Cannabidiol disrupts the reconsolidation of contextual drug-associated memories in Wistar rats

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ABSTRACT

In addicts, craving and relapse are frequently induced by the recall of memories related to a drug experience. Several studies have demonstrated that drug-related memories are reactivated after exposure to environmental cues and may undergo reconsolidation, a process that can strengthen memories. Thus, reactivation of mnemonic traces provides an opportunity for disrupting memories that contribute to the pathological cycle of addiction. Here we used drug-induced conditioned place preference (CPP) to investigate whether cannabidiol (CBD), a phytocannabinoid, given just after reactivation sessions, would affect reconsolidation of drug-reward memory, reinstatement of morphine-CPP, or conditioned place aversion precipitated by naltrexone in Wistar rats. We found that CBD impaired the reconsolidation of preference for the environment previously paired with both morphine and cocaine. This disruption seems to be persistent, as the preference did not return after further reinstatement induced by priming drug and stress reinstatement. Moreover, in an established morphine-CPP, an injection of CBD after the exposure to a conditioning session led to a significant reduction of both morphine-CPP and subsequent conditioned place aversion precipitated by naltrexone in the same context. Thus, established memories induced by a drug of abuse can be blocked after reactivation of the drug experience. Taken together, these results provide evidence for the disruptive effect of CBD on reconsolidation of contextual drug-related memories and highlight its therapeutic potential to attenuate contextual memories associated with drugs of abuse and consequently to reduce the risk of relapse.

Keywords cannabidiol, conditioned place preference, memory reconsolidation.

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INTRODUCTION

In human addicts, exposure to environmental cues previously associated with drug-taking elicits craving and strongly increases the risk of relapse (O'Brien *et al.* 1992). Several studies have indicated that learning and memory, and particularly contextual memories, play a critical role in establishing conditioned responses in addiction (Nestler 2002; Robbins & Everitt 2002). Indeed, a growing body of evidence indicates that memory and addiction share both neural circuitry and molecular mechanisms (Nestler 2002; Tronson & Taylor 2007; Milton & Everitt 2010). The ability to attenuate drug-associated memories in drug addicts is important because this attenuation is expected to suppress the cycle of relapse to drugs. Persistent drug-taking behavior involves consolidation of memory for the drug and drug-associated cues and contexts (Tronson & Taylor 2007; Milton & Everitt 2010). Thus, given that several aspects of addiction depend on mnemonic processes induced by the drug experience, it is reasonable to hypothesize that disrupting these memories may contribute to the prevention of relapse and disruption of the addiction cycle (Tronson & Taylor 2013). Memory reconsolidation, the process by which memories are re-stabilized after retrieval, may have special relevance for addiction both in terms of treatment potential and as a mechanism for maintaining and strengthening the cue–drug relationship over time (Tronson & Taylor 2013).

Δ9-Tetrahydrocannabinol (THC) is a major constituent of the *Cannabis* plant and acts as a partial agonist of the cannabinoid receptors CB1 and CB2. The second major constituent of *Cannabis* extract is cannabidiol (CBD), which does not have significant intrinsic activity over cannabinoid receptors (Howlett *et al.* 2002), but unexpectedly displays high potency as an antagonist of CB1/CB2 receptor agonists in CB1-expressing and CB2-expressing cells or tissues (Thomas *et al.* 2007). CBD interacts with many other non-endocannabinoid

signaling systems as a 'multi-target' drug (Izzo *et al.* 2009). In fact, the mechanism that mediates the actions of CBD is complex and not fully understood. Unlike THC, CBD has antipsychotic properties (Izzo *et al.* 2009) and a favorable side-effect profile in humans (Nurmikko *et al.* 2007). Furthermore, CBD has a large-spectrum therapeutic potential to treat many neuropsychiatric disorders (Hill *et al.* 2012), including addiction (Morgan *et al.* 2010, 2013).

Pre-clinical studies investigating the effects of CBD on animal models of addiction are still scarce despite the promise of CBD as a treatment for drug addiction (Parker *et al.* 2004; Ren *et al.* 2009; Katsidoni *et al.* 2013). For example, CBD seems to lack hedonic properties because it does not induce conditioned place preference (CPP; Parker *et al.* 2004). Furthermore, an interesting study examining the effects of CBD on heroin self-administration in rats found that CBD attenuated cue-induced reinstatement of heroin-seeking behavior (Ren *et al.* 2009).

CBD has been shown to impair reconsolidation and to facilitate extinction of contextual fear in rats (Bitencourt *et al.* 2008; Stern *et al.* 2012). In addition, anxiolytic properties (Bergamaschi *et al.* 2011; de Mello Schier *et al.* 2014) and the enhancement of consolidation of extinction learning (Das *et al.* 2013) have been described in humans treated with CBD. Thus, CBD may have high potential as an adjunct to cue exposure therapies for disorders such as post-traumatic stress disorder and addiction, which are characterized by persistent maladaptive memories.

To the best of our knowledge, there are no studies on the effects of CBD on reconsolidation of drug-related memories (i.e. hedonic or aversive contextual memories). The aim of this study was to investigate whether an established drug-induced memory can be disrupted by acute systemic administration of CBD after its reactivation. To this end, we used a classic CPP task, in which the animal learns to associate a context with the pleasurable or rewarding effects of a drug [morphine (MOR) or cocaine (COC)] or conditioned place aversion (CPA), elicited by a single naltrexone–place pairing in rats previously trained for MOR-CPP.

MATERIALS AND METHODS

Animals

A total of 295 male Wistar rats (2 months old and 180–220 g at the beginning of the experiment) were provided by the animal facility of the Universidade Federal de Santa Catarina. Animals were maintained in a room under controlled temperature ($21 \pm 2°C$) on a 12-hour light/dark cycle (lights on at 7:00 AM) with standard laboratory chow and water freely available throughout the study. Each behavioral test was conducted during the light phase of the cycle. All procedures were conducted with strict adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local Committee on Animal Care and Use (protocol number PP00505/2011CEUA).

Drugs and injections

Cocaine (Sigma-Aldrich, Missouri, USA) and morphine hydrochloride (Merck, Darmstadt, Germany) were both dissolved in 0.9 percent sodium chloride (saline) and given at a volume of 1 ml/kg to a final concentration of 10 mg/kg for initial CPP training and at 2.5 mg/kg as the priming drug for reinstatement of MOR-CPP. CBD (Tocris Bioscience, Missouri, USA) was dissolved in a vehicle (VEH) solution consisting of 10 percent dimethyl sulfoxide plus 0.1 percent Tween 80 in saline and injected at a volume of 2 ml/kg of body weight.

CPP

The CPP task was conducted under low illumination (10 lux) in four identical rectangular wooden boxes covered with Formica, composed of three distinctive compartments separated by guillotine doors. The two conditioning compartments ($30 \times 25 \times 40$ cm) had different tactile and visual cues: one chamber was black with a smooth wooden floor and the other was black with vertical white stripes and aluminum floor. The central compartment ($15 \times 25 \times 40$ cm) was gray with a smooth wooden floor and had openings (10×10 cm) that provided access to the two other chambers (de Carvalho *et al.* 2010). The amount of time each animal spent in each conditioning compartment was recorded by an observer blind to the treatments using a video monitor.

Acquisition of CPP

We performed three experiments using a CPP procedure described previously (de Carvalho *et al.* 2014). The acquisition of CPP consisted of three phases: the pre-conditioning test, conditioning trials and the initial preference test. On day 1, the pre-conditioning test (pre-test), rats were placed in the central chamber and were allowed to freely explore the three compartments for 15 minutes. The time spent in each large chamber was recorded. Approximately 5 percent of rats were excluded because they displayed a strong (< 540 seconds in large chamber) unconditioned preference (de Carvalho *et al.* 2014).

For conditioning trials, the MOR-CPP animals received MOR (10 mg/kg s.c.) and were immediately confined to the conditioning compartment for 30 minutes. On the same day, each animal also received saline in the contralateral compartment. The two conditioning sessions on the same day were carried out 6 hours apart. For COC-CPP, rats alternately received an injection of COC

(10 mg/kg i.p.) or saline, one conditioning session per day for 8 days. Overall, each rat received a total of four 30-minute pairings with the drug (MOR or COC) in one compartment and four 30-minute pairings with saline in the other. The order of injection and the compartment paired with the drug was counterbalanced within each group. Rats in the saline groups underwent the same CPP procedure described previously, but received only saline injections before being placed in either chamber.

One day after the last conditioning trial, an initial preference test was given. The post-conditioning tests consisted of placing the animal in the center choice compartment and allowing it to freely access the entire apparatus for 15 minutes. The amount of time each animal spent in each large compartment was recorded. In our study, we excluded the rats that display < 90 seconds in the drug-paired chamber (initial place preference test–pre-conditioning test). Approximately 7 percent of rats were excluded from this study. Culling rats was performed to assure that only those rats who clearly acquired CPP were subsequently examined for pharmacological interventions to change the previously established preference (Paolone *et al.* 2009). The CPP or CPA score was calculated for each rat as the difference (in sec) between the time spent in the drug-paired chamber and the saline-paired chamber on the same day (Fang *et al.* 2011).

Reactivation of place preference

Following conditioning and the initial CPP test, rats showing robust preference for the drug-paired chamber were subjected to a place preference reactivation trial. A 10-minute re-exposure to the drug-paired chamber was designed to serve as a retrieval trial to reactivate the memory of drug cue association (Wang *et al.* 2008) acquired during the conditioning phase. Except for experiment 3, in which drug memory reactivation consisted of a single MOR conditioning session (reinforced trial), which was required for development of subsequent nal trexone-precipitated CPA (Taubenfeld *et al.* 2010). All pharmacological interventions with CBD were performed immediately after the memory reactivation.

Extinction and reinstatement of place preference

During extinction sessions, rats were confined to one of the conditioning chambers in a drug-free state for eight consecutive days (days 35 through 42). Sixteen 30-minute counterbalanced sessions were performed, eight in the drug-paired chamber and eight in the drug-unpaired chamber, without any injection. Following the last extinction session, an extinction test (day 43) was conducted to determine if each animal's preference for the MOR-paired chamber was successfully extinguished.

The extinction of CPP occurs when there is no significant difference in the time spent in the MOR-paired versus saline-paired chambers among rats previously exhibiting MOR-CPP (Paolone *et al.* 2009).

The reinstatement of drug-seeking behavior refers to the resumption of a previously drug-reinforced behavior by non-contingent exposure to drug or non-drug stimuli, such as certain stressors, after extinction (Sanchis-Segura & Spanagel 2006). Here we used this approach to confirm whether CBD interfered with memory reconsolidation rather than extinction in our experimental conditions. On day 44, all rats received the priming injection of MOR (2.5 mg/kg, s.c.) immediately prior to the test (priming test) to reinstate the extinguished CPP (Mueller *et al.* 2002).

Stress-induced reinstatement was performed using a protocol adapted from Ribeiro Do Couto *et al.* (2006), seven days after the drug-evoked reinstatement test. Rats were exposed to a 15-minute immobilization stress during which time they were placed within a PVC cylinder restrainer. Following the restraint, the rats were immediately placed in the CPP apparatus and their time spent in both chambers was recorded for 15 minutes.

Naltrexone-precipitated motivational withdrawal

The motivational withdrawal protocol adapted from Taubenfeld *et al.* (2010) consisted of naltrexone administration 4 hours after a single MOR conditioning in rats previously trained for MOR-CPP. This protocol elicits a significant CPA, which is transient, lasting no more than 2 weeks, and resembles the aversive motivational consequences of withdrawal (White *et al.* 2005; Taubenfeld *et al.* 2010).

Experimental design

Experiment 1: effects of CBD on reconsolidation of a drug-induced CPP

One week after drug-induced CPP acquisition, reward memory was reactivated by confining the rats to the drug-paired chamber for 10 minutes. First, we evaluated the effects of CBD on the reconsolidation of MOR-reward memory. Three MOR-trained groups of rats ($n = 10$ per group) were given injections of different doses of CBD (5 or 10 mg/kg, s.c.; CBD5 and CBD10) or VEH immediately after reactivation. Animals remained drug-free in their home cage, and re-testing of MOR-induced CPP was performed on the following days: 1, 7 and 14 days. Second, an independent group of rats were trained to acquire COC-CPP (COC) and were subsequently subjected to an identical experimental procedure of memory reactivation. One and two weeks later, rats were re-tested to evaluate the effects of CBD (10 mg/kg) on the reconsolidation of COC -associated memory.

Experiment 2: effects of CBD on reinstatement of morphine-reward memory

This experiment was performed to examine whether CBD could induce a persistent disruption of drug-reward memory through a block specific to the reconsolidation process and to ensure that CBD interfered with memory reconsolidation rather than extinction of memory. Acquisition, extinction and reinstatement of MOR-CPP were accomplished over a 51-day protocol. The acquisition of MOR-CPP and the memory reactivation protocol were conducted in the same way as in Experiment 1, except that MOR-trained rats ($n = 8$–10/group) received an acute injection of CBD (10 mg/kg s.c.; CBD) or VEH immediately after reactivation and were tested 21 days later to examine the long-term effects of CBD on the reconsolidation of MOR-CPP.

Subsequently, both groups underwent the extinction sessions (16 sessions), and 24 hours after the extinction test, all rats received a priming dose of MOR and were immediately tested for CPP again (priming-drug reinstatement test). Seven days later, the test of stress-induced reinstatement was performed 15 minutes after restraint in four experimental groups: MOR/VEH/non-stress, MOR/VEH/stress, MOR/CBD/non-stress and MOR/CBD/stress (section 0.0.0).

Experiment 3: effects of CBD on subsequent development of naltrexone-precipitated motivational withdrawal

The aim of this experiment was to assess whether the disruption of MOR-CPP with a post-reactivation injection of 10 mg/kg of CBD affected the subsequent motivational withdrawal. Rats were trained to acquire MOR-CPP using a protocol identical to the previous experiments except in this experiment, MOR-CPP was reactivated through a single reinforced (MOR-10 mg/kg) conditioning trial (1X COND) and immediately after, the rats received an injection of CBD (10 mg/kg, s.c.; CBD) or VEH.

The next day, they were subjected to a protocol of naltrexone-precipitated withdrawal. Both groups of rats were divided into two subgroups, who underwent an additional MOR conditioning trial and received an injection of naltrexone (0.3 mg/kg; NTX) or VEH 4 hours later. Subsequently, all rats were again confined to the drug-paired chamber for 30 minutes. All four groups (MOR/VEH/VEH, MOR/VEH/NTX, MOR/CBD/VEH and MOR/CBD/NTX) were re-tested for place preference or place aversion 2 and 7 days later.

Statistical analysis

All data were analyzed with Statistica 7 (Statsoft, Inc., USA), and graphs were designed with GraphPad 5 (GraphPad Inc., USA). CPP or CPA data were analyzed by one-way or two-way ANOVAs with repeated measures

when appropriated, followed by a Bonferroni *post hoc* test for multiple comparisons. Analyses specific to each experiment are outlined in the text. Significance was set at $\alpha = 0.05$ for all tests.

RESULTS

Experiment 1: CBD disrupts the reconsolidation of place preference induced by different drugs of abuse

A one-way repeated measures ANOVA comparing preference scores of groups (Saline versus MOR/VEH) over trials (i.e. repetition factor) revealed a main effect of group ($F_{1, 16} = 367.58$, $P < 0.000001$; Fig. 1a). Compared with the saline group, the MOR/VEH group acquired CPP and spent significantly more time in the MOR-paired chamber across the tests.
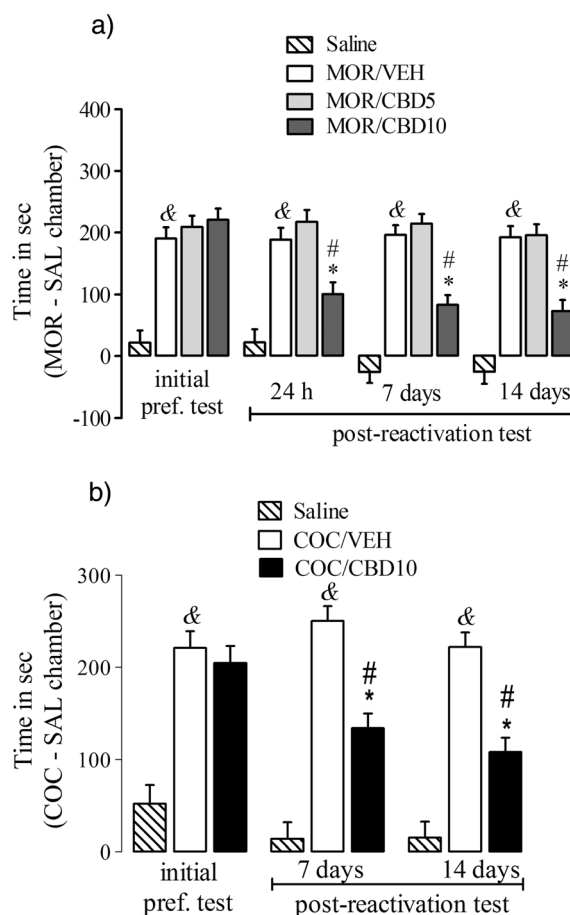
Figure 1 CBD disrupts the reconsolidation of place preference in rats previously trained for CPP task. Rats previously conditioned to morphine (a) or cocaine (b), were exposed to the drug-paired context, and immediately after received CBD (5 or 10 mg/kg; CBD5 or CBD10) or vehicle (VEH) and were retested until 14 days later. Data are presented as means + SEM (n = 10–11/group) for CPP or CPA scores in seconds (the time spent in the drug-paired chamber minus the time spent in the saline-paired chamber). Symbols indicate P < 0.05 (&) vs. saline group; (*) vs. to the initial preference test or (#) vs. vehicle-treated group for the same test

For MOR-trained rats, a two-way ANOVA across treatment (CBD) and trials revealed significant effects of treatment ($F_{2, 108} = 32.09$, $P < 0.00001$), trials ($F_{3, 108} = 4.50$; $P < 0.005$) and treatment–trials interaction ($F_{6, 108} = 5.48$; $P < 0.0001$; Fig. 1a). Initially, *post hoc* comparisons indicated no significant differences among all MOR-trained groups during the initial preference test. On the other hand, rats treated with CBD (10 mg/kg) presented a significant disruption of MOR-CPP 24-hour post-reactivation ($P < 0.03$; Fig. 1a), compared with the MOR/VEH group. This disruption was persistent at 7 ($P < 0.003$) and 14 ($P < 0.01$) days after treatment, suggesting that reduction of CPP is persistent and not state-dependent.

Next, we evaluated whether CBD (10 mg/kg) also affects the COC-CCP in rats. One-way ANOVA with repeated measures comparing preference scores for groups (Saline versus COC/VEH) over trials revealed a significant effect of group ($F_{1, 18} = 209.80$; $P < 0.000001$; Fig. 1b). Compared with the saline group, the COC/VEH group acquired CPP and spent significantly more time in the COC-paired chamber over trials.

For COC-trained rats, a two-way ANOVA across treatment (CBD) and trials revealed significant effects of treatment ($F_{1, 60} = 33.28$; $P < 0.00001$), trials ($F_{2, 60} = 3.76$; $P < 0.03$) and treatment-trials interaction ($F_{2, 60} = 5.38$; $P < 0.008$; Fig. 1b). *Post hoc* analyses indicated no significant differences between COC-trained groups for the initial preference test. However, the COC/CBD10 group had a significantly lower CPP score (Fig. 1b) compared with the COC/VEH at 7 days after reactivation ($P < 0.01$) and this disruption persisted 1 week later ($P < 0.03$). Moreover, the COC/CBD10 group had a significant decrease in COC-CPP scores over both re-tests compared with the initial preference test ($P < 0.05$). Taken together, these findings indicate that CBD impaired the reconsolidation of preference for environments previously paired with MOR or COC.

Experiment 2: CBD prevents the spontaneous recovery and the reinstatement of morphine-reward memory

The results for experiment 2 are illustrated in Fig. 2. A two-way ANOVA with repeated measures for the MOR-trained
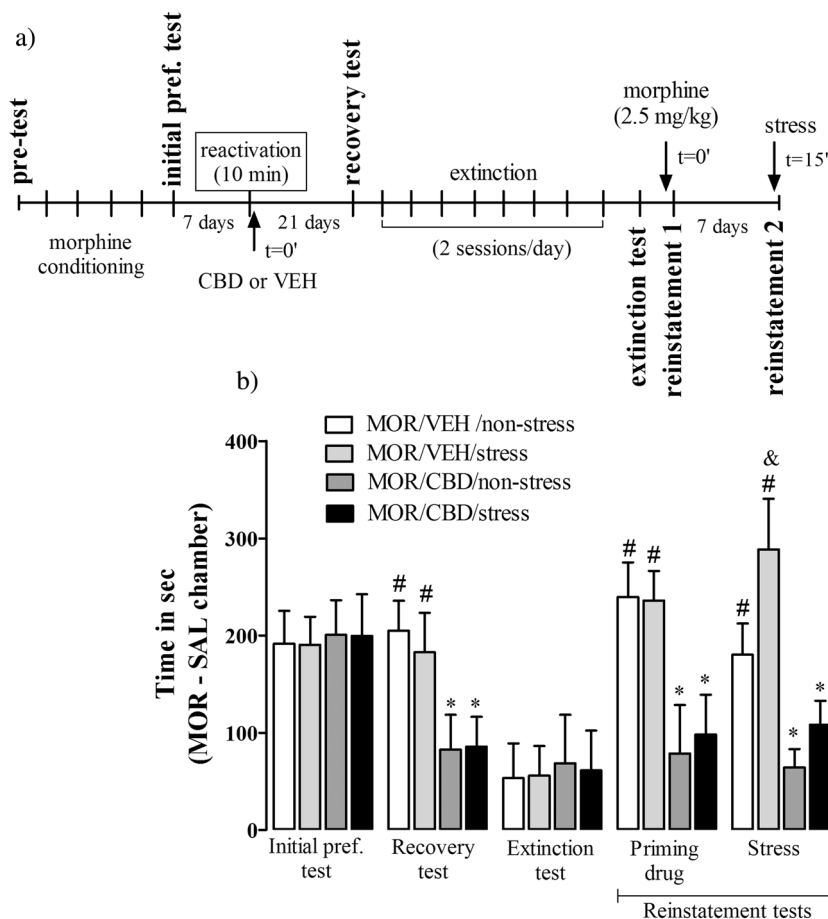
Figure 2 CBD injection after reactivation session in the CPP chamber prevents spontaneous recovery and the reinstatement of morphine-related memory in rats. (a) Timeline of experimental protocol (see section 2.4.2 for details). (b) Data are presented as means + SEM (n = 10–11/group) for CPP or CPA scores in seconds (the time spent in the drug-paired chamber minus the time spent in the saline-paired chamber). Symbols indicate P < 0.05: (*) vs. vehicle-treated group of the same test, (#) within the same group during extinction test and (&) MOR/VEH/non-stress vs. MOR/VEH/stress at stress-induced reinstatement test

rats across treatment (CBD) and stress over trials (repetition factor) revealed significant main effects of treatment ($F_{1, 37} = 153.48$; $P < 0.00001$), repetition ($F_{4, 148} = 137.49$; $P < 0.00001$), treatment–trials interaction ($F_{4, 148} = 47.68$; $P < 0.00001$) and stress–trials interaction ($F_{4, 148} = 3.21$; $P < 0.02$). *Post hoc* comparisons indicated no significant differences in CPP scores among the four experimental groups during the initial preference test. Bonferroni's test revealed that MOR/CBD/non-stress and MOR/CBD/stress groups spent significantly less time in the MOR-paired chamber during the recovery test when compared with the initial preference test ($P < 0.0001$, for both). Further, both MOR/CBD/non-stress ($P < 0.00001$) and MOR/CBD/stress ($P < 0.00001$) groups also showed a significant decrease in their CPP scores on recovery test day compared with control groups, indicating that CBD did not produce a spontaneous recovery until 21 days after treatment.

Concerning the extinction, Bonferroni test indicated that only MOR/VEH/non-stress and MOR/VEH/stress groups showed a significant ($P < 0.00001$) decline in time spent in the MOR-chamber from the recovery test to the extinction test (232.6 versus 79.1 seconds and 215.5 versus 75.2 seconds, respectively) and acquired the extinction criteria. There were no differences in preference scores among the MOR/VEH/non-stress, MOR/VEH/stress, MOR/CBD/non-stress and MOR/CBD/stress groups for this test.

Post hoc comparisons revealed that the priming dose of MOR was able to reinstate the extinguished MOR-induced CPP only for the MOR/VEH/non-stress and MOR/VEH/stress groups, who showed a significant increase in their MOR-CPP scores compared with the extinction test ($P < 0.00001$, for both). In contrast, no significant differences in CPP scores were found for either of the CBD-treated groups from extinction test to priming drug reinstatement test. Additionally, both VEH-treated groups displayed higher CPP scores when compared with the MOR/CBD/non-stress and MOR/CBD/stress groups ($P < 0.00001$, for both). This suggests that only MOR/VEH/non-stress and MOR/VEH/stress groups reinstated place preference.

Post hoc analyses also indicated that the MOR/VEH/stress group displayed a significant increase in CPP score compared with the MOR/VEH/non-stress group ($P < 0.05$), which suggests that place preference was reinstated after stress exposure. The MOR/VEH/stress group also spent more time in the MOR-paired chamber at the stress-induced reinstatement versus extinction ($P < 0.000001$) and versus recovery ($P < 0.05$) tests. Additionally, although no significant differences were found between the MOR/CBD/non-stress and MOR/CBD/stress groups, both groups maintained a lower CPP score compared with VEH-treated groups ($P < 0.00001$, for both). Together, these results suggest that a single administration of CBD post-reactivation persistently prevented both drug- and stress-induced reinstatement of MOR-CPP.

Experiment 3: CBD disrupts the reconsolidation of morphine-CPP and suppresses subsequent naltrexone-precipitated CPA

The results for experiment 3 are illustrated in Fig. 3. A two-way ANOVA with repeated measures carried out for MOR-trained rats across pre-treatment (CBD), post-treatment (NTX) over trials (repetition factor), revealed main effects of post-treatment ($F_{1, 31} = 135.51$; $P < 0.00001$), repetition ($F_{2, 62} = 217.13$; $P < 0.0001$), pre-treatment–post-treatment interaction ($F_{1, 31} = 52.51$; $P < 0.0001$) and pre-treatment versus post-treatment versus repetition interaction ($F_{2, 62} = 27.38$; $P < 0.0001$). Overall, *post hoc* tests revealed that CBD-treatment significantly attenuated place preference (MOR/VEH/VEH versus MOR/CBD/VEH) and aversion (MOR/VEH/NTX versus MOR/CBD/NTX) to the MOR-paired chamber during re-test 1 ($P < 0.001$), and this effect persisted at re-test 2 ($P < 0.001$). Furthermore, both groups MOR/CBD/VEH and MOR/CBD/NTX displayed a significant ($P < 0.0001$) decrease in their CPP scores from the initial preference test to re-tests 1 and 2, performed 3 and 10 days after
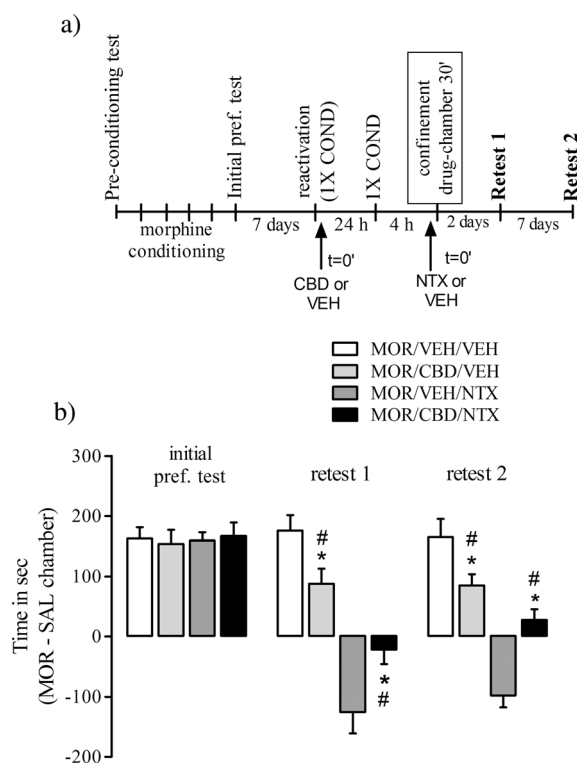
Figure 3 CBD disrupts the reconsolidation of contextual memory evoked by morphine and suppresses subsequent naltrexone-precipitated withdrawal in the same context. (a) Timeline of experimental protocol (see section 2.4.3 for details). (b) Data are presented as means + SEM (n = 10–11/group) for CPP or CPA scores in seconds (the time spent in the morphine-paired chamber minus the time spent in the saline-paired chamber) and depicted as means + SEM (n = 8–9/group). Symbols indicate (P < 0.05): (*) vs. MOR/VEH/VEH group and (#) vs. MOR/NTX/VEH for the same test

treatment. Taken together, these results suggest that CBD post-reactivation treatment disrupts place preference and the subsequent conditioned aversion to the same context.

DISCUSSION

This study investigated the effects of CBD on reconsolidation of drug-induced CPP in rats using pharmacological approaches. The main findings from this study were the following: (1) CBD impairs the reconsolidation of place preference associated with both opioid and psychostimulant drugs; (2) the long-lasting suppression of place preference behavior by CBD treatment after a brief re-exposure to the drug-conditioning context prevents the reinstatement of MOR-extinguished CPP; (3) the disruption of the reconsolidation of a MOR place preference by a single re-experience of a reinforced trial (reactivation) followed by administration of CBD leads to a subsequent weakening of naltrexone-precipitated (MOR-conditioned) withdrawal in the same place context. All together, these findings demonstrate that CBD persistently disrupts the reconsolidation of drug-related memory in rats.

Our results provide evidence that associative memories induced by a drug of abuse can become labile and be disrupted by CBD after their reactivation required by, mostly, a brief exposure to the conditioning stimulus alone or by the re-experience of a reinforced trial. We induced CPP by the administration of an opioid or a psychostimulant, in order to pair contextual cues with the perception of a drug-related reward. Afterwards, the animals were placed in the previously paired context in order to retrieve this place preference (memory reactivation). Post-reactivation administration of CBD (10 mg/kg, s.c.) disrupted the reconsolidation of MOR-induced and COC-induced CPP (lasting at least 2 weeks). Previous studies have shown that several types of memories, including appetitive memories, can be disrupted by pharmacological interventions after their reactivation (Nader *et al.* 2000; Dudai & Eisenberg 2004; Alberini 2005; Tronson & Taylor 2013).

There are a number of hypotheses to account for the disruption of reconsolidation in our CPP studies. The first hypothesis is that CBD caused a general memory loss. However, this appears unlikely because MOR-trained rats that underwent the same conditioning and testing schedule but received an injection of 10 mg/kg of CBD in their home cage, in the absence of the reactivating trial, had normal MOR-CPP and MOR-CPA (Supporting Information Fig. S1). One of the purposes of reconsolidation would be to integrate new information or new experiences to previously consolidated memories (Nader *et al.* 2000; Sara 2000; Rossato *et al.* 2007). Thus, we also tested whether CBD affected the reconsolidation of a non-emotional memory using a reconsolidation of object recognition protocol (refer to Supporting Information) previously reported by Rossato

et al. (2007). CBD did not impair the reconsolidation of a neutral memory. Administration of CBD did not affect the memory of either novel or familiar objects, nor did it affect the memory of the familiar object that was not presented during the reactivation phase in the object recognition task (Supporting Information Fig. S2).

A second hypothesis is that CBD facilitated the extinction process. This also appears unlikely because extinction involves new learning that temporally suppresses the expression of the original conditioned stimulus–unconditioned association (Bouton 2004), which is characterized by the ability of the unconditioned stimulus (drug or stress) to reinstate the conditioned behavioral response or the spontaneous recovery of the original memory (Myers & Davis 2002). In this study, reinstatement was significantly suppressed compared with controls when CBD (10 mg/kg) was used. Our results indicate that the disruption of MOR-induced CPP by an acute injection of CBD after reactivation was persistent. There was no behavioral evidence for the spontaneous recovery of contextual drug-related memory over time for CBD-treated rats. Further, CBD treatment resulted in long-lasting retrieval impairment, as the MOR-induced CPP did not re-emerge after 21 days in a test for spontaneous recovery or after a priming injection of MOR or an acute restraint stress exposure. Because the passage of time itself did not reinstate the CPP, we conclude that retrieval of the original association between MOR and the conditioning context was disrupted. These results are in agreement with studies showing the absence of spontaneous recovery within 7–28 days following disruption of memory reconsolidation in CPP tasks (Yu *et al.* 2009; Taubenfeld *et al.* 2010; de Carvalho *et al.* 2014). They also reinforce the suggestion that CBD interfered with the reconsolidation of contextual drug-related memory rather than its extinction in our experimental conditions.

A third alternative is that a possible anxiolytic effect of CBD could potentially contribute to the behavioral effects observed. However, our results indicate that acute treatment with CBD impaired the subsequent reinstatement of MOR-CPP, and it did not affect anxiety-like behavior in the elevated plus maze (Supporting Information Table S1) nor the plasma levels of corticosterone (Supporting Information Fig. S3) after the stress-induced reinstatement test. This evidence, together with our present findings, argues against involvement of CBD anxiolytic properties in the main effects observed in this study.

In general, our results on the effects of CBD on reconsolidation of drug-related memories are in agreement with the view that CBD may attenuate the rewarding effects of drugs of abuse in animal models (Parker *et al.* 2004; Ren *et al.* 2009; Katsidoni *et al.* 2013). Importantly, a previous report showed that CBD did not induce place preference, nor did it affect the reinforcing efficacy of brain stimulation on the intracranial self-stimulation

paradigm and therefore lacked hedonic properties in rats (Parker *et al.* 2004; Katsidoni *et al.* 2013). Further, CBD specifically decreased cue-induced reinstatement of heroin-seeking behavior, but did not alter the extinction of heroin-seeking behavior in rats (Ren *et al.* 2009). Another study showed that CBD facilitated the extinction of COC-induced and amphetamine-induced place preference without affecting learning or retrieval, and that these effects were not mediated by CB1 cannabinoid receptors (Parker *et al.* 2004). Notably, systemic administration of CBD blocked the reward-facilitating effect of MOR, but not COC, in the intracranial self-stimulation paradigm (Katsidoni *et al.* 2013). In our study, it appeared that CBD specifically disrupted the reconsolidation of drug-related memories associated with different classes of drugs of abuse independent of its emotional nature (hedonic or aversive), but did not affect the reconsolidation of non-emotional memory in rats.

It is noteworthy that a pilot, randomized, double-blind, placebo-controlled study found that the inhalation of CBD during a one-week period reduced the number of cigarettes smoked by tobacco smokers who wished to stop smoking (Morgan *et al.* 2013). The authors suggested that CBD may weaken the attentional bias of smokers to associative learning around drug cues (Morgan *et al.* 2010, 2013), suggesting that CBD may have potential as an adjunct to extinction-based therapies, including those for addiction. Overall, the evidence reviewed indicates substantial support for a role of CBD in modulating the reinforcing properties of different drugs of abuse.

In agreement with the pioneer study by Taubenfeld *et al.* (2010), our results demonstrate that CBD impaired the reconsolidation of contextual memory evoked by MOR and subsequently weakened the negative motivational component of opiate withdrawal in the same context. Our findings are supported to some extent by those of other authors (O'Brien *et al.* 1992; Schulteis *et al.* 2005) that opiate withdrawal symptoms reflect classically conditioned responses and that disrupting the reconsolidation of drug-induced memories may represent an interesting strategy for attenuating context-dependent withdrawal in opiate addicts (Taubenfeld *et al.* 2010). In opioid-dependent individuals, withdrawal is characterized by the emergence of a negative emotional state and by many somatic signs, and both contribute to the maintenance of drug-taking and relapse (Koob & Le Moal 2005). To our knowledge, this is the first study that has determined the effect of CBD on reconsolidation of naltrexone-precipitated CPA. However, phytocannabinoids, including CBD and THC, and endocannabinoid catabolic enzyme inhibitors have been known to ameliorate somatic MOR withdrawal signs in rodents (Hine *et al.* 1975; Bhargava 1976; Gamage *et al.* 2015). Conversely, neither THC nor endocannabinoid catabolic

enzyme inhibitors affect the motivational aversive aspects in the classical CPA task (Gamage *et al.* 2015).

CBD has been shown to impair reconsolidation and facilitate extinction of contextual fear memory in rats by a mechanism that involves indirect activation of CB1 cannabinoid receptors (Bitencourt *et al.* 2008; Stern *et al.* 2012), possibly acting as a fatty acid amide hydrolase inhibitor and increasing the levels of endocannabinoid anandamide (Bisogno *et al.* 2001). On the other hand, boosting endocannabinoid signaling by inhibiting anandamide metabolism promoted a transient and CB1 receptor-dependent increase in reconsolidation of MOR-CPP (de Carvalho *et al.* 2014), whereas the blockade of cannabinoid CB1 receptors by rimonabant disrupts the reconsolidation of methamphetamine- (Yu *et al.* 2009), nicotine- (Fang *et al.* 2011) and MOR-induced CPP in rats (de Carvalho *et al.* 2014). Thus, despite similarities in the inhibitory effects of CBD on reconsolidation of Pavlovian aversive memories and the results of this study, it is unlikely that the effects of CBD involve the same mechanism (CB1 receptor activation). At present it is not possible to indicate which of the many documented mechanistic actions of CBD underlie the changes reported in this study; the precise mechanisms by which CBD modulates the reconsolidation of drug-reward memory remain to be elucidated.

In conclusion, we provide for the first time evidence for the disruptive effect of acute injection of CBD on reconsolidation of contextual drug-related memories in rats. In translational terms, these findings suggest that CBD could be a useful pharmacological adjuvant to cue-exposure therapy used as novel treatments for weakening contextual memories associated with drugs of abuse and consequently reducing the risk of drug relapse.

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Authors Contribution

CRC and RNT were responsible for the study concept and design. CRC performed the experiments and analyzed the data. RNT assisted with data analysis and interpretation of findings. CRC drafted the manuscript and RNT provided critical revision of the manuscript for important

intellectual content. All authors critically reviewed the content and approved the final version submitted for publication.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Anxiety-like behavior of morphine-trained Wistar rats evaluated in the elevated plus maze (for 5 min) after exposure to stress-induced reinstatement test.

Figure S1 Memory reactivation is required for the disruptive effects of CBD on the reconsolidation of both morphine-CPP and withdrawal.

Figure S2 Administration of CBD after a reactivation session involving exposition to a novel and a familiar object does not affect memory reconsolidation of these objects in rats.

Figure S3 Corticosterone plasma levels of morphine-trained rats immediately after exposure to the elevated plus maze test.