

**Cannabidiol regulation of emotion and emotional memory processing: relevance
for treating anxiety-related and substance abuse disorders**

Jonathan L. C. Lee¹, Leandro J. Bertoglio², Francisco S. Guimarães³, *Carl W. Stevenson⁴

¹School of Psychology, University of Birmingham, Birmingham, UK

²Department of Pharmacology, Federal University of Santa Catarina, Florianopolis, SC, Brazil

³Department of Pharmacology, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

⁴School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, UK

Running title: CBD regulation of fear & drug reward processing

Article type: Review

Number of words: 4769

Number of tables: 3

Number of figures: 1

***Corresponding Author:**

Carl Stevenson

School of Biosciences

University of Nottingham

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bph.13724

Sutton Bonington Campus

Loughborough

LE12 5RD

UK

Email: carl.stevenson@nottingham.ac.uk

Abstract

Learning to associate cues or contexts with potential threats or rewards is adaptive and enhances survival. Both aversive and appetitive memories are therefore powerful drivers of behaviour but the inappropriate expression of conditioned responding to fear- and drug-related stimuli can develop into anxiety-related and substance abuse disorders, respectively. These disorders are associated with abnormally persistent emotional memories and inadequate treatment, often leading to symptom relapse. Studies show that cannabidiol, the main non-psychotomimetic phytocannabinoid found in *Cannabis sativa*, reduces anxiety via serotonin_{1A} and (indirect) cannabinoid receptor activation in paradigms assessing innate responses to threat. Accumulating evidence from animal studies investigating the effects of cannabidiol on fear memory processing also indicates that it reduces learned fear in paradigms that are translationally relevant to phobias and post-traumatic stress disorder. Cannabidiol does so by reducing fear expression acutely, and by disrupting fear memory reconsolidation and enhancing fear extinction, both of which can result in the lasting reduction of learned fear. Recent studies have also begun to determine the effects of cannabidiol on drug memory expression using paradigms with translational relevance to addiction. Emerging evidence suggests that cannabidiol reduces the expression of drug memories acutely and by disrupting their reconsolidation. Here we review the literature demonstrating the anxiolytic effects of cannabidiol before focusing on studies investigating its effects on various fear and drug memory processes. Understanding how cannabidiol regulates emotion and emotional memory processing may eventually lead to its use in treating anxiety-related and substance abuse disorders.

TARGETS
GPCRs^a
<u>5-HT_{1A} receptor</u>
Enzymes^b
<u>Fatty acid amide hydrolase</u>
Other protein targets^c
<u>Fatty acid-binding proteins</u>

LIGANDS
<u>Cannabidiol</u>

Abbreviations

5-HT_{1A}: serotonin_{1A}

AFC: auditory fear conditioning

BNST: bed nucleus of the stria terminalis

BZD: benzodiazepine

CB₁: cannabinoid type 1

CB₂: cannabinoid type 2

CBD: cannabidiol

CFC: contextual fear conditioning

CS: conditioned stimulus

CUS: chronic unpredictable stress

dIPAG: dorsolateral periaqueductal gray

EPM: elevated plus-maze

ETM: elevated T-maze

FAAH: fatty acid amide hydrolase

FAPBs: fatty acid-binding proteins

fMRI: functional magnetic resonance imaging

ICR: Institute of Cancer Research

i.c.v.: intracerebroventricular

IL: infralimbic

i.p.: intraperitoneal

LDT: light-dark test

MBT: marble burying test

OFC: olfactory fear conditioning

NSF: novelty suppressed feeding

PAG: periaqueductal grey

PL: prelimbic

PPAR_γ: peroxisome proliferator-activated receptor gamma

PTSD: post-traumatic stress disorder

SCR: skin conductance response

SHR: spontaneously hypertensive rat

SPECT: single-photon emission computed tomography

THC: delta-9-tetrahydrocannabinol

TRP: transient receptor potential

TRPV₁: transient receptor potential vanilloid1

US: unconditioned stimulus

Introduction

Anxiety (e.g. generalized and social anxiety, panic, phobias), trauma-related (i.e. post-traumatic stress disorder (PTSD)), and substance abuse disorders are serious forms of mental illness associated with a significant lifetime prevalence. These disorders pose an enormous social and financial burden as they are often chronic in nature and inadequately treated (Di Luca et al., 2011). Certain anxiety-related disorders (i.e. phobias, PTSD) and addiction are characterized by aberrant and persistent emotional memories of fear- and drug-related stimuli. These discrete or contextual cues can trigger the emergence of symptoms or even their re-emergence after treatment, highlighting the limited effectiveness of currently available psychological and pharmacological therapies to curtail symptom relapse over the long-term (Tronson and Taylor, 2013; Everitt, 2014; Kindt, 2014; Singewald et al., 2015). Moreover, there is also significant co-morbidity between substance abuse disorders and PTSD, which can further complicate how PTSD develops and is treated. For example, the learning and memory processes involved in the psychological therapies that are used for treating PTSD can be adversely affected by different abused drugs, which may also have complex drug-drug interactions with pharmacological treatments for PTSD (Tipps et al., 2014). Thus there is an urgent need to improve the treatment of these disorders.

An area of real promise in this field concerns the use of existing or novel medications as adjuncts to psychological therapies to enhance the efficacy of treatment. Cannabidiol (CBD) is one such drug that shows therapeutic potential in a broad range of neurological and psychiatric diseases (Campos et al., 2012b). This phytocannabinoid is the main non-psychoactive constituent of the *Cannabis sativa* plant and mounting evidence indicates that CBD has anxiolytic properties (Blessing et al., 2015). Emerging preclinical and clinical evidence also indicates that CBD regulates different aversive and appetitive memory processes (Prud'homme et al., 2015; Jurkus et al., 2016), in keeping with the findings of recent studies showing a role for CBD in modulating other types of memory, such as novel object and social recognition, in cognitively impaired animals (Fagherazzi et al., 2012; Cheng et al., 2014). In this paper we begin with a brief historical account of the discovery of CBD and touch on the first studies that investigated its behavioural effects in rodents and humans. We then review the literature on CBD regulation of anxiety and the pharmacological and brain mechanisms involved. The bulk of the paper focuses on discussing the findings from the growing number of mostly preclinical studies that have examined the regulation of

learned fear and, more recently, addictive drug memory processing by CBD. Importantly, these studies have used experimental procedures with clinical relevance for understanding the psychological and neurobiological mechanisms involved in the pathophysiology and treatment of anxiety-related and substance abuse disorders.

CBD discovery and initial studies on its behavioural effects

The *Cannabis sativa* plant contains more than 100 chemically related terpenophenol components called phytocannabinoids (Izzo et al., 2009; Gold, 2015). Since the seminal work of Raphael Mechoulam's group in the 1960s, Delta-9-tetrahydrocannabinol (THC) is considered the main component responsible for the pharmacological effects of the plant (Gaoni and Mechoulam, 1964). The second major component of most samples of *Cannabis sativa* is CBD. Originally isolated by Adams and co-workers in 1940 (Adams et al., 1940), its structure was elucidated by Mechoulam and Shvo in 1963. Although the CBD molecule is similar to THC, it has a distinct spatial conformation that could help to explain their different pharmacological properties. Whereas THC has a planar conformation, CBD presents a "bent" structure with two rings at a right angle to each other (Burstein, 2015).

Initial studies performed in the 1970s, mostly in Brazil, indicated that CBD could block some effects induced by THC in rodents (Karniol and Carlini, 1973; Russo and Guy, 2006). Following these initial studies, Zuardi and collaborators investigated if CBD could prevent the effects of high doses of THC in healthy human volunteers. They found that it attenuates the psychotomimetic and anxiogenic effects of THC (Zuardi et al., 1982).

Although the mechanisms of action of these two drugs were completely unknown at that time, the fact that not all effects of THC were blocked by CBD indicated that the latter was not simply an antagonist of a putative THC receptor. On the contrary, the study suggested that CBD possesses its own antipsychotic and anxiolytic properties (Zuardi et al., 1982).

Laboratory animal tests used to assess the anxiolytic properties of CBD

The potential anxiolytic effect of CBD was initially investigated in preclinical studies. Several animal tests have been employed to explore the effects of putative anxiolytic drugs and the neurobiology of anxiety, which can be defined separately to fear as the emotional response to potential or anticipated (as opposed to actual and present) threat (Tovote et al., 2015). These tests are based on the measurement of defensive behaviours (either active or inhibitory) expressed in response to a threatening or unpleasant stimulus (Campos et al., 2013a). The initial preclinical studies investigating the possible anxiolytic-like effects of

CBD were performed in learning-based models and produced mixed results. These apparently conflicting results were later explained by Guimaraes et al. (1990) using the elevated plus-maze (EPM). This is a commonly used test to investigate anxiety-like behaviour in preclinical studies and is based on the natural aversion that rodents show to open spaces (Handley and Mithani, 1984; Pellow et al., 1985; Treit et al., 1993; Carobrez and Bertoglio, 2005).

Using the EPM, and performing a full dose-response curve in rats, Guimaraes and co-workers showed that acute systemic administration of CBD produces a typical 'bell-shaped' dose-response curve, being anxiolytic at low and intermediate doses but not at high doses. Although some contradictory results exist in the literature, most studies using unlearned or operant conditioning models of anxiety have confirmed these initial findings and the studies investigating CBD effects in classical (Pavlovian) conditioning models also go in the same direction, which will be discussed separately below (summarized in Tables 1-2). Moreover, these anxiolytic effects of CBD in animals have been replicated in human studies using healthy subjects exposed to anxiety-provoking stimuli or situations (Zuardi et al., 1982, 1993; Crippa et al., 2004; Fusar-Poli et al., 2009, 2010) and in patients with anxiety, and possibly also substance abuse, disorders (Bergamaschi et al., 2011, Crippa et al., 2011; Hurd et al., 2015; Shannon and Opila-Lehman, 2016; summarized in Table 3).

Pharmacological mechanisms and brain sites involved in the anxiolytic effects of CBD

The potential therapeutic effects of CBD have been related to multiple pharmacological mechanisms, including the agonism of serotonin 5-HT_{1A} receptors, inhibition of re-uptake and/or metabolism of the endocannabinoid anandamide (resulting indirectly in cannabinoid receptor activation), activation of transient receptor potential vanilloid 1 (TRPV1) channels, inhibition of adenosine re-uptake, antagonism of GPR55, agonism of PPAR γ receptors, intracellular Ca²⁺ increase, and anti-oxidative effects, among others (summarized in Fig 1; the pharmacological nomenclature used throughout conforms with The Concise Guide to PHARMACOLOGY (Alexander et al., 2013)). These pharmacological mechanisms have been discussed recently in several reviews (Izzo et al., 2009; Campos et al., 2012a; Ibeas Bih, et al., 2015; McPartland et al., 2015), to which the reader is referred. So far, however, only two of these mechanisms - 5-HT_{1A} receptor activation and indirect potentiation of endocannabinoid transmission - have been implicated in the attenuation of defensive responses to threatening or stressful stimuli.

Two primary brain systems organize defensive responses to threatening stimuli: one responsive to innate threats and the other responsible for the association between neutral and aversive stimuli, although the neural circuit mechanisms underlying the regulation of anxiety and learned fear show considerable overlap (for reviews see McNaughton and Corr, 2004; Canteras et al., 2010; Gross & Canteras, 2012; Tovote et al., 2015). The brain areas implicated in the anxiolytic effects of cannabidiol include certain medial prefrontal cortical subregions (e.g. prelimbic (PL) and infralimbic (IL) cortex), the bed nucleus of the stria terminalis (BNST), periaqueductal grey (PAG), and amygdala. This evidence comes from preclinical studies and functional imaging studies in humans, which have confirmed the involvement of some of these brain areas. For example, CBD reduced amygdala activation in both mice and humans (Todd and Arnold, 2016; Crippa et al., 2004). Activity in and functional connectivity between the amygdala and anterior cingulate cortex, the homologous region to the rodent dorsomedial prefrontal cortex, were both also decreased by CBD when viewing fearful facial expressions (Fusar-Poli et al., 2009, 2010).

In an initial preclinical study using the EPM test, Campos and Guimarães (2008) showed that the anxiolytic-like effects of CBD injected into the dorsolateral PAG (dlPAG) were prevented by local treatment with the 5-HT_{1A} receptor antagonist WAY100635. Even if this drug can also activate D₄ receptors (Chemel et al., 2006), the anti-aversive effects of CBD were similar to other 5-HT_{1A} receptor agonists infused into the dlPAG (Graeff, 2002). 5-HT_{1A} receptor involvement in the acute anxiolytic/anti-stress effect of CBD was further demonstrated in other relevant brain regions, including the BNST (Gomes et al., 2011) and IL cortex (Marinho et al., 2015). Moreover, systemic treatment with 5-HT_{1A} receptor antagonists was also able to prevent this CBD-induced anxiolysis (see Table 1).

In the marble-burying test and after repeated administration, however, CBD effects on anxiety seem to depend on cannabinoid type 1 (CB₁) receptors rather than 5-HT_{1A} receptors (Casarotto et al., 2010; Campos et al., 2013; Nardo et al., 2014). Even if the (+)-CBD enantiomer shows affinity for CB₁ receptors, the naturally occurring (-)-CBD does not bind to these receptors (Hanus et al., 2005), indicating that the CB₁ receptor-mediated anti-aversive effects of CBD are probably indirect. Bisogno et al. (2001) showed that CBD blocked the reuptake and metabolism of anandamide *in vitro*. In the same direction, using embryonic hippocampal cells, Campos et al. (2013b) showed that the increase in cell proliferation induced by CBD is prevented by antagonism of either CB₁ or cannabinoid type 2 (CB₂) receptors, as well as by overexpression of fatty acid amide hydrolase (FAAH), the enzyme responsible for anandamide metabolism. More recently, Dale Deutsch's group demonstrated

that CBD binds to fatty acid binding proteins necessary for the transport of anandamide from the plasma membrane to intracellular FAAH, which might be a primary mechanism by which CBD decreases anandamide uptake/metabolism (Elmes et al., 2015). Consistent with these *in vitro* studies, the anti-stress (in mice) and antipsychotic (in humans) effects of repeated CBD administration were associated with increased hippocampal and serum levels, respectively, of anandamide (Campos et al., 2013b, Leweke et al., 2012).

Emotional learning and memory processing

We first summarize the psychological mechanisms involved in classical conditioning, a type of associative learning whereby discrete cues or contexts come to predict the occurrence of threatening or rewarding stimuli, before reviewing the evidence demonstrating a role for CBD in regulating different fear and drug memory processes. During conditioning an innocuous conditioned stimulus (CS), which can be a discrete cue (e.g. sound, light, odor) or a context (e.g. testing chamber/arena), becomes associated with an aversive (e.g. footshock) or appetitive (e.g. drug reward availability) unconditioned stimulus (US). After conditioning, the CS-US association undergoes consolidation into long-term memory and later presentation of or re-exposure to the CS alone initially elicits conditioned fear (e.g. freezing, avoidance) or drug-seeking (e.g. lever pressing, place preference) responses (Peters et al., 2009). Retrieval of the CS can make emotional memories labile by destabilizing the memory trace, which allows for these memories to be maintained or updated through the process of reconsolidation (Lee, 2009). Repeated presentations of or prolonged exposure to the CS causes the extinction of emotional memories, resulting in the formation of a new CS-no US association which competes with the original emotional memory to suppress conditioned responding to the CS (Peters et al., 2009). Understanding how behavioural and/or pharmacological interventions can attenuate conditioned responding, disrupt memory reconsolidation, and/or enhance extinction has clinical relevance given that all of these mechanisms are potential therapeutic strategies for alleviating the symptoms of PTSD (i.e. pathological fear) and addiction (i.e. drug craving) (Tronson and Taylor, 2013; Everitt, 2014; Kindt, 2014; Singewald et al., 2015).

CBD effects on fear memory processing

As alluded to above, growing evidence indicates that CBD also regulates learned fear (see Table 2). Systemic CBD administration has been shown to reduce the expression of fear memory when given acutely (Zuardi and Karniol, 1983; Resstel et al., 2006; Lemos et al., 2010; Jurkus et al., 2016). CBD has also been reported to impair the acquisition of fear learning, given that acute systemic administration before fear conditioning resulted in attenuated fear expression during later memory retrieval testing (Levin et al., 2012). Fear memory consolidation is equally impaired by acute systemic administration of CBD when given after conditioning (Stern et al., 2016). In contrast, the reported effects of repeated CBD administration on fear memory expression are still scarce and conflicting. In one study, daily injections for 14 days prior to conditioning enhanced fear expression during retrieval testing, suggesting that chronic CBD facilitated fear learning (ElBatsh et al., 2012), whereas another study showed no effect of CBD on fear conditioning when administered for 21 days (Cheng et al., 2014).

Studies indicate that CBD also modulates the extinction and reconsolidation of conditioned fear, leading to lasting effects on learned fear expression. Intracerebroventricular infusions of CBD given before three extinction sessions resulted in enhanced contextual fear extinction (Bitencourt et al., 2008). Systemic administration of CBD given acutely before extinction has been shown to affect contextual fear extinction depending on the strength of fear conditioning beforehand. CBD impaired extinction after weak conditioning but enhanced extinction after strong conditioning (Song et al., 2016). However, CBD given systemically before auditory fear extinction reduced fear expression acutely without affecting extinction memory (Jurkus et al., 2016). Interestingly, a study in humans also showed that CBD had no effect on the extinction of visual fear memory when given before extinction but it did enhance extinction memory when given immediately after extinction (Das et al., 2013).

Contrary to the reported facilitatory effects of CBD on fear extinction, this drug has been shown to disrupt the reconsolidation of contextual fear memory after its brief retrieval (Stern et al., 2012; Gazarini et al., 2015; Stern et al., 2015), although these contrasting effects of CBD on fear extinction and memory reconsolidation both result in the lasting reduction of learned fear expression. The disruptive effect of systemic CBD administration on reconsolidation required that it was given immediately after memory retrieval as CBD had no effect if it was given without, or six hours after, retrieval. CBD was also able to disrupt the reconsolidation of both newer and older fear memories. Moreover, the subsequent reduction of learned fear expression lasted for over 21 days and was not reinstated by later shock

presentation, indicating that the effects of CBD were due to disrupted memory reconsolidation and not enhanced extinction (Stern et al., 2012).

In another study, CBD given immediately after retrieval disrupted the reconsolidation of an abnormally persistent fear memory when the partial NMDA receptor agonist D-cycloserine was first administered before retrieval to facilitate memory destabilization. Fear memory was strengthened pharmacologically by enhancing adrenergic transmission immediately after conditioning, resulting in generalized fear expression and impaired fear suppression by extinction (Gazarini et al., 2015). Understanding the mechanisms underlying reconsolidation disruption of such fear memories is important because evidence indicates that strong fear memories can show resistance to pharmacological disruption of reconsolidation (Lee, 2009), which has implications for using this potential therapeutic approach to weaken traumatic memories in the treatment of PTSD.

Pharmacological mechanisms and brain sites involved in the effects of CBD on learned fear

Just as the anxiolytic effects of CBD involve a direct effect on 5-HT_{1A} receptors and an indirect effect on cannabinoid receptors via elevated endocannabinoid levels, so too do its effects on different fear memory processes. Similarly, there is overlap in the neural circuitry involved in mediating the effects of CBD on anxiety and learned fear. The reduction in conditioned fear expression induced by CBD was accompanied by attenuated c-Fos expression in the PL and IL cortices and the BSNT. Moreover, CBD infusion into the BNST or PL cortex reduced fear memory expression, although infusing CBD into the IL cortex enhanced the expression of learned fear (Lemos et al., 2010). This discrepancy between the effects of CBD infused into the PL or IL cortex is likely due to these medial prefrontal cortical subregions exerting opposing influences on learned fear, with the former facilitating its expression and the latter being involved in its suppression and/or extinction (Fenton et al., 2014; Giustino and Maren, 2015). The regulation of conditioned fear expression by CBD in these brain areas was shown to be 5-HT_{1A} receptor-dependent (Gomes et al., 2012; Fogaça et al., 2014; Marinho et al., 2015). The inhibitory effect of CBD on the acquisition of fear conditioning has also been shown to depend on 5-HT_{1A} receptor activation in the nucleus accumbens shell (Norris et al., 2016).

In contrast to the acquisition and expression of fear memory, the consolidation, reconsolidation, and extinction of learned fear involves (indirect) cannabinoid receptor activation. The inhibitory effect of CBD on fear memory consolidation was blocked by CB₁ or CB₂ receptor antagonist pretreatment (Stern et al., 2016). The facilitatory effect of intracerebroventricular CBD infusion on fear extinction was inhibited by prior CB₁ receptor antagonism but not TRPV1 channel blockade (Bitencourt et al., 2008). CBD was shown to act in the IL cortex to facilitate fear extinction as infusing CBD into this region enhanced extinction, an effect which also depended on CB₁ receptors (Do Monte et al., 2013). The disruptive effect of CBD on fear memory reconsolidation was blocked by pretreatment with a CB₁ receptor antagonist given systemically or infused into the PL cortex, whereas prior 5-HT_{1A} receptor antagonism had no effect on the disruption of reconsolidation by CBD (Stern et al., 2012, 2014).

CBD effects on addictive drug memory processing

In contrast to the study of fear memories, to date there has been a much more limited exploration of the effects of CBD on addictive drug-related memories. This necessitates a narrative review of the relevant literature, which follows below. Moreover, the small number of studies has been conducted across a variety of experimental paradigms and with different drugs of abuse. These drugs can elicit sensitized responses with intermittent repeated administration, which is context-dependent and thereby reliant upon context-drug associations. Similarly, the acquisition and expression of conditioned place preference behaviour depends upon the integrity of context-drug and/or cue-drug associations. Finally, cue-drug associations can precipitate cue-induced relapse of drug seeking in rodents previously trained to self-administer drug (Aguilar et al., 2009; Steketee and Kalivas, 2011). Each of these paradigms can be studied using stimulants (e.g. cocaine, amphetamine), opiates (e.g. heroin, morphine), and other drugs (e.g. alcohol, nicotine, etc.).

Unlike THC, studies have shown that CBD lacks any rewarding effects of its own given that it fails to induce conditioned place preference or enhance the reinforcing effects of electrical brain self-stimulation (Parker et al., 2004; Vann et al., 2008; Katsidoni et al., 2013). In a study of amphetamine-induced locomotor sensitization, infusions of CBD (100 ng) into the shell subregion of the nucleus accumbens attenuated the development of locomotor sensitization (Renard et al., 2016). While this might suggest that CBD impaired the formation of an amphetamine memory that supports locomotor sensitization, these findings were within the context of mesolimbic mechanisms involved in the potential antipsychotic action of CBD.

Moreover, even though the attenuation of locomotor sensitization was paralleled by modulation of cellular mechanisms of synaptic plasticity, it remains a challenge to disambiguate learning-related behavioural effects from modulation of drug reward (c.f. Katsidoni et al., 2013; Prud'homme et al., 2015), which would impact upon reward-dependent learning. The non-mnemonic interpretation is supported by a failure of CBD to prevent the acquisition of amphetamine place preference (Parker et al., 2004). However, while it appears that CBD does not disrupt the formation of amphetamine-related memories, this does not rule out potential effects on memories formed in relation to other drugs of abuse.

Subsequent to their acquisition, CBD might affect the expression of drug memories. Here there appears to be a contrast depending upon the drug reward under study. Acute administration of CBD (5 and 10 mg kg⁻¹) did not alter cocaine self-administration or cue-induced relapse to cocaine seeking (Mahmud et al., 2016), and so failed to replicate an earlier study of heroin self-administration (Ren et al., 2009). While CBD (5 and 20 mg kg⁻¹) similarly did not alter heroin self-administration, there were effects on cue-induced relapse to heroin seeking (Ren et al., 2009), a measure of cue-heroin memory expression. CBD (5 mg kg⁻¹) reduced responding at a cue-induced relapse test, but only when given 24 hr, and not 30 min, prior to the test. This long-lasting impact upon the expression of the cue-heroin memory was even more persistent (up to 14 days) when three consecutive daily injections of 5 mg kg⁻¹ CBD were given. This ability of CBD to have such long-lasting effects may be mediated by an upregulation of AMPA GluR1 receptors in the nucleus accumbens (Ren et al., 2009).

The impaired expression of cue-heroin relapse in response to CBD administration in animals suggests that this drug might have anti-relapse properties in opiate addiction in humans. This has been explored in a preliminary study of heroin addicts, in which participants were given daily doses of CBD (400 or 800 mg) or placebo for 3 days (Hurd et al., 2015). CBD reduced craving both 24 hr and 7 days later, mirroring the preclinical rodent study (Ren et al., 2009). This beneficial effect of CBD may not be limited to opiate addiction as a conceptually similar, albeit more modest, effect was also previously observed in tobacco smokers (Morgan et al., 2013). In this small week-long study, smokers were instructed to inhale a metered dose of CBD (400 µg) or placebo when they felt like smoking. CBD acutely reduced the number of cigarettes smoked but this effect was not maintained after the cessation of CBD administration. Interestingly, and in contrast to the heroin study, CBD did not alter craving, either acutely or persistently. Therefore, it is not clear whether CBD has generalised effects on the expression of cue-drug memory to elicit craving and precipitate relapse, or whether its effects are specific to certain classes of addictive drugs.

For the maintenance (i.e. reconsolidation) of drug-related memories, there is a single study on morphine and cocaine conditioned place preference. When the place preference memory was briefly reactivated in order to trigger reconsolidation, CBD administration (10 mg kg^{-1}) immediately thereafter led to an impairment in the subsequent maintenance of both cocaine and morphine memories to reduce place preference at test (de Carvalho and Takahashi, 2016). This was a long-lasting effect, which is usually evidence for reconsolidation impairments. However, the study lacked a true non-reactivation control, and so the long-lasting impairment, especially for morphine place preference, is not dissimilar to the aforementioned persistent reduction in the expression of cue-heroin memories in the self-administration setting (Ren et al., 2009). Therefore, it remains unclear whether CBD indeed impairs the reconsolidation of drug memories. Nevertheless, there are indications from the comparison between the place preference and self-administration studies to suggest that their results might be underpinned by qualitatively different processes. For example, while the CBD-induced impairment failed to ameliorate heroin-primed reinstatement of drug seeking (Ren et al., 2009), post-reactivation CBD did prevent morphine-primed reinstatement of place preference (de Carvalho and Takahashi, 2016). Moreover, the contrasting effects of post-reactivation CBD and acute CBD treatment on the subsequent expression of cocaine memories (de Carvalho and Takahashi, 2016) suggests that the impairment in cocaine place preference is not simply explained by long-lasting modulation of drug memory expression.

Similarly, there is a single study of CBD and drug memory extinction. Injection of CBD (5 mg kg^{-1}) prior to an extinction trial enhanced the subsequent reduction in cocaine and amphetamine place preference (Parker et al., 2004). Despite the lack of a no-extinction control, the observation that CBD reduces the expression of stimulant-induced place preference again suggests that such a reduction was, at least in part, due to the concomitant extinction trial. Interestingly, the effect of CBD to reduce cocaine and amphetamine place preference in this extinction study (Parker et al., 2004) is rather similar to the previous observation that CBD impairs the reconsolidation of morphine and cocaine memories in the same place preference setting (de Carvalho and Takahashi, 2016). Indeed, while there was a difference in the timing of CBD administration between the two studies, the single behavioural trial that served to extinguish (Parker et al., 2004) or destabilise (de Carvalho and Takahashi, 2016) the drug memory did not differ greatly. The extinction trial was 15 min in duration, compared to a 10 min reactivation trial, although the former was a confinement to the drug-paired chamber, whereas the latter was a test. Moreover, the conditioning parameters were rather similar across the two studies, and also in prior studies of

reconsolidation that have used 30-min confined reactivation trials for amphetamine place preference (Sakurai et al., 2007), 20-min confined reactivation trials for cocaine place preference (Valjent et al., 2006) and 10-min confined reactivation trials for morphine and nicotine place preference (Wang et al., 2008; Fang et al., 2011). Given also that the parameters of appetitive memory reconsolidation and extinction are usually well distinguished, such that they are each typically defined by much different durations of context re-exposure or numbers of cue presentations (Flavell and Lee, 2013), it is unclear if CBD both enhances extinction and impairs reconsolidation of drug memories. It is perhaps more likely that the two observed effects of CBD to reduce later drug place preference (Parker et al., 2004; de Carvalho and Takahashi, 2016) instead reflect qualitatively similar processes. Appealing simply to the parametric comparisons presented above, we would conclude that there is stronger evidence for CBD impairing drug memory reconsolidation than there is for it enhancing drug memory extinction. Furthermore, given that pharmacological enhancement of extinction is usually dependent upon appreciable extinction-mediated memory reduction (Webber et al., 2007; Bouton et al., 2008), and there was no evidence for any such reduction in the CBD study (Parker et al., 2004), it remains unclear if CBD actually enhances drug memory extinction.

Concluding remarks and future directions

Converging lines of evidence have established that acute CBD treatment is anxiolytic in both animals and humans. A growing number of preclinical studies also indicate that this drug reduces fear memory expression when given acutely. Importantly, CBD results in an enduring reduction in learned fear expression when given in conjunction with fear memory reconsolidation or extinction by disrupting the former and facilitating the latter. This makes CBD a potential candidate for testing as a pharmacological adjunct to psychological therapies or behavioural interventions used in treating PTSD and phobias. These effects of CBD are mediated at least in part by 5-HT_{1A} receptors and indirectly via endocannabinoid-mediated action on cannabinoid receptors, although the involvement of other possible pharmacological mechanisms has not yet been investigated. Studies have begun to elucidate the neural circuit mechanisms underlying the effects of CBD on anxiety and learned fear. The recent functional imaging studies in humans that have examined the alterations in brain activity that accompany the anxiolytic effects of CBD may inform future preclinical and clinical studies investigating the wider neural circuitry involved in mediating its effects on learned fear. In contrast to anxiety and learned fear, research on CBD regulation of addictive drug memory

processing is still in its infancy. Further studies are therefore needed to determine the psychological, pharmacological and brain mechanisms involved in the attenuation of drug memory expression by CBD in relation to different classes of abused drugs. Given the significant co-morbidity between anxiety-related and substance abuse disorders, CBD could also be investigated as a common treatment for such disorders. An outstanding issue is to determine the effects of chronic CBD treatment on different emotional memory processes. For example, one potential therapeutic strategy is to use CBD chronically to reduce symptoms by dampening fear and/or drug memory expression. However, CBD given acutely during the psychological therapy session to impair memory reconsolidation or enhance extinction might be sufficient for facilitating such treatment. Another important consideration is how CBD would be delivered in the treatment of these disorders. Most of the recreationally used cannabis available today contains low levels of CBD and high levels of THC, which can exacerbate symptoms, although cannabis strains containing a more favourable CBD:THC ratio might be an option (Hurd et al., 2015). Similarly, novel formulations of CBD containing only trace amounts of other phytocannabinoids have recently become available for the putative treatment of childhood epileptic disorders (e.g. Epidiolex, GW Pharmaceuticals; Gofshteyn et al., 2016). In summary, this line of research may lead to the potential development of CBD for use in treating anxiety-related and substance abuse disorders in the future.

Acknowledgements

FSG, JLCL, and CWS were funded jointly by a FAPESP-University of Birmingham-University of Nottingham pump-priming award (2012/50896-8). LJB was funded by a Brazilian CNPq research fellowship (307895/2013-0). The funders had no other involvement in any aspect of this work. FSG is co-inventor of the patent “Fluorinated CBD compounds, compositions and uses thereof. Pub. No.: WO/2014/108899. International Application No.: PCT/IL2014/050023”; Def. US no. Reg. 62193296; 29/07/2015; INPI in 19/08/2015 (BR1120150164927). The authors have no other potential conflicts of interest to declare.

References

- Adams R, Hunt M, Clark JH (1940). Structure of cannabidiol, a product isolated from the marihuana extract of minnesota wild hemp. *J Am Chem Soc* 62:196-200.
- Aguilar MA, Rodríguez-Arias M, Miñarro J (2009). Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. *Brain Res Rev* 59:253-277.
- Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M et al. (2013). The Concise Guide to PHARMACOLOGY 2013/14: G Protein-Coupled Receptors. *Br J Pharmacol* 170:1459-1581.
- Almeida V, Levin R, Peres FF, Niigaki ST, Calzavara MB, Zuardi AW et al. (2013). Cannabidiol exhibits anxiolytic but not antipsychotic property evaluated in the social interaction test. *Prog Neuropsychopharmacol Biol Psychiatry* 41:30-35.
- Bergamaschi MM, Queiroz RH, Chagas MH, de Oliveira DC, De Martinis BS, Kapczinski F et al. (2011). Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naïve social phobia patients. *Neuropsychopharmacology* 36:1219-1226.
- Bitencourt RM, Pamplona FA, Takahashi RN (2008). Facilitation of contextual fear memory extinction and anti-anxiogenic effects of AM404 and cannabidiol in conditioned rats. *Eur Neuropsychopharmacol* 18:849-59.
- Blessing EM, Steenkamp MM, Manzanares J, Marmar CR (2015). Cannabidiol as a potential treatment for anxiety disorders. *Neurotherapeutics* 12:825-836.
- Bouton ME, Vurbic D, Woods AM (2008). D-cycloserine facilitates context-specific fear extinction learning. *Neurobiol Learn Mem.* 90:504-510.
- Burstein S (2015). Cannabidiol (CBD) and its analogs: a review of their effects on inflammation. *Bioorg Med Chem* 23:1377-1385.
- Campos AC, de Paula Soares V, Carvalho MC, Ferreira FR, Vicente MA, Brandão ML et al. (2013a). Involvement of serotonin-mediated neurotransmission in the dorsal periaqueductal gray matter on cannabidiol chronic effects in panic-like responses in rats. *Psychopharmacology (Berl)* 226:13-24.
- Campos AC, Ferreira FR, Guimarães FS (2012a). Cannabidiol blocks long-lasting behavioral consequences of predator threat stress: possible involvement of 5HT1A receptors. *J Psychiatr Res* 46:1501-1510.
- Campos AC, Guimarães FS (2009). Evidence for a potential role for TRPV1 receptors in the dorsolateral periaqueductal gray in the attenuation of the anxiolytic effects of cannabinoids. *Prog Neuropsychopharmacol Biol Psychiatry* 33:1517-1521.

- Campos AC, Guimarães FS (2008). Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. *Psychopharmacology (Berl)* 199:223-230.
- Campos AC, Moreira FA, Gomes FV, Del Bel EA, Guimarães FS (2012b). Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. *Philos Trans R Soc Lond B Biol Sci* 367:3364-3378.
- Campos AC, Ortega Z, Palazuelos J, Fogaça MV, Aguiar DC, Díaz-Alonso J et al. (2013b). The anxiolytic effect of cannabidiol on chronically stressed mice depends on hippocampal neurogenesis: involvement of the endocannabinoid system. *Int J Neuropsychopharmacol* 16:1407-1419.
- Canteras NS, Resstel LB, Bertoglio LJ, Carobrez Ade P, Guimarães FS (2010). Neuroanatomy of anxiety. *Curr Top Behav Neurosci* 2:77-96.
- Carobrez AP, Bertoglio LJ (2005). Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. *Neurosci Biobehav Rev* 29:1193-1205.
- Casarotto PC, Gomes FV, Resstel LB, Guimarães FS (2010). Cannabidiol inhibitory effect on marble-burying behaviour: involvement of CB1 receptors. *Behav Pharmacol* 21:353-358.
- Chemel BR, Roth BL, Armbruster B, Watts VJ, Nichols DE (2006). WAY-100635 is a potent dopamine D4 receptor agonist. *Psychopharmacology (Berl)* 188:244-251.
- Cheng D, Low JK, Logge W, Garner B, Karl T (2014). Chronic cannabidiol treatment improves social and object recognition in double transgenic APP^{swe}/PS1 Δ E9 mice. *Psychopharmacology (Berl)* 231:3009-3017.
- Crippa JA, Zuardi AW, Garrido GE, Wichert-Ana L, Guarnieri R, Ferrari L et al. (2004). Effects of cannabidiol (CBD) on regional cerebral blood flow. *Neuropsychopharmacology* 29:417-426.
- Crippa JA, Derenusson GN, Ferrari TB, Wichert-Ana L, Duran FL, Martin-Santos R et al. (2011). Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. *J Psychopharmacol* 25:121-130.
- Das RK, Kamboj SK, Ramadas M, Yogan K, Gupta V, Redman E et al. (2013). Cannabidiol enhances consolidation of explicit fear extinction in humans. *Psychopharmacology* 226:781-792.
- Deiana S, Watanabe A, Yamasaki Y, Amada N, Arthur M, Fleming S et al. (2012) Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarin (CBDV), Δ^9 -tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral

- and intraperitoneal administration and CBD action on obsessive-compulsive behaviour. *Psychopharmacology (Berl)* 219:859-873.
- de Carvalho CR, Takahashi RN (2016). Cannabidiol disrupts the reconsolidation of contextual drug-associated memories in Wistar rats. *Addict Biol* DOI: 10.1111/adb.12366.
- Di Luca M, Baker M, Corradetti R, Kettenmann H, Mendlewicz J, Olesen J et al. (2011). Consensus document on European brain research. *Eur J Neurosci* 33:768-818.
- Do Monte FH, Souza RR, Bitencourt RM, Kroon JA, Takahashi RN (2013). Infusion of cannabidiol into infralimbic cortex facilitates fear extinction via CB1 receptors. *Behav Brain Res* 250:23-27.
- ElBatsh MM, Assareh N, Marsden CA, Kendall DA (2012). Anxiogenic-like effects of chronic cannabidiol administration in rats. *Psychopharmacology (Berl)* 221:239-247.
- Elmes MW, Kaczocha M, Berger WT, Leung K, Ralph BP, Wang L et al. (2015). Fatty acid-binding proteins (FABPs) are intracellular carriers for Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD). *J Biol Chem* 290:8711-8721.
- Everitt BJ (2014). Neural and psychological mechanisms underlying compulsive drug seeking habits and drug memories--indications for novel treatments of addiction. *Eur J Neurosci* 40:2163-2182.
- Fagherazzi EV, Garcia VA, Maurmann N, Bervanger T, Halmenschlager LH, Busato SB et al. (2012). Memory-rescuing effects of cannabidiol in an animal model of cognitive impairment relevant to neurodegenerative disorders. *Psychopharmacology (Berl)* 219:1133-1140.
- Fang Q, Li FQ, Li YQ, Xue YX, He YY, Liu JF et al. (2011). Cannabinoid CB1 receptor antagonist rimonabant disrupts nicotine reward-associated memory in rats. *Pharmacol Biochem Behav* 99:738-742.
- Flavell CR, Lee JLC (2013). Reconsolidation and extinction of an appetitive pavlovian memory. *Neurobiol Learn Mem* 104:25-31.
- Fogaça MV, Reis FM, Campos AC, Guimarães FS (2014). Effects of intra-prelimbic prefrontal cortex injection of cannabidiol on anxiety-like behavior: involvement of 5HT1A receptors and previous stressful experience. *Eur Neuropsychopharmacol* 24:410-419.
- Fusar-Poli P, Allen P, Bhattacharyya S, Crippa JA, Mechelli A, Borgwardt S et al. (2010). Modulation of effective connectivity during emotional processing by Delta 9-tetrahydrocannabinol and cannabidiol. *Int J Neuropsychopharmacol* 13:421-432.

- Fusar-Poli P, Crippa JA, Bhattacharyya S, Borgwardt SJ, Allen P, Martin-Santos R et al. (2009). Distinct effects of Δ^9 -tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Arch Gen Psychiatry* 66:95-105.
- Gaoni Y, Mechoulam R (1964). Isolation, structure and partial synthesis of an active constituent of hashish. *J Am Chem Soc* 86:1646.
- Gazarini L, Stern CA, Piornedo RR, Takahashi RN, Bertoglio LJ (2015). PTSD-like memory generated through enhanced noradrenergic activity is mitigated by a dual step pharmacological intervention targeting its reconsolidation. *Int J Neuropsychopharmacol* 18(1): DOI: 10.1093/ijnp/pyu026.
- Giustino TF, Maren S (2015). The role of the medial prefrontal cortex in the conditioning and extinction of fear. *Front Behav Neurosci* 9:298.
- Gofshteyn JS, Wilfong A, Devinsky O, Bluvstein J, Charuta J, Ciliberto MA et al. (2016) Cannabidiol as a potential treatment for febrile infection-related epilepsy syndrome (FIRES) in the acute and chronic phases. *J Child Neurol* 32:35-40.
- Gomes FV, Reis DG, Alves FH, Corrêa FM, Guimarães FS, Resstel LB (2012). Cannabidiol injected into the bed nucleus of the stria terminalis reduces the expression of contextual fear conditioning via 5-HT_{1A} receptors. *J Psychopharmacol* 26:104-113.
- Gomes FV, Resstel LB, Guimarães FS (2011). The anxiolytic-like effects of cannabidiol injected into the bed nucleus of the stria terminalis are mediated by 5-HT_{1A} receptors. *Psychopharmacology (Berl)* 213:465-473.
- Gould J (2015) The Cannabis crop. *Nature* 525:S2-S3.
- Graeff FG (2002). On serotonin and experimental anxiety. *Psychopharmacology (Berl)* 163:467-476.
- Granjeiro EM, Gomes FV, Guimarães FS, Corrêa FM, Resstel LB (2011). Effects of intracisternal administration of cannabidiol on the cardiovascular and behavioral responses to acute restraint stress. *Pharmacol Biochem Behav* 99:743-748.
- Gross CT, Canteras NS (2012). The many paths to fear. *Nat Rev Neurosci* 13:651-658.
- Guimarães FS, Chiaretti TM, Graeff FG, Zuardi AW (1990). Antianxiety effect of cannabidiol in the elevated plus-maze. *Psychopharmacology (Berl)* 100:558-559.
- Guimarães FS, de Aguiar JC, Mechoulam R, Breuer A (1994). Anxiolytic effect of cannabidiol derivatives in the elevated plus-maze. *Gen Pharmacol* 25:161-164.
- Handley SL, Mithani S (1984). Effects of alpha₂-adrenoceptor agonists and antagonists in a maze-exploration model of fear-motivated behavior. *Naunyn Schmiedeberg Arch Pharmacol* 327:1-5.

- Hanuš LO, Tchilibon S, Ponde DE, Breuer A, Fride E, Mechoulam R (2005). Enantiomeric cannabidiol derivatives: synthesis and binding to cannabinoid receptors. *Org Biomol Chem* 3:1116-1123.
- Hsiao YT, Yi PL, Li CL, Chang FC (2012). Effect of cannabidiol on sleep disruption induced by the repeated combination tests consisting of open field and elevated plus-maze in rats. *Neuropharmacology* 62:373-384.
- Hurd YL, Yoon M, Manini AF, Hernandez S, Olmedo R, Ostman M et al. (2015). Early phase in the development of cannabidiol as a treatment for addiction: opioid relapse takes initial center stage. *Neurotherapeutics* 12:807-815.
- Ibeas Bih C, Chen T, Nunn AV, Bazet M, Dallas M, Whalley BJ (2015). Molecular targets of cannabidiol in neurological disorders. *Neurotherapeutics* 12:699-730.
- Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R (2009). Non-psychoactive plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol Sci* 30:515-527.
- Jurkus R, Day HL, Guimarães FS, Lee JL, Bertoglio LJ, Stevenson CW (2016). Cannabidiol regulation of learned fear: implications for treating anxiety-related disorders. *Front Pharmacol* 7:454.
- Karniol IG, Carlini EA (1973). Pharmacological interaction between cannabidiol and delta-9-tetrahydrocannabinol. *Psychopharmacologia* 33:53-70.
- Katsidoni V, Anagnostou I, Panagis G (2013). Cannabidiol inhibits the reward-facilitating effect of morphine: involvement of 5-HT1A receptors in the dorsal raphe nucleus. *Addict Biol* 18:286-296.
- Kindt M (2014). A behavioural neuroscience perspective on the aetiology and treatment of anxiety disorders. *Behav Res Ther* 62:24-36.
- Lee JL (2009). Reconsolidation: maintaining memory relevance. *Trends Neurosci* 32:413-20.
- Lemos JJ, Resstel LB, Guimarães FS (2010). Involvement of the prelimbic prefrontal cortex on cannabidiol-induced attenuation of contextual conditioned fear in rats. *Behav Brain Res* 207:105-11.
- Levin R, Almeida V, Peres FF, Calzavara MB, da Silva ND, Suiama MA et al. (2012). Antipsychotic profile of cannabidiol and rimonabant in an animal model of emotional context processing in schizophrenia. *Curr Pharm Des* 18:4960-4965.
- Leweke FM, Piomelli D, Pahlisch F, Muhl D, Gerth CW, Hoyer C et al. (2012). Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. *Transl Psychiatry* 2:e94.

- Long LE, Chesworth R, Huang XF, McGregor IS, Arnold JC, Karl T (2010). A behavioural comparison of acute and chronic Delta9-tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice. *Int J Neuropsychopharmacol* 13:861-876.
- Long LE, Chesworth R, Huang XF, Wong A, Spiro A, McGregor IS et al. (2012). Distinct neurobehavioural effects of cannabidiol in transmembrane domain neuregulin 1 mutant mice. *PLoS One* 7:e34129.
- Mahmud A, Gallant S, Sedki F, D'Cunha T, Shalev U (2016). Effects of an acute cannabidiol treatment on cocaine self-administration and cue-induced cocaine seeking in male rats. *J Psychopharmacol* DOI: 10.1177/0269881116667706.
- Malone DT, Jongejan D, Taylor DA (2009). Cannabidiol reverses the reduction in social interaction produced by low dose Delta(9)-tetrahydrocannabinol in rats. *Pharmacol Biochem Behav* 93:91-96.
- Marinho AL, Vila-Verde C, Fogaça MV, Guimarães FS (2015). Effects of intra-infralimbic prefrontal cortex injections of cannabidiol in the modulation of emotional behaviors in rats: contribution of 5HT_{1A} receptors and stressful experiences. *Behav Brain Res* 286:49-56.
- McNaughton N, Corr PJ (2004). A two-dimensional neuropsychology of defense: fear/anxiety and defensive distance. *Neurosci Biobehav Rev* 28:285-305.
- McPartland JM, Duncan M, Di Marzo V, Pertwee RG (2015). Are cannabidiol and Δ(9) - tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. *Br J Pharmacol* 172:737-53.
- Mechoulam R, Shvo Y (1963) 1. Structure of cannabidiol. *Tetrahedron* 19: 2073-2078.
- Moreira FA, Aguiar DC, Guimarães FS (2006). Anxiolytic-like effect of cannabidiol in the rat Vogel conflict test. *Prog Neuropsychopharmacol Biol Psychiatry* 30:1466-71.
- Morgan CJ, Das RK, Joye A, Curran HV, Kamboj SK (2013). Cannabidiol reduces cigarette consumption in tobacco smokers: preliminary findings. *Addict Behav* 38:2433-2436.
- Musty RE, Conti LH, Mechoulam R (1985). Anxiolytic properties of cannabidiol. In *Marihuana '84*. Ed Harvey, D IRL Press: Oxford, pp.713-719.
- Nardo M, Casarotto PC, Gomes FV, Guimarães FS (2014). Cannabidiol reverses the mCPP-induced increase in marble-burying behavior. *Fundam Clin Pharmacol* 28:544-50.
- Norris C, Loureiro M, Kramar C, Zunder J, Renard J, Rushlow W et al. (2016). Cannabidiol modulates fear memory formation through interactions with serotonergic transmission in the mesolimbic system. *Neuropsychopharmacology* 41:2839-2850.

O'Brien LD, Wills KL, Segsworth B, Dashney B, Rock EM, Limebeer CL et al. (2013).

Effect of chronic exposure to rimonabant and phytocannabinoids on anxiety-like behavior and saccharin palatability. *Pharmacol Biochem Behav* 103:597-602.

Onaivi ES, Green MR, Martin BR (1990). Pharmacological characterization of cannabinoids in the elevated plus maze. *J Pharmacol Exp Ther* 253:1002-1009.

Parker LA, Burton P, Sorge RE, Yakiwchuk C, Mechoulam R (2004). Effect of low doses of delta9-tetrahydrocannabinol and cannabidiol on the extinction of cocaine-induced and amphetamine-induced conditioned place preference learning in rats. *Psychopharmacology (Berl)* 175:360-366.

Pellow S, Chopin P, File SE, Briley M (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Meth* 14:149-167.

Peters J, Kalivas PW, Quirk GJ (2009). Extinction circuits for fear and addiction overlap in prefrontal cortex. *Learn Mem* 16:279-288.

Prud'homme M, Cata R, Jutras-Aswad D (2015). Cannabidiol as an intervention for addictive behaviors: a systematic review of the evidence. *Subst Abuse* 9:33-38.

Ren Y, Whittard J, Higuera-Matas A, Morris CV, Hurd YL (2009). Cannabidiol, a nonpsychotropic component of cannabis, inhibits cue-induced heroin seeking and normalizes discrete mesolimbic neuronal disturbances. *J Neurosci* 29:14764-14769.

Renard J, Loureiro M, Rosen LG, Zunder J, de Oliveira C, Schmid S et al. (2016). Cannabidiol counteracts amphetamine-induced neuronal and behavioral sensitization of the mesolimbic dopamine pathway through a novel mTOR/p70S6 kinase signaling pathway. *J Neurosci* 36:5160-5169.

Resstel LB, Joca SR, Moreira FA, Corrêa FM, Guimarães FS (2006). Effects of cannabidiol and diazepam on behavioral and cardiovascular responses induced by contextual conditioned fear in rats. *Behav Brain Res* 172:294-298.

Resstel LB, Tavares RF, Lisboa SF, Joca SR, Corrêa FM, Guimarães FS (2009). 5-HT1A receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats. *Br J Pharmacol* 156:181-188.

Russo E, Guy GW (2006). A tale of two cannabinoids: the therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. *Med Hypotheses* 66:234-246.

Sakurai S, Yu L, Tan SE (2007). Roles of hippocampal N-methyl-D-aspartate receptors and calcium/calmodulin-dependent protein kinase II in amphetamine-produced conditioned place preference in rats. *Behav Pharmacol* 18:497-506.

- Schiavon AP, Bonato JM, Milani H, Guimarães FS, Weffort de Oliveira RM (2016). Influence of single and repeated cannabidiol administration on emotional behavior and markers of cell proliferation and neurogenesis in non-stressed mice. *Prog Neuropsychopharmacol Biol Psychiatry* 64:27-34.
- Shannon S, Opila-Lehman J (2016). Effectiveness of cannabidiol oil for pediatric anxiety and insomnia as part of posttraumatic stress disorder: a case report. *Perm J* 12;20(4).
- Silveira Filho NG, Tufik S (1981). Comparative effects between cannabidiol and diazepam on neophobia, food intake and conflict behavior. *Res Commun Psychol Psychiatr Behav* 6:25-66.
- Singewald N, Schmuckermair C, Whittle N, Holmes A, Ressler KJ (2015) Pharmacology of cognitive enhancers for exposure-based therapy of fear, anxiety and trauma-related disorders. *Pharmacol Ther* 149:150-190.
- Soares Vde P, Campos AC, Bortoli VC, Zangrossi H Jr, Guimarães FS, Zuardi AW (2010). Intra-dorsal periaqueductal gray administration of cannabidiol blocks panic-like response by activating 5-HT1A receptors. *Behav Brain Res* 213:225-229.
- Song C, Stevenson CW, Guimarães FS, Lee JL (2016). Bidirectional effects of cannabidiol on contextual fear memory extinction. *Front Pharmacol* 7:493.
- Steketee JD, Kalivas PW (2011). Drug wanting: behavioral sensitization and relapse to drug-seeking behavior. *Pharmacol Rev* 63:348-365.
- Stern CA, da Silva TR, Pasquini C, Kato L, Guimaraes FS, Andreatini R et al. Cannabidiol attenuates normal and abnormal fear memory formation and related behavioral outcomes through consolidation disruption. *Neuropharmacology*, submitted.
- Stern CA, Gazarini L, Takahashi RN, Guimarães FS, Bertoglio LJ (2012). On disruption of fear memory by reconsolidation blockade: evidence from cannabidiol treatment. *Neuropsychopharmacology* 37:2132-2142.
- Stern CA, Gazarini L, Vanvossen AC, Zuardi AW, Guimaraes FS, Takahashi RN et al. (2014). Involvement of the prelimbic cortex in the disruptive effect of cannabidiol on fear memory reconsolidation. *Eur Neuropsychopharmacol* 24:S322.
- Stern CA, Gazarini L, Vanvossen AC, Zuardi AW, Galve-Roperh I, Guimaraes FS et al. (2015). Δ 9-Tetrahydrocannabinol alone and combined with cannabidiol mitigate fear memory through reconsolidation disruption. *Eur Neuropsychopharmacol* 25:958-965.
- Tipps ME, Raybuck JD, Lattal KM (2014). Substance abuse, memory, and post-traumatic stress disorder. *Neurobiol Learn Mem* 112:87-100.

- Todd SM, Arnold JC (2016). Neural correlates of interactions between cannabidiol and $\Delta(9)$ -tetrahydrocannabinol in mice: implications for medical cannabis. *Br J Pharmacol* 173:53-65.
- Tovote P, Fadok JP, Lüthi A (2015). Neuronal circuits for fear and anxiety. *Nat Rev Neurosci* 16:317-331.
- Treit D, Menard J, Royan C (1993). Anxiogenic stimuli in the elevated plus-maze. *Pharmacol Biochem Behav* 44:463-469.
- Tronson NC, Taylor JR (2013). Addiction: a drug-induced disorder of memory reconsolidation. *Curr Opin Neurobiol* 23:573-580.
- Twardowschy A, Castiblanco-Urbina MA, Uribe-Mariño A, Biagioni AF, Salgado-Rohner CJ, Crippa JA et al. (2013). The role of 5-HT_{1A} receptors in the anti-aversive effects of cannabidiol on panic attack-like behaviors evoked in the presence of the wild snake *Epicrates cenchria crassus* (Reptilia, Boidae). *J Psychopharmacol* 27:1149-59.
- Uribe-Mariño A, Francisco A, Castiblanco-Urbina MA, Twardowschy A, Salgado-Rohner CJ, Crippa JA et al. (2012). Anti-aversive effects of cannabidiol on innate fear-induced behaviors evoked by an ethological model of panic attacks based on a prey vs the wild snake *Epicrates cenchria crassus* confrontation paradigm. *Neuropsychopharmacology* 37:412-421.
- Valjent E, Corbille AG, Bertran-Gonzalez J, Herve D, Girault JA (2006). Inhibition of ERK pathway or protein synthesis during reexposure to drugs of abuse erases previously learned place preference. *Proc Natl Acad Sci U S A* 103:2932-2937.
- Vann RE, Gamage TF, Warner JA, Marshall EM, Taylor NL, Martin BR et al. (2008). Divergent effects of cannabidiol on the discriminative stimulus and place conditioning effects of Delta(9)-tetrahydrocannabinol. *Drug Alcohol Depend* 94:191-198.
- Wang XY, Zhao M, Ghitza UE, Li YQ, Lu L (2008). Stress impairs reconsolidation of drug memory via glucocorticoid receptors in the basolateral amygdala. *J Neurosci* 28:5602-5610.
- Weber M, Hart J, Richardson R (2007). Effects of D-cycloserine on extinction of learned fear to an olfactory cue. *Neurobiol Learn Mem* 87:476-82.
- Zuardi AW, Cosme RA, Graeff FG, Guimarães FS (1993). Effects of ipsapirone and cannabidiol on human experimental anxiety. *J Psychopharmacol* 7(1 Suppl):82-88.
- Zuardi AW, Karniol IG (1983). Changes in the conditioned emotional response of rats induced by 9-THC, CBD and mixture of the two cannabinoids. *Arq Biol TecnoI* 26:391-397.

Zuardi AW, Shirakawa I, Finkelfarb E, Karniol IG (1982). Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects. *Psychopharmacology (Berl)* 76:245-250.

Accepted Article

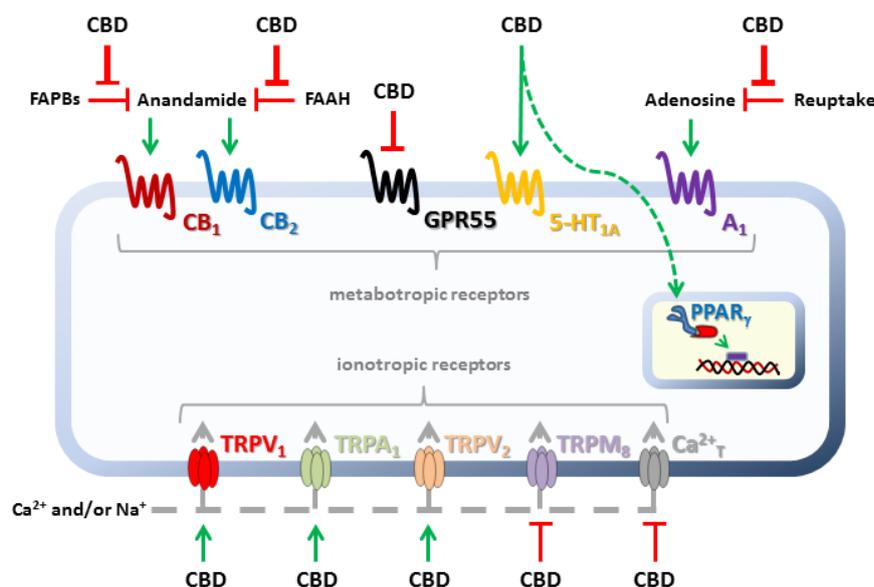


Fig 1. The main molecular targets and potential mechanisms of action of CBD. This drug inhibits both fatty acid amide hydrolase (FAAH), the enzyme which metabolizes anandamide, and fatty acid-binding proteins (FAPBs), which mediate the transport of anandamide to FAAH; both mechanisms ultimately result in the indirect activation of cannabinoid type 1 (CB₁) and/or type 2 (CB₂) receptors. CBD also activates the serotonin_{1A} (5-HT_{1A}) receptor, peroxisome proliferator-activated receptor gamma (PPAR_γ), and the transient receptor potential (TRP) channels TRPV₁, TRPA₁, and TRPV₂. Finally, CBD inhibits adenosine reuptake and antagonizes GPR55, TRPM₈ and T-type Ca²⁺ channels. 5-HT_{1A} and (indirect) cannabinoid receptor activation are the mechanisms that have been implicated in the anxiolytic effects of CBD to date (see Ibeas Bih et al. (2015) and McPartland et al. (2015) for further details).

Table 1. CBD effects on anxiety-like behaviour in male animals. 5-HT_{1A}: serotonin_{1A}; BNST: bed nucleus of the stria terminalis; BZD: benzodiazepine; CB₁: cannabinoid type 1; CUS: chronic unpredictable stress, dlPAG: dorsolateral periaqueductal grey; EPM: elevated plus-maze, ETM: elevated T-maze; ICR: Institute of Cancer Research; i.c.v.: intracerebroventricular; IL: infralimbic, i.p.: intraperitoneal; LDT: light-dark test, MBT: marble burying test, NSF: novelty suppressed feeding, PL: prelimbic; SHR: spontaneously hypertensive rats; THC: delta-9-tetrahydrocannabinol; TRPV1: transient receptor potential vanilloid 1.

Reference	Test used	Strain, species, effective dose, and route/site of administration	Effect	Pharmacological mechanism
Guimarães et al., 1990	EPM	Wistar rats, 2.5-10 mg kg ⁻¹ , i.p.	Anxiolytic (bell-shaped dose-response curve)	Not tested
Onaivi et al., 1990	EPM	ICR mice, 1 and 10 mg kg ⁻¹ , i.p.	Anxiolytic (bell-shaped dose-response curve)	BZD (blocked by flumazenil)
Guimarães et al., 1994	EPM	Wistar rats, 5 mg kg ⁻¹ , i.p.	Anxiolytic	Not tested
Bitencourt et al., 2008	Fear-potentiated EPM	Wistar rats, 6.4 nmol, i.c.v.	Anxiolytic	Not tested
Campos and Guimarães, 2008	EPM	Wistar rats, 30 nmol, intra-dlPAG	Anxiolytic (bell-shaped dose-response curve)	5-HT _{1A} receptor activation
Campos and Guimarães, 2009	EPM	Wistar rats, 30 nmol, intra-dlPAG (60 nmol effective when combined with a TRPV1 channel antagonist)	Anxiolytic	Lack of anxiolytic effect of high doses associated with TRPV1 channel activation
Malone et al., 2009	THC-induced decrease in social interaction	Sprague Dawley rats, 20 mg kg ⁻¹ , i.p.	Anxiolytic	Not tested
Resstel et al., 2009	Restraint stress, autonomic changes, delayed (24 h) anxiogenic effect in EPM	Wistar rats, 10 mg kg ⁻¹ , i.p.	Anti-stress	5-HT _{1A} receptor activation
Casarotto et al., 2010	MBT	C57BL/6 mice, 15-60 mg kg ⁻¹ , i.p.	Anti-compulsive	Indirect CB1 receptor activation
Soares et al., 2010	ETM, electrical stimulation of dlPAG	Wistar rats, 15-60 nmol, intra-dlPAG	Anxiolytic/panicolytic	5-HT _{1A} receptor activation

Long et al., 2010	Open field and light-dark tests	C57BL/6 mice, 1 mg kg ⁻¹ (light-dark test) and 50 mg kg ⁻¹ (open-field), i.p., daily for 21 days	Anxiolytic	Not tested
Gomes et al., 2011	EPM	Wistar rats, 30 nmol, intra-BNST	Anxiolytic	5-HT _{1A} receptor activation
Granjeiro et al., 2011	Restraint stress, autonomic reactivity, delayed (24 h) anxiogenic effect in EPM	Wistar rats, 30 nmol, intra-cisterna magna	Anti-stress	Not tested
Campos et al., 2012	EPM after predator (cat) exposure	Wistar rats, 5 mg kg ⁻¹ , i.p., daily for 7 days	Anxiolytic	5-HT _{1A} receptor activation
Deiana et al., 2012	MBT	Swiss mice, 120 mg kg ⁻¹ , orally or i.p.	Anticompulsive	Not tested
Long et al., 2012	Open field and light-dark tests	C57BL/6 Arc mice, 1 and 100 mg kg ⁻¹ , i.p. daily for 13 days	Anxiolytic (open-field only)	Not tested
Uribe-Mariño et al., 2012	Snake exposure	Swiss mice, 0.3-30 mg kg ⁻¹ , i.p.	Panicolytic	Not tested
Hsiao et al., 2012	Repeated EPM and open-field	Wistar rats, 3.2 nmol, intra-central amygdaloid nucleus	Anxiolytic	Not tested
Campos et al., 2013	EPM and NSF	C57BL/6 mice, 30 mg kg ⁻¹ , daily for 14 days (CUS-exposed animals)	Anti-stress	CB1 receptor-mediated facilitation of hippocampal neurogenesis
O'Brien et al., 2013	Light-dark test	Sprague Dawley rats, 2.5 mg kg ⁻¹ , i.p. for 14 days	No effect	Not tested
Twardowschy et al., 2013	Snake exposure	Swiss mice, 3.0 mg kg ⁻¹ , i.p.	Panicolytic	5-HT _{1A} receptor activation
Almeida et al., 2013	Social interaction test	Wistar and SHR rats, 1 mg kg ⁻¹ , i.p.	Increased social interaction (Wistar rats only)	Not tested
Cheng et al., 2014	EPM	C57BL/6J mice, 20 mg kg ⁻¹ , i.p. daily for 21 days	No effect	Not tested
Fogaça et al., 2014	EPM	Wistar rats, 30 nmol, intra-PL cortex	Anxiogenic (bell-shaped dose response curve), anxiolytic 24 hr after restraint stress	5-HT _{1A} receptor activation

Nardo et al., 2014	MBT	Swiss mice, 30 mg kg ⁻¹ , i.p.	Attenuated mCPP-induced increase in marble-burying (bell-shaped dose response curve)	Indirect CB1 receptor activation
Marinho et al., 2015	EPM	Wistar rats, 15-30 nmol, intra-IL cortex	Anxiolytic (bell-shaped dose response curve), no effect 24 hr after restraint stress	5-HT _{1A} receptor activation
Todd and Arnold, 2016	Open-field	C57BL/6 mice, 10 mg kg ⁻¹ , i.p.	Prevented THC- induced anxiogenesis	Not tested
Schiavon et al., 2016	EPM	Swiss mice, 3 mg kg ⁻¹ , i.p.	Anxiolytic	Not tested

Table 2. CBD effects on learned fear processing in male animals. 5-HT_{1A}: serotonin_{1A}; AFC: auditory fear conditioning; BNST: bed nucleus of the stria terminalis; BZD: benzodiazepine; CB₁: cannabinoid type 1; CB₂: cannabinoid type 2; CFC: contextual fear conditioning; i.c.v.: intracerebroventricular; IL: infralimbic; i.p.: intraperitoneal; OFC: olfactory fear conditioning; PL: prelimbic; THC: delta-9-tetrahydrocannabinol.

Reference	Test used	Strain, species, effective dose and route/site of administration	Effect	Pharmacological mechanism
<i>Studies conducted in operant conditioning paradigms</i>				
Silveira Filho and Tufik, 1981	Geller-Seifter conflict test	Wistar rats, 100 mg kg ⁻¹ , i.p.	No effect	Not tested
Musty et al., 1985	Vogel punished licking test	Sprague-Dawley rats, 5-10 mg kg ⁻¹ , i.p.	Anxiolytic (bell-shaped dose-response curve)	Not tested
Moreira et al., 2006	Vogel punished licking test	Wistar rats, 10 mg kg ⁻¹ , i.p.	Anxiolytic	Not blocked by BZD antagonism (flumazenil)
Gomes et al., 2011	Vogel punished licking test	Wistar rats, 30-60 nmol, intra-BNST	Anxiolytic	5-HT _{1A} receptor activation
<i>Studies conducted in classical (Pavlovian) conditioning paradigms</i>				
Zuardi and Karniol, 1983	AFC	Wistar rats, 10 mg kg ⁻¹ , i.p.	Anxiolytic (decreased fear expression)	Not tested
Resstel et al., 2006	CFC	Wistar rats, 10 mg kg ⁻¹ , i.p.	Anxiolytic (decreased fear expression)	Not tested
Bitencourt et al., 2008	CFC	Wistar rats, 6.4 nmol, i.c.v.	Facilitated fear memory extinction	Indirect CB ₁ receptor activation
Lemos et al., 2010	CFC	Wistar rats, 10 mg kg ⁻¹ , i.p.	Anxiolytic (decreased fear expression)	Not tested
Lemos et al., 2010	CFC	Wistar rats, 30 nmol, intra-PL cortex	Anxiolytic (decreased fear expression)	Not tested
Lemos et al., 2010	CFC	Wistar rats, 30 nmol, intra-IL cortex	Anxiogenic (increased fear expression)	Not tested
ElBatsh et al., 2012	CFC	Lister-hooded rats, 10 mg kg ⁻¹ , i.p. daily for 14 days	Anxiogenic (increased fear expression)	Decreased hippocampal BDNF and TrkB, reduced

				frontal cortex phospho-ERK1/2 expression
Gomes et al., 2012	CFC	Wistar rats, 30-60 nmol, intra-BNST	Anxiolytic (decreased fear expression)	5-HT _{1A} receptor activation
Levin et al., 2012	CFC	Wistar and SHR rats, 1-15 mg kg ⁻¹ , i.p.	Anxiolytic (decreased fear expression) and/or disrupted fear memory formation (Wistar rats only)	Not tested
Stern et al., 2012	CFC	Wistar rats, 3-30 mg kg ⁻¹ , i.p.	Disrupted fear memory reconsolidation (bell-shaped dose response curve)	Indirect CB1 receptor activation
Do Monte et al., 2013	CFC	Long-Evans hooded rats, 1.3 nmol, intra-IL cortex	Facilitated fear memory extinction	Indirect CB1 receptor activation
Cheng et al., 2014	AFC	C57BL/6J mice, 20 mg kg ⁻¹ , i.p. daily for 21 days	No effect	Not tested
Fogaca et al., 2014	CFC	Wistar rats, 30 nmol, intra-PL cortex	Anxiolytic (decreased fear expression)	5-HT _{1A} receptor activation
Gazarini et al., 2014	CFC	Wistar rats, 10 mg kg ⁻¹ , i.p.	Disrupted fear memory reconsolidation	Not tested
Stern et al., 2014	CFC	Wistar rats, 10 mg kg ⁻¹ , i.p.	Disrupted fear memory reconsolidation	Indirect CB1 receptor activation in PL cortex
Marinho et al., 2015	CFC	Wistar rats, 30 nmol, intra-IL cortex	Anxiogenic (increased fear expression)	5-HT _{1A} receptor activation
Stern et al., 2015	CFC	Wistar rats, 1 mg kg ⁻¹ + THC 0.1 mg kg ⁻¹ , i.p.	Disrupted fear memory reconsolidation	Not tested
Norris et al., 2016	OFC	Sprague Dawley rats, 0.03-0.32 nmol, intra-nucleus accumbens shell	Disrupted fear memory formation (acquisition)	5-HT _{1A} receptor activation
Song et al., 2016	CFC	Lister hooded rats, 10 mg kg ⁻¹ , i.p., before extinction (after weak or strong conditioning)	Impaired or enhanced extinction after weak or strong conditioning, respectively	Not tested
Jurkus et al., 2016	AFC	Lister hooded rats, 5-20 mg kg ⁻¹ , i.p.	Anxiolytic (decreased fear expression) at highest dose, no effect on extinction	Not tested
Stern et al., 2016	CFC	Wistar rats, 10-30 mg kg ⁻¹ , i.p.	Disrupted fear memory consolidation	Indirect CB1 or CB2 receptor activation

Table 3. CBD effects on anxiety in humans. 5-HT_{1A}: serotonin_{1A}; fMRI: functional magnetic resonance imaging; PTSD: post-traumatic stress disorder; SCR: skin conductance response; SPECT: single-photon emission computed tomography; THC: delta-9-tetrahydrocannabinol.

Reference	Subjects and test(s) used	Effective dose and route of administration	Effect	Possible pharmacological or neural mechanism
Zuardi et al., 1982	Healthy subjects, THC-induced anxiety	~70 mg (1 mg kg ⁻¹) orally	Prevented the anxiogenic effects of THC	Not tested
Zuardi et al., 1993	Healthy subjects, simulated public speaking-induced anxiety	300 mg orally	Prevented public speaking-induced increase in anxiety	Not tested (effects similar to the 5-HT _{1A} receptor partial agonist ipsapirone)
Crippa et al., 2004	Healthy subjects, SPECT	400 mg orally	Anxiolytic	Decreased blood flow in medial temporal structures and posterior cingulate gyrus
Fusar-Poli et al., 2009, 2010	Healthy subjects, fearful faces, fMRI	600 mg orally	Anxiolytic (trend)	Decreased blood flow in amygdala and anterior cingulate cortex that correlated with a reduced SCR to fearful faces
Bergamaschi et al., 2011	Social anxiety disorder patients, simulated public speaking-induced anxiety	600 mg orally	Anxiolytic	Not tested
Crippa et al., 2011	Generalized anxiety disorders patients, SPECT	400 mg orally	Decreased subjective anxiety	Altered blood flow in limbic and paralimbic brain areas
Hurd et al., 2015	Abstinent heroin abusers, heroin cue-induced anxiety	400 or 800 mg orally	Decreased subjective anxiety (preliminary data)	Not tested
Shannon and Opila-Lehman, 2016	A 10 year-old girl with PTSD (case report)	At least 25 mg daily for 5 months	Reduced anxiety and improved sleep	Not tested