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## Divergent effects of cannabidiol on the discriminative stimulus and place conditioning effects of $\Delta^9$ -tetrahydrocannabinol

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### Abstract

*Cannabis sativa* (marijuana plant) contains myriad cannabinoid compounds; yet, investigative attention has focused almost exclusively on  $\Delta^9$ -tetrahydrocannabinol (THC), its primary psychoactive constituent. Interest in modulation of THC's effects by these other cannabinoids [e.g., cannabidiol (CBD)] has been stimulated anew by recent approval by Canada of Sativex (a 1:1 dose ratio combination of CBD:THC) for the treatment of multiple sclerosis. The goal of this study was to determine the degree to which THC's abuse-related effects were altered by co-administration of CBD. To this end, CBD and THC were assessed alone and in combination in a two-lever THC discrimination procedure in Long-Evans rats and in a conditioned place preference/aversion (CPP/A) model in ICR mice. CBD did not alter the discriminative stimulus effects of THC at any CBD:THC dose ratio tested. In contrast, CBD, at CBD:THC dose ratios of 1:1 and 1:10, reversed CPA produced by acute injection with 10 mg/kg THC. When administered alone, CBD did not produce effects in either procedure. These results suggest that CBD, when administered with THC at therapeutically relevant ratios, may ameliorate aversive effects (e.g., dysphoria) often associated with initial use of THC alone. While this effect may be beneficial for therapeutic usage of a CBD:THC combination medication, our discrimination results showing that CBD did not alter THC's discriminative stimulus effects suggest that CBD:THC combination medications may also produce THC-like subjective effects at these dose ratios.

### Keywords

$\Delta^9$ -tetrahydrocannabinol; cannabidiol; marijuana; drug discrimination; conditioned place aversion

### 1. Introduction

The medicinal and recreational properties of *Cannabis sativa* (marijuana plant) have been recognized for thousands of years. Nearly 70 cannabinoids have been found in marijuana, including  $\Delta^9$ -tetrahydrocannabinol (THC) [its primary psychoactive constituent], cannabidiol (CBD), cannabinal, cannabigerol, and cannabichromene (see review Elsohly and Slade, 2005). Yet, until recently, only THC had been formulated in an oral form for medical use. THC and nabilone, a synthetic derivative of THC, have been marketed as appetite stimulants and antiemetics in chronic diseases such as AIDS and cancer; however, therapeutic success of these

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drugs has been hampered by adverse side effects, including reports of negative subjective effects such as dysphoria (see review Ben Amar, 2006). Recent findings with CBD, a cannabinoid without THC-like psychoactivity, have suggested that it may have potential therapeutically useful effects when administered alone (e.g., as an antipsychotic, see Zuardi et al., 2006). In addition, it has been proposed that CBD may complement or attenuate various effects of THC (see Russo and McPartland, 2003).

### 1.1. Preclinical and clinical interactions between THC and CBD

Preclinical research has yielded inconsistent results regarding the influence of CBD on a battery of four pharmacological effects in mice that are characteristic of THC and other THC-like cannabinoids (Martin et al., 1991): suppression of spontaneous activity, antinociception, hypothermia and catalepsy. For example, some investigators report that high doses of CBD increased the antinociceptive, cataleptic, and hypothermic effects of THC in mice (Fernandes et al., 1974; Karniol and Carlini, 1973), while other investigations reported CBD antagonized them (Welburn et al., 1976) (Karniol and Carlini, 1973) (Borgen and Davis, 1974) or had no effect (Sanders et al., 1979) (Jones and Pertwee, 1972b) (Ham and De Jong, 1975). Recently, Varvel et al. (2006) demonstrated that equivalent doses of CBD did not substantially modify the effects of THC on locomotor activity, nociception (tail flick), rectal temperature, and catalepsy.

The putative beneficial effects of combined THC and CBD also have been investigated recently in several clinical trials for multiple sclerosis, neuropathic pain, and varied neurogenic symptoms (Berman et al., 2004; Brady et al., 2004; Rog et al., 2005; Wade et al., 2004; Wade et al., 2003). In addition, Sativex®, a 1:1 THC: CBD ratio oromucosal spray formulation, is currently marketed in Canada for treatment of neuropathic pain associated with multiple sclerosis. Indeed, separate clinical studies have demonstrated that dronabinol (oral THC) (Svendsen et al., 2004), GW-2000-02 (oromucosal spray containing primarily THC), and GW-1000-02 (Sativex) (Berman et al., 2004) were marginally, yet significantly, effective against pain symptoms associated with multiple sclerosis, although each drug produced greater adverse events than placebo treatment. In that study comparisons were only made to placebo treatment. Thus inferences regarding therapeutic differences between administration of THC alone (GW-2000-02) and THC combined with CBD (GW-1000-02) can be made. Nevertheless, anecdotal reports that smoked marijuana is considered more favorably as a medication by some patients than is synthetic oral THC persist. While the conflicting body of scientific literature has not clearly demonstrated that CBD markedly alters the characteristic, but non-selective (Wiley and Martin, 2003), preclinical effects of THC in mice nor that it enhances therapeutic effects of THC in the clinic, modulation of the selective subjective effects of THC by CBD and/or other similar cannabinoids might affect patient perception.

### 1.2. CBD and THC interactions in drug discrimination

THC's discriminative stimulus effects are mediated via CB1 cannabinoid receptors (Wiley et al., 1995) and are pharmacologically selective for cannabinoids that possess THC-like psychoactivity, including plant-derived cannabinoids, classical tricyclic analogs, and other non-structurally related synthetic cannabinoids (Wiley, 1999). Further, these effects serve as an animal model of the subjective effects of marijuana in humans (Balster and Prescott, 1992). In drug discrimination studies, combined administration of THC and CBD has resulted in varied effects including no effect, lack of antagonism, or time course potentiation. For example, co-administration of 17.5 mg/kg CBD and several doses of THC (0.1, 0.3, 0.56 mg/kg), produced no change in THC appropriate responding in pigeons compared to that of THC alone (Hiltunen and Järbe, 1986a). The time course of THC's stimulus effects was unchanged as well. Additionally, CBD failed to substitute for THC in pigeons trained to discriminate THC from vehicle (Järbe et al., 1977). On the contrary, in rats, CBD 30 mg/kg potentiated the time

course effects of low doses of THC (0.3 and 1.0 mg/kg) (Hiltunen and Järbe, 1986a). Metabolic interference is one possible explanation for this prolongation of THC's time course effects by CBD (Bornheim et al., 1993b; Bornheim et al., 1995b; Jones and Pertwee, 1972a). Since all of these results were obtained with doses of CBD that were 30-fold and 100-fold higher than the co-administered THC doses, these effects, or lack of effects, may be associated with high ratios of CBD to THC, only.

### 1.3. Purpose of study

To this end, the first objective of this study was to determine the effects of CBD on THC drug discrimination over a greater range of CBD to THC ratios. Secondly, the effects of CBD alone and in combination with THC were evaluated using the place conditioning paradigm. Place conditioning is a learning paradigm that can be used to investigate associations between preference/aversive properties of psychoactive drugs and contextual cues (see review, Tzschentke, 1998). Together, these experiments will assess CBD's ability to produce marijuana-like discriminative stimulus effects in rats and its effectiveness as a rewarding or aversive stimulus in a place conditioning procedure in mice in THC to CBD ratios similar to those found in marijuana and Sativex. In addition, the ability of CBD to modulate the effects of THC in these mice and rat models will be evaluated.

## 2. Methods

### 2.1. Subjects

Male ICR mice (25–32 g), used in the place conditioning experiments, were purchased from Harlan (Dublin, VA) and were housed in groups of four. All mice were kept in a temperature-controlled (20–22°C) environment with a 14:10-h light:dark cycle and received food and water ad libitum. Male Long Evans rats (Harlan) were used in the drug discrimination experiments. They were individually housed in a temperature-controlled (20–22°C) vivarium with a 12-hour light/dark cycle. During the drug discrimination studies, rats were maintained within the indicated weight range (400–450g) by restricted post-session feeding and had ad libitum water in their home cages. All animals used in this study were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Virginia Commonwealth University and the 'Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy Press, 1996).

### 2.2. Apparatus

Place conditioning chambers (ENV-3013), interface and software were purchased from Med Associates, Inc. (St. Albans, VT). The overall inside dimensions of the conditioning apparatus were 47 cm L × 13 cm W × 18 cm H and consisted of three distinct compartments (separated by manual doors). The center compartment 11 cm long was gray with a smooth PVC floor. The choice compartments each measured 18 cm long. One compartment was all black with a stainless steel grid rod floor consisting of 3 mm rods placed on 8 mm centers. The other compartment was all white with a stainless steel mesh floor. All chambers had hinged clear polycarbonate lids for animal loading. Data were collected by a PC, which was interfaced to infrared photobeam strips that were located within each chamber.

For the drug discrimination studies, rats were trained and tested in standard operant conditioning chambers (BRS/LVE Inc., Laurel, MD or Lafayette Instruments Co., Lafayette, IN) housed in sound-attenuated cubicles. Pellet dispensers delivered 45-mg BIO SERV (Frenchtown, NJ) food pellets to a food cup on the front wall of the chamber between two response levers. Fan motors provided ventilation and masking noise for each chamber. House lights located above the food cup were illuminated during training and testing sessions. A

micro-computer with Logic '1' interface (MED Associates, Georgia, Vermont) and MED-PC software (MED Associates) were used to control schedule contingencies and to record data.

## 2.3. Procedure

### 2.3.1 Place Conditioning

**2.3.1.1 Pre-conditioning:** Prior to conditioning, mice were tested to determine time spent in each compartment. Specifically, following a 5-min acclimation period in the center, gray compartment, doors were lifted and animals allowed access to all three compartments. Time spent in each compartment during a 15-min access period was recorded (baseline measure). Mice with extreme bias for a single side (i.e. greater than 600 s) were removed from these studies (< 10 %). The remaining mice were rank ordered and assigned to treatment groups in a counterbalanced order (i.e. unbiased design).

**2.3.1.2 Conditioning:** Following the pre-conditioning day, conditioning trials commenced. Half of the mice in each group were injected with drug and half were injected with vehicle and immediately confined to one of the choice compartments for 30 min. Pairing of drug or vehicle (non-drug) with each compartment was varied among groups to counter environmental bias. After each conditioning trial, the floors and walls of each chamber were cleaned with a dilute cleaning solution. The following day animals were placed in the opposite condition, receiving either drug or vehicle, and were conditioned in the other compartment for 30 min. This daily alternation continued for a total of 10 days (5 vehicle and 5 drug). Initially, conditioning trials were undertaken with vehicle versus saline, THC (1 and 10 mg/kg), morphine (5 mg/kg) and CBD (1, 3, 10 and 30 mg/kg) to determine the effects of each drug alone. Morphine served as a well-established positive control in the place conditioning task (Blander et al., 1984). Then, interaction studies were conducted to test whether CBD would alter the effects of THC. Specifically, 1 mg/kg THC was administered with 0.1, 1, and 3 mg/kg CBD and 10 mg/kg THC was administered with 1, 10, and 30 mg/kg CBD.

**2.3.1.3 Testing:** On the day following the final conditioning trial, no injections were administered and each mouse was placed in the chamber for 15 min. The post-conditioning test procedure was identical to the pre-conditioning tests. Time spent in each compartment was recorded.

**2.3.2. Drug Discrimination—**As described previously (Vann et al., 2007), rats were trained to press one lever following administration of 3 mg/kg  $\Delta^9$ -THC and to press another lever after injection with vehicle (1:1:18 ratio of emulphor:ethanol:saline), each according to a fixed-ratio 10 (FR-10) schedule of food reinforcement. Completion of 10 consecutive responses on the injection-appropriate lever resulted in delivery of a food reinforcer. Each response on the incorrect lever reset the response requirement on the correct lever. The position of the drug lever was varied among the group of rats. The daily injections for each rat were administered in a double alternation sequence of training drug and vehicle. Rats were injected and returned to their home cages until the start of the experimental session. Training occurred during 15-min sessions conducted five days a week (Monday-Friday) until the rats had met three criteria during eight of ten consecutive sessions: (1) first completed FR-10 on the correct lever; (2) percentage of correct-lever responding  $\geq$  80% for the entire session; and (3) response rate  $\geq$  0.4 responses/sec.

Following successful acquisition of the discrimination, stimulus substitution tests were conducted on Tuesdays and Fridays during 15-min test sessions. Training continued on Mondays, Wednesdays, and Thursdays. During test sessions, responses on either lever delivered reinforcement according to a FR-10 schedule. In order to be tested, rats must have completed the first FR and made at least 80% of all responses on the injection-appropriate lever

on the preceding day's training session. In addition, the rat must have met these same criteria during at least one of the training sessions with the alternate training compound (THC or vehicle) earlier in the week.

Dose-effect determinations with THC and then with CBD were conducted in each rat. Doses of each compound were administered in ascending order. Subsequently, interaction studies were conducted to test whether CBD would alter the discriminative stimulus effects of THC. Specifically, each of three doses of CBD (0.3, 3, and 30 mg/kg) was tested in combination with each of three THC (0.3, 1, and 3 mg/kg) doses. To test the effects of two injections, control points consisting of THC/vehicle injections and vehicle/vehicle injections were performed the week prior to start of the interaction curves.

## 2.4. Drugs

THC and CBD (National Institute on Drug Abuse, Rockville, MD) were suspended in a vehicle of absolute ethanol, Emulphor-620 (Rhône-Poulenc, Inc., Princeton, NJ), and saline in a ratio of 1:1:18. Results from vehicle alone tests in the present study suggest that 5% ETOH is not active in all of these procedures by itself; however, we did not directly assess possible interactions among ethanol and THC and/or CBD. Pre-session injection intervals for each drug were chosen based upon previous research with these drugs in our lab or on values obtained in the literature. For the drug discrimination study, THC and/or CBD, alone and in combination, were injected intraperitoneally (i.p.), 30 min prior to testing. For place conditioning tests, mice were injected i.p. with THC, CBD, or vehicle or combinations of drugs and immediately placed in the appropriate compartment. For mice, all drugs were administered (i.p.) at a volume of 0.1 ml/kg and for rats all drugs were administered at a volume of 1 ml/kg.

## 2.5. Data analysis

For the place conditioning studies, data were expressed as Preference Scores, calculated as time (s) in drug-paired compartment during test - time (s) in drug-paired compartment during baseline. Data for two mice (from different groups) were discarded due to extreme side biases; i.e., preference scores for these mice were over 2 standard deviations from the mean preference score and one mouse remained in a single compartment for almost the entire session. Data were analyzed using one way analyses of variance (ANOVAs), followed by Fisher's PLSD post hoc tests ( $p < 0.05$ ).

For the drug discrimination studies, percentage of responses on the drug lever and response rate (responses/s) were calculated. Full substitution was defined as  $\geq 80\%$  THC-lever responding. Potency ratio and  $ED_{50}$  values were calculated separately for each drug using least squares linear regression analysis, followed by calculation of 95% confidence limits. Repeated measures ANOVAs with Dunnett's post hoc tests ( $\alpha = 0.05$ ) were used to determine differences in drug lever responding during antagonism tests and response rates, both compared to vehicle control. Since rats that responded less than 10 times during a test session did not press either lever a sufficient number of times to earn a reinforcer, their drug lever selection data were excluded from analysis, but their response rate data were included in mean response rate.

## 3. Results

### 3.1. Conditioned Place

Tests of the ability of 1 and 10 mg/kg THC, and 5 mg/kg morphine to produce conditioned place preference (CPP) or conditioned place aversion (CPA) in ICR mice are presented in Figure 1, panel A. A significant difference in mean time spent in the drug paired compartment was observed as a function of treatment, ( $F(3,50)=12.92, p = 0.0001$ ). Post hoc tests revealed that, compared to saline controls, 5 mg/kg morphine resulted in a significant conditioned place

preference (CPP), suggesting that the procedural parameters used in the present study were sufficiently sensitive for detection of place preference of this positive control. In contrast, ICR mice conditioned with 10 mg/kg THC displayed a significant conditioned place aversion (CPA) whereas mice treated with 1 mg/kg THC or with 1 or 10 mg/kg CBD displayed neither significant CPP nor CPA [Figure 1, panel A]. Male C57/B16 mice also showed CPA when conditioned with 10 mg/kg THC without pre-exposure (unpublished data).

In order to assess the ability of CBD to potentiate a non-effective dose of THC, various doses of CBD were co-administered with 1 mg/kg THC to ICR mice during conditioning trials. Results of subsequent tests indicate that none of the various CBD doses altered the Preference Scores of mice treated with 1 mg/kg THC [Figure 1, panel B]. In contrast, CBD (1 and 10 mg/kg) reversed the CPA observed in ICR mice treated with 10 mg/kg THC ( $F(4,32)=2.74$ ,  $p=0.046$ ) [Figure 1, panel C]. A higher dose of CBD (30 mg/kg) did not affect the CPA produced by 10 mg/kg THC (i.e., was the only condition that was still significantly decreased compared to vehicle). These results indicate that, at certain ratios to THC, CBD reverses THC-induced CPA.

### 3.2. Drug Discrimination

THC fully and dose-dependently substituted for THC with an  $ED_{50} = 0.9$  mg/kg (95% CL: 0.6 – 1.5), whereas CBD alone did not produce THC-lever responding at any dose (Figure 2, top panel). Compared with responding following VEH injections, response rates were significantly decreased by 10 mg/kg THC ( $F(4,24)=10.04$ ,  $p < 0.05$ ). No significant changes in response rates were observed following CBD administration (Figure 2, bottom panel).

Figure 3 shows the results of combination tests with the various THC and CBD doses in drug discrimination. The CBD-THC combination did not alter drug-lever responding (compared to THC alone) at any of the dose combinations tested (Figure 3, left column). In addition, CBD did not significantly alter  $ED_{50}$  values of the THC dose effect curve when co-administered at doses of 0.3, 3, or 30 mg/kg (Table 1). Hence, THC substitution patterns were not altered by any dose of CBD. No significant changes in response rates were observed following CBD and THC co-administration.

## 4. Discussion

The purpose of this study was to investigate the degree to which CBD altered the subjective effects of THC related to its abuse liability. As in numerous previous studies [for a review, see (Wiley, 1999)], THC served as an effective discriminative stimulus here, producing dose-dependent substitution for the training dose. CBD, however, failed to substitute for THC (Hiltunen and Jarbe, 1986a; Jarbe et al., 1977; Jarbe et al., 1986), confirming that CBD does not exhibit THC-like discriminative stimulus effects. These results are consistent with the finding that CBD has minimal affinity for CB1 receptors (Pertwee, 1997a). When co-administered with THC at equivalent doses (Levy and McCallum, 1975) or at CBD:THC dose ratios similar to those contained in Sativex and found in marijuana (present study), CBD also did not alter THC's discriminative stimulus effects. These results suggest that the subjective effects of THC in marijuana are not affected by CBD; however, they also suggest that CBD:THC combination medications would produce subjective effects similar to those of THC alone.

### 4.1. Place conditioning effects of THC and CBD

While the THC drug discrimination represents an excellent model of the subjective intoxication produced by marijuana (Balster and Prescott, 1992), THC has other types of stimulus effects that may not be assessed adequately in this procedure. Specifically, THC may produce

rewarding or aversive effects. These effects, which may be associated with contextual cues, are better modeled in animals by place conditioning procedures (see review, Tzschentke, 1998). Previous reports have found that lower doses of THC are not effective as conditioning stimuli in place conditioning procedures (Mallet and Beninger, 1998; Parker and Gillies, 1995; Robinson et al., 2003). In contrast, higher doses of cannabinoids such as CP 55,940 (McGregor et al., 1996), WIN 55212-2 (Chaperon et al., 1998), THC (Sanudo-Pena et al., 1997); (Cheer et al., 2000); (Hutcheson et al., 1998); (Parker and Gillies, 1995), and HU210 (Cheer et al., 2000) produced aversion in place conditioning procedures in both rats and mice. Further, the aversive effects produced by THC were blocked by the cannabinoid receptor antagonist, SR141716A (Chaperon et al., 1998), suggesting that these effects were CB1 receptor-mediated. Taken together, across strains and species, most studies have reported that cannabinoids produce conditioned place aversion rather than conditioned place preference. On the other hand, THC-induced conditioned place preference has been observed under discrete conditions. For example, Lepore et al (1995) demonstrated conditioned place preference for a low dose of THC through use of a procedural modification ( i.e., THC pretreatment prior to conditioning) intended to eliminate THC's initial aversive effects. In the same study, however, these effects were observed at high doses of THC, despite this procedural modification. Additionally, at low doses, THC-induced place preference was found when initial aversive effects were reduced with pretreatment of THC prior to the first conditioning session (see Valjent and Maldonado, 2000; Valjent et al., 2002). High doses of THC continued to produce aversion (Soria et al., 2004). Overall, then, these studies demonstrate that high doses of THC reliably produce aversion in both rats and mice whereas low doses of THC may induce preference under conditions in which initial aversive effects are reduced. Interestingly, clinical reports suggest that HIV+ patients who had previously smoked marijuana were less likely to report feelings of dysphoria associated with oral marinol than were drug naïve patients, although oral marinol was effective at enhancing appetite in both groups (Haney, 2002).

The results of the present study are consistent with those of the clinical and preclinical studies described above. That is, a lower dose of THC (1 mg/kg) produced no effect whereas a higher dose (10 mg/kg) produced conditioned place aversion in mice not previously exposed to cannabinoids. Alone, CBD, a cannabinoid void of THC-like psychoactivity, produced no preference or aversion at any of the four doses tested. These results are consistent with a previous report in which 5 mg/kg CBD alone failed to alter preference in a place conditioning procedure in rats (Parker et al., 2004). However, in that study both CBD and THC potentiated cocaine and amphetamine induced extinction of place preference learning. The present study extends previous findings by demonstrating that CBD, when administered concomitantly with THC in CBD:THC ratios of 1:10 and 10:10, inhibited the aversive properties of 10 mg/kg THC. Parker and Gillies (1995) suggested that THC-induced place aversion may be related to its anxiogenic effects. If CBD produces anxiolytic effects as has been suggested (Parker and Gillies, 1995), then the present study may provide support for such a behavioral mechanism. In contrast, a 3-fold larger dose (30 mg/kg) of CBD failed to reverse the aversion produced by 10 mg/kg THC. This latter effect may be related to interference with the metabolism of THC by larger doses of CBD (Bornheim et al., 1993b; Bornheim et al., 1995b; Jones and Pertwee, 1972a).

#### 4.2. Putative mechanisms for THC and CBD interactions

The neural mechanism by which CBD exerts its modulatory effects on THC-induced CPA is unclear. CBD has minimal affinity for CB1 receptors (Pertwee, 1997b), although it appears to interact with the endocannabinoid system via fatty acid amide hydrolase (Watanabe et al., 1996) or through the putative anandamide membrane transporter (Bisogno et al., 2001). CBD also has been found to have agonist activity at TRPV1 (Bisogno et al., 2001) and at 5-HT1A receptors (Russo et al., 2005). In addition, prior research has suggested that metabolic factors

may account for interactions between CBD and THC, as inhibition of the metabolism of THC via interactions with P450 enzymes has been observed following large doses of CBD (Bornheim et al., 1993a; Bornheim et al., 1995a). In support of this latter hypothesis, concomitant administrations of THC and CBD in much higher ratios (30:1 and 100:1 CBD:THC) than those tested in the present study resulted in prolongation of THC's discriminative stimulus effects in rats (Hiltunen and Jarbe, 1986b).

### 4.3. Conclusions

In conclusion, the present results show that CBD alters some, but not all, of the stimulus properties of THC. As shown in previous studies, CBD did not induce THC-like discriminative stimulus effects when administered alone nor did it alter THC's discriminative stimulus effects when it was co-administered with THC at several CBD:THC ratios. In contrast, CBD (1 and 10 mg/kg) attenuated the aversive effects of 10 mg/kg THC in a place conditioning procedure without producing either aversion or preference on its own. These results suggest that CBD does not alter the subjective effects of THC in marijuana, but may attenuate its initial aversive effects. They also suggest that CBD:THC combination medications would produce subjective effects similar to those of THC alone if the dose of THC were sufficient. Further, the finding that certain CBD:THC combinations produce less aversion than THC alone provides a possible explanation of anecdotal reports which suggests that some patients prefer marijuana (vs. oral THC) for symptom alleviation. At a higher CBD:THC ratios (3:1), however, CBD failed to attenuate THC-induced aversive effects, suggesting that the ameliorative effect of CBD may be biphasic. Overall, the findings of this study suggest that combining lower doses of CBD with THC may alleviate the initial aversive effects associated with initial administration of THC, but will probably not alter THC's intoxicating properties.

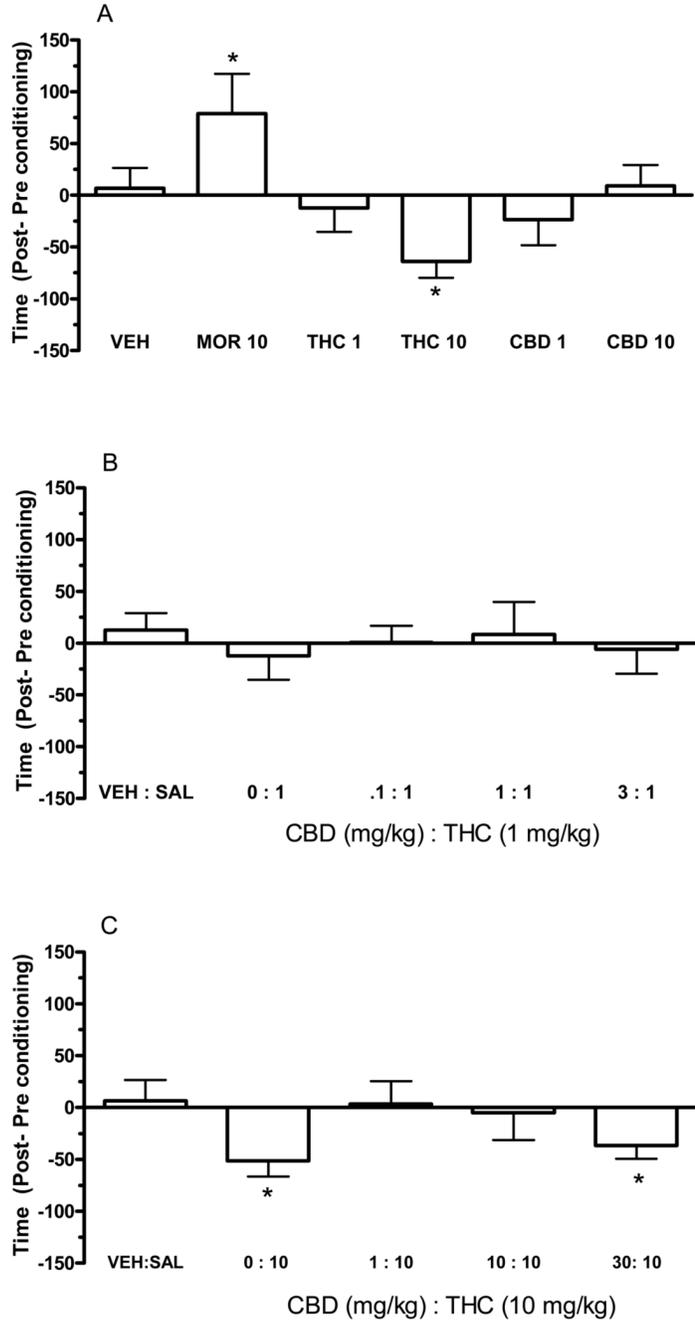
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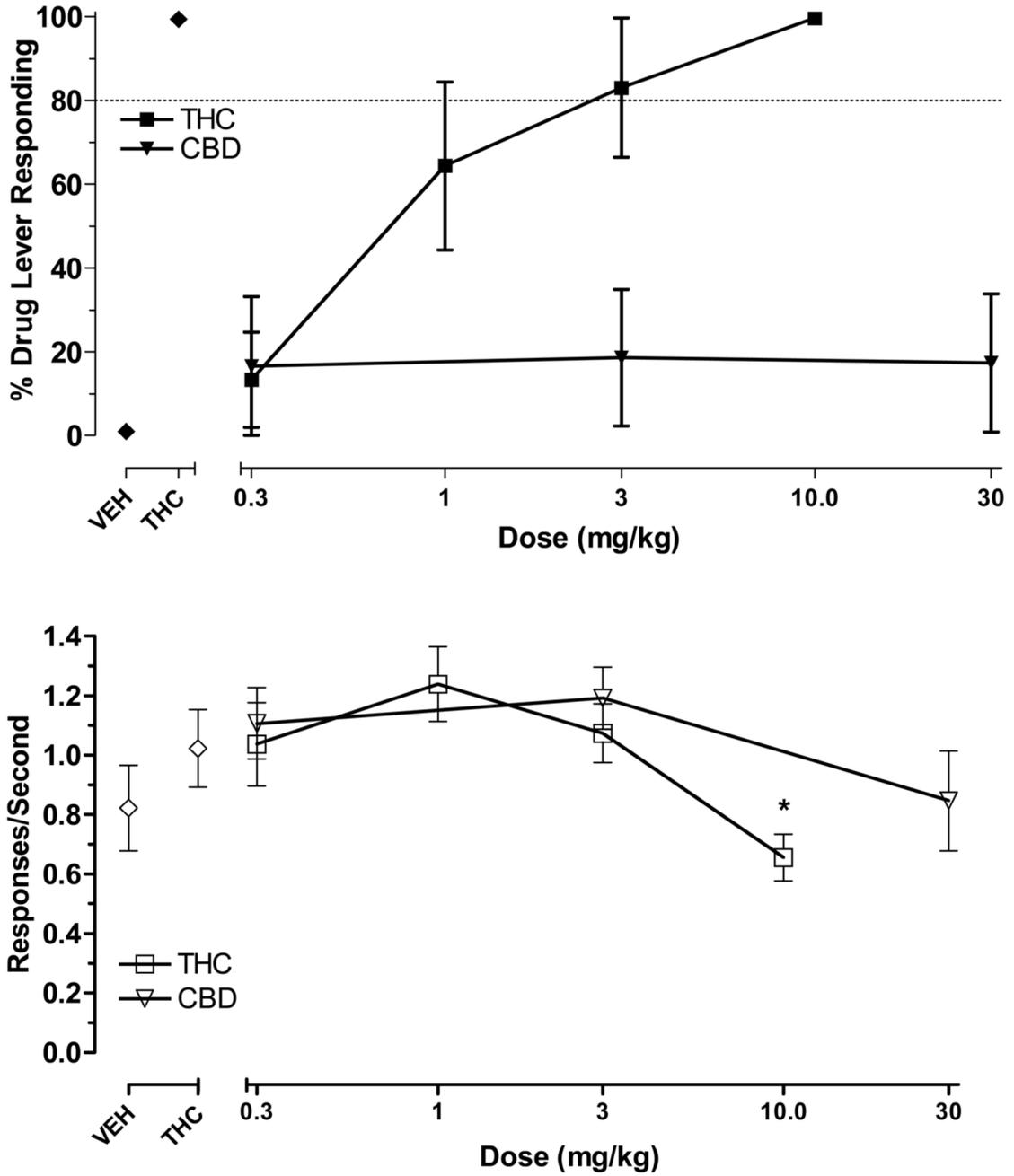
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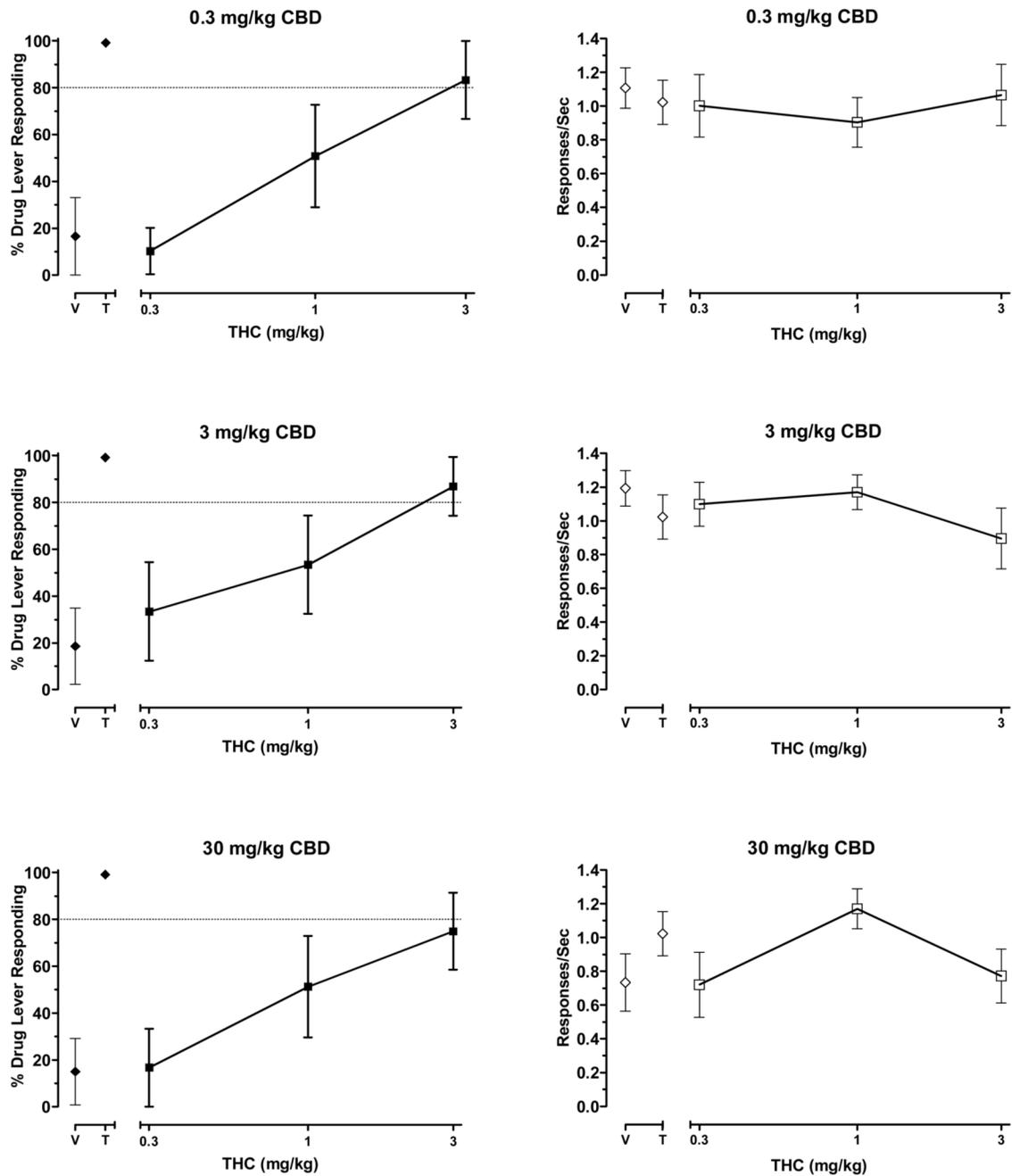
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**Figure 1.** Panel A, The ability of VEH, 1, and 10 mg/kg THC, 1 and 10 mg/kg CBD, and 5 mg/kg MOR to produce place conditioning effects. Panel B, Effects of various doses of CBD upon a dose of THC (1 mg/kg) incapable of producing CPP or CPA when administered alone. Panel C, Effects of various doses of CBD upon a dose of THC (10 mg/kg) that produces CPA when administered alone. Values represent the mean ( $\pm$ SEM) of 9–12 mice per group and are expressed as the difference between postconditioning and preconditioning time spent in the drug-paired compartment.



**Figure 2.** Effects of CBD and THC on percentage of THC-lever responding (upper panel) and response rates (lower panel) in rats trained to discriminate 3 mg/kg THC from vehicle. Points above VEH and THC represent the results of control tests with vehicle and 3 mg/kg THC conducted before each dose-effect determination. For each dose-effect curve determination, values represent the mean ( $\pm$ SEM) of 6–7 rats.



**Figure 3.** Effects of various doses of CBD and on percentage of THC-lever responding (left panels) and response rates (right panels) in rats trained to discriminate 3 mg/kg THC from vehicle. Points above V and T represent the results of control tests with vehicle and 3 mg/kg THC conducted before each dose-effect determination. For each dose-effect curve determination, values represent the mean (±SEM) of 6-7 rats.

**Table 1**

ED<sub>50</sub>s and potency ratios for THC and combinations of THC and CBD in rats trained to discriminate 3 mg/kg THC from vehicle

THC Dose effect &	VEH	0.3 mg/kg CBD	3 mg/kg CBD	30 mg/kg CBD
ED <sub>50</sub> mg/kg (CL)	0.8 (0.6 – 1.2)	1.1 (0.6 – 2.1)	0.7 (0.4 – 1.3)	1.1 (0.53 – 2.2)
Potency Ratio CBD : THC (CL)	ND	0.8 (0.4 – 1.4)	1.1 (0.4 – 2.8)	0.8 (0.3 – 1.7)

ED<sub>50</sub> values are derived from the THC dose effect (0.3, 1, 3 mg/kg) plus the respective dose of CBD (column headings). Potency ratios (and 95% confidence limits [CL]) for each drug were calculated with respect to the ED<sub>50</sub> for THC (VEH column).

\* indicates that potency for the measure is significantly different than potency for producing THC discriminative stimulus when administered with vehicle. ( $p < 0.05$ ). ND=not determined.