased dendritic spine number in prelimbic (PL; **b**) and infralimbic (IL; **c**) regions ($n = 5$ mice per group). **e** Experimental scheme for sustained treatment. Seven days after CBD injection (ip), the number of

dendritic spines did not change in PFC (PL and IL, **f**, **g**, respectively; $n = 4$ mice per group). In hippocampus (dentate gyrus (DG)), systemic CBD administration did not change the dendritic spines number (acute, **d**; 7 days, **h**; $n = 5$ mice per group). The data are presented as mean \pm SEM. * $p < 0.05$ compared to VEH mice

its effects in two different animal models sensitive to antidepressant drugs, the learned helplessness and the FSL animals. One-way ANOVA showed that administration of CBD immediately after PT, but not 1 h before T, reduced the number of escape failures (Fig. 5a; one-way ANOVA, $F_{4,47} = 2.818$, $p < 0.05$) and increased the number of escapes (Fig. 5b; $F_{4,47} = 2.134$), without changing the number of crossings (Fig. 5c; $F_{4,47} = 1.681$, $p > 0.05$). These data are indicative of a rapid antidepressant-like effect of CBD since the LH is irresponsible to acute (single) treatment with conventional antidepressants.

In Sprague-Dawley rats, the control animals of FRL and FSL rats, the injection of CBD at the dose of 30 mg kg^{-1} significantly reduced the immobility time in FST (Fig. 5d; $F_{3,23} = 3.348$, $p < 0.05$). The same effect was found to the positive-control ketamine (Fig. 5d; $t_{11} = 2.732$, $p < 0.05$). Neither ketamine nor CBD modified the exploratory activity of the animals in the OFT (Fig. 5e; $F_{2,23} = 1178$, $p > 0.05$). Similarly, as shown in fig. 5f, g, CBD (10 and 30 mg kg^{-1}) and ketamine (15 mg kg^{-1}) significantly reduced the immobility time in FSL rats (Fig. 5f; $F_{3,25} = 8.441$, $p < 0.05$). When the animals were re-exposed to FST, the groups treated with CBD did not show any change in the immobility time (Fig. 5g; $F_{3,24} = 3873$, $p > 0.05$) whereas this effect was still significant to the positive-control ketamine (Fig. 5g; $t_{12} = 2.941$, $p <$

0.05). In FRL rats, injection of CBD and ketamine did not reduce the immobility time in FST (Fig. 5g; $F_{3,22} = 1.085$, $p > 0.05$). Seven days after, the animals were re-exposed to the FST and CBD reduced the immobility significantly. (Figure 5h; $t_{13} = 2.689$, $p < 0.05$). Neither ketamine or CBD modified the exploratory activity of the animals in the OFT (Fig. 5h; FSL, $F_{3,28} = 0.0965$; FRL; $F_{3,22} = 0.3544$, $p > 0.05$ for both).

Discussion

The main finding of the present study is that CBD induces not only a robust acute, but also sustained, antidepressant-like effect in different species and distinct animal models involving stress exposure (FST, LH) and selective breeding (FSL/FRL). The rapid effect was accompanied by increased BDNF levels in the HPC and mPFC, and markers of synaptic plasticity in the mPFC. Moreover, CBD effect was blocked by icv injection of TrkB receptor antagonist or mTOR inhibitor. Additionally, the sustained effects of CBD were accompanied by increases in PSD95 and SYP expression in the PFC, without any change in the number of dendritic spines. Altogether, our results suggest that the rapid antidepressant-like effect induced by acute CBD injection involves BDNF/TrkB/mTOR signaling and increased

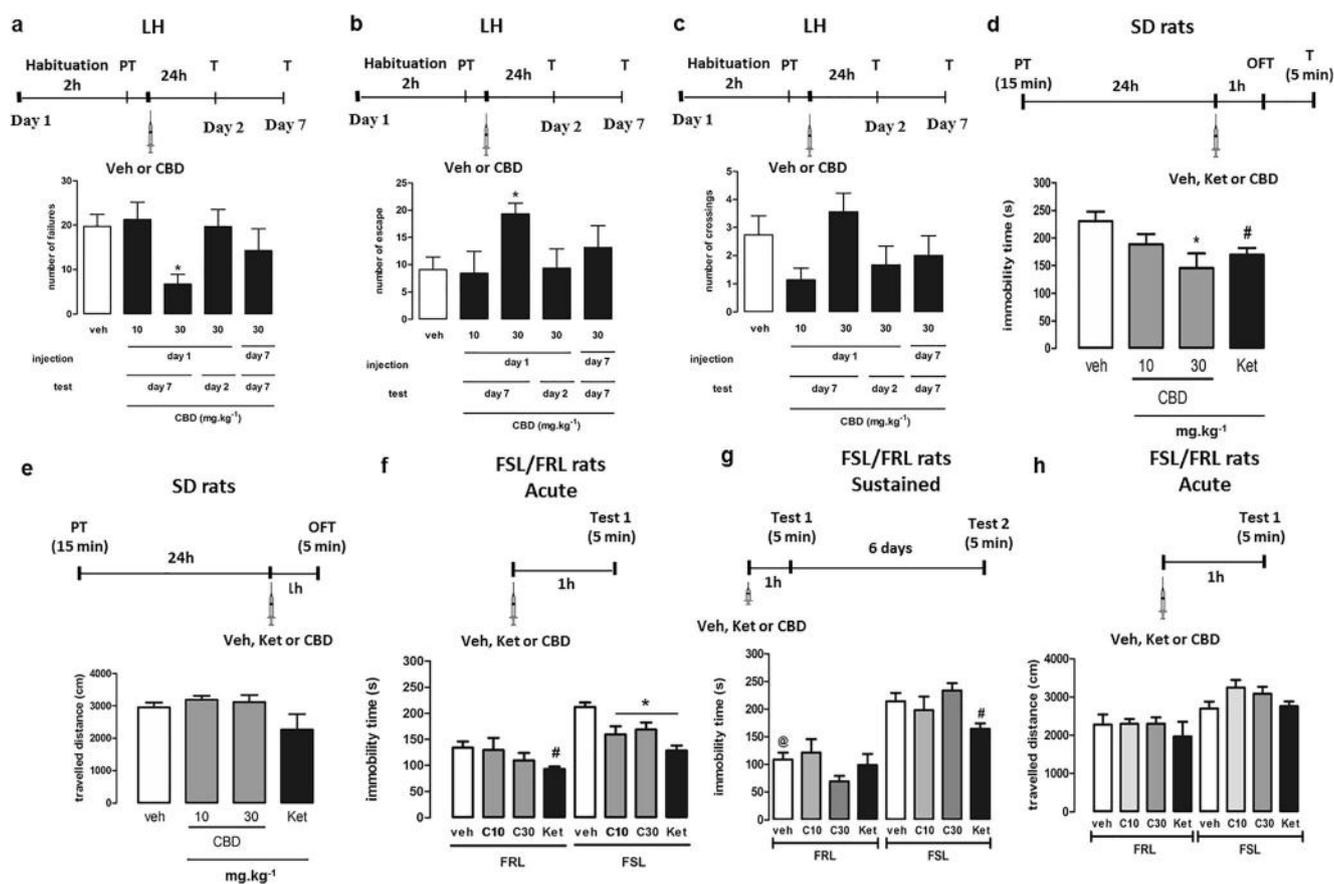


Fig. 5 Effect of cannabidiol (CBD) or vehicle (VEH) administration in different animal models. In learned helplessness (LH), acute CBD treatment (ip) induced sustained reduction in number of failures (**a**), increase in the number of escape (**b**), and did not change the number of crossings (**c**; $n = 10\text{--}13$ rats per group). In *Sprague-Dawley* rats, CBD and ketamine (ket) treatment reduced the immobility time in forced swimming test (FST; **d**; $n = 6\text{--}7$ rats per group) and did not change the number of crossing in open field test (OFT; **e**; $n =$

$6\text{--}7$ rats per group). **f** Injection of CBD and ket in FSL rats reduced the immobility time in FST ($n = 4\text{--}8$ per group). **g** The re-exposition of FSL rats treated with CBD to the FST did not change immobility time ($n = 4\text{--}8$ per group). **h** The traveled distance did not differ between treatment groups ($n = 4\text{--}8$ rats per group). The data are presented as mean \pm SEM. * $p < 0.05$ compared to VEH mice; # $p < 0.05$ compared to the VEH mice; @ $p < 0.05$ compared VEH FRL X VEH FSL

dendritic spine density in the medial PFC, whereas the long-lasting effect may be associated with an enhancement of synaptic function in this same brain area.

The behavioral effects of CBD observed herein agree with previous reports describing its antidepressant-like effects [25–30]. For instance, systemic CBD treatment reduced the immobility time in mice submitted to the FST [25–27] and the tail suspension test (TST; [30]). In another study, CBD attenuated the behavioral deficits induced by olfactory bulbectomy within 24 h, indicating a similar fast-acting antidepressant profile [28]. Chronic treatment with CBD was also effective in animals subjected to chronic unpredictable stress [46]. Interestingly, a recent study showed that CBD induces a prohedonic effect in the saccharin preference test in the Wistar-Kyoto, a genetic rat model of depression [47]. This finding is in full accordance with our observation that CBD produced an antidepressant-like effect in the FSL/FRL rats, another genetic rat model of depression based on selective breeding [44].

Since fast-acting antidepressants, such as ketamine, Glyx-13 (NMDA receptor partial agonist), and scopolamine (muscarinic receptor antagonist), induce behavioral effects through rapid BDNF-mediated signaling and increased synaptogenesis in the PFC [11, 48, 49], we hypothesized that this mechanism is essential for the behavioral effects induced by CBD. BDNF is recognized as playing important roles in neuronal survival, differentiation, outgrowth, and synaptogenesis, during development and in the adult brain (for review see [55]). Stress exposure is able to negatively change BDNF levels whereas the behavioral effect of fast-acting antidepressants has been related to the rapid and long-lasting increases in synaptogenesis in response to increased BDNF levels in PFC [56, 57]. In the hippocampus, Garcia and coworkers demonstrated that 1 h after one single injection of ketamine also increases BDNF levels [58]. Moreover, the fast-acting behavioral antidepressant-like effects induced by ketamine and other NMDA antagonist in mouse models was shown to

be dependent on the rapid synthesis of BDNF due to de-repression of its translation in the hippocampus [59]. Therefore, BDNF signaling in both PFC and hippocampus seems to play an important role the behavioral effect induced by fast-acting antidepressants.

Supporting our initial hypothesis, acute administration of CBD (10 mg kg⁻¹) rapidly increased BDNF levels in both mPFC and HPC, an effect not observed 7 days later. Since icv administration of k252a (Trk antagonist) or rapamycin (mTOR inhibitor) blocked the behavioral effect induced by systemic CBD administration, it is probable that the fast antidepressant-like effect of CBD depends on intact BDNF-TrkB-mTOR signaling in the brain. In support of our data, repeated CBD treatment increased BDNF and mTOR levels in the spinal cord [35] and brain of rodents exposed to models of neurodegeneration [36]. In contrast, unaltered BDNF levels in the PFC and HPC after acute CBD administration have also been reported [25, 27]. However, differences in the age and species used or CBD dose (30 vs. 10 mg kg⁻¹; [25]) might have contributed to the contradictory findings. Based on the present study, we suggest that CBD can rapidly increase BDNF levels in the PFC and HPC, an effect associated with its behavioral effects in the FST. The rapid upregulation of BDNF protein and subsequent activation of its receptor, TrkB, could trigger several intracellular signaling pathways that ultimately lead to mTOR activation (see [22]), which mediates protein synthesis and synaptogenesis.

The mechanism responsible for CBD effects resulting in increased BDNF and mTOR signaling are not yet clear. It is known, however, that CBD increases serotonin levels in the PFC [28] and that administration of CBD into the same brain region induces antidepressant-like effects that are dependent on local 5-HT_{1A} activation [29]. Evidence indicates that treatment with 5-HT_{1A} agonists rapidly increases BDNF mRNA and protein levels in PFC and hippocampus [60] and in cultured neurons [61]. Moreover, decreased BDNF levels and TrkB activation are described in the brain of 5-HT_{1A} knockout mice [62], thus suggesting that proper 5-HT_{1A} activation positively regulates BDNF levels in the brain. This mechanism seems to participate in the behavioral effect of fast-acting antidepressants, since activation of 5-HT_{1A} receptors in the medial PFC was shown to induce acute and sustained antidepressant-like and the sustained effect induced by systemic injection of ketamine was attenuated by intramedial PFC injection of a 5-HT_{1A} receptor antagonist, WAY100635. Altogether, it is possible to suggest that 5-HT_{1A} activation by CBD, either by direct binding or by indirect increase in serotonin levels, can lead to increased BDNF levels and TrkB activation, which would ultimately lead mTOR activation and synaptogenesis, as we observed herein. This hypothesis, however, warrants further investigation.

Alternatively, recent evidence indicates that chronic CBD treatment induces behavioral and neuroplastic effects in

stressed animals due to a facilitation of endocannabinoid neurotransmission and consequent CB₁/CB₂ receptor activation, which could recruit intracellular/synaptic proteins involved in neurogenesis and dendritic remodeling [63]. It remains to be investigated if this could participate in acute and sustained effects induced by the drug.

Based on the findings about BDNF and mTOR involvement in CBD effects, we further investigated if CBD effects would also be associated with increased levels of synaptic proteins and dendritic spines, since chronic antidepressant treatment prevents or reverses dendritic spine alterations caused by stress [50, 51]. In addition, drugs with a fast-acting antidepressant effect, such as ketamine, rapidly increase the number of dendritic spines in the PFC and reverse the effects of chronic stress via increased BDNF regulation of synaptic protein synthesis [39, 52]. Our data indicate that CBD increases the number of dendritic spines in the medial PFC (PL and IL) 30 min after the administration. Although we did not observe this effect 7 days later, there was an increase of PSD95 and SYP in the PFC, suggesting an enhancement of dendritic function. One possible explanation for the discrepant results in the number of dendritic spines and synaptic proteins at 30 min and 7 days after CBD administration could rely on the fact that CBD would actually rapidly favor synaptogenesis to substantiate an activity-driven selection of the appropriate synaptic contacts, thus allowing dendrite turnover to take place [64]. Therefore, the number of dendrites would not be increased after 7 days, only increased synaptic function as revealed by increased SYP and PSD95.

Supporting our data, depressed subjects present reduced PSD95 levels in the PFC, which is reversed by ketamine [53]. Additionally, administration of rapamycin (icv and intra-mPFC) blocks the antidepressant behavioral effects and the increase in dendritic spines induced by ketamine [39]. Similarly, icv rapamycin administration blocked CBD-induced behavioral effects in the present study. Altogether, these results suggest that CBD induces fast effects on dendritic remodeling in the mPFC, possibly involving BDNF-mediated signaling. On the other hand, its sustained effect could rely on increased synaptogenesis in the same brain region. Experiments specifically targeting the mPFC or the HPC could help clarifying the differential involvement of these two brain regions in CBD-induced effects.

Finally, to further substantiate our behavioral findings in the FST, we investigated CBD effects in two different animal models with higher face, construct, and predictive validity, the learned helplessness and FRL/FSL animals. Both models fail to respond to the acute effects of conventional antidepressants, but they positively identify the acute antidepressant effect of ketamine [20, 44]. In our study, a single injection of CBD reduced the number of escape failures in the LH and the immobility time in the FSL animals. Additionally, the effects in the LH and FRL animals were present 7 days after the first

injection. These results further support the proposal that CBD possesses fast-acting antidepressant properties. The use of more than one behavioral test can provide convergent validity and increase confidence in studies aimed at identifying new fast-acting antidepressant drugs [54]. Nevertheless, the fact that all molecular analysis was performed with brain tissue from only one species (mice) submitted to the forced swimming test remains a limitation of the present study. Further analysis using different animal models and treatment intervals could reveal new important information about CBD effects.

In conclusion, this study demonstrates that CBD induces a rapid antidepressant effect, probably by increasing BDNF signaling in the PFC and synaptic dendritic spine density. Moreover, a long-lasting enhancement of synaptic efficacy could mediate CBD-sustained antidepressant effects.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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