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REVIEW ARTICLE

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

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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 5-HT_{1A} and (indirect) cannabinoid receptor activation in paradigms assessing innate responses to threat. There is also accumulating evidence from animal studies investigating the effects of cannabidiol on fear memory processing indicating 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 a lasting reduction of learned fear. Recent studies have also begun to elucidate the effects of cannabidiol on drug memory expression using paradigms with translational relevance to addiction. The findings suggest 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 as a treatment for anxiety-related and substance abuse disorders.

LINKED ARTICLES

This article is part of a themed section on Pharmacology of Cognition: a Panacea for Neuropsychiatric Disease? To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v174.19/issuetoc

Abbreviations

BNST, bed nucleus of the stria terminalis; CBD, cannabidiol; CS, conditioned stimulus; dlPAG, dorsolateral periaqueductal gray; EPM, elevated plus-maze; IL, infralimbic; PAG, periaqueductal gray; PL, prelimbic; PTSD, post-traumatic stress disorder; THC, Δ^9-tetrahydrocannabinol; US, unconditioned stimulus

DOI:10.1111/bph.13724

Tables of Links

TARGETS	
Other protein targets[a]	**Voltage-gated ion channels**[d]
Fatty acid binding proteins (FABPs)	TRPA1
GPCRs[b]	TRPM8
5-HT_{1A} receptor	TRPV1
A_1 receptor	TRPV2
CB_1 receptor	**Nuclear hormone receptors**[e]
CB_2 receptor	PPARγ
D_4 receptor	**Catalytic receptors**[f]
GPR55	TrkB
Ligand-gated ion channels[c]	**Enzymes**[g]
GluA1 receptor	Fatty acid amide hydrolase (FAAH)

LIGANDS
Adenosine
Anandamide
Cannabidiol
Cocaine
Morphine
THC

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 ([a,b,c,d,e,f,g]Alexander *et al.*, 2015a,b,c,d,e,f,g).

Introduction

Anxiety (e.g. generalized and social anxiety, panic and 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 (e.g. phobias and 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 the psychological and pharmacological therapies currently available 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 drugs of abuse, 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 involves 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-psychotomimetic 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 studies, mostly preclinical, 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 *C. sativa* plant contains more than 100 chemically related terpenephenol components called phytocannabinoids (Izzo *et al.*, 2009; Gould, 2015). Since the seminal work of Raphael Mechoulam's group in the 1960s, Δ^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 *C. sativa* is CBD. Originally isolated by Adams and co-workers in 1940 (Adams *et al.*, 1940), its structure was elucidated by Mechoulam and Shvo (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 from 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 Guimarães *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, Guimarães 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 and 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 5-HT_{1A} receptors, inhibition of reuptake 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 reuptake, antagonism of GPR55, agonism of PPARγ receptors, intracellular Ca^{2+} increase, and anti-oxidative effects, among others (summarized in Figure 1). 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 gray (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 D_4 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). The involvement of the 5-HT_{1A} receptor in the acute anxiolytic/anti-stress effect of

Table 1

CBD effects on anxiety-like behaviour in male animals

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 CB$_1$ receptor activation
Soares Vde *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.* (2012a)	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

continues

Table 1 (Continued)

Reference	Test used	Strain, species, effective dose, and route/site of administration	Effect	Pharmacological mechanism
Campos *et al.* (2013a,b)	EPM and NSF	C57BL/6 mice, 30 $mg \cdot kg^{-1}$, daily for 14 days (CUS-exposed animals)	Anti-stress	CB_1 receptor-mediated facilitation of hippocampal neurogenesis
O'Brien *et al.* (2013)	Light–dark test	Sprague Dawley rats, 2.5 $mg \cdot kg^{-1}$, i.p. for 14 days	No effect	Not tested
Twardowschy *et al.* (2013)	Snake exposure	Swiss mice, 3.0 $mg \cdot kg^{-1}$, i.p.	Panicolytic	$5\text{-}HT_{1A}$ receptor activation
Almeida *et al.* (2013)	Social interaction test	Wistar and SHR rats, 1 $mg \cdot kg^{-1}$, i.p.	Increased social interaction (Wistar rats only)	Not tested
Cheng *et al.* (2014)	EPM	C57BL/6 J mice, 20 $mg \cdot kg^{-1}$, 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 h after restraint stress	$5\text{-}HT_{1A}$ receptor activation
Nardo *et al.* (2014)	MBT	Swiss mice, 30 $mg \cdot kg^{-1}$, i.p.	Attenuated mCPP-induced increase in marble-burying (bell-shaped dose response curve)	Indirect CB_1 receptor activation
Marinho *et al.* (2015)	EPM	Wistar rats, 15–30 nmol, intra-IL cortex	Anxiolytic (bell-shaped dose response curve), no effect 24 h after restraint stress	$5\text{-}HT_{1A}$ receptor activation
Todd and Arnold (2016)	Open-field	C57BL/6 mice, 10 $mg \cdot kg^{-1}$, i.p.	Prevented THC- induced anxiogenesis	Not tested
Schiavon *et al.* (2016)	EPM	Swiss mice, 3 $mg \cdot kg^{-1}$, i.p.	Anxiolytic	Not tested

BZD, benzodiazepine; CUS, chronic unpredictable stress; ETM, elevated T-maze; ICR, Institute of Cancer; MBT, marble burying test; NSF, novelty suppressed feeding; SHR, spontaneously hypertensive rats.

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 $5\text{-}HT_{1A}$ 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 CB_1 receptors rather than $5\text{-}HT_{1A}$ receptors (Casarotto *et al.*, 2010; Campos *et al.*, 2013a; Nardo *et al.*, 2014). Even if the (+)-CBD enantiomer shows affinity for CB_1 receptors, the naturally occurring (−) CBD does not bind to these receptors (Hanuš *et al.*, 2005), indicating that the CB_1 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*. Correspondingly, using embryonic hippocampal cells, Campos *et al.* (2013b) showed that the increase in cell proliferation induced by CBD is prevented by antagonism of either CB_1 or CB_2 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 (FABPs) 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 (Leweke *et al.*, 2012, Campos *et al.*, 2013b).

Emotional learning and memory processing

We will 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,

Table 2

CBD effects on learned fear processing in male animals

Reference	Test used	Strain, species, effective dose and route/site of administration	Effect	Pharmacological mechanism
Studies conducted in operant conditioning paradigms				
Silveira Filho and Tufik, 1981	Geller-Seifter conflict test	Wistar rats, 100 $mg{\cdot}kg^{-1}$, i.p.	No effect	Not tested
Musty *et al.* (1985)	Vogel punished licking test	Sprague–Dawley rats, 5–10 $mg{\cdot}kg^{-1}$, i.p.	Anxiolytic (bell-shaped dose–response curve)	Not tested
Moreira *et al.* (2006)	Vogel punished licking test	Wistar rats, 10 $mg{\cdot}kg^{-1}$, 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
Studies conducted in classical (Pavlovian) conditioning paradigms				
Zuardi and Karniol, 1983	AFC	Wistar rats, 10 $mg{\cdot}kg^{-1}$, i.p.	Anxiolytic (decreased fear expression)	Not tested
Resstel *et al.* (2006)	CFC	Wistar rats, 10 $mg{\cdot}kg^{-1}$, 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 $mg{\cdot}kg^{-1}$, 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 $mg{\cdot}kg^{-1}$, i.p. daily for 14 days	Anxiogenic (increased f ear 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 f ear expression)	5-HT_{1A} receptor activation
Levin *et al.* (2012)	CFC	Wistar and SHR rats, 1–15 $mg{\cdot}kg^{-1}$, 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 $mg{\cdot}kg^{-1}$, i.p.	Disrupted fear memory reconsolidation (bell-shaped dose response curve)	Indirect CB_1 receptor activation
Do Monte *et al.* (2013)	CFC	Long–Evans hooded rats, 1.3 nmol, intra-IL cortex	Facilitated fear memory extinction	Indirect CB_1 receptor activation
Cheng *et al.* (2014)	AFC	C57BL/6 J mice, 20 $mg{\cdot}kg^{-1}$, i.p. daily for 21 days	No effect	Not tested
Fogaça *et al.* (2014)	CFC	Wistar rats, 30 nmol, intra-PL cortex	Anxiolytic (decreased fear expression)	5-HT_{1A} receptor activation
Gazarini *et al.* (2015)	CFC	Wistar rats, 10 $mg{\cdot}kg^{-1}$, i.p.	Disrupted fear memory reconsolidation	Not tested
Stern *et al.* (2014)	CFC	Wistar rats, 10 $mg{\cdot}kg^{-1}$, i.p.	Disrupted fear memory reconsolidation	Indirect CB_1 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

continues

Table 2 (Continued)

Reference	Test used	Strain, species, effective dose and route/site of administration	Effect	Pharmacological mechanism
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 CB_1 or CB_2 receptor activation

AFC, auditory fear conditioning; BZD, benzodiazepine; CFC, contextual fear conditioning; OFC, olfactory fear conditioning.

Table 3

CBD effects on anxiety in humans

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

fMRI, functional magnetic resonance imaging; SCR, skin conductance response; SPECT, single-photon emission computed tomography.

an innocuous conditioned stimulus (CS), which can be a discrete cue (e.g. sound, light or odour) 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

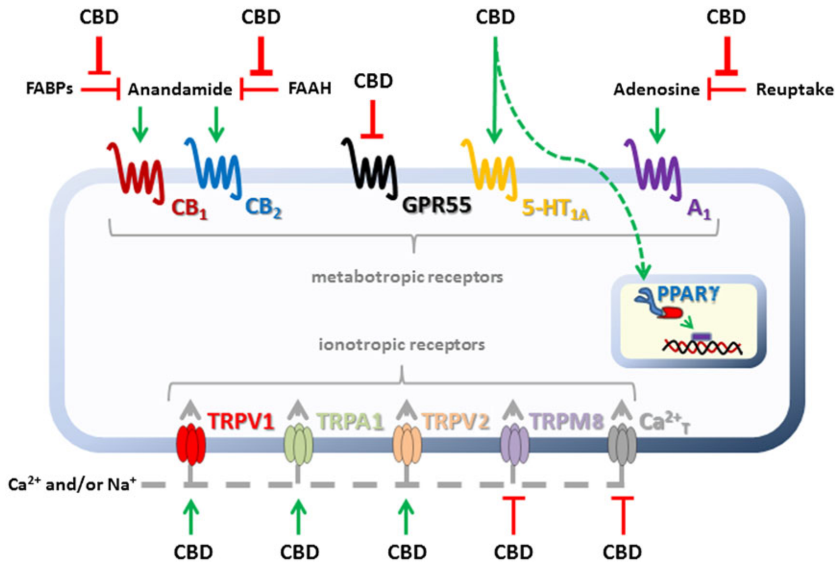

Figure 1

The main molecular targets and potential mechanisms of action of CBD. This drug inhibits both FAAH, the enzyme which metabolizes anandamide, and FABPs, which mediate the transport of anandamide to FAAH; both mechanisms ultimately result in the indirect activation of CB_1 and/or CB_2 receptors. CBD also activates the 5-HT_{1A} receptor, PPARγ and the transient receptor potential channels TRPV1, TRPA1 and TRPV2. Finally, CBD inhibits adenosine reuptake and antagonizes GPR55, TRPM8 and T-type Ca^{2+} 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).

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, there is growing evidence indicating 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; acute systemic administration before fear conditioning resulted in attenuated fear expression during later memory retrieval testing (Levin *et al.*, 2012). In contrast, there are few reported effects of repeated CBD administration on fear memory expression and those that exist are conflicting. In one study, daily injections of CBD 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 it was administered for 21 days (Cheng *et al.*, 2014).

The results of several studies indicate that CBD also modulates the extinction and reconsolidation of conditioned fear, leading to lasting effects on learned fear expression. I.c.v. 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; 2015; Gazarini *et al.*, 2015), although these contrasting effects of CBD on fear extinction and memory reconsolidation both result in a 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 6 h 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 there is evidence indicating 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 probably 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 dependent on 5-HT_{1A} receptors (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 reconsolidation and extinction of learned fear involve (indirect) cannabinoid receptor activation. The facilitatory effect of i.c.v. CBD infusion on fear extinction was inhibited by prior CB_1 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 CB_1 receptors (Do Monte *et al.*, 2013). The disruptive effect of CBD on fear memory reconsolidation was blocked by pretreatment with a CB_1 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 a drug (Aguilar *et al.*, 2009; Steketee and Kalivas, 2011). Each of these paradigms can be studied using stimulants (e.g. cocaine and amphetamine), opiates (e.g. heroin and morphine), and other drugs (e.g. alcohol and nicotine).

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 distinguish learning-related behavioural effects from modulation of drug reward (cf. 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 disparity depending upon the drug reward under study. Acute administration of CBD (5 and 10 $mg \cdot kg^{-1}$) 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, it did have an effect 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 in a cue-induced relapse test but only when given 24 h, and not 30 min, prior to the test. This long-lasting effect on 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 up-regulation of AMPA GluA1 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 h 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 has also been 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 generalized 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 is still 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) suggest 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 on the effect of CBD on 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 ability of CBD to reduce cocaine and amphetamine place preference in this extinction study (Parker *et al.*, 2004) is 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 destabilize (de Carvalho and Takahashi, 2016) the drug memory did not differ greatly. The extinction trial was 15 min in duration, compared with a 10 min reactivation trial, although the former was confined to the drug-paired chamber, whereas the latter was a test. Moreover, the conditioning parameters were similar across the two studies, and also to previous 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). Also, given that the parameters of appetitive memory reconsolidation and extinction are usually well distinguished, such that they are each typically defined by very 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 ability of CBD to reduce later drug place preference observed in these two studies (Parker *et al.*, 2004; de Carvalho and Takahashi, 2016) instead reflects qualitatively similar processes. By simply considering the parametric comparisons presented above, we 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 (Weber *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 produces 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, which 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 into the effects of CBD on addictive drug memory processing is still in its infancy. Therefore, further studies are 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 should also be investigated as a common treatment for such disorders. One outstanding issue that needs to be addressed is determining 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 aimed at impairing memory reconsolidation or enhancing extinction might be sufficient to facilitate this effect. Another important consideration is how CBD would be delivered for treating these disorders. Most of the recreationally used cannabis available today contains low levels of CBD and high levels of THC, which can exacerbate symptoms; however, 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 development of a formulation of CBD for use as a treatment for anxiety-related and substance abuse disorders in the future.

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Conflict of interest

F.S.G. 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).

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