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Cannabidiol prevents several of the behavioral alterations related to cocaine addiction in mice

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ABSTRACT

Cocaine dependence is a highly prevalent disease in modern society and lacks an effective treatment. Cannabidiol (CBD), a major non-psychoactive constituent of *Cannabis sativa*, has been shown to be a promising tool in the management of some neuropsychiatric disorders, including cocaine abuse. However, its therapeutic effects on the behavioral outcomes related to cocaine addiction remain unclear. The present research evaluates the effects of CBD (30, 60 and 120 mg/kg; injected intraperitoneally) on the acquisition, expression, extinction and reinstatement of cocaine (10 mg/kg)-induced conditioned place preference (CPP; Study 1); cocaine (25 mg/kg)-induced locomotor stimulation (Study 2); and cocaine withdrawal symptoms (Study 3) in male C57BL/6 J mice. The results show that CBD does not possess motivational properties in itself and does not modify the acquisition, expression or extinction of cocaine-induced CPP. Interestingly, when administered during the extinction phase of the cocaine-induced CPP, CBD (30 and 60 mg/kg) prevented priming-induced reinstatement of CPP. Moreover, CBD abolished cocaine-induced hyperactivity without altering the spontaneous locomotion of the animals. Furthermore, CBD (120 mg/kg) reduced the memory deficits induced by cocaine withdrawal in the object recognition test, though it did not reverse depressive-like symptoms measured in the tail suspension test. Overall, our data suggest that CBD can prevent the development of cocaine addiction, and, when administered during cocaine abstinence, may be of help in avoiding relapse to drug-seeking and in ameliorating the memory disturbances provoked by chronic consumption of cocaine.

1. Introduction

Cocaine is the second most commonly consumed illicit drug in Europe and the United States. In the European Union around 18 million adults aged 15–64 (5.4%) have used cocaine at least once in their lifetime, and, among those aged 15–34, nearly 3 million (2.4% of this age group) are estimated to have used the drug in the last year (EMCDDA-European Monitoring Centre for Drugs and Drug Addiction, 2020). In the United States, there are approximately 2.2 million regular consumers of cocaine, of whom 1 million have been diagnosed with cocaine use disorder (CUD) in the past year (SAMHSA-Substance Abuse Mental Health Services Administration, 2018). CUD is a serious public health problem, as it is associated with substantial morbidity, social decline, criminality, a heavy economic burden to society (including increased emergency room admissions and elevated incidences of health care), high rates of HIV, hepatitis B and hepatitis C (among others), and a high number of deaths (Butler et al., 2017; Peacock et al., 2020; Sturgess et al., 2011). Given that no successful therapies against CUD have yet been approved, the development of effective treatments for cocaine addiction is a clinical priority.

Animal models can help us to understand the neurobiological substrates of CUD, and to find new therapeutic targets. As with almost all of drugs of abuse, cocaine administration is able to elicit conditioned place preference (CPP) (Baskin et al., 2020; Bennett et al., 2020; Calpe-López et al., 2020). In the CPP procedure, one context is paired with drug injections, while a different context is paired with a vehicle. Acquisition of drug-induced CPP is considered to have occurred when an animal spends more time in the drug-paired context during a subsequent drug-free test in which the animal can choose between both environments (Golden et al., 2019; Tzschentke, 2007). The CPP paradigm has been

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used for assessing the positive affective memories induced by drugs of abuse and cue-eliciting drug-seeking behaviors (Ledesma and Aragon, 2013; Tzschentke, 1998). Furthermore, by applying CPP extinction/reinstatement protocols, this procedure can be used to model drug relapse in animals (Aguilar et al., 2008). In this way, it is possible to carry out manipulations that can accelerate the elimination of cue-eliciting drug-seeking behaviors and impede the reinstatement of a previously extinguished drug-induced CPP (Aguilar et al., 2008; Cunningham et al., 2011; Ribeiro Do Couto et al., 2012; Titomanlio et al., 2013).

Another common effect that cocaine shares with the majority of drugs with abuse potential is its ability to produce locomotor stimulation (Badiani et al., 2000; Wise and Bozarth, 1987). It has been suggested that there is some overlap between the neurobiological substrates of drug-induced behavioral activation and those underlying the motivational effects of drugs of abuse (i.e., mesocorticolimbic dopamine pathways) (Amalric and Koob, 1993; Tzschentke and Schmidt, 2000). This effect has commonly been used as an indirect measure of the presence of neural changes thought to play a role in the development of addiction (Berridge and Robinson, 2016; Piazza and Deroche-Gamonet, 2013; Wise and Koob, 2014). Coupled with the loss of medial prefrontal inhibitory control of striatal systems, drug-induced hyper-locomotion has been considered the result of the hyper-responsiveness of dopaminergic mesocorticolimbic neurons, which is thought to be a key contributor to the shift from voluntary to compulsive drug consumption (Everitt, 2014). Hence, it has been proposed that treatments that block cocaine-induced locomotor stimulation represent a useful strategy to prevent and/or reverse some of the effects that lead to the development of cocaine addiction.

Animal models have also been used to explore the characteristic features of cocaine abstinence and its underlying neural substrates (Barker and Rebec, 2016; Zhang et al., 2013). It has been demonstrated that cocaine withdrawal in dependent animals causes behavioral alterations such as memory disturbances and depression-like symptoms (Aguilar et al., 2017; Perrine et al., 2008), which may contribute to high rates of relapse and enhance the risk of addiction. In fact, negative feelings experienced by addicts during withdrawal constitute a potent driver for craving and relapse (Chavkin and Koob, 2016). In addition, cognitive dysfunction is frequent in cocaine addicts, and has been associated with poor treatment outcomes (Mahoney, 2019). Thus, prevention of the emotional and cognitive sequels of cocaine abstinence needs to be a priority in the development of effective pharmacotherapies versus CUD.

Cannabidiol (CBD), the second most abundant phytocannabinoid component of the cannabis plant (Pertwee, 2007), has recently been proposed as a promising treatment for substance abuse disorders such as cannabis, opioid, alcohol, and nicotine dependence (Chye et al., 2019; Crippa et al., 2018; Prud'homme et al., 2015). Interestingly, there is some evidence that CBD modulates various neural circuits and behavioral outcomes involved in drug addiction (Calpe-López et al., 2019). For example, CBD raises the threshold for the activation of the brain reward mechanisms mediated by the increase of mesocorticolimbic dopamine (DA) release after drug administration (Katsidoni et al., 2013; Mijangos-Moreno et al., 2014; Murillo-Rodríguez et al., 2011). Intra-accumbal infusion of CBD reverses the increase in the firing frequency of DA in the ventral tegmental area (VTA), psychomotor sensitization effects, and deficits in pre-pulse inhibition after an amphetamine challenge in rats (Pedrazzi et al., 2015; Renard et al., 2016). Moreover, exposure to CBD attenuates the alterations of the endocannabinoid and glutamatergic neurotransmission in the nucleus accumbens (NAcc) associated with cue-induced reinstatement of heroin self-administration in rats (Ren et al., 2009). In addition, CBD pre-treatment can reverse increases of brain-derived neurotrophic factor in the hippocampus of an animal model of mania induced by chronic d-amphetamine dispensation (Valvassori et al., 2011).

With regard to CUD, several studies suggest that CBD treatment is an effective pharmacological adjuvant to therapy for individuals with problems related to cocaine addiction (for review see Rodrigues et al., 2020). In this way, it has been shown that acute CBD administration inhibits the reinstatement of context-induced cocaine-seeking behavior, and anxiety- and impulsive-like behaviors in rats with a previous history of chronic cocaine self-administration (Gonzalez-Cuevas et al., 2018). Moreover, systemic administration of CBD dose-dependently reduces cocaine self-administration and attenuates the enhancement of the activity of the brain reward system induced by this drug in rats (Galaj et al., 2020). Furthermore, CBD treatment during conditioning reduces cocaine-induced CPP when measured 20 days after drug cessation, and also modifies the locomotor-stimulating effects of cocaine (Chesworth and Karl, 2020). It has also been demonstrated that pre-treatment with CBD 30-min before administration of a priming dose of cocaine abrogates the reinstatement of a previously extinguished cocaine-induced CPP in mice (Calpe-López et al., 2021).

Although there are several studies evaluating the effect of CBD on some of the behavioral outcomes related to cocaine addiction in mice, further investigation is needed to clarify its potential therapeutic actions against CUD. Thus, through a series of three separate studies, the current research project aimed to assess the potential role of CBD administration on: the conditioning properties of cocaine (by using different CPP procedures; namely, acquisition, expression, extinction and reinstatement of cocaine-induced CPP; Study 1); the locomotor-stimulating properties of cocaine (Study 2); and memory deficits and depression-like symptoms following cocaine withdrawal (Study 3). Given that CBD has been shown to reverse some of the addiction-like effects elicited by cocaine, we hypothesized that CBD would reduce the conditioning effects of the drug, its locomotor-enhancing actions, and memory-related and depressive-like symptoms in mice undergoing cocaine withdrawal.

2. Methods

2.1. Animals

A total of 275 male mice of the C57BL/6 J strain were purchased from Charles River (France) for the present study. The subjects were 21 days old upon arrival at the laboratory and were all housed under standard conditions in groups of four, in plastic cages (25 × 25 × 14.5 cm), at a constant temperature (21 ± 2 °C) and relative humidity (60%), with a reversed light schedule (white lights on 20:00–8:00). Food and water were provided ad libitum throughout the study. All the procedures involving the mice and their care complied with national, regional and local laws and regulations, which are in accordance with the Directive 2010/63/EU of the European Parliament and the Council of 22 September 2010 on the protection of animals used for scientific purposes. The protocol was approved by the Ethical Committee for Animal Experiments of the University of Valencia and Generalitat Valenciana (2016/VSC/PEA/00176).

2.2. Drugs

Cannabidiol (CBD) was commercially acquired from THC Pharm GmbH (Frankfurt, Germany). Prior to intraperitoneal (i.p.) injection at doses of 30, 60, and 120 mg/kg it was dissolved in a vehicle solution consisting of physiological saline (NaCl 0.9% *w/v*; Sal) containing 4% of dimethyl sulfoxide (DMSO). Cocaine hydrochloride (Coc) was supplied by Laboratorios Alcaliber S.A. (Madrid, Spain). It was diluted in Sal and injected i.p. at doses of 10 and 5 mg/kg (for the acquisition and reinstatement of conditioned place preference, respectively); 25 mg/kg (for the locomotor stimulation experiment); and 5, 10, 15 and 20 mg/kg (for the study of the behavioral alterations induced by cocaine withdrawal). All the drug doses, administration schedules and routes, and preparations were based on previous published reports (Aguilar et al., 2017; Calpe-López et al., 2021; Deiana et al., 2012; Ledesma et al., 2017; Titomanlio et al., 2013; Viudez-Martínez et al., 2018).

2.3. Experimental procedure

2.3.1. Study 1: effects of CBD on cocaine-induced conditioned place preference (CPP)

For drug-induced place conditioning, we employed 12 identical Plexiglas boxes with two equal-sized compartments (30.7 × 31.5 × 34.5 cm) separated by a gray central area (13.8 × 31.5 × 34.5 cm). The compartments had different colored walls (black vs. white) and distinct floor textures (fine grid in the black compartment and wide grid in the white one). Four infrared light beams in each compartment of the box and six in the central area allowed the recording of the position of the animals and their crossings from one compartment to the other. The equipment was controlled by three IBM PC computers using MONPRE 2Z software (Cibertec SA, Madrid, Spain).

In all the CPP experiments, the protocol followed was unbiased in terms of initial spontaneous preference, and mice always underwent a pre-conditioning phase (Pre-C) during which they were allowed access to both compartments of the apparatus for 15 min (900 s) per day, over 2 days. On day 3, the time spent in each compartment during a 900 s-period was recorded. Animals showing a strong unconditioned aversion (less than 33% of the session time, i.e., 300 s) or preference (more than 66%, i.e., 600 s) for any compartment were discarded ($n = 5$). In each experimental group, half of the animals received Coc in one compartment, and the other half received it in the second compartment. After assigning animals to the compartments, an analysis of variance (ANOVA) revealed no significant differences between the time spent in the drug-paired and Sal-paired compartments during Pre-C. This is an important step in this experimental paradigm that avoids any preference bias prior to conditioning. All the CPP procedures described in the following sections (Study 1; experiments 1 to 3) were initiated on postnatal day 50 (PND50) and are summarized in Table 1.

2.3.1.1. Experiment 1: effects of CBD on the acquisition of cocaine-induced CPP. After Pre-C, animals ($n = 10$–12 per group) were exposed to the conditioning phase. During conditioning, mice were pre-treated with CBD (0, 30 or 60 mg/kg) 60 min prior to Sal or Coc (10 mg/kg) administration, and, immediately after, were placed in its corresponding conditioning chamber for 30 min. After an interval of 4 h, all animals received Sal immediately before being confined to the other compartment for 30 min. As a result, for each condition subjects underwent a total of 2 pairings per day (one with the drug, and the other with saline) separated by 4-h intervals, on 4 consecutive days. For each of the 30-min conditioning trials, the central area was made inaccessible by closing the guillotine doors. The order of conditioning sessions with drugs (CBD and Coc) or Sal was alternated each day. Five experimental groups were constituted according to the treatment received before conditioning in the drug-paired compartment: CBD 0 + Coc, CBD 30 + Coc, CBD 60 + Coc, CBD 30 + Sal and CBD 60 + Sal.

The preference test (post-conditioning; Post-C) took place 24 h after the last conditioning session. During Post-C, the guillotine doors separating the two cubicles were removed and the time spent by the untreated mice in each chamber was recorded during a 900-s period.

2.3.1.2. Experiment 2: effects of CBD on the expression of cocaine-induced CPP. The procedure followed in this experiment was the same as the previous one, with the exception that animals ($n = 10$–12 per group) were treated only with Coc during the conditioning phase (and those in the opposite compartment with Sal), and received an injection of CBD (0, 30, or 60 mg/kg) 60 min prior to the Post-C test. As a result, three experimental groups were formed: Coc + CBD 0, Coc + CBD 30 and Coc + CBD 60.

Table 1
Summary of the experimental design of Study 1: "Effects of CBD on cocaine-induced conditioned place preference (CPP)". In experiment 1 (Exp. 1, Acquisition), during the conditioning phase mice were injected with cannabidiol (CBD) at doses of 0, 30 or 60 mg/kg (CBD 0, 30, and 60; respectively) 60 min prior to the administration of 10 mg/kg of cocaine (Coc 10 mg/kg) or saline (NaCl 0.9%, *w/v*; Sal) during 4 consecutive days (see details in the text). Twenty-four hours after the last conditioning session, mice were exposed to the preference test (post-conditioning; Post-C). Five experimental groups were constituted according to the treatment received before conditioning in the drug-paired compartment: CBD 0 + Coc, CBD 30 + Coc, CBD 60 + Coc, CBD 30 + Sal, and CBD 60 + Sal. In experiment 2 (Exp. 2, Expression), during the conditioning phase all mice received cocaine (Coc 10 mg/kg). Three experimental groups were constituted according to the treatment received one hour before the Post-C test: 0, 30 or 60 mg/kg of CBD (CBD 0, 30, and 60; respectively). In experiment 3 (Exp. 3, Extinction/Reinstatement), all animals were conditioned with cocaine and performed the Post-C test free of any treatment. Then, they initiated the extinction phase (two weekly extinction sessions) during which mice were injected with CBD (0, 30 or 60 mg/kg) 60 min before each extinction assay. Twenty-four hours after the confirmation of the extinction of the CPP, all mice were treated with a priming dose of 5 mg/kg of cocaine (Coc 5 mg/kg) fifteen min before the reinstatement test. Three experimental groups were formed according to the dose of CBD received before the extinction sessions: 0, 30 or 60 mg/kg of CBD (CBD 0 + RCoc, CBD 30 + RCoc, and CBD 60 + RCoc; respectively).

CPP procedure	Experimental group	Conditioning phase			Post-C			Extinction			Reinstatement		
		Pre-treatment	*Time*	*Treatment*	*Treatment*	*Time*		*Treatment*	*Time*		Treatment	*Time*	
Exp. 1: Acquisition	CBD 0 + Coc	CBD (0 mg/kg)	60 min	Coc (10 mg/kg)	–	–	Test	–	–	–	–	–	–
	CBD 30 + Coc	CBD (30 mg/kg)		Coc (10 mg/kg)									
	CBD 60 + Coc	CBD (60 mg/kg)		Coc (10 mg/kg)									
	CBD 30 + Sal	CBD (30 mg/kg)		Sal									
	CBD 60 + Sal	CBD (60 mg/kg)		Sal									
Exp. 2: Expression	CBD 0	–	–	Coc (10 mg/kg)	CBD (0 mg/kg)	60 min	Test	–	–	–	–	–	–
	CBD 30			Coc (10 mg/kg)	CBD (30 mg/kg)								
	CBD 60			Coc (10 mg/kg)	CBD (60 mg/kg)								
Exp. 3: Extinction/ Reinstatement	CBD 0 + RCoc	–	–	Coc (10 mg/kg)	–	–	Test	CBD (0 mg/kg)	60 min	Tests (4–6)	RCoc (5 mg/kg)	15 min	Test
	CBD 30 + RCoc			Coc (10 mg/kg)				CBD (30 mg/kg)			RCoc (5 mg/kg)		
	CBD 60 + RCoc			Coc (10 mg/kg)				CBD (60 mg/kg)			RCoc (5 mg/kg)		

2.3.1.3. Experiment 3: effects of CBD administration during the extinction of cocaine-induced CPP and priming-induced reinstatement. The CPP extinction procedure followed herein was based on previous published reports (Calpe-López et al., 2021; Guerrero-Bautista et al., 2019). In brief, after being conditioned with cocaine as in the preceding experiment, and following the Post-C test during which they were free of any treatment, mice (n = 8–10 per group) underwent a CPP extinction protocol of two weekly extinction sessions in which they were placed in the apparatus (without the guillotine doors separating the compartments) for 900 s, until the time spent in the drug-paired compartment by each group was similar to that of Pre-C and different from that of the Post-C test. To evaluate the effect of CBD during this phase, mice were injected with CBD (0, 30, or 60 mg/kg) 60 min before each extinction assay. Extinction of CPP was always confirmed in a subsequent trial 24 h after the session in which extinction was achieved. In these groups, the effect of a priming dose of cocaine (RCoc; 5 mg/kg) on the reinstatement of CPP was also evaluated twenty-four hours after confirmation of extinction. Based on our previous investigations (Calpe-López et al., 2021), the reinstatement test was the same as that for Post-C (free ambulation for 900 s), except that the animals were tested 15 min after the non-contingent administration of cocaine priming. In this experiment, three experimental groups were formed according to the dose of CBD received before the extinction sessions: 0, 30 or, 60 mg/kg of CBD (CBD 0 + RCoc, CBD 30 + RCoc and CBD 60 + RCoc; respectively).

2.3.2. Study 2: effects of CBD and cocaine on locomotion

2.3.2.1. Experiment 4: effects of CBD on cocaine-induced locomotor stimulation. Locomotor activity was measured automatically by an actimeter (CIBERTEC S.A., Spain) consisting of eight cages (33 × 15 × 13 cm), each with eight infrared lights located in a frame around the cage. In this apparatus, beams were positioned on the horizontal axis 2 cm apart, at a height just above the bottom of the cage (body level of the mice). The different frames were separated from each other at a distance of 4 cm, and, since they were opaque, prevented the animals from seeing conspecifics. To evaluate the effect of our treatments on locomotor activity, subjects (n = 11–12 per group, PND 50) were divided into six experimental groups depending on the dose of CBD (0, 30, or 60 mg/kg) administered 60 min prior to the treatment with Coc (25 mg/kg) or Sal as follows: CBD 0 + Coc, CBD 30 + Coc, CBD 60 + Coc, CBD 0 + Sal, CBD 30 + Sal and CBD 60 + Sal (see Table 2). Mice were introduced into the actimeter cages immediately after the administration of the first injection (CBD 0, 30, or 60 mg/kg) and remained there for 60 min, until they received the second injection, in order to habituate them to the test environment. Locomotor activity was recorded during an additional period of 60 min, initiated immediately after the second injection (Coc or Sal).

Table 2
Summary of the experimental design of Experiment 5: "Effects of CBD on cocaine-induced locomotor stimulation". Cannabidiol (CBD) was i.p. injected at doses of 0, 30, or 60 mg/kg (CBD 0, 30 or 60; respectively) and mice were immediately introduced into the actimeter cages for one hour (Habituation phase) prior to the dispensation of 25 mg/kg of cocaine (Coc) or saline (NaCl 0.9%, w/v; Sal). Locomotor activity was recorded during 60 min just after the second injection (Test phase). The experimental groups formed were: CBD 0 + Coc, CBD 30 + Coc, CBD 60 + Coc, CBD 0 + Sal, CBD 30 + Sal, and CBD 60 + Sal. For more details see the materials and methods section.

Experimental group	Habituation phase		Test phase	
	Pre-treatment	*Time*	*Treatment*	*Time*
CBD 0 + Coc	CBD (0 mg/kg)	60 min	Coc (25 mg/kg)	60 min
CBD 30 + Coc	CBD (30 mg/kg)		Coc (25 mg/kg)	
CBD 60 + Coc	CBD (60 mg/kg)		Coc (25 mg/kg)	
CBD 0 + Sal	CBD (0 mg/kg)		Sal	
CBD 30 + Sal	CBD (30 mg/kg)		Sal	
CBD 60 + Sal	CBD (60 mg/kg)		Sal	

2.3.3. Study 3: effects of CBD on the behavioral alterations induced by cocaine withdrawal

In this study, we attempted to assess the effect of CDB on different behavioral disturbances in mice experiencing withdrawal from cocaine after chronic dispensation. The procedure of cocaine administration was a variation of that described by Black and colleagues, which has been proven to induce long-lasting alterations in rodent behavior (Black et al., 2006; Ledesma et al., 2017). In short, cocaine (or Sal, at the same volume as in control animals) was administered to mice in ascending doses (binge pattern) over a period of 19 days starting on PND 68. During this period the animals received three injections per day, separated by 60-min intervals, of either Sal or 5 mg/kg of cocaine (Coc 5) on PND 68 and 69; 10 mg/kg (Coc 10) on PND 70, 71 and 72 (followed by a 2-day drug-free period); 15 mg/kg (Coc 15) on PND 75, 76, 77, 78 and 79; and after another 2 drug-free days, 20 mg/kg (Coc 20) on PND 82, 83, 84, 85 and 86.

The behavioral tests were performed from PND 89 to PND 92. These time intervals between cocaine cessation and behavioral testing were based on previously published reports, which showed that the effects of cocaine abstinence on several behaviors in mice can be detected between the first and the fourteenth days after cocaine removal (Aguilar et al., 2017; Georgiou et al., 2016; Ledesma et al., 2017). In all the experiments carried out in Study 3, mice (n = 12–15 per group) were treated with CBD (0, 60, or 120 mg/kg) 60 min before initiating each experiment, and the following experimental groups were composed: Sal+CBD 0, Sal+CBD 60, Sal+CBD 120, Coc + CBD 0, Coc + CBD 60 and Coc + CBD 120 (see Table 3). These doses were based on a report in which it was demonstrated that only a dose of 60 mg/kg (and not that of 30 mg/kg) of CBD was able to ameliorate the DI disturbances induced by cocaine in C57 mice (Gomes et al., 2015). For this reason, we decide to administer doses of CBD over 30 mg/kg in the present study.

2.3.3.1. Experiment 5: effects of CBD on the Open Field after cocaine withdrawal. The open field (OF) experiment was performed on PND 89 with the aim of assessing whether CBD alters changes in motor behavior induced by cocaine withdrawal. This experiment also aims to determine whether CBD induces any unspecific effect on the normal spontaneous locomotor activity of mice that could mask the results obtained in the rest of the experiments. For this purpose, animals were introduced individually into locomotor activity chambers consisting of a square arena (30 × 30 × 35 cm) illuminated by a dim white light (40 lx). Horizontal locomotion was recorded by a computerized video-tracking system and measured as cm traveled in 10 min. The movement of the mice inside the open-field chamber was registered and sent automatically to the computer using the software package Ethovision 2.0 (Noldus, The Netherlands).

2.3.3.2. Experiment 6: effects of CBD on the object recognition test after cocaine withdrawal. To estimate the effect of our treatments on memory, we employed the Object Recognition (OR) paradigm. The novel object recognition test is considered a suitable model to investigate the effects of pharmacological manipulations on learning and memory (Ennaceur and Delacour, 1988). It has been used in rodents to evaluate episodic memory as a measure of cognitive dysfunction, according to deficits in object-context identification (Broadbent et al., 2004). In the present study, the OR was performed in an open box (30 × 30 × 15 cm) divided by cardboard into four equally sized arenas. A camera recorder fixed to the ceiling enabled the four arenas to be visualized simultaneously. To implement the OR test, we followed the same methodology as in earlier investigations performed in our laboratory (Aguilar et al., 2017; Ledesma et al., 2017). For the test, two types of objects were used: two small river stones and a small non-toxic plastic toy. This task consists of three phases: habituation, training session (T1), and test session (T2). In the habituation phase (performed on PND 90), mice were placed in the center of the empty open field box and allowed to explore it freely for 2

Table 3
Summary of the experimental design of Study 3: "Effects of CBD on the behavioral alterations induced by cocaine withdrawal". Cocaine (Coc) was i.p. administered for three daily injections separated by a 60-min interval over a period of 19 subsequent days in ascending doses as follows: two days of 5 mg/kg, three days of 10 mg/kg, two-day period of abstinence, five days of 15 mg/kg, a second period of two-day abstinence, and five days of 20 mg/kg. Sal = saline (NaCl 0.9%, *w/v*) was given at the same treatment conditions as cocaine. Cannabidiol (CBD) was dispensed one hour prior to each behavioral test at doses of 0, 60 or 120 mg/kg (CBD 0, 60, and 120; respectively). PND = Post-natal day. OF = Open Field, OR = Object Recognition test, TST = Tail Suspension Test. The experimental groups constituted were: Sal+CBD 0, Sal+CBD 60, Sal+CBD 120, Coc + CBD 0, Coc + CBD 60, and Coc + CBD 120. For more details see the materials and methods section.

Experimental group	Cocaine "binge-pattern"				Behavioral testing								
	PND 68–69	*PND 70–72*	*PND 75–79*	*PND 82–86*	*PND 89*	*Time*	*Test*	*PND 91*	*Time*	*Test*	*PND 92*	*Time*	*Test*
Sal+CBD 0	Sal	Sal	Sal	Sal	CBD (0 mg/kg)	60 min	OF	CBD (0 mg/kg)	60 min	OR	CBD (0 mg/kg)	60 min	TST
Sal+CBD 60	Sal	Sal	Sal	Sal	CBD (60 mg/kg)			CBD (60 mg/kg)			CBD (60 mg/kg)		
Sal+CBD 120	Sal	Sal	Sal	Sal	CBD (120 mg/kg)			CBD (120 mg/kg)			CBD (120 mg/kg)		
Coc + CBD 0	Coc (5 mg/kg)	Coc (10 mg/kg)	Coc (15 mg/kg)	Coc (20 mg/kg)	CBD (0 mg/kg)			CBD (0 mg/kg)			CBD (0 mg/kg)		
Coc + CBD 60	Coc (5 mg/kg)	Coc (10 mg/kg)	Coc (15 mg/kg)	Coc (20 mg/kg)	CBD (60 mg/kg)			CBD (60 mg/kg)			CBD (60 mg/kg)		
Coc + CBD 120	Coc (5 mg/kg)	Coc (10 mg/kg)	Coc (15 mg/kg)	Coc (20 mg/kg)	CBD (120 mg/kg)			CBD (120 mg/kg)			CBD (120 mg/kg)		

min. Twenty-four hours later (on PND 91), during T1, subjects were treated with CBD (0, 60 and 120 mg/kg) and 60 min later were placed for 3 min in the open-field arena containing the stones (placed in opposite corners), after which the mice were returned to their home cage for 1 min (memory retention interval). For T2, we replaced one of the stones with a toy, and, after the memory retention interval, the animals were placed once again in the open-field arena for another 3 min to evaluate their exploration of the novel object. Object exploration was defined as intentional contact of the mouse's snout or front paws with the object from a distance of 2 cm or less. The following behaviors were scored: sum of the total time (t, seconds); exploring the two stones in T1; sum of the time exploring the stone and the toy in T2; and the difference between the time exploring the stone versus the toy in T2. The basic measure of memory acquisition in the OR test is the discrimination index (DI), calculated as: DI = [(tnovel - tfamiliar)/(tnovel + tfamiliar)] × 100.

2.3.3.3. Experiment 7: effects of CBD on the Tail Suspension Test after cocaine withdrawal. The Tail Suspension Test (TST) measures the time that a mouse is immobile, which is considered to represent behavioral despair (Pollak et al., 2010). It is based on the observation that rodents, after initial escape-oriented movements, develop an immobile posture when placed in an inescapable stressful situation. In the case of the TST, the stressful situation involves the hemodynamic stress of being hung by their tail, a position in which they have no control (Cryan et al., 2005). This has been used as a measure of behavioral depression because, when antidepressant treatments are given prior to the test, the subjects engage in escape-directed behaviors for longer periods of time (Pollak et al., 2010). Following the protocol described by Vaugeois and colleagues (Vaugeois et al., 1997), on PND 92 mice were suspended from a hook by the tail using adhesive tape while connected to a strain gauge that recorded their movements during a 6-min test. The behavior displayed by the mice was video recorded and later analyzed by a 'blind' observer using a computerized method. The parameter considered in the statistical analyses was the total time (*sec*) spent immobile.

2.4. Statistical analysis

To evaluate the acquisition of CPP, the time spent in the drug-paired compartment during Pre-and Post-C tests was analyzed with a mixed ANOVA with a between-subjects variable - Treatment, with five levels (CBD 0 + Coc, CBD 30 + Coc, CBD 60 + Coc, CBD 30 + Sal and CBD 60 + Sal) - and a within-subjects variable: Days, with two levels (Pre-C and Post-C).

To evaluate the effects of CBD on the expression of cocaine-induced CPP the same mixed ANOVA was used, with the exception that the variable Treatment had three levels (Coc + CBD 0, Coc + CBD 30 and Coc + CBD 60).

During extinction, differences between the time spent in the drug-paired compartment during Pre-C and each extinction session were analyzed using paired Student's *t*-tests. Extinction was considered to have been achieved when there were no significant differences between the time spent in the drug-paired compartment in Pre-C and in the extinction session on two consecutive days. To evaluate if CBD is capable of impairing reinstatement of a previously extinguished cocaine-induced CPP, the difference between the time spent in the drug-paired compartment during the reinstatement test versus Pre-C was also analyzed using paired Student's *t*-tests.

To assess the effect of CBD on cocaine-induced locomotor stimulation, the data were analyzed by means of a two-way ANOVA with the variables Pre-treatment, with three levels (CBD 0, 30 and 60), and Treatment, with two levels (Sal or Coc).

To evaluate the effects of CBD on symptoms of cocaine abstinence, we performed a two-way ANOVA with two between-subjects variables: Treatment (with two levels, Sal and Coc) and CBD (with three levels, CBD 0, 60 and 120 mg/kg).

In all cases, post-hoc comparisons were performed with Bonferroni tests.

3. Results

3.1. Study 1: effects of CBD on cocaine-induced conditioned place preference (CPP)

3.1.1. Experiment 1: effects of CBD on the acquisition of cocaine-induced CPP

In the first experiment, we found that none of the doses of CBD administered (30 and 60 mg/kg) was able to impede the acquisition of cocaine-induced CPP. Moreover, at these doses, CBD did not evoke any conditioning effect by itself. For this reason, and given that we had observed that CBD (30 and 60 mg/kg) did not induce any conditioning effect in experiment 1 (i.e., groups CBD 30+ and 60 + Sal), we decided it was unnecessary to incorporate an additional control group - namely, a CBD 0 + Sal group that would not receive any pharmacological treatment) - because it would not provide any relevant information in relation to the working hypothesis. This was decided with the aim of complying with regulations of the national committee for research ethics in science and technology regarding the use of animals in research

required, which restrict the number of experimental animals to the minimum required to provide reliable data.

The ANOVA of the data regarding the time spent in the drug-paired compartment revealed that the variables Days [$F(1, 50) = 10.67$; $p < 0.01$] and Treatment [$F(4, 50) = 3.65$; $p < 0.05$], as well as the interaction Days X Treatment [$F(4, 50) = 9.01$; $p < 0.01$], were significant. As depicted in Fig. 1, all mice treated with cocaine (irrespective of the dose of CBD administered) spent more time in the drug-paired compartment during Post-C in comparison to Pre-C ($p < 0.001$ for the groups CBD 0 + Coc and CBD 60 + Coc, $p < 0.05$ for the group CBD 30 + Coc). Animals treated with CBD (30 or 60) + Sal did not show significant changes in the time spent in this compartment between Pre-C and Post-C.

3.1.2. Experiment 2: effects of CBD on the expression of cocaine-induced CPP

Similarly to experiment 1, in experiment 2 we observed that pre-treatment with CBD (30 and 60 mg/kg) did not modify the expression of CPP in animals previously exposed to our cocaine-induced CPP procedure. The ANOVA of the data for the time spent in the drug-paired compartment revealed that only the variable Days was significant [$F(1, 29) = 33.8$; $p < 0.01$]. As can be seen in Fig. 2, all mice expressed cocaine-induced CPP, irrespective of the dose of CBD received before the Post-C test ($p < 0.01$ for the Coc + CBD 0, $p < 0.001$ for Coc + CBD 30, and $p < 0.05$ for the Coc + CBD 60).

3.1.3. Experiment 3: effects of CBD administration during the extinction of cocaine-induced CPP and priming-induced reinstatement

As expected, all groups acquired cocaine-induced CPP (i.e., the animals spent more time in the Coc-paired compartment in Post-C versus Pre-C; Fig. 3). In relation to the extinction of cocaine-induced CPP, all the experimental groups required a similar number of extinction sessions to achieve it, independently of the dose of CDB administered (6 sessions for the groups that received CBD 0 and 60 mg/kg, and 4 sessions for the group injected with CBD 30 mg/kg). Therefore, it seems that, under our experimental conditions, CBD (30 and 60 mg/kg) does not modulate the extinction of cocaine-induced CPP. However, CBD treatment before each of the trials carried out to extinguish the previously acquired CPP did block the reinstatement of cocaine-induced CPP.

The ANOVA for the time spent in the drug-paired compartment during Pre-C and Post-C revealed that only the variable Days was significant [$F(1, 24) = 58$; $p < 0.001$]. Nevertheless, as can be seen in Fig. 3, only the group treated with CBD 0 mg/kg before each extinction trial showed reinstatement of CPP after injection of the priming dose of Coc (CBD 0 + RCoc group; Student's-*t*-test, $p < 0.05$; significant differences detected when comparing the time spent in the drug-paired compartment in the reinstatement test versus Pre-C).

3.2. Study 2: effects of CBD and cocaine on locomotion

3.2.1. Experiment 4: effects of CBD on cocaine-induced locomotor stimulation

In this experiment, we observed that pre-treatment with 30 mg/kg of CBD reduced the locomotor stimulating effects of Coc.

The ANOVA of the accumulated activity during the Test period showed that the variable Treatment [$F(1, 65) = 19.42$; $p < 0.01$] and the interaction Pre-treatment X Treatment [$F(2, 65) = 3.34$; $p < 0.05$] were significant. Post-hoc comparisons indicated that cocaine was able to induce locomotor stimulation (Fig. 4), since the groups CBD 0 + Coc and CBD 60 + Coc displayed increased locomotion with regard to their control groups (CBD 0 + Sal and CBD 60 + Sal, respectively; $p < 0.01$). However, pre-treatment with CBD 30 mg/kg blocked cocaine-induced hyperlocomotion, as locomotor activity in the CBD 30 + Coc group did not differ from that in the CBD 30 + Sal group. In addition, the CBD 30 + Coc group exhibited less locomotion than the CBD 0 + Coc group ($p < 0.05$). Furthermore, CBD (30 and 60 mg/kg) did not have an effect by itself on spontaneous locomotion under our experimental conditions,

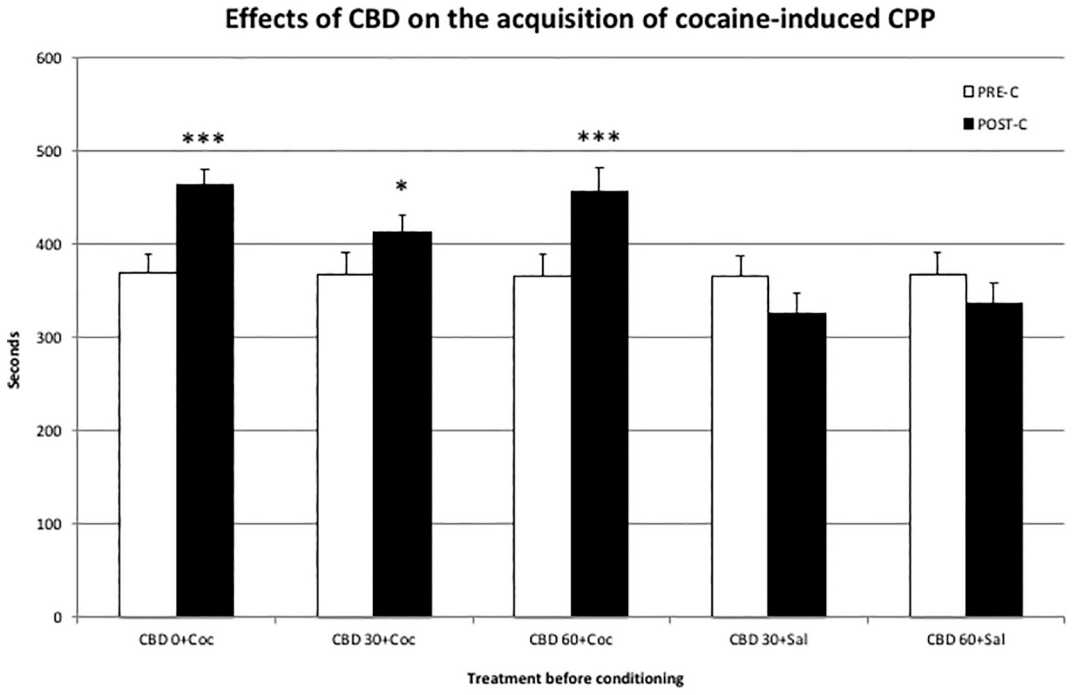

Fig. 1. Effects of CBD on the acquisition of cocaine-induced CPP. Five groups were formed according to the treatment received before confinement to the drug-paired compartment during four conditioning sessions. The first group received one injection of vehicle and, sixty min later, one injection of 10 mg/kg of cocaine (CBD 0 + Coc, $n = 12$). The second and third groups received one injection of CBD, 30 or 60 mg/kg respectively, and sixty min later, one injection of 10 mg/kg of cocaine (CBD 30 + Coc, $n = 10$; CBD 60 + Coc, n = 12). The fourth and fifth groups received one injection of CBD, 30 or 60 mg/kg respectively, and sixty min later, one injection of saline (CBD 30 + Sal, $n = 11$; CBD 60 + Sal, n = 10). Bars represent the mean (and vertical lines ± SEM) time spent (in seconds) in the drug-paired compartment before conditioning (Pre-C, white bars) and after conditioning (Post-C, black bars) (*, and ***; $p < 0.05$ and 0.001 respectively, significant difference between Post-C and Pre-C).

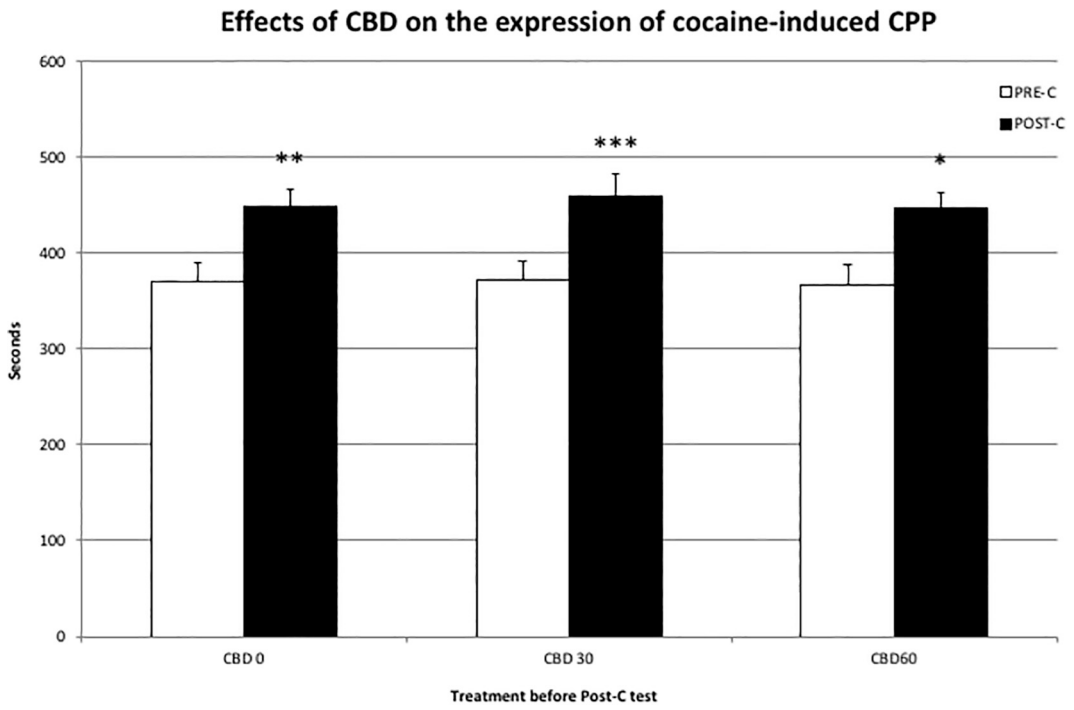

Fig. 2. Effects of CBD on the expression of cocaine-induced CPP. Three groups were formed according to the treatment received before the Post-C test. All groups were conditioned with cocaine (10 mg/kg) during four conditioning sessions. Sixty min before the Post-C test the first group received vehicle (CBD 0, n = 10) and the second and third groups 30 or 60 mg/kg of CBD, respectively (CBD 30, n = 12; CBD 60, n = 10). Bars represent the mean (and vertical lines ± SEM) time spent (in seconds) in the drug-paired compartment before conditioning (Pre-C, white bars) and after conditioning (Post-C, black bars) (*, **, and ***; $p < 0.05$, 0.01, and 0.001 respectively; significant difference between Post-C and Pre-C).

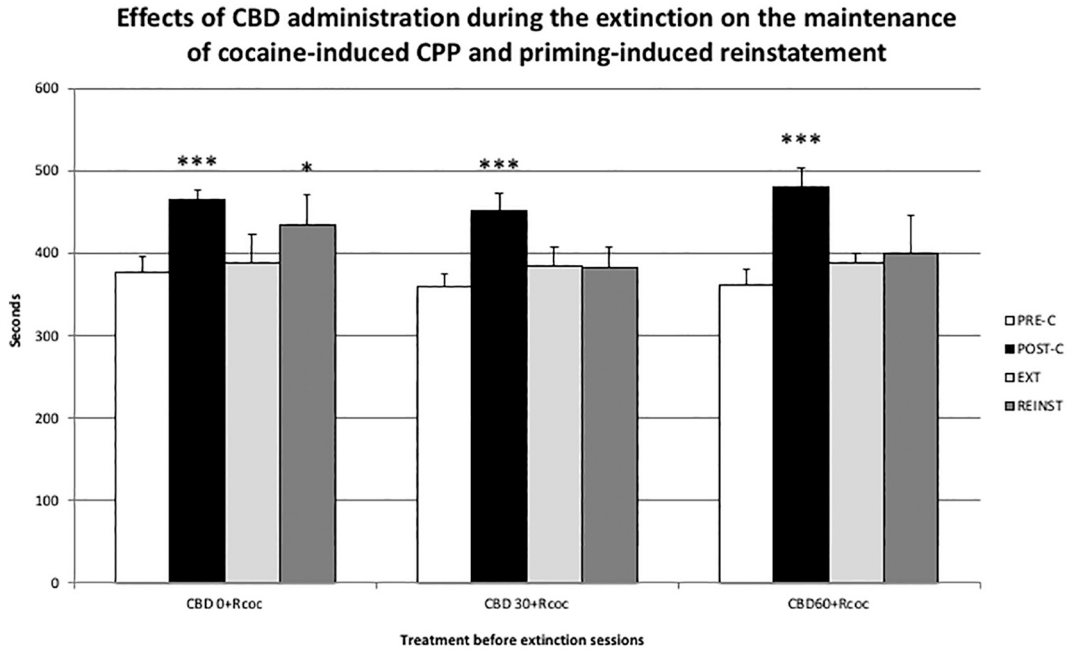

Fig. 3. Effects of CBD administration during extinction on the maintenance of cocaine-induced CPP and priming-induced reinstatement. Three groups were formed according to the treatment received before the extinction of the CPP. All groups were conditioned with cocaine (10 mg/kg) during four conditioning sessions. Sixty min before each extinction session of CPP, the first group received vehicle (CBD 0 + RCoc, $n = 8$) and the second and third groups 30 or 60 mg/kg of CBD, respectively (CBD 30 + RCoc, n = 10; CBD 60 + RCoc, $n = 9$). After extinction of CPP, all groups received a priming injection of cocaine (5 mg/kg) and were tested for reinstatement of CPP. Bars represent the means (and vertical lines ± SEM) of the time spent (in seconds) in the drug-paired compartment before conditioning (Pre-C, white bars), after conditioning (Post-C, black bars), in the last extinction session (EXT, light grey bars), and in the reinstatement test (REINST, dark grey bars) (***, and *; $p < 0.001$ and 0.05 respectively, significant difference with respect to Pre-C).

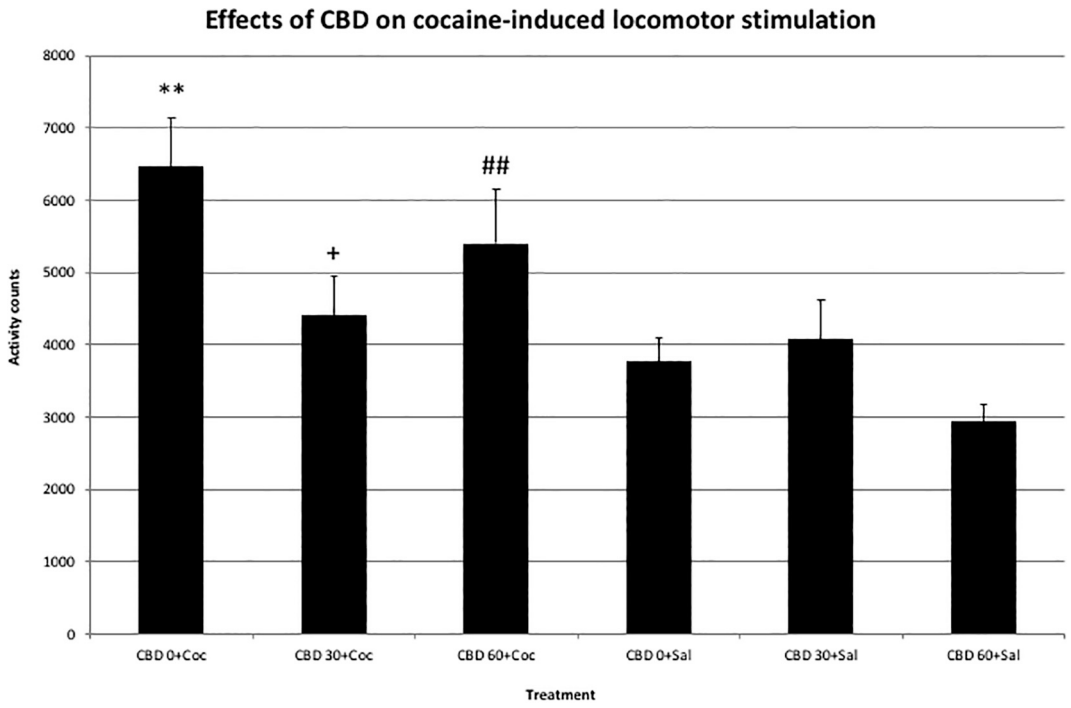

Fig. 4. Effects of CBD on cocaine-induced locomotor stimulation. Six groups were formed according to the treatment administered before the assessment of locomotor stimulation. All mice (n = 12; except for the group CBD 60 + Coc, n = 11) received two injections separated by a period of 60 min (habituation to the actimeter, data not shown). Immediately after the second injection, locomotor behavior was registered during a period of 60 min. The first group received an injection of the vehicle and 60 min later an injection of 25 mg/kg of cocaine (group CBD 0 + Coc). The second and third groups received an injection of 30 or 60 mg/kg of CBD and 60 min later an injection of 25 mg/kg cocaine (group CBD 30 + Coc and group CBD 60 + Coc, respectively). The fourth group received an injection of the vehicle and 60 min later an injection of saline (group CBD 0 + Sal). The fifth and the sixth groups received an injection of 30 or 60 mg/kg of CBD and 60 min later an injection of saline (group CBD 30 + Sal and group CBD 60 + Sal, respectively). Bars depict the accumulated activity counts during the whole period of the test (60 min) (**, $p < 0.01$ significantly different with respect to CBD 0 + Sal; ##, $p < 0.01$ significantly different with respect to CBD 60 + Sal; +, $p < 0.05$ significantly different with respect to CBD 0 + Coc).

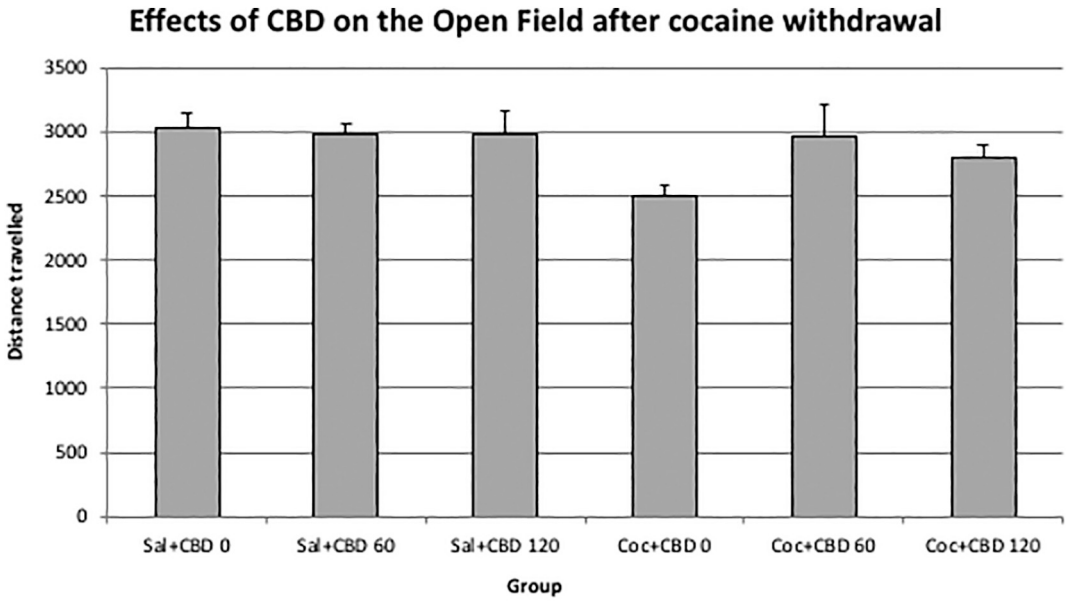

Fig. 5. Effects of CBD on the Open Field after cocaine withdrawal. Six groups were formed according to the treatment received during the induction of cocaine dependence, and before the test. Three groups received injections of saline (following the same schedule as the cocaine binges) and were treated with vehicle, 60 or 120 mg/kg of CBD, 60 min before the Open Field test (groups Sal+CBD 0, Sal+CBD 60, and Sal+CBD 120; respectively). The other three groups received binges of cocaine (see details of doses and schedule of administration in the text) and were treated with vehicle, 30 or 60 mg/kg of CBD, 60 min before the Open Field test (groups Coc + CBD 0, Coc + CBD 60, and Coc + CBD 120; respectively). Bars represent the distance travelled in cm during the 10-min test period.

as demonstrated by the lack of significant differences between the two groups treated with CBD (CBD 30+ and CBD 60 + Sal) and the group that did not receive CBD (CBD 0 + Sal).

3.3. Study 3: effects of CBD on the behavioral alterations induced by cocaine withdrawal

3.3.1. Experiment 5: effects of CBD on the open field after cocaine withdrawal

In relation to the spontaneous locomotor activity of mice under cocaine withdrawal, we did not observe any effect of either Coc and CBD in the OF.

As can be observed in Fig. 5, all the groups exhibited similar locomotor activity levels. The ANOVA for the results of the OF experiment demonstrated that the variables Treatment [$F(1, 83) = 3.222$, $p = 0.08$] and CBD [$F(1, 83) = 0.874$, $p = 0.42$], as well as their interaction [$F(2, 82) = 1.316$, $p = 0.27$], were not significant.

3.3.2. Experiment 6: effects of CBD on the object recognition test after cocaine withdrawal

The major relevant finding of this experiment is that administration of the dose of 120 mg/kg of CBD before exposure to OR reduced the memory impairment elicited by cocaine abstinence in mice.

The ANOVA for the data of the OR (Fig. 6) indicated that there was a significant effect of the variable Treatment [$F(1, 82) = 108.48$, $p < 0.01$], and of the interaction between Treatment and CBD [$F(2, 82) = 13.51$, $p < 0.01$]. Post-hoc comparisons revealed a lower DI in the Sal+CBD 120 group than in the Sal+CBD 0 group ($p < 0.05$). In addition, the groups Coc + CBD 0 and Coc + CBD 60 showed a lower DI than their respective control groups (Sal+CBD 0 and Sal+CBD 60, respectively) ($p < 0.01$), while the Coc + CBD 120 group presented a higher DI than the Coc + CBD 0 and Coc + CBD 60 groups ($p < 0.01$).

3.3.3. Experiment 7: effects of CBD on the Tail Suspension Test after cocaine withdrawal

In relation to the depressive-like symptoms measured in mice under cocaine withdrawal in the TST, our data revealed that CBD did not have any effect.

The ANOVA for the results of the TST (Fig. 7) showed that only the variable Treatment was significant [$F(1, 80) = 6.92$, $p < 0.01$]. Mice treated with cocaine binges spent more time immobile than mice treated with Sal ($p < 0.01$).

4. Discussion

The results presented herein provide information about the effects of CBD administration on some of the main behavioral outcomes related to cocaine addiction in mice. Specifically, CBD was effective in preventing reinstatement, but did not affect acquisition, expression or extinction of cocaine CPP (Study 1). Furthermore, CBD reduced the locomotor-stimulating actions of cocaine (Study 2) and the memory deficits (but not the depressive-like symptoms) associated with cocaine withdrawal (Study 3).

4.1. Study 1: effects of CBD on cocaine-induced conditioned place preference (CPP)

In Study 1, we evaluated the effects of CBD on the conditioned rewarding properties of cocaine by exposing mice to different phases of the CPP paradigm (i.e., acquisition, expression, extinction and reinstatement). In line with previous reports, in the first experiment, we found that cocaine (10 mg/kg) induced CPP in mice (Calpe-López et al.,

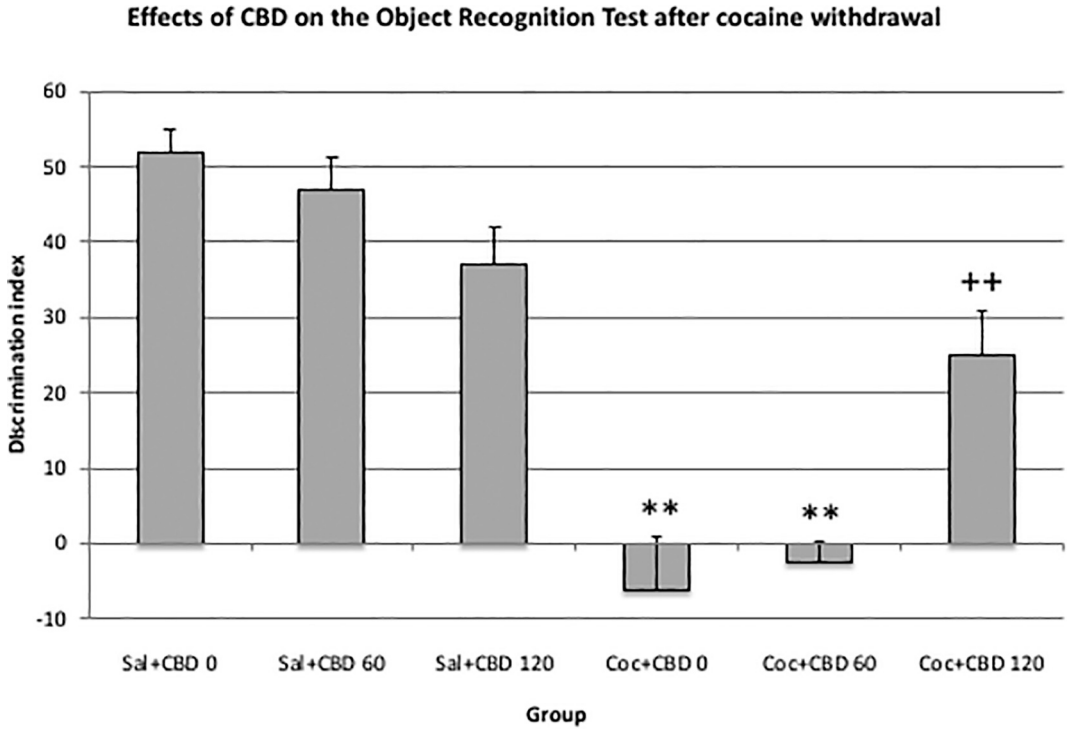

Fig. 6. Effects of CBD on the Object Recognition Test after cocaine withdrawal. Six groups were formed according to the treatment received during induction of cocaine dependence, and before the test. Three groups received injections of saline (following the same schedule as the cocaine binges) and were treated with vehicle, 60 or 120 mg/kg of CBD, 60 min before the Object Recognition test (groups Sal+CBD 0, Sal+CBD 60, and Sal+CBD 120; respectively). The other three groups received binges of cocaine (see details of doses and schedule of administration in the text) and were treated with vehicle, 60 or 120 mg/kg of CBD, 60 min before the Object Recognition test (groups Coc + CBD 0, Coc + CBD 60, and Coc + CBD 120; respectively). Bars represent the discrimination index (see details in the text) (**, $p < 0.01$ significantly different comparing the Coc + CBD 0 and Coc + CBD 60 with respect to its corresponding control group, Sal+CBD 0 and Sal+CBD 60 respectively; ++, $p < 0.01$ significantly different from the Coc + CBD 0 and Coc + CBD 60 groups).

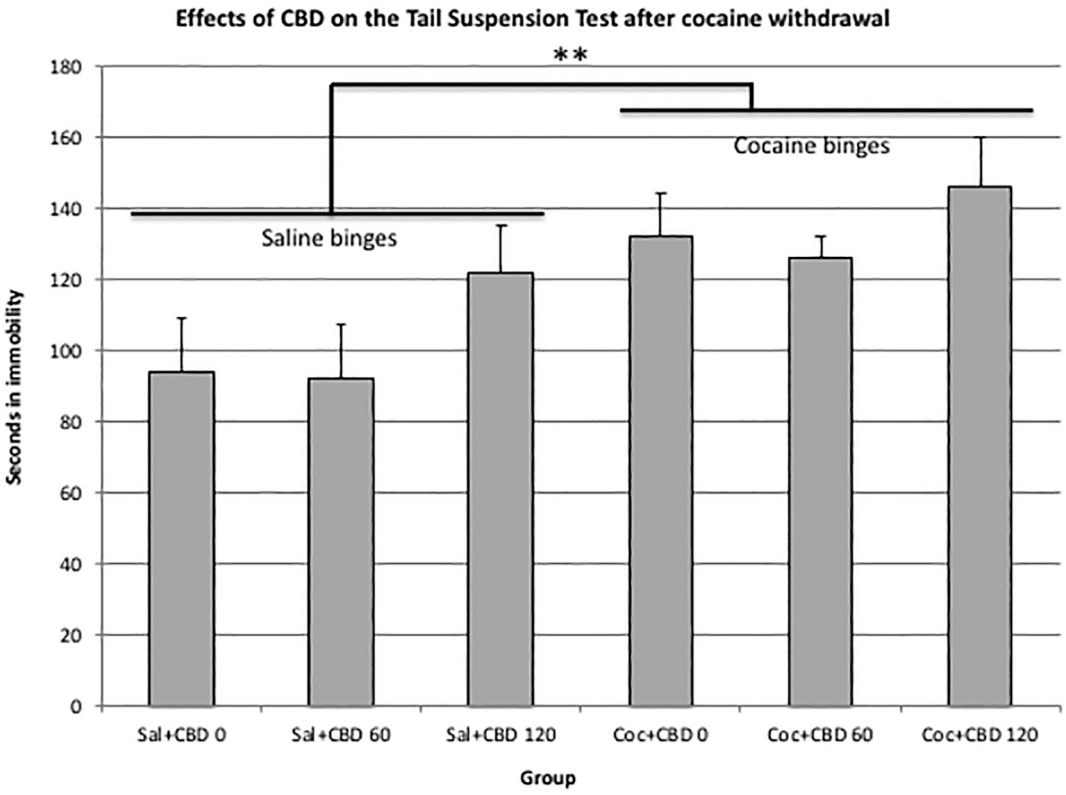

Fig. 7. Effects of CBD on the Tail Suspension Test after cocaine withdrawal. Six groups were formed according to the treatment received during the induction of cocaine dependence, and before the test. Three groups received injections of saline (following the same schedule as the cocaine binges) and were treated with vehicle, 60 or 120 mg/kg of CBD, 60 min before the Tail Suspension Test (groups Sal+CBD 0, Sal+CBD 60, and Sal+CBD 120; respectively). The other three groups received binges of cocaine (see details of doses and schedule of administration in the text) and were treated with vehicle, 60 or 120 mg/kg of CBD, 60 min before the Tail Suspension Test (groups Coc + CBD 0, Coc + CBD 60, and Coc + CBD 120; respectively). Bars represent the time (seconds) spent immobile (**, $p < 0.01$ significant difference between the groups receiving binges of cocaine and the groups treated with saline).

2020; Li and Wolf, 2015) and that CBD did not produce motivational effects by itself (Asth et al., 2021; Markos et al., 2018; Viudez-Martínez et al., 2018; Yang et al., 2020). Moreover, we observed that pre-treatment with CBD did not modify acquisition of cocaine-induced CPP. These results are similar to those obtained in an earlier investigation, in which it was found that CBD (30 mg/kg) pre-treatment did not block acquisition of the CPP elicited by cocaine (10 mg/kg) in mice (Luján et al., 2018). Nonetheless, in the same study it was shown that CBD was capable of impairing the acquisition of cocaine-induced CPP when administered at doses of 10 and 20 mg/kg. These results seem to indicate that CBD only undermines the rewarding actions mediated by cocaine at doses lower than 30 mg/kg. However, in another recent study, CBD administered at a dose of 10 mg/kg, 30 min before each conditioning session with cocaine (15 mg/kg), did not alter the acquisition of cocaine-induced CPP (Chesworth and Karl, 2020). In our opinion, the discrepancies in the results observed between studies could be due to differences in the experimental procedures (e.g., the strain of mice used, or the duration and number of CPP sessions, among others). A key factor seems to be the time at which the Post-C test is performed; 24 h after the last CPP training session (as in the present study, and that of Chesworth and Karl, 2020), or 5 days after the last CPP session (as in the experiments carried out Luján et al., 2018). It cannot be ruled out that this 5-day interval between the last conditioning trial and the Post-C test reduces the capacity of mice to recall the previously acquired CPP. Given that we have not observed differences between vehicle and CBD pre-treated animals in the acquisition of cocaine-induced CPP, we suspect that this treatment is unable to impair the conditioned rewarding actions mediated by cocaine, at least under our experimental conditions; that is, when tested CPP 24 h after the last conditioning session.

In the second experiment, we found that acute administration of CBD (0, 30 and 60 mg/kg) before the Post-C test did not modify the expression of the previously acquired cocaine-induced CPP. Testing the effects of a drug on the expression rather than the acquisition of CPP offers the advantage of allowing its influence on previously acquired conditioned rewarding effects to be evaluated (pairing the compartment with cocaine without any additional treatment during conditioning) (Gremel and Cunningham, 2008). Arguably, treatments that impair drug-seeking during CPP tests might have significant translational relevance in terms of identifying potential therapies for drug addiction (Cunningham et al., 2011). To our knowledge, this is the first study to evaluate the effects of CBD on the expression of cocaine-induced CPP. Our results suggest that CBD is unable to impair the conditioned preference for environmental cues previously associated with the rewarding effects evoked by cocaine.

In the third experiment, we observed that CBD does not modulate the extinction of cocaine-induced CPP, in accordance with the results of a recent investigation (Chesworth and Karl, 2020), but in contrast with those of an earlier study in which it was shown that CBD pre-treatment potentiated the extinction of cocaine-induced CPP in rats (Parker et al., 2004). However, given diverging results between rats and mice in response to the reinforcing effects of several psychoactive drugs, such as alcohol (Green and Grahame, 2008), it is reasonable to attribute the inconsistencies between our results and those of Parker and colleagues (Parker et al., 2004) to the use of different rodent models. Besides the species used, there are other important differences concerning the CPP paradigm between our study and that of Parker et al. (2004). In particular, Parker et al. (2004) used only one Pre-C trial and a different schedule of conditioning (two or four cycles, every 48–72 h, consisting of one cocaine trial and one saline trial separated by intervals of 24 h), and they employed forced extinction (one trial of confinement in the previously treatment-paired compartment without receiving any drug).

Thus, we can conclude that, at least under our experimental conditions, CBD does not accelerate the extinction of conditioned behavior. The most interesting finding of this experiment is in relation to the reinstatement of CPP. We found that administration of 30 or 60 mg/kg of CBD prior to each extinction trial subsequently blocked the priming-induced reinstatement of cocaine CPP. These data are in accordance with a very recent investigation conducted in our laboratory, in which we demonstrated that acute administration of CBD 60 min prior to the priming dose of cocaine was also effective in abrogating the reinstatement of cocaine-induced CPP in CD1 mice (Calpe-López et al., 2021).

In our opinion, the lack of an effect of CBD on both the acquisition and expression of CPP suggests that CBD is not effective in preventing the formation of associations between the positive effects of cocaine and contextual cues that are present during its consumption in the initial phases of drug use. Furthermore, CBD appears not to facilitate extinction of drug-seeking behavior elicited by cues previously associated with exposure to cocaine. In contrast, CBD is effective in preventing reinstatement of drug-seeking behavior. Taking into the account that relapse to drug seeking and drug intake is a significant problem in human addict populations, manipulations that impede the recovery of CPP after its extinction are a useful and widely used model of relapse to drug-seeking in rodents (Aguilar et al., 2008). Given that CBD treatment during the extinction phase of cocaine-induced CPP is able to blunt its later reinstatement, CBD should be considered a potentially useful medication in the management of cocaine addiction during withdrawal. Therefore, we tentatively propose that CBD could be used in early stages of cocaine addiction to help prevent neuroadaptations which lead to substance abuse and dependence.

At the molecular level, multiple signal functional assays in the brain have indicated that CBD acts as a negative allosteric modulator of the cannabinoid type 1 receptors (CB1) and a partial agonist of cannabinoid type 2 receptors (CB2) (Laprairie et al., 2015; Tham et al., 2019). Moreover, it functions as an agonist of 5-hydroxytryptamine (serotonin) type 1a receptors (5-HT1a) and the vanilloid receptor 1 (TRPV1) (Bisogno et al., 2001; Russo et al., 2005). In addition to the effect of CBD on D2 receptors, its actions on CB2, 5HT1a and TRPV1 have been related to its capacity to block the activation of the brain's reward circuitry after administration of different drugs of abuse, including cocaine (for review see Galaj and Xi, 2020). Specifically, the reduction of cocaine self-administration elicited by CBD treatment is inhibited by administration of AM630, WAY100135 and capsazepine (CB2, 5HT1a and TRPV1 antagonists, respectively) (Galaj et al., 2020), thus suggesting that the central mechanism by which CBD attenuates the behavioral alterations related to cocaine addiction is mediated by such receptors. Furthermore, a recent study in our laboratory has shown that the increased gene expression of the dopamine transporter in the ventral tegmental area induced by an acute injection of cocaine is reversed by administration of CBD (Calpe-López et al., 2021).

4.2. Study 2: effects of CBD and cocaine on locomotion

In Experiment 4, we demonstrated that CBD does not induce changes in spontaneous locomotion and that the lowest dose of CBD (30 mg/kg) employed blocks the locomotor stimulating effects provoked by acute administration of cocaine.

As occurs with other psychoactive substances, the locomotor-stimulating properties of cocaine have been related to an activation of the mesolimbic dopamine system (Crawford et al., 2019; Kalafateli et al., 2021). This effect is due to the ability of cocaine to inhibit the neuronal plasma membrane transporter for dopamine, leading to an augmentation of extracellular dopamine levels (Song et al., 2012; Uhl et al., 2002). The mesolimbic dopamine system, which originates in the ventral tegmental area and projects to the nucleus accumbens, has been shown to be a critical mediator of many psychiatric diseases, including CUD. Although, to date, no medication has yet been approved for the treatment of CUD, some of the most promising pharmacotherapeutic agents include dopamine agonists. Ideally, the use of partial agonists for the receptor of the same neurotransmitter activated by drugs of abuse (e.g., dopamine D2 receptors in the case of cocaine), are considered to be the most effective treatments against diverse addictive disorders such as CUD (Kampman, 2019). Interestingly, CBD has been shown to be a partial agonist of D2 receptors (Seeman, 2016). It has been demonstrated that, when administered alone, CBD (10 and 20 mg/kg, i.p.) does not alter mesolimbic dopamine levels, and that it significantly attenuates the increase in extracellular concentrations of dopamine elicited by an acute challenge of cocaine (10 mg/kg) (Galaj et al., 2019). As noted in the Introduction section, treatments that block cocaine-induced locomotor stimulation by abrogating the increased response that this drug elicits in dopaminergic mesocorticolimbic neurons have been put forward as a useful strategy to prevent some of the effects that lead to the development of cocaine addiction. Therefore, we tentatively propose CBD as a promising ally in the fight against CUD.

4.3. Study 3: effects of CBD on the behavioral alterations induced by cocaine withdrawal

In Study 3, we focused on the possible therapeutic effects of CBD in mice exposed to cocaine withdrawal after chronic administration, emulating a binge-pattern regime like that seen in human addicts (see Table 3 of Supplementary material). Firstly, in Experiment 5, we carried out the OF with the aim of assessing whether CBD alters the changes in motor behavior induced by cocaine abstinence, and also to evaluate if this treatment produces any non-specific effect on the normal spontaneous locomotor activity of mice that could have masked the results obtained in the rest of the experiments. In the same line as that observed in Experiment 4 (CBD did not modify spontaneous locomotion measured in the actimeter), none of the doses of CBD had an effect per se on locomotion in the OF. Furthermore, there were no significant differences in locomotor activity levels between mice treated with binges of cocaine or saline. This result is in accordance with earlier research performed in our laboratory, in which we found that mice exposed to cocaine abstinence did not display locomotor alterations in the OF (Aguilar et al., 2017; Ledesma et al., 2017).

In Experiment 6, we assessed the effect of CBD on the cognitive deficits evoked by cocaine withdrawal. Our results show that the groups Coc + CBD 0 and Coc + CBD 60 exhibited memory disturbances when measured in the OR paradigm. These results are in line with previous reports demonstrating memory impairments in mice performing the OR task during cocaine abstinence (Aguilar et al., 2017; Borkar et al., 2019; Briand et al., 2008; Ladrón de Guevara-Miranda et al., 2017; Ledesma et al., 2017; Morisot et al., 2014). Notwithstanding, our results suggest that the high dose of CBD used reduces the memory alterations elicited by cocaine withdrawal, since the DI of the Coc + CBD 120 group was significantly higher than that of the Coc + CBD 0 and Coc + CBD 60 groups. As mentioned above, the memory disturbances associated with CUD have been related to high rates of relapse and an enhanced risk of addiction (Aguilar et al., 2017; Perrine et al., 2008). We consider this to be a very important finding, as this is the first report confirming this putative therapeutic effect of CBD in the treatment of one of the major psychiatric consequences of chronic cocaine intake.

Finally, in Experiment 7 we assessed the consequences of cocaine withdrawal with respect to depression-like symptoms measured in the TST. As Fig. 7 depicts, the animals in all the experimental groups previously treated with cocaine binges remained immobile in the TST for longer than controls, thereby indicating that cocaine abstinence induces depressive-like behavior in mice. Previous investigations have demonstrated that rats exhibit depression-like behavior when cocaine is removed after its chronic administration, as measured in the forced swimming test (Chartoff et al., 2021; Filip et al., 2006; Hall et al., 2010; Perrine et al., 2008; Philogene-Khalid et al., 2017; Valenza et al., 2017). Nevertheless, to our knowledge, ours is the first study to demonstrate that cocaine withdrawal induces depressive-like symptoms in mice. In

addition, our results indicate that CBD is unable to block this effect of cocaine abstinence.

4.4. General conclusions

The main original findings derived from the current research are as follows: first, CBD administered during the extinction of cocaine-induced CPP blocks later priming-induced reinstatement; second, CBD is effective in inhibiting the locomotor-stimulating action of cocaine; and third, CBD treatment prevents the memory deficit elicited by cocaine withdrawal. Additionally, we show for the first time that this compound does not alter the expression of cocaine-induced CPP and is unable to counteract the depressive-like symptoms related to cocaine abstinence.

Overall, our data endorse CBD as a promising treatment of cocaine addiction, at least at the pre-clinical level. CBD may be an effective pharmacological tool in the management of some of the behavioral alterations seen in CUD, in particular by avoiding relapse to cocaine consumption in addicted patients and reducing the memory disturbances evoked by chronic consumption of this drug. Moreover, the fact that CBD blocks cocaine-induced locomotor stimulation in mice, and considering that this effect is mediated by the same molecular mechanisms involved in cocaine addiction, we believe that CBD can prevent the development of cocaine dependence. Future pre-clinical studies should evaluate in more depth the efficacy of CBD to revert other behavioral outcomes related to CUD.

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Author contributions

MAA review the literature and design the experiments. CM and JCL performed the experimental work. MAA and JCL performed the statistical analyses and wrote the first draft of the paper. All the authors provided critical input to the final version of the paper.

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Welfare of animals

All the procedures involving the mice and their care complied with national, regional, and local laws and regulations, which are in accordance with the Directive 2010/63/EU of the European Parliament and the Council of 22 September 2010 on the protection of animals used for scientific purposes. The protocol was approved by the Ethical Committee for Animal Experiments of the University of Valencia and Generalitat Valenciana (2016/VSC/PEA/00176).

Declaration of Competing Interest

None of the authors report any biomedical financial interests or potential conflicts of interest.

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