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ORIGINAL INVESTIGATION

Effect of prior foot shock stress and Δ^9-tetrahydrocannabinol, cannabidiolic acid, and cannabidiol on anxiety-like responding in the light-dark emergence test in rats

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Abstract
Rationale Cannabis is commonly used by humans to relieve stress.
Objectives and methods Here, we evaluate the potential of intraperitoneally (i.p.) administered Δ^9-tetrahydrocannabiol (THC) and cannabidiolic acid (CBDA, the precursor of cannabidiol [CBD]) to produce dose-dependent effects on anxiety-like responding in the light-dark (LD) emergence test of anxiety-like responding in rats, when administered acutely or chronically (21 days). As well, we evaluate the potential of THC, CBDA, and CBD to reduce anxiogenic responding produced by foot shock (FS) stress 24 h prior to the LD test.
Results In the absence of the explicit FS stressor, THC (1 and 10 mg/kg) produced anxiogenic-like responding when administered acutely or chronically, but CBDA produced neither anxiogenic- nor anxiolytic-like responding. Administration of FS stress 24 h prior to the LD test enhanced anxiogenic-like responding (reduced time spent and increased latency to enter the light compartment) in rats pretreated with either vehicle (VEH) or THC (1 mg/kg); however, administration of CBDA (0.1–100 μg/kg) or CBD (5 mg/kg) prevented the FS-induced anxiogenic-like responding (an anxiolytic-like effect). The 5-hydroxytryptamine 1A (5-HT_{1A}) receptor antagonist, WAY100635, reversed CBDA's anxiolytic effect (1 μg/kg). Combining an anxiolytic dose of CBDA (1 μg/kg) or CBD (5 mg/kg) with an anxiogenic dose of THC (1 mg/kg) did not modify THC's anxiogenic effect.
Conclusion These results suggest the anxiolytic effects of CBDA and CBD may require the presence of a specific stressor.

Keywords Anxiety · Anxiolytic · Δ^9-tetrahydrocannabiol · Cannabidiolic acid · Cannabidiol · Foot shock · Stress · Light-dark emergence test · Rat

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Introduction

Cannabis is commonly used for relaxation, yet reasons reported for cessation of cannabis use are often increased anxiety and panic reactions (Thomas 1993; Reilly et al. 1998; Schofield et al. 2006). Cannabis contains not only the psychoactive cannabinoid Δ^9-tetrahydrocannabinol (THC), but also over 100 other cannabinoids that are not intoxicating. Among these non-intoxicating cannabinoids is cannabidiol (CBD). Both THC and CBD have been found to have a dose-dependent effect on anxiety-like responding in preclinical studies with rats and mice (see Patel et al. 2014). Preclinical tests for anxiety-like responding in rats generally show that low doses of THC (and other agonists of cannabinoid 1 [CB_1] receptors) are anxiolytic (e.g., Berrendero and Maldonado 2002; Rubino et al. 2007), but high doses (greater than 1 mg/kg, intraperitoneal [i.p.]) tend to be anxiogenic (Onaivi et al. 1990; Valjent et al. 2002; Schramm-Sapyta et al. 2007; Klein et al. 2011). However, higher anxiogenic doses of THC also tend to reduce general activity levels of rats (Rock et al. 2015) which may interact with tests of anxiety-like behavior.

In contrast, CBD is generally shown to be anxiolytic within a dose range of 1–10 mg/kg without modifying general activity levels (Espejo-Porras et al. 2013), but may produce no effect at higher or lower doses (Onaivi et al. 1990; Guimarães et al. 1994; Moreira et al. 2006, but see O'Brien

et al. 2013; Todd and Arnold 2016). Recent findings also suggest that CBD can attenuate the acute autonomic response to stress and its subsequent elevation of anxiety-like responding in rats (Resstel et al. 2009). The anxiolytic effects of THC are mediated by its action on the CB_1 receptor (e.g., Onaivi et al. 1990), while the anxiolytic effects of CBD appear to be mediated by its action on the serotonin 1A ($5\text{-}HT_{1A}$) receptor (Guimarães et al. 1990; Russo et al. 2005).

The acidic precursor of CBD, cannabidiolic acid (CBDA; Mechoulam and Gaoni 1965), which is converted to CBD upon the application of heat, is 100 times more potent than CBD in displacing the $5\text{-}HT_{1A}$ receptor agonist, 8-hydroxy-2-(dipropylamino)tetralin hydrobromide (8-0H-DPAT), in an in vitro model of rat brainstem activation (Bolognini et al. 2013). In vivo testing revealed that CBDA is also 100–1000 times more potent than CBD in reducing acute and anticipatory nausea in rat gaping models and vomiting in a shrew model (Bolognini et al. 2013; Rock and Parker 2013). It is interesting to note that these potent effects of CBDA are seen even though no dose of CBDA tested (0.1–1 mg/kg) produced changes in locomotor activity in a 15-min test in a novel environment (Rock et al. 2015). Unlike CBD, however, there is little work on CBDA's potential anti-anxiety effects. Brierley et al. (2016) reported that acute administration of CBDA (5 mg/kg, po) had a moderate anxiolytic effect in the novelty feeding suppression test of anxiety-like behavior and number of seconds spent in the center of an open field, but consistent with our reports had no effect on general motor activity across a range of doses from 0.5 to 5 mg/kg, po.

Here, we evaluate the dose-dependent effects of both acute and chronic (21 days) i.p. exposure to THC (0.1–10 mg/kg) or CBDA (0.1–100 μg/kg) on anxiety-like responding in the LD emergence test in Sprague-Dawley rats. The LD emergence test of anxiety-like responding provides a less stressful baseline of responding than paradigms that employ foot shock (FS) and therefore may be more sensitive to both increases and decreases in anxiety-like behavior (Holmes 2001; Bourin and Hascoët 2003). This test relies upon the natural tendency of rodents to prefer dark compartments, but also to simultaneously explore novel environments. Following placement in the dark compartment (Rodgers et al. 1999; Long et al. 2010; Klein et al. 2011), the latency to enter the light compartment and the amount of time spent in the light compartment are measures of anxiety-like responding. Subsequent experiments evaluated the anxiolytic-like responding produced by THC, CBDA, and CBD in the LD emergence test in rats tested 24 h following foot shock (FS) stress, a procedure that has been shown to enhance anxiety-like responding in this test in mice (Bluett et al. 2014). The potential of the $5\text{-}HT_{1A}$ antagonist, WAY100635, to reverse the anxiolytic effect of CBDA (1 μg/kg, i.p.) evaluated the mechanism of the effect. The final experiments evaluated the potential of combined treatment with low doses of THC and CBDA to reduce anxiety-like responding in the LD emergence test in rats.

Materials and methods

Animals

All animal procedures complied with the Canadian Council on Animal Care and were approved by the Institutional Animal Care Committee (accredited by the Canadian Council on Animal Care). Naïve male Sprague-Dawley rats obtained from Charles River Laboratories (St Constant, Quebec) arrived at the facility at 6–8 weeks of age. Rats weighing between 288 and 398 g on the day of the initial LD test were used for assessment of anxiety-like behavior. The rats were pair-housed in opaque plastic shoebox cages (48 × 26 × 20 cm) containing a bed-o-cob bedding from Harlan Laboratories, Inc. (Mississauga, Ontario), a brown paper towel, and a Crink-l'Nest™ (The Andersons, Inc., Maumee, OH). Additionally, the rats were provided with a soft white paper container that was 14 cm long and 12 cm in diameter. The colony room was kept at an ambient temperature of 21 °C with a 12/12-h light-dark schedule (lights off at 8 a.m.). The rats were tested in their dark cycle and were maintained on food (Highland Rat Chow [8640]) and water ad libitum.

Drugs

All drugs were administered intraperitoneally (i.p.) at a volume of 1 ml/kg. THC and CBDA (kindly provided by Prairie Plant Systems Inc.) and CBD (provided by Dr. Raphael Mechoulam) were dissolved in ethanol. The drugs were prepared in a graduated cylinder to ensure the appropriate final concentration of drug (vehicle (VEH) in a ratio of 1:9; Tween80:saline [SAL]) following evaporation of the ethanol. The ethanol/drug solution was measured into the graduated cylinder, the Tween80 was added and the mixture vortexed. The ethanol was evaporated using a nitrogen stream (complete evaporation determined by volume of Tween80 left in the cylinder) after which saline was added. For combined doses of CBDA or CBD and THC, the drugs were mixed in a cocktail in VEH.

Apparatus

The LD emergence apparatus consisted of an opaque white plastic rectangular box that was divided into two compartments: a small (25 cm wide × 20.5 cm long × 20.5 cm high) enclosed dark box built of opaque black plastic with a door

(8 cm wide × 10 cm high) leading to a larger (39.5 cm long × 25 cm wide) open lit box. The open lit box was illuminated by one lamp (with a 60-W bulb, 180 lx in the light chamber) positioned 115 cm above the center of the lit box. A video camera was mounted over the top of the light-dark box and the videotapes were analyzed by Ethovision software (Noldus Information Technology, Leesburg, VA, USA) for the duration of time spent in the light box and the latency to emerge from the dark box into the light box for the 5-min test.

For the FS session, the rats were placed in sound attenuating MED Associates fear conditioning chambers (St. Albans, VT, USA). The 6-min FS session consisted of six 0.8 mA foot shocks delivered 1 min apart. Each 0.5-s shock was preceded by a 30-s auditory tone (90 dB, 5000 Hz) as described by Bluett et al. (2014).

Procedures

All rats were acclimatized to the facility for 13 days prior to experimental manipulations, with weighing and handling occurring for eight of these days.

Experiment 1: effect of acute and chronic exposure to THC or CBDA on anxiety-like responding in the LD emergence test After acclimatizing, for experiment 1, the rats were pretreated with VEH, THC (0.1–10 mg/kg, i.p.; experiment 1a), or CBDA (0.1–100 μg/kg, i.p. [Bolognini et al. 2013; Rock and Parker 2013]; experiment 1b) 45 min prior to the first LD test (day 1). For this test, rats were placed in the corner of the dark chamber, facing away from the opening between the light and dark chambers and the movement of the rat was tracked during the 5-min test. After the test, the rats were returned to their home cage. Following this first LD emergence test, the rats were weighed daily and then injected with their respective treatment for 20 additional days. On Day 21, the rats were again pretreated with VEH, THC (experiment 1a), or CBDA (experiment 1b) 45 min prior to returning to the LD emergence test. The groups were as follows for experiment 1a (THC): VEH (n = 13), 0.1 mg/kg (n = 8), 1 mg/kg (n = 6), and 10 mg/kg (n = 6). The groups were as follows for experiment 1b (CBDA): VEH (n = 11), 0.1 μg/kg (n = 7), 1 μg/kg (n = 10), 10 μg/kg (n = 6), and 100 μg/kg (n = 6).

Experiment 2: effect of acute exposure to THC, CBDA, or CBD following foot shock stress on anxiety-like responding in the LD emergence test For experiment 2, following acclimatizing, the rats received a single FS stress session (lasting 6 min), or no FS stress session (denoted no FS) occurring 24 h before the LD emergence test (Bluett et al. 2014). On the LD emergence test day, the rats were pretreated with THC (VEH or 0.1 or 1 mg/kg, i.p.; experiment 2a), CBDA (VEH, 0.1, 1, 100 μg/kg, i.p.; experiment 2b), or CBD (VEH, 5 mg/kg, i.p. [Guimarães et al. 1990]; experiment 2c). Forty-five minutes later, they were placed in the dark chamber of the LD box, and tested as in experiment 1. The groups were as follows for experiment 2a: no FS-VEH (n = 9), FS-VEH (n = 12), no FS-1 THC (n = 6), and FS-1 THC (n = 6). The groups were as follows for experiment 2b: no FS-VEH (n = 9), FS-VEH (n = 12), no FS 0.1 CBDA (n = 7), FS-0.1 CBDA (n = 8), no FS-1 CBDA (n = 7), FS-1 CBDA (n = 7), no FS-100 CBDA (n = 8), and FS-100 CBDA (n = 7). The groups were as follows for experiment 2c: no FS-VEH (n = 9), FS-VEH (n = 15), no FS-5 CBD (n = 7), and FS-5 CBD (n = 6).

Experiment 3: the potential of a 5-HT_{1A} receptor antagonist, WAY100635 (0.1 mg/kg, i.p. [Bolognini et al. 2013]), to reverse the anxiolytic effect of CBDA was evaluated The rats were treated the same as experiment 2b, except that they were also pretreated with the 5-HT_{1A} receptor antagonist, WAY100635 (0.1 mg/kg) or VEH, 15 min prior to VEH or CBDA (1 μg/kg). The groups included the following: no FS-VEH-VEH (n = 8), FS-VEH-VEH (n = 8), no FS-VEH-CBDA (n = 6), FS-VEH-CBDA (n = 6), no FS-WAY-VEH (n = 8), FS-WAY-VEH (n = 8), no FS-WAY-CBDA (n = 8), and FS-WAY-CBDA (n = 8).

Experiment 4: potential of CBDA and CBD to reverse the anxiogenic effect of THC Since groups injected with 1 mg/kg THC displayed anxiogenic-like responding that was potentiated by FS stress, experiment 4 evaluated the potential of an anxiolytic dose of CBDA (1 μg/kg, which produced an equivalent anxiolytic-like effect as that produced by a higher dose in experiment 2b) or CBD (5 mg/kg) to reduce the anxiety-like responding produced by THC (1 mg/kg) under conditions of FS stress and no FS stress. The groups were as follows: no FS-VEH (n = 9), FS-VEH (n = 12), no FS-1 THC (n = 6), FS-1 THC (n = 6), no FS-1 THC + 1 CBDA (n = 8), FS 1 THC + 1 CBDA (n = 9), no FS-1 THC + 5 CBD (n = 8), and FS 1 THC + 5 CBD (n = 8).

Experiment 5: potential of combined low doses of CBDA and THC to modify stress-induced anxiogenic responding Experiment 5 evaluated the potential of low doses of both CBDA (0.1 μg/kg) and THC (0.1 mg/kg) combined to enhance the anxiolytic responding following FS stress and no FS stress. The groups were as follows: no FS-VEH (n = 9), FS-VEH (n = 12), no FS-0.1 THC (n = 7), FS-0.1 THC (n = 8), no FS 0.1 CBDA (n = 7), FS 0.1 CBDA (n = 8), no FS-0.1 THC + 0.1 CBDA (n = 8), and FS-0.1 THC + 0.1 CBDA (n = 9).

Statistical analysis

In each experiment, the amount of time spent in the light box and the latency to enter the light box during the LD emergence

test were entered into a factorial analysis of variance (ANOVA) as appropriate. For all statistical analyses, significance was defined as $p < 0.05$.

Results

Experiment 1: effect of acute and chronic administration of THC or CBDA on anxiety-like behavior

Experiment 1a: THC At a dose of 1 or 10 mg/kg, THC decreased the amount of time spent in the light box on days 1 and 21, suggesting an anxiogenic-like effect. As well, at a dose of 10 mg/kg only, THC increased the latency to enter the light box, but only on day 1. The mean number of seconds spent in the light box by the various groups is presented in section a of Fig. 1. The ANOVA for the time spent in the light box revealed significant main effects of pretreatment dose, $F(3, 29) = 9.9$, $p < 0.001$, and trial, $F(1, 29) = 19.0$, $p < 0.001$, but no dose x trial interaction. Subsequent single factor ANOVAs between pretreatment groups on each of day 1 and day 21 revealed a significant dose effect ($p < 0.05$); least significant differences (LSD) pairwise comparison tests revealed that those rats pretreated with 1 or 10 mg/kg THC spent less time in the light compartment compared to VEH controls on both day 1 and day 21 ($p < 0.05$).

The rats pretreated with THC (10 mg/kg) also took significantly longer to enter the light box on day 1 only, suggesting an anxiogenic-like effect as seen in section b of Fig. 1. The ANOVA for latency to enter the light compartment revealed a significant main effect of trial, $F(1, 29) = 13.1$, $p = 0.001$, a significant main effect of dose, $F(3, 29) = 7.9$, $p = 0.001$, and a significant trial x dose interaction, $F(3, 29) = 11.9$, $p < 0.001$. Subsequent single factor ANOVAs revealed a significant effect for day 1 only, $F(3, 29) = 12.1$, $p < 0.001$. The LSD post hoc comparison tests revealed that those rats pretreated with 10 mg/kg THC took longer to enter the light box compared to all other pretreatment groups on day 1 only ($p < 0.001$).

Experiment 1b: CBDA In experiment 1b, CBDA did not produce anxiety-like behavior in the LD emergence test at any dose whether administered acutely or chronically. The section c of Fig. 1 presents the mean number of seconds spent in the light box on day 1 and on day 21 by each of the pretreatment groups. The ANOVA for time spent in

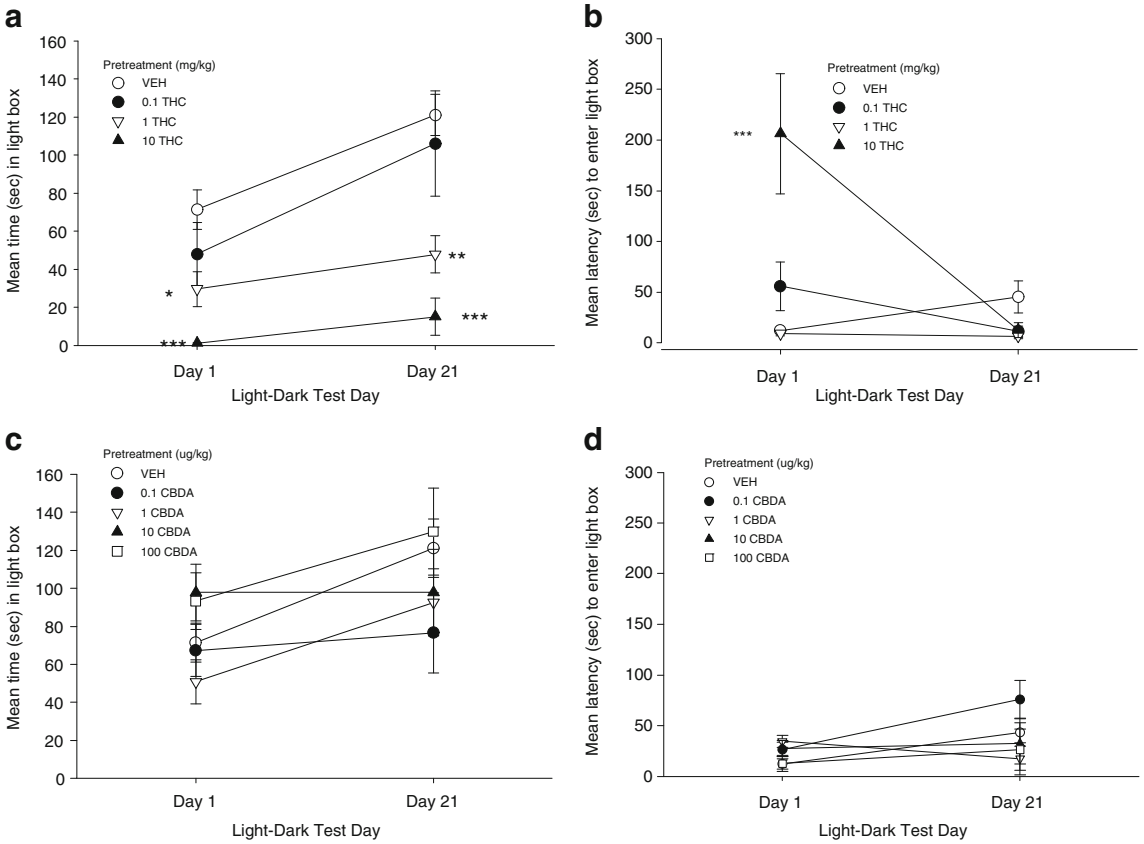

Fig. 1 The mean (± sem) number of seconds spent in the light box and the mean (± sem) latency (sec) to enter the light box on day 1 and day 21 in experiment 1 following THC (sections **a** and **b**: VEH, 0.1, 1, and 10 mg/kg, i.p.) or CBDA (sections **c** and **d**: VEH 0.1, 1, 10, 100 μg/kg, i.p.) in experiment 1. The *asterisks* indicate a significant difference from those rats treated with VEH (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

the light compartment revealed only a significant main effect of trial, F (1, 37) = 13.1, p = 0.001; rats spent more time in the light box on day 21 than on day 1. CBDA also did not modify the latency to enter the light compartment, presented in section d of Fig. 1; the ANOVA revealed no significant effects.

Experiment 2: effect of THC, CBDA, or CBD on anxiety-like behavior when preceded by a FS stressor

Experiment 2a: THC The FS stress dramatically enhanced the anxiety-like responding in both measures of time spent in the light box and latency to enter the light box among the VEH-treated rats and rats treated with 1 mg/kg THC. The section a of Fig. 2 presents the mean (± sem) number of seconds that the rats spent in the light box. The ANOVA of time spent in the light box revealed a significant main effect of FS stress, F (1, 29) = 49.6, $p < 0.001$; pretreatment dose, F (1, 29) = 21.1, $p < 0.01$; and a FS stress x pretreatment dose interaction, F (1, 29) = 11.2, $p < 0.01$. The subsequent LSD pairwise comparison tests revealed that the groups pretreated with both VEH and 1 mg/kg THC spent less time in the light box following FS in comparison to the no FS groups ($p < 0.01$). As well, among the no FS groups, the rats

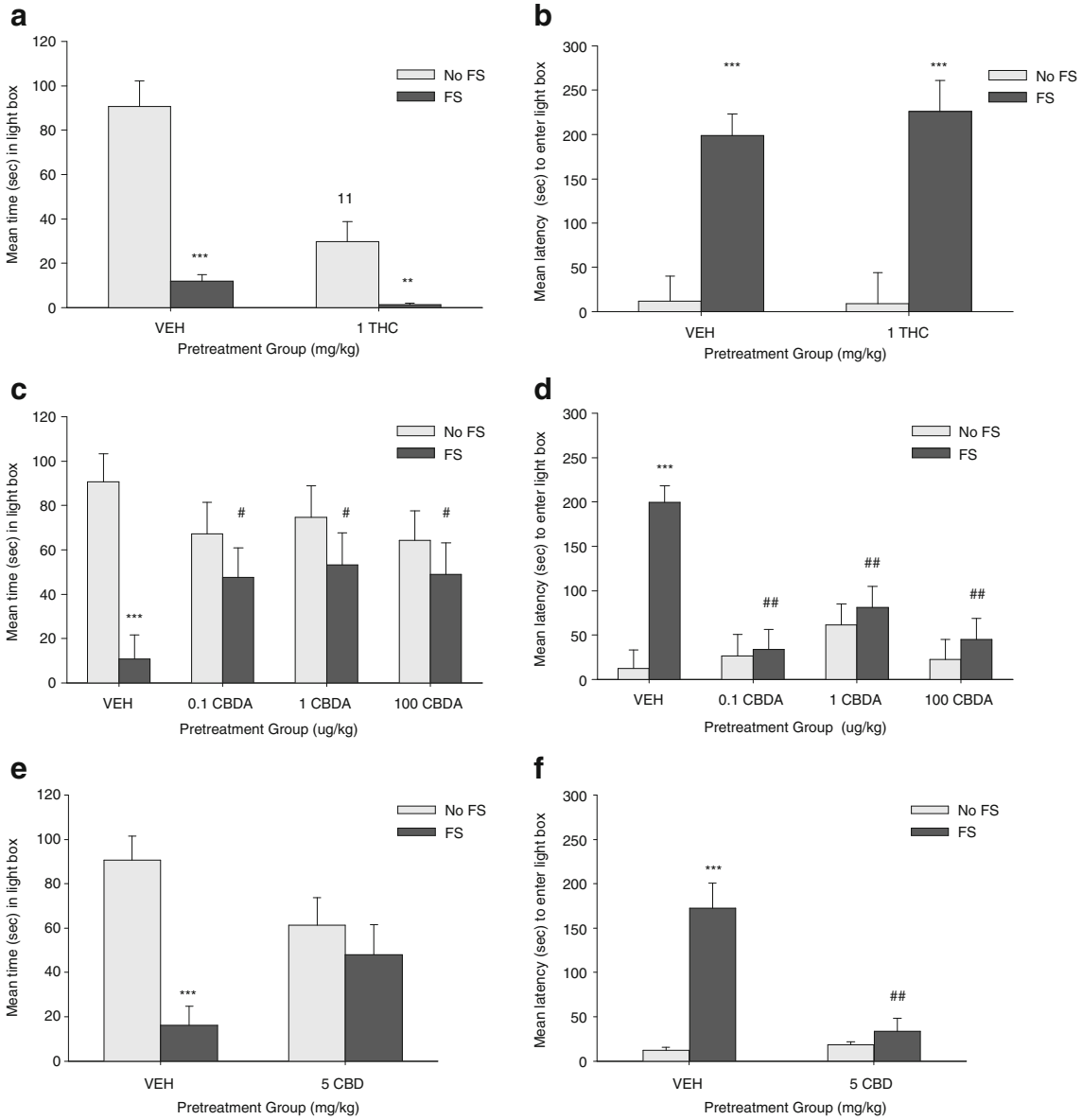

Fig. 2 The mean (± sem) number of seconds spent in the light box and the mean (± sem) latency (sec) to enter the light box 24 h following exposure to no FS (*gray bars*) or FS (*black bars*) by rats injected i.p. with THC (**a** and **b**: VEH or 1 mg/kg), CBDA (**c** and **d**: VEH, 0.1, 1, and 100 μg/kg), or CBD (**e** and **f**: VEH or 5 mg/kg, i.p.) in experiment 2. The *asterisks* indicate a significant difference between FS and no FS (**$p < 0.01$, ***$p < 0.001$) for each group. The *11* represents a significant difference from VEH in the no FS group in section **a** ($^{11}p < 0.01$) and the *number sign* indicates a significant difference from VEH in the FS group in sections **c**, **d**, and **f** ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$)

pretreated with 1 mg/kg THC also spent less time in the light box than those of group VEH ($p < 0.01$), replicating the effect found in experiment 1a.

The section b of Fig. 2 presents the mean number of seconds to enter the light box. Among the groups pretreated with VEH or 1 mg/kg THC, the FS stress significantly enhanced the latency to enter the light box. The ANOVA for the latency measure revealed significant effects of FS stress, F (1, 29) = 58.5, $p < 0.001$; both VEH- and THC-treated rats showed enhanced latency to enter the light box following FS stress.

Experiment 2b: CBDA In VEH-treated animals, the prior stress (FS) produced anxiogenic-like behavior, but administration of CBDA abolished this effect as seen in section c of Fig. 2. The ANOVA for time spent in the light compartment revealed a significant main effect of FS stress, F (1, 57) = 12.7, $p < 0.001$ and a FS stress x pretreatment dose interaction, F (3, 57) = 3.1, $p = 0.035$. By subsequent LSD pairwise comparison tests for each pretreatment group, the FS stress potentiated anxiogenic-like responding in the VEH group only ($p < 0.001$). As well, among the FS-stressed rats, group VEH spent less time in the light box than any CBDA-treated group ($p < 0.025$).

The FS stress enhanced the latency to enter the light box in the VEH-treated group, but not in the CBDA-treated groups as seen in section d of Fig. 2. The ANOVA for latency to enter the light compartment revealed a significant main effect of FS stress, F (1, 57) = 13.5, $p < 0.001$, pretreatment dose, F (3, 57) = 5.5, $p < 0.01$, and a significant FS stress x pretreatment dose interaction, F (3, 57) = 8.5, $p < 0.001$; subsequent LSD pairwise comparison tests revealed that only the VEH group showed an enhanced latency to enter the light box following FS stress ($p < 0.001$). As well, among the FS-stressed groups, group VEH displayed a longer latency to enter the light box than any CBDA-pretreated group ($p < 0.01$).

Experiment 2c: CBD In VEH-treated animals, the prior stress (FS) produced anxiogenic-like behavior, but administration of CBD abolished this effect as seen in section e of Fig. 2. The ANOVA for time spent in the light compartment revealed a significant main effect of FS stress, F (1, 33) = 14.6, $p = 0.001$, and a significant FS stress x pretreatment interaction, F (1, 33) = 7.1, $p < 0.5$. The VEH-pretreated rats who received FS spent significantly less time in the light compartment than VEH rats who did not receive FS ($p < 0.001$), whereas no such difference was seen in the 5 mg/kg CBD rats. As well, among the rats receiving FS stress, CBD-pretreated rats spent more time in the light box than did VEH-pretreated rats ($p < 0.01$).

The ANOVA for latency to enter the light compartment revealed a significant FS stress effect, F (1, 33) = 12.0, $p = 0.01$, a significant effect of pretreatment, F (1, 33) = 6.8, $p < 0.05$, and a significant FS stress x pretreatment interaction, F (1, 33) = 8.2, $p < 0.01$. As seen in section f of Fig. 2, the VEH rats who received FS took significantly longer to enter the light compartment than VEH rats who did not receive FS ($p < 0.001$), whereas no such difference was seen in the 5 mg/kg CBD-pretreated rats. As well, CBD reduced the latency to enter the light box following FS compared with that of VEH pretreatment ($p < 0.01$).

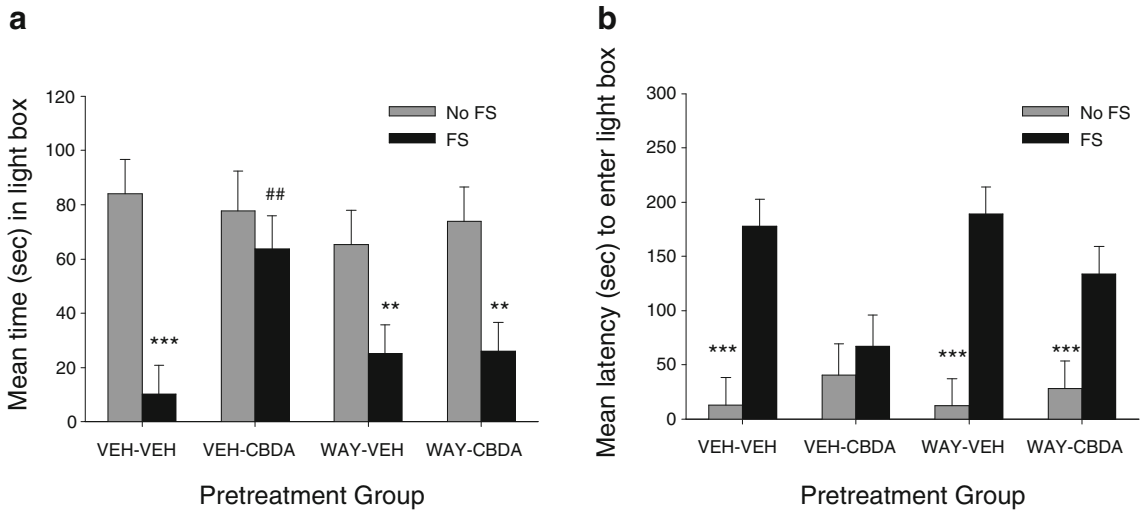

Fig. 3 The mean (± sem) amount of time spent in the light compartment (section **a**) and mean (± sem) latency to enter the light compartment (section **b**) 24 h following exposure to FS (*black bars*) or no FS (*gray bars*) for various pretreatment groups in experiment 3. The *asterisks* indicate a significant different between FS and no FS for each group (***$p < 0.01$, **$p < 0.025$). The *number signs* indicate a significant difference among the FS-treated rats between group VEH-CBDA and all other groups ($^{\#\#}p < 0.025$)

Experiment 3: potential of WAY100635 to reverse the anxiolytic effect of 1 μg/kg CBDA following FS stress

As is evident in Fig. 3, WAY100635 reversed the anxiolytic effect of 1 μg/kg CBDA as assessed by both time in the light box and latency to enter the light box. The ANOVA for time spent in the light box revealed a significant effect of FS stress, F (1, 52) = 25.9; $p < 0.001$ and a significant 3-way interaction, F (1, 52) = 4.0; $p = 0.05$. As indicated in Fig. 3a, the FS stress reduced the time spent in the light box for all groups except VEH-CBDA ($p < 0.025$). As well, among the FS-stressed rats, group VEH-CBDA spent more time in the light box than any other group ($p < 0.025$).

The ANOVA for latency to enter the light box revealed a significant effect of FS stress, F (1, 52) = 38.6; $p < 0.001$ and a significant VEH/CBDA x FS stress interaction, F (1, 52) = 7.5; $p < 0.01$. In Fig. 3b, the FS stress enhanced the latency to enter the light box in all groups other than that of VEH-CBDA ($p < 0.001$).

Experiment 4: potential of anxiolytic doses of CBDA or CBD to reverse the anxiogenic effect of THC following FS stress

Neither CBDA (0.1 μg/kg) nor CBD (5 mg/kg) reduced the anxiogenic effects of THC (1 mg/kg). The section a of Fig. 4 presents the time spent in the light box among groups in experiment 4. The between groups ANOVA revealed a significant effect of FS stress, F (1, 60) = 46.3, $p < 0.001$, pretreatment group, F (3, 60) = 10.1, $p < 0.001$, and a FS stress x pretreatment interaction, F (3, 60) = 6.3; $p < 0.001$. The rats spent less time in the light box following FS stress than those following no FS stress, regardless of pretreatment condition ($p < 0.05$). As well, among the no FS groups, group VEH spent more time in the light box than all other groups ($p < 0.001$). The analysis of the latency data was similar to that of the time in the light box as seen in section b of Fig. 4; the FS stress enhanced the

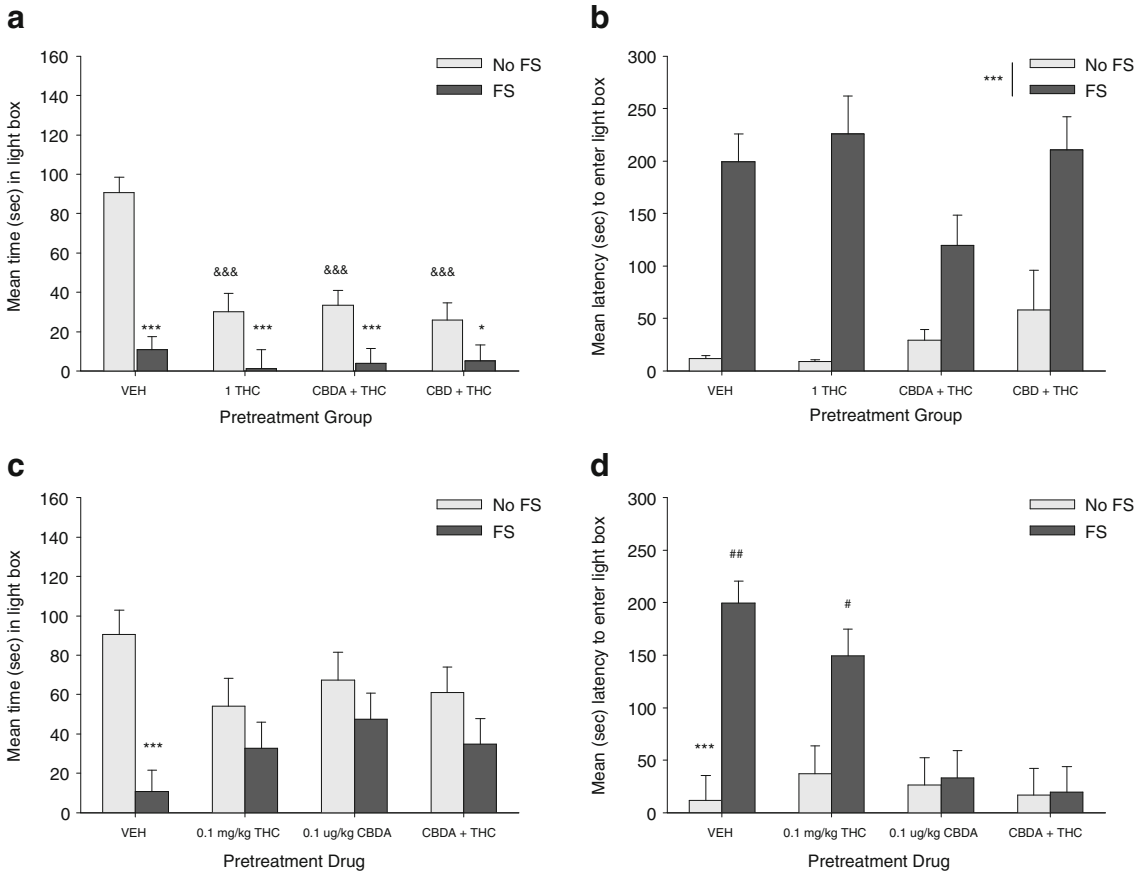

Fig. 4 The mean (± sem) amount of time spent in the light compartment and mean (± sem) latency to enter the light compartment 24 h following exposure to FS (*black bars*) or no FS (*gray bars*) for rats in experiment 4 (sections **a** and **b:** in which rats were injected with VEH, 1 mg/kg THC, combined 1 μg/kg CBDA +1 mg/kg THC or combined 5 mg/kg CBD + 1 mg/kg THC, i.p.) and experiment 5 (sections **c** and **d**: in which rats were injected with VEH, 0.1 mg/kg THC, 0.1 μg/kg CBDA, or combined 0.1 μg/kg CBDA +0.1 mg/kg THC, i.p.). The *asterisks* indicate a significant difference between FS stress and no FS stress for each group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). The *111* symbols in section **a** indicate a significant difference between VEH and other groups among the no FS groups ($^{111}p < 0.001$) and the *number signs* in section **d** indicate a significant difference ($^{\#\#}p < 0.01$, $^{\#}p < 0.05$) among the FS-stressed groups from rats treated with CBDA or the combination of CBDA + THC

latency to enter the light box in all groups as revealed by a significant effect of FS stress, F (1, 60) = 51.7, $p < 0.001$, with no other significant effects.

Experiment 5: effect of combined low doses of CBDA and THC on anxiety-like responding

The FS stress reduced the time spent in the light box in the VEH-treated rats, but pretreatment with 0.1 μg/kg CBDA, 0.1 mg/kg THC, or combination of the two compounds prevented this effect as seen in section c of Fig. 4. The FS stress also increased the latency to enter the light box among both VEH- and THC- (0.1 mg/kg, i.p.) treated rats, but 0.1 μg/kg CBDA and combined doses of 0.1 μg/kg CBDA + 0.1 mg/kg THC prevented this effect, as seen in section d of Fig. 4. The ANOVA of the time spent in the light box revealed significant effects of FS stress, F (1, 60) = 16.2, $p < 0.001$, and a FS stress x pretreatment group interaction, F (3, 61) = 2.8, $p < 0.05$. The subsequent LSD pairwise comparison tests revealed that the FS stress reduced the time spent in the light box only among the VEH-pretreated group ($p < 0.001$).

The ANOVA of the latency data revealed significant effects of FS stress, F (1, 60) = 19.6, $p < 0.001$, pretreatment group, F (3, 60) = 6.7, $p < 0.001$, and a FS stress x pretreatment group interaction, F (3, 60) = 67.2, $p < 0.001$; subsequent LSD pairwise comparison tests revealed that groups VEH ($p < 0.001$) and 0.1 mg/kg THC ($p < 0.05$) showed a significantly enhanced latency to enter the light box following FS stress. As well, among the FS-stressed rats, both groups VEH ($p < 0.001$) and 0.1 THC ($p < 0.01$) displayed a longer latency to enter the light box than group 0.1 CBDA and group 0.1 CBDA + 0.1 THC ($p < 0.01$), suggesting that the CBDA not only reduces FS stress-induced anxiety alone but also when an ineffective dose of THC is *on board*.

Discussion

CBDA (0.1–100 μg/kg, i.p.) administered acutely or chronically did not modify anxiety-like behavior in the LD emergence test under low-stress conditions; however, when administered to previously stressed (foot shock) animals, CBDA prevented the stress-induced enhancement of anxiogenic-like behavior in the LD emergence test. CBDA has been previously shown to have no effects on locomotor behavior at doses within the range of the current study (Rock et al. 2015) and at doses as high as 5 mg/kg, po. (Brierley et al. 2016). Similarly, CBD (5 mg/kg, i.p.) blocked the FS-induced enhancement of anxiogenic-like behavior in the LD emergence test, but did not differ from VEH in the no FS condition. Together, these results suggest that CBDA and CBD (at least at a dose of 5 mg/kg) may be more effective anxiolytic agents under conditions of stress than under conditions of minimal stress. Indeed, a recent report by Song et al. (2016) suggests that CBD is more effective in enhancing contextual fear memory extinction under high fear conditions than under low fear conditions. As has been demonstrated for the anxiolytic effects of CBD (Campos et al. 2012; Gomes et al. 2012; Fogaça et al. 2014; Resstel et al. 2009), the anxiolytic effect of CBDA was also reversed by the 5-HT_{1A} receptor antagonist, WAY100635.

In contrast to CBDA and CBD, THC (1 and 10 mg/kg) when administered acutely or chronically, produced anxiogenic-like behavior in non-stressed rats in the LD emergence test and did not alter the FS-induced enhancement of anxiogenic-like behavior. Chronic administration of THC prevented the enhanced latency to enter the light box after 21 days of exposure, possibly as a result of tolerance to its anxiogenic effects; however, such tolerance was not evident in the time spent in the light box. Although the anxiogenic-like effect of 10 mg/kg THC might be explained by reduced locomotor behavior, doses of 1 mg/kg or even 0.1 mg/kg THC (which did not modify the enhanced latency to enter the light box following FS stress in experiment 4) are below the threshold to modify locomotor activity (Järbe et al. 2002; Wiley and Martin 2003; Le Foll et al. 2006; Polissidis et al. 2010; Klein et al. 2011; Rock et al. 2015). It is also noteworthy, that a low dose of THC (0.1 mg/kg) prevented the anxiogenic-like effect of prior FS stress on the time spent in the light box in experiment 5, but not on latency to enter the light box.

The anxiolytic effects of CBD were first revealed by studies suggesting that it reversed the anxiogenic effects of THC in humans without having an effect on its own (Karniol et al. 1974; Zuardi et al. 1993). However, neither an anxiolytic dose of CBD (5 mg/kg, i.p.) nor CBDA (1 μg/kg, i.p.) reversed the anxiogenic effects of THC (1 mg/kg, i.p.) when assessed as the time spent in the light box or latency to enter the light box. This finding is consistent with a similar recent report by Todd et al. (2017). It is interesting to note that despite the human work suggesting that CBD reduces the anxiogenic effects of THC, Klein et al. (2011) report that CBD actually augmented the anxiogenic effects of THC in Wistar rats assessed by the LD emergence test and the elevated plus maze. Therefore, evaluation of the effects of combined CBD + THC in different strains and/or doses is warranted.

Our finding that CBDA did not impact the amount of time spent in the light compartment when administered acutely or chronically to unstressed animals is in agreement with findings from Brierley et al. (2016) indicating that CBDA does not modify the number of entries or time spent in the light chamber in the LD emergence test in rats. Similarly, when administered acutely or chronically (14 days), we have previously reported that CBD (2.5 mg/kg, i.p.) has no impact on time spent in the light chamber of the LD emergence test in rats

(O'Brien et al. 2013). However, Long et al. (2010) demonstrated that daily administration of CBD for 21 days to mice produced an anxiolytic effect in the LD test, but only at a very low dose of 1 mg/kg.

Anxiogenic-like responses at higher doses of THC have been previously reported (Onaivi et al. 1990; Valjent et al. 2002; Patel and Hillard 2006; Klein et al. 2011). In fact, our finding that 1 and 10 mg/kg THC produced anxiogenic-like effects is in accordance with those reported by Onaivi et al. (1990). This group found that THC (1–10 mg/kg) reduced the time spent in the open arms of the elevated plus maze in rats. In addition, THC (1 mg/kg) has been shown to increase the time spent in the dark chamber in the LD emergence test and a trend towards reduced time spent in the open arms of the elevated plus maze in rats, indicating anxiogenic-like effects (Klein et al. 2011).

We have shown that prior stress (FS) produces anxiogenic-like behavior, but administration of CBDA or CBD abolishes this anxiogenic-like behavior. Previous work has also shown that prior stress (exposure to a predator, FS, or restraint) produces anxiogenic-like behavior, but administration of CBD prevents this anxiogenic-like behavior in the elevated plus maze (Bitencourt et al. 2008; Resstel et al. 2009; Campos et al. 2012). Conversely, we have shown that administration of THC (1 mg/kg) does not abolish this anxiogenic-like behavior produced by prior stress. This finding is in accordance with Hill and Gorzalka (2004), who showed that in stressed animals (subjected to chronic unpredictable stress), HU-210 (10, 50 μg/kg, i.p.) induced anxiogenic-like behavior in the elevated plus maze. However, this finding is contradictory to that of Fokos and Panagis (2010) indicating that THC (1 mg/kg, i.p.) induced an anxiolytic-like effect in rats subjected to chronic unpredictable stress for 10 days, as measured in the elevated plus maze. This difference in findings may be a result of differences such as intensity of the stressor, or the paradigms to induce stress or to assess anxiety (elevated plus maze versus LD emergence test).

Human studies confirm the anxiolytic effects of CBD in preclinical animal studies. Consistent with the present findings, CBD potently reduced experimentally induced anxiety (Martin-Santos et al. 2012; Hindocha et al. 2015) including anxiety associated with simulated public speaking in both healthy subjects (Zuardi et al. 1982; Zuardi et al. 1993) and in subjects with social anxiety disorder (Bergamaschi et al. 2011). Although the effects of THC in modulating anxiety are CB_1 receptor mediated (Patel and Hillard 2006), the anxiolytic-like effects of CBD (Campos et al. 2012; Gomes et al. 2012; Fogaça et al. 2014) and CBDA appear to be mediated by 5-HT_{1A} receptor activation. As well, the anti-nausea and anti-emetic effects of CBDA are also 5-HT_{1A} receptor mediated (Bolognini et al. 2013).

The results of the present study suggest that CBD and CBDA may be highly effective treatments for the reduction of anxiety, but only among individuals in a high state of stress. Neither compound produced anxiolytic-like responding in animals which did not receive prior FS stress in the LD emergence test, a relatively less stressful test used to assess anxiety-like responding.

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Compliance with ethical standards All animal procedures complied with the Canadian Council on Animal Care and were approved by the Institutional Animal Care Committee (accredited by the Canadian Council on Animal Care).

Conflict of interest The authors declare that they have no conflict of interest.

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