Psychopharmacology (2013) 226:781–792
DOI 10.1007/s00213-012-2955-y

ORIGINAL INVESTIGATION

Cannabidiol enhances consolidation of explicit fear extinction in humans

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Received: 24 September 2012 / Accepted: 6 December 2012 / Published online: 10 January 2013

Abstract
Rationale Whilst Cannabidiol (CBD), a non-psychotomimetic cannabinoid, has been shown to enhance extinction learning in rats, its effects on fear memory in humans have not previously been studied.
Objectives We employed a Pavlovian fear-conditioning paradigm in order to assess the effects of CBD on extinction and consolidation.
Method Forty-eight participants were conditioned to a coloured box (CS) with electric shocks (UCS) in one context and were extinguished in a second context. Participants received 32 mg of CBD either following before or after extinction in a double-blind, placebo-controlled design. At recall, 48 h later, participants were exposed to CSs and conditioning contexts before (recall) and after (reinstatement) exposure to the UCS. Skin conductance and shock expectancy measures of conditioned responding were recorded throughout.
Results Successful conditioning, extinction and recall were found in all three treatment groups. CBD given post-extinction enhanced consolidation of extinction learning as assessed by shock expectancy. CBD administered at either time produced trend level reduction in reinstatement of autonomic contextual responding. No acute effects of CBD were found on extinction.
Conclusions These findings provide the first evidence that CBD can enhance consolidation of extinction learning in humans and suggest that CBD may have potential as an adjunct to extinction-based therapies for anxiety disorders.

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Keywords Cannabidiol · Cannabinoids · Conditioning · Extinction · Consolidation · Pavlovian · Anxiety

Introduction

Pavlovian fear learning mechanisms (Pavlov 1927) are important in anxiety disorders (Watson and Rayner 1920; Mineka and Zinbarg 1996). Impaired naturalistic extinction is thought to be a major contributor to fear persistence in these disorders. Pharmacological treatments that acutely enhance extinction learning or potentiate its consolidation (post-learning stabilisation; Müller and Pilzecker 1900; Dudai 2004) therefore hold significant promise in improving the efficacy of exposure-based psychological treatments for anxiety disorders.

Recently, the endocannabinoid (eCB) system has gained attention for its role in learning and memory. The eCB system is comprised of two classes of receptors: CB_1 and CB_2, endogenous cannabinoids (eCBs); anandamide and 2-arachidonylglycerol (2-AG) and enzymes for their hydrolysis. Pre-synaptic CB_1 receptors (CB_1Rs) are expressed most densely in the hippocampus, amygdala and prefrontal cortex (Freund et al. 2003) and modulate the firing of both excitatory glutamatergic and inhibitory GABAergic neurons in the amygdala. They thus play an important role in emotional learning.

However, the role of the eCB system on extinction learning remains poorly understood. Despite cognitive impairing effects of Δ9-THC, both CB_1 agonist WIN 55,212-2 (Pamplona et al. 2006; Bitencourt et al. 2008; but see Chhatwal et al. 2005) and AM404, an anandamide reuptake inhibitor (Beltramo et al. 1997; Fowler et al. 2004), potentiate extinction of conditioned fear. Conversely, CB_1R knockout or antagonism generally impairs fear extinction (Kaplan and Moore 2011; Pamplona et al. 2006)

and increases memory rigidity (as evidenced by failures in reversal learning; Lutz 2007; Varvel and Lichtman 2002), leaving fear acquisition intact. These findings suggest specific and dissociable roles of CB_1Rs in fear conditioning and extinction (Marsicano et al. 2002). Thus, CB_1R blockade may impair *acquisition* of extinction learning (Chhatwal et al. 2005; Marsicano et al. 2002; Suzuki et al. 2004; Niyuhire et al. 2007). However, glucocorticoid-induced eCB synthesis and downstream disinhibition of noradrenergic neurons in the basolateral amygdala (BLA) may also enhance memory *consolidation* (Hill and McEwen 2009; de Bitencourt et al. 2013). Consistent with this, WIN 55,212-2 enhances memory consolidation, and intra-BLA infusions of the CB_1 receptor antagonist rimonabant prevent the facilitation of fear memory consolidation by systemic corticosterone in vivo (Campolongo et al. 2009). ECBs may thus be critical in both the acquisition and maintenance of conditioned fearful responding. As such, the eCB system may be an important target for improving the efficacy of treatment for anxiety disorders.

Cannabidiol (CBD), a non-psychoactive constituent of cannabis with effects on the eCB system, is a promising compound in this respect and has been shown to enhance extinction in rat models of conditioned fear (Bitencourt et al. 2008). However, the action of CBD is complex and not fully characterised. It has been shown to have some antagonistic action at the CB_1 and CB_2 receptor (Thomas et al. 2007; Pertwee 2008), but binding affinity at these receptors is generally low (Petitet et al. 1998). Recent evidence suggests that it exerts its behavioural effects via inhibition of fatty acid amide hydrolase (FAAH), an enzyme that hydrolyses endogenous cannabinoids (Leweke et al. 2012). CBD may thus increase anandamide levels by reducing intracellular anandamide breakdown. It may therefore be expected to exert similar effects to AM404 on memory. Indeed, previous research comparing the two compounds supports this hypothesis, with both CBD and AM404 similarly potentiating extinction when administered immediately prior to training (independently of any anxiolytic effects) in animal models of anxiety (Bitencourt et al. 2008). Given its excellent safety profile in humans (Nurmikko et al. 2007; Bergamaschi et al. 2011), absence of acute subjective effects at low doses and promising findings on enhancement of extinction learning in animals, CBD may have a significant clinical potential as an adjunct to exposure therapies for anxiety disorders. However, no study to date has examined the effects of CBD on fear memory in humans.

The present study therefore aimed to assess whether CBD has potential to help in the treatment of pathological fear memories by examining its effects on fear memory in healthy volunteers. A contextual fear-conditioning paradigm was employed in order to assess the potential effects of CBD on the acquisition or consolidation of extinction. We tested the effect of a sub-anxiolytic dose of CBD in two groups who received the drug either before (so that CBD would be active during acquisition and consolidation of extinction) or after (active during consolidation only) extinction in order to dissociate the contribution of acquisition and consolidation mechanisms. Based on the hypothesised action of CBD as an FAAH inhibitor, we predicted that it would potentiate the acquisition (when given before) and consolidation (when given after) of extinction. Since conditioned fear responding may reflect explicit, propositional knowledge as well as relatively automatic processes (Lovibond and Shanks 2002; Grillon 2009; Bechara et al. 1995), we examined both the explicit (expectancy ratings) and autonomic (skin conductance response) aspects of conditioned responding. Such assessment is relevant as our previous findings suggest that CBD's ameliorating effects on neuropsychological functioning were evident in measures of explicit, declarative memory (Morgan et al. 2010).

Methods

Design and participants

In a double-blind, placebo-controlled between-subjects design, 48 participants were randomised to three groups (each $n=16$) to receive either (1) 32 mg of inhaled CBD prior to extinction (CBD pre-extinction group), (2) 32 mg of inhaled CBD following extinction (CBD post-extinction group) or (3) placebo (placebo group). The participants were recruited via community advertisement and word of mouth; the inclusion criteria were: 18–35 years old, fluent in English, no history of serious mental or physical health problems, no substance misuse problems, normal or corrected-to-normal colour vision, no learning impairments or neurological history, not pregnant. The study was approved by the University College London research ethics committee.

Procedure

After collecting written, witnessed, informed consent, basic demographic data were collected. The Beck Depression Inventory (Beck et al. 1996), Spielberger State-Trait Anxiety Inventory (Spielberger et al. 1983), Spot-the-Word (Baddeley et al. 1993) and prose recall task then provided measures of depressive symptoms, trait anxiety, verbal IQ, and non-emotional explicit memory, respectively. A drug-use interview assessed participants' use of psychoactive substances.

The participants then underwent fear conditioning (described below) after which the Mood Rating Scale

(MRS) (Bond and Lader 1974) and Bodily Symptoms Scale (BSS) were used to assess anxiety, current mood and physical symptoms. The first drug 'balloon' was then administered. The participants in the CBD pre-extinction group inhaled 32 mg of CBD at this point, while members of the other two groups inhaled placebo. Five minutes following drug administration, the MRS and BSS were again given to the participants, who then completed the fear extinction task. Finally, the second drug balloon was administered. Those in the CBD post-extinction group then inhaled 32 mg of CBD, the other groups inhaled placebo, and final mood rating and subjective anxiety measures were given. This concluded the first day of testing.

Twenty-four hours after the first testing session, the participants first completed the MRS and BSS and delayed prose recall. Participants then completed the fear recall task (described in full under the "Tasks" section). Following this, the participants were debriefed and testing was concluded.

Drug administration

CBD and placebo ethanol were provided by STI Pharmaceuticals (UK). All drugs were vaporised at 210 °C and administered via a Volcano Medic vaporiser (Storz & Bickel, Tuttlingen, Germany). Thirty-two milligrammes of CBD in 0.08 mg ethanol vehicle was administered to those in the CBD groups, while 0.08 mg ethanol vehicle only was used as placebo. This dose of vaporised CBD was chosen based on extensive pilot work with the vaporiser as an administration medium and evidence from a previous study in our lab where 32 mg of CBD was not shown to affect anxiety levels (Morgan et al. in preparation). The anxiety subscale of the MRS was used to confirm that this dose of CBD was sub-anxiolytic.

The participants were told to fast for 4 h prior to testing in order to facilitate CBD absorption (CBD is lipid soluble) and maximise peak plasma levels of CBD during the learning and consolidation windows. In order to maintain the drug blinding, drugs were prepared and administered by a separate experimenter to the one collecting behavioural data. Pharmacological interaction between CBD and ethanol in the preparation was highly unlikely given the extremely low dose of ethanol. Previous use of this CBD preparation in our lab suggested no interaction between the compounds (Morgan et al. in preparation).

Drugs were administered on a 10-s inhalation cycle. Participants were instructed to fully exhale, then fully inhale from the Volcano Medic balloon, hold their breath for 10 s then fully exhale. This procedure was repeated until the balloon was empty. All the participants were familiarised with the inhalation protocol using a placebo balloon before actual drug administration. In order to maintain the drug blinding, drugs were prepared and administered by a separate experimenter to the one collecting behavioural data.

Tasks

Day 1

Conditioning Fear conditioning took place in a virtual 'context' presented on a 17-inch computer monitor: 'Room A', defined by screen background which depicted a white-walled room (Fig. 1). The unconditioned stimulus (UCS) was a 250-ms, 4-mA electric shock generated by a Psychlab generator (Contact Precision Instruments). Conditioned stimuli (CSs) were red and yellow boxes that appeared on the context background. One of the boxes (the CS+) co-terminated with the UCS on 50 % of trials. The other CS (CS−) was never paired with a shock. Which stimulus (i.e. the red or the yellow box) acted as the CS+ and CS− was counterbalanced across participants and drug groups. The participants were first habituated to both CSs and the context by two presentations of each CS in the conditioning context. They also received a single test shock before the start of the task. Each conditioning trial consisted of context background alone for 10 s, followed by CS in the context for between 10 and 14 s (mean=12 s). Every trial was followed by a plain grey screen inter-trial interval of 15 s.

Thirty-two conditioning trials (16 of each CS) were randomised with the stipulation that no more than two of one CS type could appear in succession. At the beginning of the task, the participants were instructed that they would see red and yellow boxes in 'Room A' and that they would occasionally receive shocks. They were instructed that they should try to discern the relationship between the boxes and the shocks. Throughout the conditioning trials, skin conductance response (SCR) was measured via silver/silver chloride electrodes attached to the medial phalanges of the index and middle fingers of participants' non-dominant hands. SCR was amplified and recorded by a Psychlab modular skin conductance amplifier unit (Contact Precision Instruments) with a 0.02 μS low-pass filter. In order to assess explicit learning of the contingency between the CS+ and UCS, shock expectancy ratings were collected on every trial. When the CS appeared, the participants rated (with their free hand) how much they expected to receive a shock from 0 (certain there will *not* be a shock) to 5 (certain there *will* be a shock).

Extinction Fear extinction was carried out in 'Room B', defined by screen background (Fig. 1). Prior to the extinction task, participants were told that they would be seeing

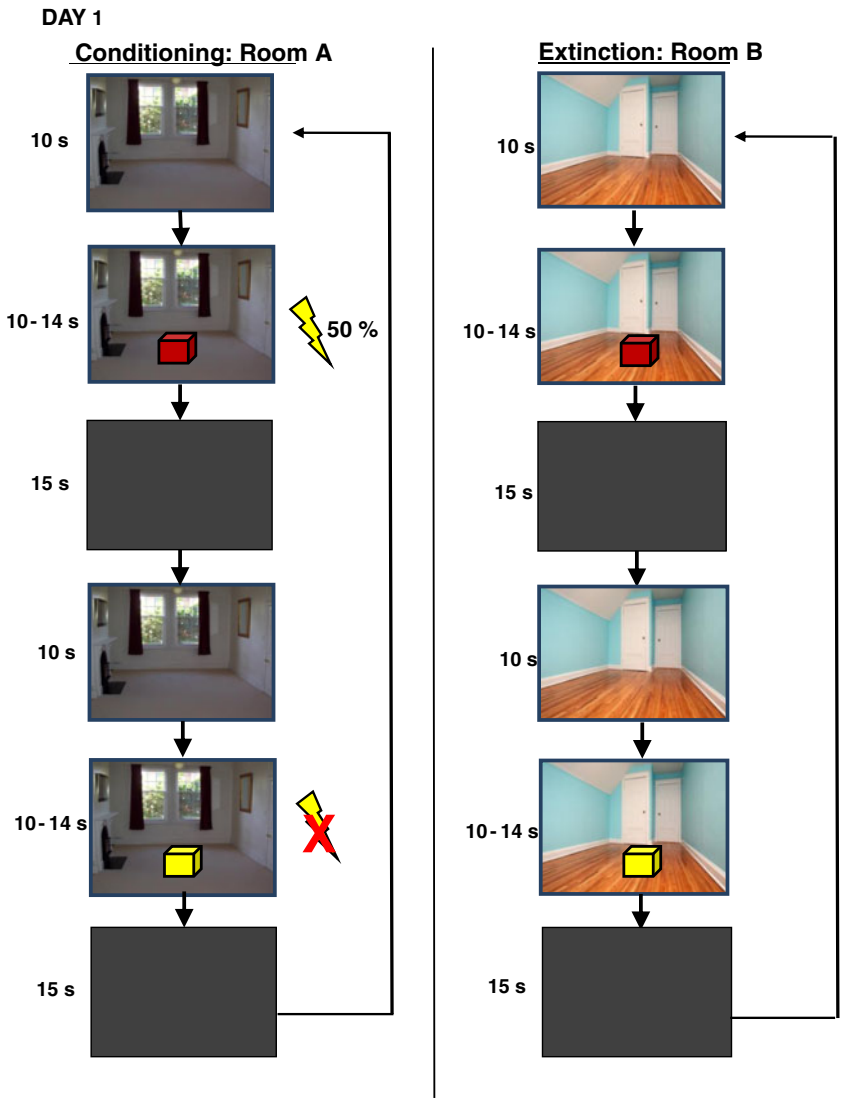

Fig. 1 Conditioning and extinction tasks. This figure represents two trials of conditioning and extinction to demonstrate trial timing and shock contingencies. For both conditioning and extinction, this cycle is repeated, in a pseudo-randomised order, until each CS had been repeated 16 times

the *same* boxes that they had seen in the conditioning task, but that they would now be appearing in 'Room B'. The extinction task was otherwise identical to conditioning, except no shocks were presented.

Recall The fear recall task, 24 h later, consisted of two phases: recall and reinstatement. In the recall phase, CSs were presented in the conditioning and extinction contexts in an alternating manner. The reinstatement phase was identical to recall, except prior to the reinstatement trials, the UCS was presented in each context once in the absence of any CSs. At the beginning of the task, participants were told that they would be seeing the *same* boxes as the first day in the *same* rooms to maximise reliance on memory from conditioning and extinction and to avoid occasion-setting effects. SCR was continuously recorded through the task and shock expectancy ratings were made twice per trial, one when the context alone was visible and one when the CS appeared. This rating protocol was used in order to examine utilisation of cue and context information (Fig. 2).

Data analyses

Skin conductance

All skin conductance records we collected at a sampling rate of 100 Hz and down-sampled to 50 Hz prior to analysis. Records were smoothed and visually inspected for movement artefacts. If artefacts were found, the trial in which they occurred was excluded from the analysis. SCRs were calculated for the last 10 s of each CS presentation during fear conditioning. Analysis was performed in this way to best capture anticipatory autonomic responses. SCRs were calculated by subtracting the skin conductance level at the start of the trial from the maximum level reached within the. If skin conductance level flat-lined or reduced throughout the block, responses were marked as 0.

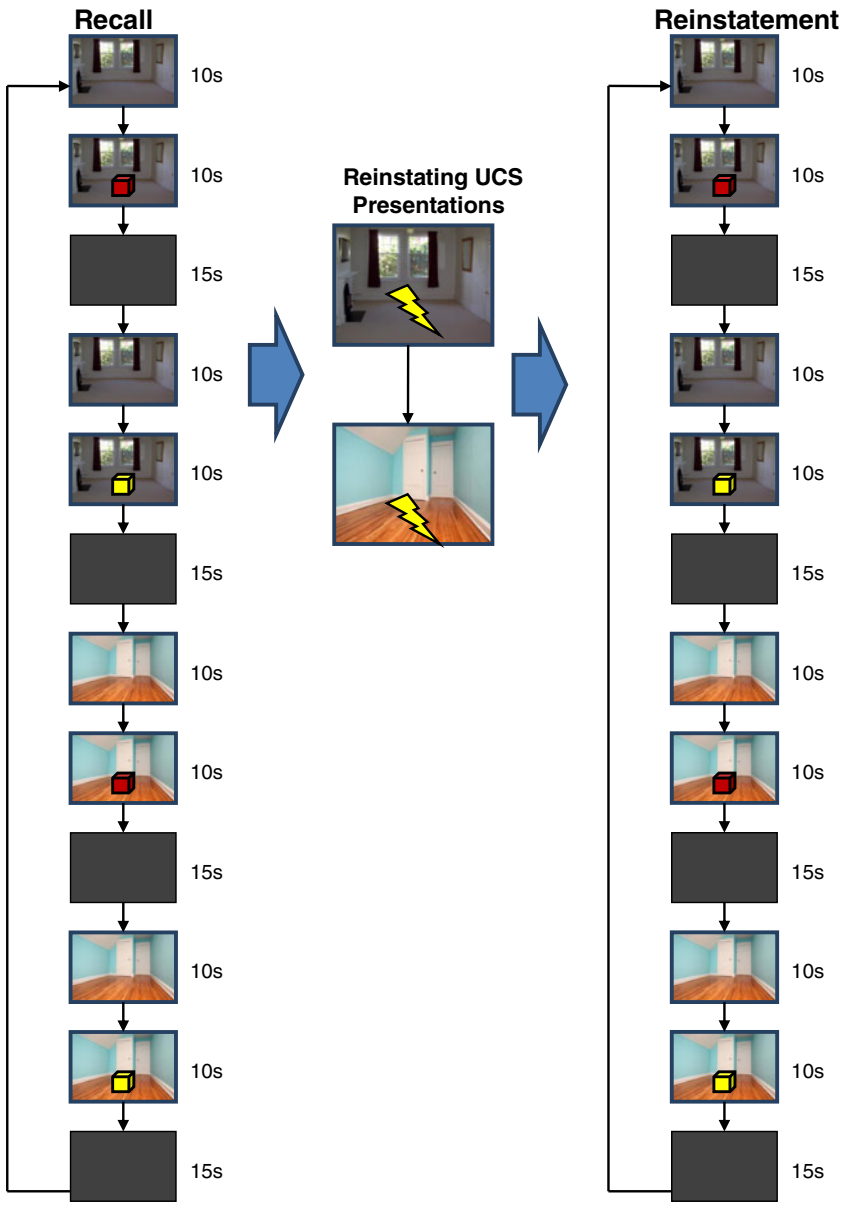

Fig. 2 In the recall task, contexts were presented in an alternating fashion, with each CS appearing in each context before context change. Two trials are presented in this figure to demonstrate this trial ordering. The reinstatement phase was identical to the recall phase but was preceded by a single UCS presentation in each context in order to reactivate contextual fear memory. In both phases, each CS appeared in each context three times

Other analyses

Data were analysed with IBM SPSS version 19 for Windows. Mixed analyses of variance (ANOVAs) were used to analyse SCR, subjective and computer-based response data. Significant and trend-level ($p<.1$) between-subjects main effects and interactions were followed up with, Dunnett's *t* post hoc tests (as we were interested in comparing effects of CBD to placebo control), *t* tests or planned Helmert contrasts, where appropriate. Where sphericity was violated, a Greenhouse-Geisser correction was applied. Adjusted dfs and *p* values are reported in this instance. Identical ANOVAs were used for shock expectancy and SCR data and for the recall and reinstatement phases. For conditioning, these were 2×4×3 RMANOVAs with within-subjects factors of CS (CS+/CS−) and block (four blocks of four trials each) and a between-subject factor of group (placebo, CBD pre-extinction, CBD post-extinction). For extinction, an extra level of block was added to compare the last block of conditioning to extinction blocks, yielding 2×5×3 RMANOVAs. For the recall and reinstatement tasks, responding to contexts alone was assessed by 2 (conditioning context/extinction context)×6 (trial 1–6)×3 (group) RMANOVAs, and responding to CSs within contexts by 2(CS+/CS−)×2(conditioning context/extinction context)×3(trial 1, 2 or 3)×3(group) RMANOVAs.

Results

Demographics The groups did not differ on any standardised questionnaire measures of mental health (anxiety, depression or verbal IQ). Some participants had used

tobacco and cannabis. There were no differences between drug groups in use, with the exception of recency of tobacco use [$F(2,20)=3.882$, $p=.038$], due to more recent tobacco use in the CBD pre-extinction group than the CBD post-extinction group [$t(12)=2.617$, $p=.023$] see Table 1 for descriptive statistics.

Associative fear learning

Excluded and missing data

A proportion of skin conductance data for extinction trials was lost due to equipment failure and participant non-response for each task. Final Ns at each stage are as follows (all given as placebo N, CBD pre-extinction N, CBD post-extinction N); conditioning $n=13$, $n=15$, $n=14$; extinction $n=8$, $n=13$, $n=12$; recall $n=12$, $n=13$, $n=13$, reinstatement $n=12$, $n=12$, $n=12$. For shock expectancy, five participants failed to make responses during the recall task, leaving $n=15$, $n=15$, $n=13$.

Habituation

No differences in SCRs to the CS+ or CS− or between groups were found during habituation (all $p>.2$). Stimuli and groups were thus well matched for responding at baseline.

Conditioning

SCRs Rapid conditioning was shown by a main effect of CS [CS+>CS−; $F(1,39)=18.50$, $p<.001$, $\eta_p^2=.322$]. Significant conditioned responding between CSs was evident from the second block onwards (all $p<0.005$) with a trend for conditioned responding in the first block [$t(40)=2.005$, $p=.052$]. As it is clearly shown in Fig. 3, successful conditioning of SCRs was achieved within the first two blocks. A main effect of block [$F(2.40,93.2)=3.85$, $p<.03$, $\eta_p^2=.084$], reflected a general decrease in SCRs over the course of the trials indicating habituation to the UCS. This was driven by a significant decrease in SCRs from the first block to later blocks only [$F(1,37)=10.266$, $p=.003$, $\eta_p^2=.217$]. Together these data suggest ceiling-level conditioning by the end of the second block.

Expectancy Conditioning was also evidenced by expectancy ratings which showed a main effect of CS (CS+>CS−); [$F(1,44)=131.845$, $p<.001$, $\eta_p^2=.75$], qualified by a CS× block interaction [$F(2.34,102.94)=5.323$, $p=.005$, $\eta_p^2=.106$], indicating a significant reduction in ratings for the CS− between blocks 1 and 2 [$t(46)=4.668$, $p<.001$] but no reduction in ratings for the CS+ (all $p>.05$). Concordant with SCR data, this shows rapid conditioning.

Extinction

SCRs Extinction was evidenced by a main effect of block [$F(2.838,79.367)=4.977$, $p=.004$, $\eta_p^2=.142$], showing a significant decrease in responding between the last block of conditioning and all extinction blocks [$F(1,30)=6.092$, $p=.02$, $\eta_p^2=.169$] and between the first and later blocks of extinction trials [$F(1,30)=12.983$, $p=.001$]. A borderline significant main effect of CS was also found [$F(1,28)=4.23$, $p=.05$, $\eta_p^2=.131$]. Helmert contrasts showed that this was driven by greater conditioned responding (CS+>CS−) in the last conditioning block compared to all extinction blocks [$F(1,28)=5.228$, $p=.03$, $\eta_p^2=.157$]. Confirmatory t tests

Table 1 Descriptive statistics for baseline demographic data

	CBD pre-extinction ($n=16$)	CBD post-extinction ($n=16$)	Placebo ($n=16$)
Gender (M/F)	8:8	10:6	12:4
No. of alcohol users	13	12	13
Alcohol days per month	8.69±5.57	7.96±5.23	8.23±5.76
Alcohol units per session	4.73±2.2	6±3.93	6.54±4.29
Days since last use	3.38±3.93	19.42±14.5	6.85±8.67
No. of cigarette smokers	8	6	9
Tobacco days per month	7.31±10.25	5.67±11.98	1.67±1.41
Cigarettes per day	1.56±1.31	1±0.89	1.11±0.93
Days since last use	9.25±9.95	330.25±325.65	171.75±234.12
No. of cannabis users	4	6	4
Cannabis days per month	3.17±3.82	1.75±2.22	0.75±0.5
Cannabis time to smoke eighth	4.5±3.54	12.33±6.81	5.75±1.5
Days since last use	55.7±59.25	223.5±320.62	67.5±95.39
Spielberger Trait Anxiety score	35.88±6.4	36±10.06	36.29±5.65
BDI score	5.63±4.3	6.27±6.2	5.88±4.33
Spot-the-Word score	47.33±5.3	48.6±3.64	50.06±3.05

BDI Beck depression inventory

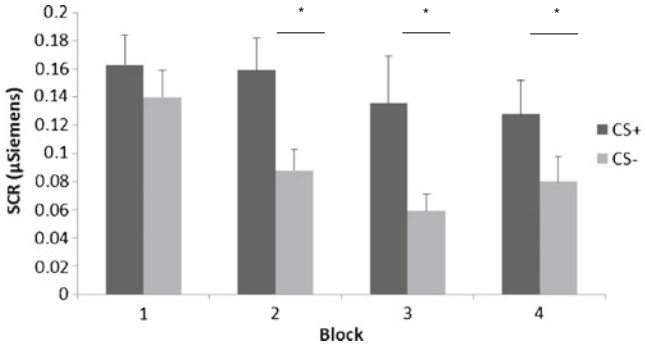

Fig. 3 Conditioned SCRs to the CS+ and CS− across blocks of conditioning (one block=four trials). *Bars* represent mean skin conductance responses±SEM. The *asterisks* indicate CS+>CS− ($p<0.05$)

showed a significant effect of CS (CS+>CS−) in the last block of conditioning only [$t(41)=3.355$, $p=.002$] with no differences between CSs during any extinction blocks. Thus successful extinction occurred within the first two blocks of extinction trials. No group effects were found.

Expectancy

A CS×block interaction was found for ratings during extinction [$F(2.289,89.268)=11.256$, $p=<0.001$, $\eta_p^2=.224$]. The interaction was driven by a significant *increase* in shock expectancy between the last block of conditioning and first block of extinction for the CS− [$t(41)=4.224$, $p<0.001$] but no change between these time points for CS+ ratings ($p>0.5$). This suggests an occasion-setting effect in the early block of extinction, with participants expecting a contingency reversal to occur. However, a main effect of block [$F(2.757,107.537)=20.981$, $p<0.001$, $\eta_p^2=.35$] showed significant reduction in ratings to both CSs across the first three blocks of extinction [all $p<.001$], but not between the third and last block ($p>.5$), suggesting that ceiling-level extinction of expectancies had occurred by the final extinction block. No group effects were found.

Recall

SCRs

Contexts An interaction was found between group, context (conditioning or extinction) and trial (1–6) [$F(6.68,110.26)=2.231$, $p=.039$, $\eta_p^2=.119$]. One-way ANOVAs revealed a trend for a group difference only on the sixth trial in the extinction context [$F(2,33)=3.625$, $p=.038$]. However, post hoc tests did not reach post-correction significance.

Conditioned stimuli Recall in all drug groups was evidenced by a context×CS interaction [$F(1,35)=6.828$, $p=.013$, $\eta_p^2=.163$; see Fig. 4], driven by significantly greater SCRs to the CS+ than the CS− in the conditioning context [$t(37)=2.796$, $p=.008$] but no differences in the extinction context. No effects of group or context were found.

Expectancy

Contexts A context×trial interaction [$F(3.06,119.34)=4.014$, $p=.009$, $\eta_p^2=.093$] showed no difference between contexts in ratings on trials 1 and 2 but greater ratings for the conditioning context for all trials thereafter (all $p<.002$).

Conditioned stimuli Explicit recall was shown by a context×CS interaction [$F(1,40)=4.509$, $p=.04$, $\eta_p^2=.101$], whereby expectancies for the CS+ were higher in the conditioning context than the extinction context [$t(42)=2.738$, $p=.009$], but no contextual discrimination was found for the CS− ($p>.4$) showed. A trend for a main effect of group was found [$F(2,40)=2.991$, $p=.062$, $\eta_p^2=.13$], reflecting lower post correction ratings in the CBD post-extinction group than placebo group [$p=.047$] but no differences between placebo and CBD pre-extinction.

Reinstatement

SCRs

Contexts A trend for a context×group interaction was found [$F(2,35)=2.545$, $p=.097$, $\eta_p^2=.132$], suggesting lower responding to the extinction context in the CBD post-extinction group than placebo (see Fig. 5). However, no significant differences emerged following post hoc tests. A main effect of trial [$F(2.696,94.348)=27.289$, $p<.001$, $\eta_p^2=.438$] indicated a decrease in overall SCR, driven by a large decrease in SCRs between the first trial and all later trials [$F(1,35)=50.348$, $p<0.001$].

Conditioned stimuli SCRs in the conditioning context were higher than the extinction context [$F(1,33)=6.206$, $p=.018$, $\eta_p^2=.158$], indicating reinstatement of contextual responding. A CS×context interaction was found [$F(1,33)=4.312$, $p=.046$, $\eta_p^2=.116$], driven by greater responses to the CS+ in the conditioning context than extinction context [$t(37)=2.707$, $p=0.01$], but no contextual difference in responding to the CS− ($ps>0.5$). A trend for a group×context interaction was also found [$F(2,35)=2.836$, $p=.073$, $\eta_p^2=.147$], driven by a trend for greater differential responding between contexts in the placebo group than CBD groups ($p=.045$), although this effect did not reach significance post-correction.

Expectancy

Contexts Expectancy ratings were higher overall in the conditioning context than extinction context [$F(1,38)=5.032$,

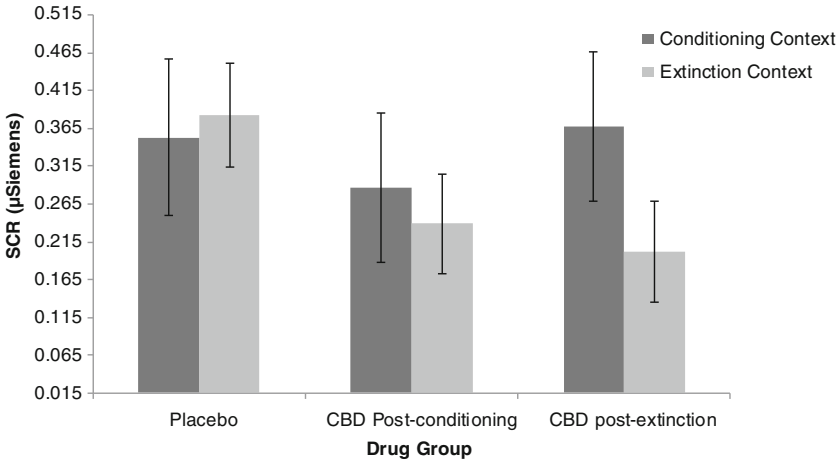

Fig. 4 SCRs to the extinction and conditioning contexts by drug group during reinstatement. *Bars* show mean ±SEM

p=.031, η_p^2=.117]. A trial×context interaction was found [F(5,190)=3.274, p=.007, η_p^2=.079], driven by a significant increase between the first and second trials of the conditioning context only [t(41)=4.471, p<.001]. This is evidence of reinstatement of contextual contingency with the UCS following the reinstating shock. A trial×group interaction was also found [F(10,190)=2.274, p=.016, η_p^2=.107]. An effect of trial was only found in the CBD post-extinction group [F(5,65)=3.5, p=.007, η_p^2=.212] driven by a significant increase in expectancy between the first and second trials only [F(1,13)=12.444, p=.003], showing a greater reactivity to contingency violation by reinstating UCS shocks paired with context only (and not CS+) in this group only.

Conditioned stimuli A main effect of group was found [F(1,40)=4.76, p=.014, η_p^2=.192], driven by lower overall ratings in the CBD post-extinction group compared to the placebo [t(26)=2.215, p=.036] and CBD pre-extinction group [t(26)=3.046, p=.005; see Fig. 6]. Overall recall was shown by an interaction between CS and context [F(1,40)=15.915, p<.001, η_p^2=.285], with greater responses to the CS+ in the conditioning context than the extinction context [t(42)=3.789, p<0.001], but no difference for the CS−.

Subjective effects of CBD

Descriptive statistics for all MRS data are given in Table 2. No effect of group was found at any time on any subscales of MRS score. A significant main effect of measurement time was found on anxiety scores [F(3,132)=2.902, p=.037, η_p^2=.062]. This was driven by a reduction in anxiety in all drug groups from before to after inhaling the first balloon, irrespective of whether the balloon contained drug or placebo [t(46)=2.347, p=0.023].

Prose recall

A main effect of group [F(2,44)=3.305, p=0.046, η_p^2=.131; see Fig. 6] reflected greater immediate [t(30)=2.456, p=.02] and delayed [t(30)=2.2946, p=.029] recall in the placebo group than the CBD pre-extinction group. Delayed recall was poorer than immediate recall time in all groups

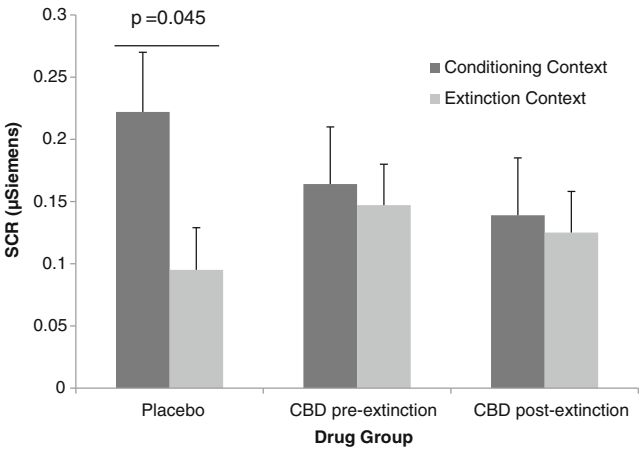

Fig. 5 SCR across groups by context during reinstatement. *Bars* show mean±SEM

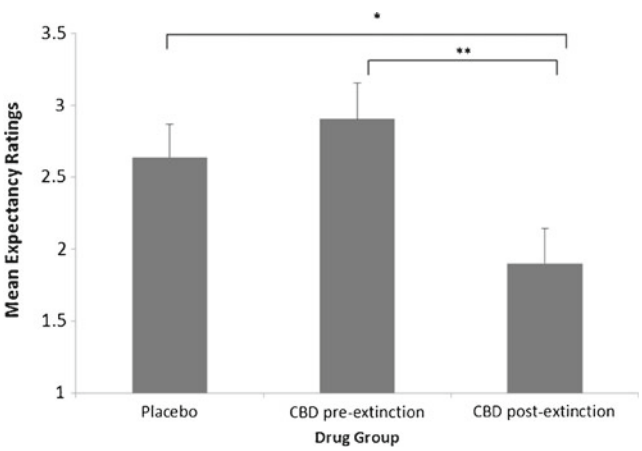

Fig. 6 Main effect of drug on overall ratings during fear reinstatement task. *Bars* show means+SEM. Significant at *p<.05; **p<.01

Table 2 Mean MRS subscale scores pre- and post-drug administration across drug groups

MRS subscale	Time	CBD pre-extinction	CBD post-extinction	Placebo
Anxiety	Pre-inhalation 1	31.17±19.54	35.67±23.52	34.15±31.82
	Post-inhalation 1	21.93±23.98	30.57±25.67	22.47±21.98
	Pre-inhalation 2	24.3±17.51	32.53±24.55	15.91±12.93
	Post-inhalation 2	21.97±16.33	38.9±26.8	18.91±19.24
Discontentedness	Pre-inhalation 1	23.61±9.12	28.95±15.42	24.45±21.49
	Post-inhalation 1	22.79±11.27	25.52±16.18	22.62±19.12
	Pre-inhalation 2	22.53±12.82	22.91±14.83	19.17±12.99
	Post-inhalation 2	22.01±10.58	29.56±18.28	16.87±13.98
Sedation	Pre-inhalation 1	26.5±13.64	28.4±16.26	24.94±13.67
	Post-inhalation 1	34.34±24.71	33.84±20.73	30.22±20.68
	Pre-inhalation 2	34.59±19.81	27.64±18.64	28.36±21.97
	Post-inhalation 2	32±18	33.94±20.65	29.12±18.97

[$F(1,44)=35.402$, $p<.001$, $\eta_p^2=.446$]. To assess whether this difference in explicit memory capacity could explain memory differences in the renewal and reinstatement tasks, prose recall scores were correlated with trial-by-trial scores on skin conductance and shock expectancy in these tasks, but no correlations were found (all ps>.2), suggesting the observed recall effects are independent of baseline differences in explicit memory capacity.

Post hoc power calculation Owing to the loss of SCR data, post hoc power calculations were performed to assess the impact of the final SCR Ns on power to detect a CBD effect during the extinction, recall and reinstatement tasks. The η_p^2 value of .192 (the observed effect size of the between-groups CBD effect on expectancies) was used as the best available estimate of a possible CBD effect size. This found that power was not overly compromised for SCR with achieved power of 0.77, 0.82 and 0.81 for extinction, recall and reinstatement tasks, respectively.

Discussion

The current study examined the effects of CBD on extinction and consolidation of contextual conditioned fear memory. CBD administered after extinction learning led to a generalised attenuation of explicit fearful responding during recall and reinstatement, accompanied by greater sensitivity to violations in learned context/CS/UCS contingencies. CBD administered either pre- *or* post-extinction also produced a trend-level reduction in reinstatement of contextual responding on SCRs. This suggests that CBD can potentiate consolidation of extinction memory in humans, leading to extinction trace dominance at recall.

This is the first study to show extinction-enhancing effects of CBD in human aversive conditioned memory. There is currently no information on how CBD may affect emotional memory in humans when administered after discrete phases of learning (e.g. conditioning and extinction). These results suggest that, consistent with the known role of eCBs in memory consolidation in rats (Campolongo et al. 2009), giving CBD after extinction preferentially enhanced consolidation of extinction memory relative to acquisition memory, leading to extinction trace dominance and the global attenuation of explicit fearful responding at test seen here. A preferential enhancement of extinction consolidation may also partly explain the dissociation between the effect of CBD given pre- and post-extinction, with peak plasma levels falling more in the consolidation window in the latter.

It is interesting that this effect should only be seen in shock expectancy ratings during recall, although a trend-level reduction in SCR responses to the extinction context alone was found during reinstatement in the CBD post-extinction group, consistent with a potentiation of extinction consolidation. Such dissociations of psychophysiological and expectancy metrics in conditioning are well documented (Mineka 1979; Bechara et al. 1995; Sevenster et al. 2012) and there is currently debate as to the role explicit and implicit associative processes play, with authors variously regarding conditioning as an automatic, low-level process, a dual-level process (Ohman and Minkea 2001), or a more propositional process, where UCS expectancy plays a central role (DeHouwer 2009). The current findings highlight the importance of collecting multiple measures of conditioning in human studies (Boddez et al. 2012), especially if conditioning and extinction models are to be applied successfully to anxiety disorders. The finding that CBD can attenuate fearful expectancy processes is therefore striking and highly encouraging for its use in anxiety patients where expectancy of fearful outcomes plays a central role in disease symptomatology (Reiss 1991).

A trend for a reduction in reinstatement of contextually discriminant responding in the presence of conditioned

stimuli was seen in both groups administered CBD, suggesting that CBD administered either before *or* after extinction may provide some protection against reinstatement of contextual memory. This may be due to some lingering action of CBD during consolidation (albeit at a lower level than CBD post-extinction) in the pre-extinction CBD group. In contrast to previous preclinical findings (Pamplona et al. 2008), no acute effects of CBD on extinction were observed, although very rapid ceiling-level extinction made seeing a subtle drug effect on extinction rate unlikely. As this rapid extinction learning is typical in humans, acute potentiation of extinction may not be a feasible or necessary mechanism for enhancing extinction-based learning in anxiety disorders, with *endurance* and *dominance* of the extinction trace during memory retrieval more likely important for long-term outcome improvement.

As noted above, these reinstatement effects may reflect enhanced memory flexibility following CBD. Activation of the eCB system is critical in behavioural flexibility and emotional re-learning, processes which underlie successful extinction learning following conditioning (Marsicano et al. 2002; Pamplona et al. 2008; Hill et al. 2006), with CB_1R knockout mice showing deficits in reversal learning (Varvel and Lichtman 2002). Consistent with the hypothesised role of CBD as an inhibitor of the transport and hydrolysis of anandamide, CBD may increase the flexibility of acquired memory traces (as evidenced by potentiated extinction learning) via increasing extracellular anandamide. This flexibility may then persist at test via glucocorticoid/eCB-mediated consolidation enhancement (Campolongo et al. 2009).

Importantly, the reinstating shocks (one administered in each context) violated learned CS/context/UCS contingencies. Before these UCS presentations, the UCS had never been presented in the absence of the CS+ and never associated with purely contextual information. That these violations reinforced differential contextual responding during reinstatement in the placebo group, demonstrates a rigidity of conditioned memory in this group that was resistant to contingency violation. That is, novel context/UCS pairings reinstated learned contextual fear contingencies, rather than shifting responding towards new learning (as was the case in the CBD groups). The lack of contextual differentiation following this contingency violation in both CBD groups may be indicative of a rapid shift in responding based on new information (i.e. greater memory flexibility), with less reactivity to stimuli that no longer predicted aversive outcomes. This resistance to reinstatement effects is consistent with a more dominant extinction trace and increased flexibility at test. The increase in responding during presentation of contexts only (when reinstating UCSs were administered) within the first two trials in the CBD post-extinction group further supports this interpretation, indicating a shift in UCS expectancy from old learned associations to new learning. Future studies should pay close attention to the point at which violations of expected and actual outcomes occurs, as this mismatch is thought to be critical for triggering extinction or reconsolidation of memory traces (Osan et al. 2011) and CBD has recently been found to have effects on the latter process (Stern et al. 2012). While disrupted reconsolidation or enhanced extinction may present the same effect at test, complicating interpretation of results, the present findings cannot be attributed to reconsolidation blockade due to the lack of sufficient time between conditioning and extinction for memory consolidation to occur.

These findings have important implications for the use of CBD as an adjunct to extinction-based pharmacotherapies for anxiety disorders, particularly PTSD, where aversive memory persistence and reinstatement are major contributors to maintenance of pathological fearful responding. Enhancing consolidation of extinction learning and increasing flexibility of memory with CBD could therefore be an excellent strategy for improving efficacy of behavioural therapy in these patients. Furthermore, reducing reinstatement of contextually fearful responding following extinction-based therapy is critical for long-term relapse prevention in anxiety disorders. Thus, while CBD has demonstrated potential for use in anxiety disorders due to its anxiolytic properties and safety profile alone, its optimal use in the treatment of anxiety disorders may be via combined use for judicious management of anxiety during exposure therapy (Rachman et al. 2008) and via enhancing consolidation of extinction learning.

In the current study, the attenuation of fearful responding in the CBD post-extinction group was unlikely to be due to anxiolysis as there was no evidence of reduced anxiety following CBD. However, as eCBs are known to interact with glucocorticoids, which vary with stress levels (Atsak et al. 2011; Akirav 2011; Campolongo et al. 2012), eCB-glucocorticoid interactions may thus be critical in the effects of CBD on memory (de Bitencourt et al. 2013). As stress can have bi-directional effects on memory consolidation (depending on severity), the mediating role of stress on CBD's mnemonic effects should be assessed in future studies.

The CBD pre-extinction group performed worse on the prose recall task at both time points than the placebo group. This is indicative of pre-existing group differences in memory capacity. It is possible that the relative lack of effects of pre-extinction CBD is related to pre-existing memory capacity in this group. However, no correlations were found between prose recall performance and any measures during conditioning, extinction, renewal or reinstatement.

Limitations

Due to the continuous learning protocol employed in the current study (extinction followed acquisition by ~15 min),

it was not possible to examine the effects of CBD on consolidation of fear acquisition, without potential interference from new learning during extinction. This may have contributed to the lack of drug effects on consolidation of conditioning. An optimal protocol for assessing these potential effects would be a delayed extinction paradigm, where acquisition memory is allowed to consolidate before extinction learning. However, due to the recently discovered effect of CBD on memory reconsolidation (Stern et al. 2012), insufficiently long extinction sessions may make any effects within this protocol difficult to interpret.

Due to equipment failure, a significant proportion of psychophysiological data was lost. This may have negatively affected test power and may explain some of the observed dissociation between explicit and autonomic memory measures. Nevertheless, the findings of extinction consolidation enhancement by low-dose CBD on expectancy are compelling and warrant further investigation of the effects of CBD on multiple measures of conditioning and extinction in a larger cohort. The current study, however, should not have been taken as evidence against effects of CBD on autonomic measure of conditioned responding.

An issue in all conditioning studies in humans is the effect of propositional mechanisms concerning task demands during novel tasks. There was evidence of these 'occasion setting' effects in the current study on initial trial of new tasks. In extinction, for example, there was an initial increase in CS− ratings, reflecting expectancy of contingency reversal. However, following these initial orienting responses, appropriate responding was achieved and maintained, suggesting that occasion setting effects did not affect learning of contingencies. These effects are somewhat unavoidable in human studies, although the collection of multiple measures of conditioning allows appraisal of the role they may play.

Conclusion

The current study found that a sub-anxiolytic dose of CBD given post-extinction potentiated the consolidation of extinction learning as evidenced by a globally reduced UCS expectancy at test. This was accompanied by an increased sensitivity to learned contingency violation in this group (i.e. increased memory flexibility), consistent with the hypothesised action of CBD as an anandamide hydrolysis and reuptake inhibitor. These preliminary findings suggest CBD may be a potentially excellent adjunct to extinction-based therapies for anxiety disorders and warrant further investigation into its mnemonic effects.

Acknowledgments This study was supported by a grant awarded to SKK, CJAM and HVC by the Medical Research Council (UK). The authors report no conflict of interest, financial or otherwise, in this research. All data were collected in compliance with UK law.

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