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ORIGINAL INVESTIGATION

Effect of combined doses of Δ^9-tetrahydrocannabinol and cannabidiol or tetrahydrocannabinolic acid and cannabidiolic acid on acute nausea in male Sprague-Dawley rats

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Abstract

Rationale This study evaluated the potential of combined cannabis constituents to reduce nausea.

Objectives Using the lithium chloride (LiCl)-induced conditioned gaping model of nausea in male rats, we aimed to:

1) Determine effective anti-nausea doses of cannabidiol (CBD)
2) Determine effectiveness and the mechanism of action of combined subthreshold doses of CBD and Δ^9-tetrahydrocannabinol (THC)
3) Determine effective doses of synthetic cannabidiolic acid (CBDA)
4) Determine effective doses of synthetic tetrahydrocannabinolic acid (THCA)
5) Determine the mechanism of action for THCA
6) Determine effectiveness and the mechanism of action of combined subthreshold doses of CBDA and THCA

Results CBD (0.5–5 mg/kg, intraperitoneal [i.p.]) reduces LiCl-induced conditioned gaping (but 0.1, 20, 40 mg/kg are ineffective). Combined subthreshold doses of CBD (0.1 mg/kg, i.p.) and THC (0.1 mg/kg, i.p.) produce suppression of conditioned gaping, and this effect is blocked by administration of either WAY100635 (a serotonin 1A [5-HT_{1A}]) receptor antagonist or SR141716 (SR; a CB_1 receptor antagonist). THCA (0.01 mg/kg, i.p.) reduces conditioned gaping and administration of MK886 (a peroxisome proliferator-activated receptor alpha [PPARα] antagonist) blocked THCA's anti-nausea effect. Combined subthreshold doses of CBDA (0.00001 mg/kg, i.p.) and THCA (0.001 mg/kg, i.p.) produce suppression of conditioned gaping, and this effect is blocked by administration of WAY100635 or MK886.

Conclusion Combinations of very low doses of CBD + THC or CBDA + THCA robustly reduce LiCl-induced conditioned gaping. Clinical trials are necessary to determine the efficacy of using single or combined cannabinoids as adjunct treatments with existing anti-emetic regimens to manage chemotherapy-induced nausea.

Keywords Δ^9-tetrahydrocannabinol · Cannabidiol · Cannabidiolic acid · Tetrahydrocannabinolic acid · Conditioned gaping · 5-HT_{1A} · PPARα · CB_1

Abbreviations

5-HT_{1A}	Serotonin 1A
ANOVA	Analysis of variance
CB_1	Cannabinoid 1
CBD	Cannabidiol
CBDA	Cannabidiolic acid
i.p.	Intraperitoneal
CTA	Conditioned taste avoidance
FAAH	Fatty acid amide hydrolase
LiCl	Lithium chloride
OEA	*N*-oleoylethanolamide
PEA	*N*- palmitoylethanolamide
PPARα	Peroxisome proliferator-activated receptor alpha
SAL	Saline
SR	SR141716
THC	Δ^9-tetrahydrocannabinol

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THCA	Tetrahydrocannabinolic acid
TR	Taste reactivity
VEH	Vehicle
WAY	WAY100635

Introduction

Chemotherapy-induced nausea and vomiting are the most reported side effects by cancer patients (Adel 2017; Gilmore et al. 2014; Schnell 2003; Sun et al. 2005). With the use of the recommended anti-emetic prophylaxis, chemotherapy-induced vomiting has been greatly reduced, but nausea is still problematic (e.g. Chow et al. 2018; Giagnuolo et al. 2019), with up to 50% of patients still experiencing acute nausea (e.g. Araz et al. 2019; Clemmons et al. 2018; Navari et al. 2018; Timaeus et al. 2018). Effective treatments for nausea are limited, highlighting the need to understand the mechanisms of nausea to develop new therapeutics.

To screen such potential treatments, we have developed the pre-clinical model of conditioned gaping. Although rats do not vomit, they do display conditioned gaping disgust reactions (Grill and Norgren 1978) to a flavour that has been previously paired with 'sickness' such as that produced by lithium chloride (LiCl). Considerable behavioural evidence shows that manipulations that produce vomiting in other species promote conditioned gaping in rats, although even non-emetic treatments produce conditioned taste avoidance (CTA) in rats. Furthermore, treatments that reduce nausea and vomiting in other species consistently prevent conditioned gaping in rats (but not CTA). Conditioned gaping in rats requires similar orofacial musculature as vomiting in emetic species (Travers and Norgren 1986) and is topographically similar to the orofacial components of retching in the shrew (Parker 2003). Therefore, conditioned gaping in rats is a selective measure of nausea (Parker 2014), useful in evaluating the anti-nausea potential of compounds.

One such candidate for potential anti-nausea compounds is the cannabis plant. The cannabis plant contains over 100 cannabinoid constituents, several of which have been shown to reduce conditioned gaping (see Rock and Parker 2016 for review). The only compound in the cannabis plant with known psychoactive effects is Δ^9-tetrahydrocannabinol (THC). THC is an approved treatment for chemotherapy-induced nausea in an oral form (dronabinol). THC (at doses as low as 1 mg/kg, intraperitoneal [i.p.]) reduces LiCl-induced conditioned gaping in rats (Rock et al. 2015a). Tetrahydrocannabinolic acid (THCA) is the acidic precursor of THC (Mechoulam et al. 1969) which is present in the fresh plant and then decarboxylates to THC upon heating or drying. THCA (0.05, 0.5 mg/kg, i.p.) reduces conditioned gaping at lower doses than THC, suggesting greater potency over that of THC (Rock et al. 2013). Interestingly, no psychotomimetic activity was observed with THCA administration to rhesus monkeys (doses ≤ 5 mg/kg, intravenously), mice (doses ≤ 20 mg/kg, i.p.) and dogs (doses ≤7 mg/kg; Grunfeld and Edery 1969). Recently, THCA (0.5 mg/kg, i.p.) did not produce the cannabimimetic responses of hypothermia or hypoactivity in rats (Rock et al. 2013). A recent analysis of 334 blood samples from those suspected of driving under the influence of cannabis ranged from 0.2 to 14 μg/L for THCA and from 0.2 to 50 μg/L for THC (Sørensen and Hasselstrøm 2017), suggesting that THCA (and THC) is indeed present and detectable in cannabis users. These findings suggest that perhaps THCA may be a more desirable treatment than THC because it is devoid of psychoactivity, possibly due to THCA's limited access to the central nervous system (see Moreno-Sanz 2016 for an excellent review of THCA).

In addition, both cannabidiol (CBD, 5 mg/kg, i.p.; Parker and Mechoulam 2003; Rock et al. 2011, 2012) and its acidic precursor cannabidiolic acid (CBDA, 0.0005 0.001, 0.005, 0.01 mg/kg, i.p.; Bolognini et al. 2013; Rock and Parker 2013; Rock et al. 2015a) are effective in reducing conditioned gaping, with CBDA being much more potent than CBD. The anti-nausea effects of both CBD and CBDA are mediated by action at the serotonin 1A (5-HT_{1A}) receptor, as administration of the 5-HT_{1A} receptor antagonist WAY100635 (WAY) blocked the effect (Bolognini et al. 2013; Rock et al. 2012).

Our group has begun to look at the potential of combinations of cannabinoids to act synergistically to reduce conditioned gaping in rats. When combined, subthreshold doses of THC (0.1 mg/kg, i.p.) and CBDA (0.00001 mg/kg, i.p.) that were ineffective independently, enhanced the suppression of conditioned gaping (Rock et al. 2015a). In addition, subthreshold anti-emetic doses of CBD (2.5 mg/kg, i.p.) or CBDA (0.05 mg/kg, i.p.), when combined with a subthreshold anti-emetic dose of THC (1 mg/kg, i.p.) in the *Suncus murinus* (house musk shrew), an animal model of vomiting, enhanced the suppression of LiCl-induced vomiting (Rock and Parker 2015). Although a synergistic relationship has been demonstrated with these combinations, it is unknown if such an effect also exists for combinations of THC and CBD or THCA and CBDA in the conditioned gaping model.

The aims of this study were (1) to establish a dose-response for the anti-nausea effects of CBD and a subthreshold dose; (2) to examine the combination of a subthreshold dose of CBD and a known subthreshold dose of THC in conditioned gaping and the mechanism of action for this combination; (3) to expand the dose-response for the anti-nausea effects of CBDA and identify a subthreshold dose; (4) to expand the dose-response for the anti-nausea effects of THCA and identify a subthreshold dose; (5) to determine the mechanism of action for THCA in conditioned gaping; and (6) to examine the combination of subthreshold doses of CBDA and THCA in conditioned gaping and the mechanism of action.

Materials and methods

Animals

All procedures complied with the legislation of the Animals for Research Act of Ontario, as well as the guidelines of the Canadian Council on Animal Care. All animal use protocols were approved by the Institutional Animal Care Committee at the University of Guelph, which is accredited by the Canadian Council on Animal Care. Male Sprague Dawley rats (236), obtained from Charles River Laboratories (St Constant, QC, Canada), were used for assessment of acute nausea. Their body weights ranged from 268 to 344 g on the day of conditioning. Rats were individually housed in opaque plastic cages (48 × 26 × 20 cm), containing bed-o-cob bedding from Harlan Laboratories, Inc. (Mississauga, ON, Canada), a brown paper towel and Crink-l'Nest™ from The Andersons, Inc. (Maumee, OH, USA). 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 maintained at an ambient temperature of 21 °C and a 12/12 h reverse light–dark schedule (lights off at 07 h) and maintained on ad libitum chow and water. All experimental manipulations occurred during the dark-phase cycle.

Drugs

Synthetic THC (98% pure; Toronto Research Chemicals), CBD (97.4% pure; Toronto Research Chemicals), THCA (95.3% pure; Toronto Research Chemicals, kindly provided by Whistler Therapeutics) and CBDA (98% pure; Toronto Research Chemicals, kindly provided by Whistler Therapeutics) were first dissolved in ethanol in a graduated cylinder. Tween 80 (Sigma) was added to the solution, and the ethanol was evaporated off with a nitrogen stream, after which saline (SAL) was added. The final vehicle (VEH) solution consisted of 1:9 Tween 80/SAL. CBD was mixed at a concentration of 0.05, 0.25, 0.5, 2.5, 10 and 20 mg/ml and administered i.p. at 2 ml/kg (0.1, 0.5, 1, 5, 20, 40 mg/kg, respectively). THC was mixed at a concentration of 0.05 mg/ml and administered i.p. at 2 ml/kg (0.1 mg/kg). CBDA was mixed at a concentration of 0.000005 and 0.00005 mg/ml and administered i.p. at 2 ml/kg (0.00001, 0.0001 mg/kg, respectively). THCA was mixed at a concentration of 0.0005 and 0.005 mg/ml and administered i.p. at 2 ml/kg (0.001, 0.01 mg/kg, respectively). The combined doses of THC + CBD or THCA + CBDA were mixed as a cocktail solution (in a VEH of 1:9 Tween80/SAL) and administered i.p. at 2 ml/kg. The selective antagonists were also mixed as above, to a final VEH solution consisting of 1:9 Tween 80/SAL and administered i.p. at 1 ml/kg. Doses of the antagonists—SR141716 (SR; 1 mg/ml, 1 mg/kg; Sequoia Research Products Ltd), WAY100635 (WAY; 0.1 mg/ml, 0.1 mg/kg; Sigma), MK886 (1 mg/ml, 1 mg/kg; Cayman Chemical Company) and AM630 (1 mg/ml, 1 mg/kg; Sigma)—were selected based upon previous work indicating that these doses had no effect on LiCl-induced conditioned gaping on their own (Bolognini et al. 2013; Pertwee et al. 2018; Rock et al. 2012, 2013, 2015b, 2016, 2017). Lithium chloride (LiCl; Sigma) was prepared in a 0.15 M solution with sterile water and was administered i.p. at a volume of 20 ml/kg (127.2 mg/kg).

Apparatus

The taste reactivity (TR) chambers were made of clear Plexiglas (22.5 × 26 × 20 cm) that sat on a table with a clear glass top. A mirror beneath the chamber at a 45° angle facilitated viewing of the ventral surface of the rat to observe orofacial responses. A Sony video camera (Handycam, Henry's Camera Waterloo, ON, Canada) was used to videotape the rats from the mirror beneath the chamber. The videotapes were later scored using 'The Observer' event recording software (Noldus Information Technology Inc., Leesburg, VA, USA).

General procedures

All rats were surgically implanted with an intraoral cannula under isoflurane anaesthesia according to the procedure described by Limebeer et al. (2010). For 3 days following surgery, rats were weighed, and the health of the animal was assessed; a visual check for urine/faeces in the home cage, activity, vocalization, dehydration, rigidity and presence of porphyrin staining around the eye was performed, as well as adjustment of the elastics and visual inspection of the surgical site. The cannulae were flushed daily, for 3 days, with chlorhexidine antiseptic.

Following recovery from surgery, the rats received an adaptation trial in which they were placed in the taste reactivity (TR) chamber with their cannulae attached to an infusion pump (Model KDS100, KD Scientific, Holliston, MA, USA) for fluid delivery. Water was infused into their intraoral cannulae for 2 min at a rate of 1 ml/min.

Twenty-four hours later, the rats received a single conditioning trial. The rats were randomly assigned to one of the pretreatment groups (refer to Table 1 for details pertaining to each experiment). Rats were injected with the appropriate pretreatment and 30 min later were individually placed in the chamber and intraorally infused with 0.1% saccharin solution for 2 min at a rate of 1 ml/min, while the orofacial responses were video recorded from the mirror beneath the chamber with the feed sent to a computer via firewire connection. Immediately after the saccharin infusion, all rats were injected with 20 ml/kg of 0.15 M LiCl and returned to their home cage. In Experiments 2, 5 and 6, to investigate the mechanism of action for the pretreatment(s), additional groups were also

Table 1 Summary of experimental procedure details

Antagonist (mg/kg, i.p.)	*Antagonist time (prior to Sac→LiCl)*	*Pretreatments (mg/kg, i.p)*	*Pretreatment time (prior to Sac→LiCl)*	*Groups (animal numbers)*
Experiment 1: CBD dose-response in acute nausea				
none	none	VEH CBD (0.1, 0.5, 1, 5, 20, 40)	30 min	VEH ($n = 8$) 0.1 CBD ($n = 8$) 0.5 CBD ($n = 8$) 1 CBD ($n = 8$) 5 CBD ($n = 8$) 20 CBD ($n = 8$) 40 CBD ($n = 8$)
Experiment 2: Combined subthreshold doses of CBD and THC and mechanism of action in acute nausea				
SR (1) WAY (0.1)	45 min	VEH CBD (0.1) THC (0.1) CBD + THC	30 min	VEH ($n = 8$) 0.1 CBD ($n = 8$) 0.1 THC ($n = 7$) CBD + THC ($n = 8$) SR-CBD + THC ($n = 8$) WAY-CBD + THC ($n = 8$)
Experiment 3: CBDA dose-response in acute nausea				
none	none	VEH CBDA (0.00001, 0.0001)	30 min	VEH ($n = 7$) 0.00001 CBDA ($n = 8$) 0.0001 CBDA ($n = 7$)
Experiment 4: THCA dose-response in acute nausea				
none	none	VEH THCA (0.001, 0.01)	30 min	VEH ($n = 8$) 0.001 THCA ($n = 8$) 0.01 THCA ($n = 8$)
Experiment 5: THCA mechanism of action in acute nausea				
SR (1) MK886 (1) AM630 (1)	45 min	VEH THCA (0.01)	30 min	VEH ($n = 8$) 0.01 THCA ($n = 8$) SR-THCA ($n = 7$) MK886 − THCA ($n = 8$) AM630 − THCA ($n = 8$)
Experiment 6: Combined subthreshold doses of THCA and CBDA and mechanism of action in acute nausea				
MK886 (1) WAY (0.1)	45 min	VEH CBDA (0.00001) THCA (0.001) CBDA + THCA	30 min	VEH ($n = 8$) 0.00001 CBDA ($n = 8$) 0.001 THCA ($n = 8$) CBDA + THCA ($n = 8$) MK886 − CBDA + THCA ($n = 8$) WAY − CBDA + THCA ($n = 8$)

injected with receptor selective antagonists (see Table 1 for details) 45 min prior to placement in the chamber.

Seventy-two hours later, the rats were tested drug-free. They were again intraorally infused with 0.1% saccharin solution for 2 min at the rate of 1 ml/min, while the orofacial reactions were video recorded. The videotapes were later scored by an observer blind to the experimental conditions using The Observer for the behaviour of gaping (large openings of the mouth and jaw, with lower incisors exposed).

To determine if the pretreatment interfered with learning per se, CTA was assessed in a single bottle test. Rats were water restricted at 15:00 h following their test session. The next morning, a bottle containing 0.1% saccharin solution was placed on the cage at 08:00 h. Measures of saccharin consumption were taken for the next 6 h.

Experiment 1: CBD dose-response in acute nausea

In Experiment 1, we expanded the dose-response for the anti-nausea effects of CBD and also determined a subthreshold dose which was ineffective in reducing LiCl-induced conditioned gaping.

Experiment 2: Combined subthreshold doses of CBD and THC and mechanism of action in acute nausea

Having established a subthreshold dose of CBD, in Experiment 2, we combined it with a known subthreshold dose of THC (0.1 mg/kg, i.p.; Rock et al. 2015a). Previous work has shown that CBD and THC's anti-nausea effects are mediated by action at the 5-HT_{1A} and CB_1 receptors,

respectively (Parker and Mechoulam 2003; Parker et al. 2003; Rock et al. 2012). Therefore, to investigate the mechanism of action for the combination, additional groups were injected with SR (1 mg/kg, i.p.) or WAY (0.1 mg/kg, i.p.) prior to placement in the chamber. We have previously shown that these pretreatments produce no effect on LiCl-induced gaping on their own (Bolognini et al. 2013; Pertwee et al. 2018; Rock et al. 2012, 2013, 2015b, 2016, 2017).

Experiment 3: CBDA dose-response in acute nausea

Although we had determined a subthreshold dose of CBDA in the extracts received from Prairie Plant Systems and GW Pharmaceuticals that were 97.7% and 97.9% pure, respectively (Rock and Parker 2013; Rock et al. 2015a), to ensure equipotency using the current synthetic cannabinoids, we also determined the subthreshold dose to reduce acute nausea with the synthetic CBDA (98% pure) used here (Toronto Research Chemicals).

Experiment 4: THCA dose-response in acute nausea

Here we expanded the dose-response for THCA, as previous work (Rock et al. 2013) had shown that 0.05 and 0.5 mg/kg THCA effectively reduced acute nausea. We aimed to establish a subthreshold dose of THCA in reducing acute nausea.

Experiment 5: THCA mechanism of action in acute nausea

Here we evaluated the anti-nausea mechanism of action for an effective dose of THCA, by co-administering a CB_1 receptor antagonist SR (1 mg/kg, i.p.), a PPARα antagonist MK886 (1 mg/kg, i.p.) or a CB_2 receptor antagonist AM630 (1 mg/kg, i.p.).

Experiment 6: Combined subthreshold doses of THCA and CBDA and mechanism of action in acute nausea

Finally, using the established subthreshold doses of THCA and CBDA, we evaluated their combined anti-nausea efficacy and evaluated its mechanism of action by administering the PPARα antagonist MK886 (1 mg/kg, i.p.) or the 5-HT_{1A} receptor antagonist WAY (0.1 mg/kg, i.p.).

Data analysis

Data were analysed using SPSS Statistics (IBM, Version 23). Statistical significance was set at $p < 0.05$. For each experiment, a single factor analysis of variance (ANOVA) was conducted on the number of gapes at test for each group, with subsequent Bonferroni post hoc comparisons of significant effects. In addition, the amount of saccharin consumed (ml) during the CTA test for each group was entered into a mixed factors ANOVA, with subsequent one-way ANOVAs of significant interactions with Bonferroni post hoc comparisons.

Results

Please refer to Table 2 for a brief summary of behavioural results from all experiments.

Experiment 1: CBD dose-response in acute nausea

CBD dose-dependently reduced LiCl-induced acute nausea. Figure 1A presents the mean number of gapes displayed by each pretreatment group. The one-way ANOVA revealed a significant main effect of pretreatment group, $F(6, 49) = 6.4$, $p < 0.01$. Bonferroni post hoc comparison tests revealed that relative to VEH-pretreated controls, only the groups pretreated with 0.5, 1 and 5 mg/kg CBD gaped significantly less (p's < 0.01).

The mean amounts of saccharin consumed during the CTA test at 30, 120 and 360 min are presented in Fig. 1B. A 3 (time) × 7 (pretreatment group) mixed factors ANOVA revealed a significant main effect of time, $F(2, 98) = 444.5$, $p < 0.001$; a non-significant main effect of pretreatment group, $F(6, 49) = 1.8$, $p > 0.05$; and a significant time x group interaction, $F(12, 98) = 2.7$, $p < 0.01$. Subsequent one-way ANOVAs at each timepoint revealed an effect only at 360 min $F(6, 49) = 2.3$, $p < 0.05$. Bonferroni post hoc comparison tests at the 360 min timepoint revealed that relative to VEH-pretreated controls, no group significantly differed (p's > 0.05). These results indicate that no pretreatments interfered with CTA learning; therefore, administration of these compounds did not interfere with learning per se.

Experiment 2: Combined subthreshold doses of CBD and THC and mechanism of action in acute nausea

Ineffective dose of CBD or THC alone, when combined, reduced LiCl-induced acute nausea, and administration of either WAY or SR blocked the combined suppression of LiCl-induced conditioned gaping. Figure 2A presents the mean number of gapes displayed by each pretreatment group. The one-way ANOVA revealed a significant main effect of pretreatment group, $F(5, 46) = 6.2$, $p < 0.001$. Bonferroni post hoc comparison tests revealed that group CBD + THC gaped significantly less than all other groups (p's < 0.05).

The mean amounts of saccharin consumed during the CTA test at 30, 120 and 360 min are presented in Fig. 2B. A 3 (time) × 6 (pretreatment group) mixed factors ANOVA revealed a significant main effect of time, $F(2, 82) = 450.4$, $p < 0.001$; a significant main effect of pretreatment group, $F(5, 41) = 2.5$, $p < 0.05$; and a significant time x group interaction, $F(10, 82) = 3.2$, $p < 0.05$. Subsequent one-way

Table 2 Summary of behavioural results

Effect on conditioned gaping		*Effect on conditioned taste avoidance*
Experiment 1: CBD dose-response in acute nausea		
0.5 CBD ↓ 1 CBD ↓ 5 CBD ↓	0.1 CBD ¤ 20 CBD ¤ 40 CBD ¤	All groups ¤
Experiment 2: Combined subthreshold doses of CBD and THC and mechanism of action in acute nausea		
CBD + THC ↓	0.1 CBD ¤ 0.1 THC ¤ WAY − CBD + THC x SR − CBD + THC x	All groups ¤
Experiment 3: CBDA dose-response in acute nausea		
0.0001 CBDA ↓	0.00001 CBDA ¤	All groups ¤
Experiment 4: THCA dose-response in acute nausea		
0.01 THCA ↓	0.001 THCA ¤	All groups ¤
Experiment 5: THCA mechanism of action in acute nausea		
0.01 THCA ↓ SR − THCA ↓ AM630 − THCA ↓	MK886 − THCA x	SR − THCA drank less saccharin All other groups ¤
Experiment 6: Combined subthreshold doses of THCA and CBDA and mechanism of action in acute nausea		
CBDA + THCA ↓	0.00001 CBDA ¤ 0.001 THCA ¤ WAY – CBDA + THCA x MK886 – CBDA + THCA x	all groups ¤

↓ reduced conditioned gaping; ¤ no effect on behaviour; x antagonist blocked the effect

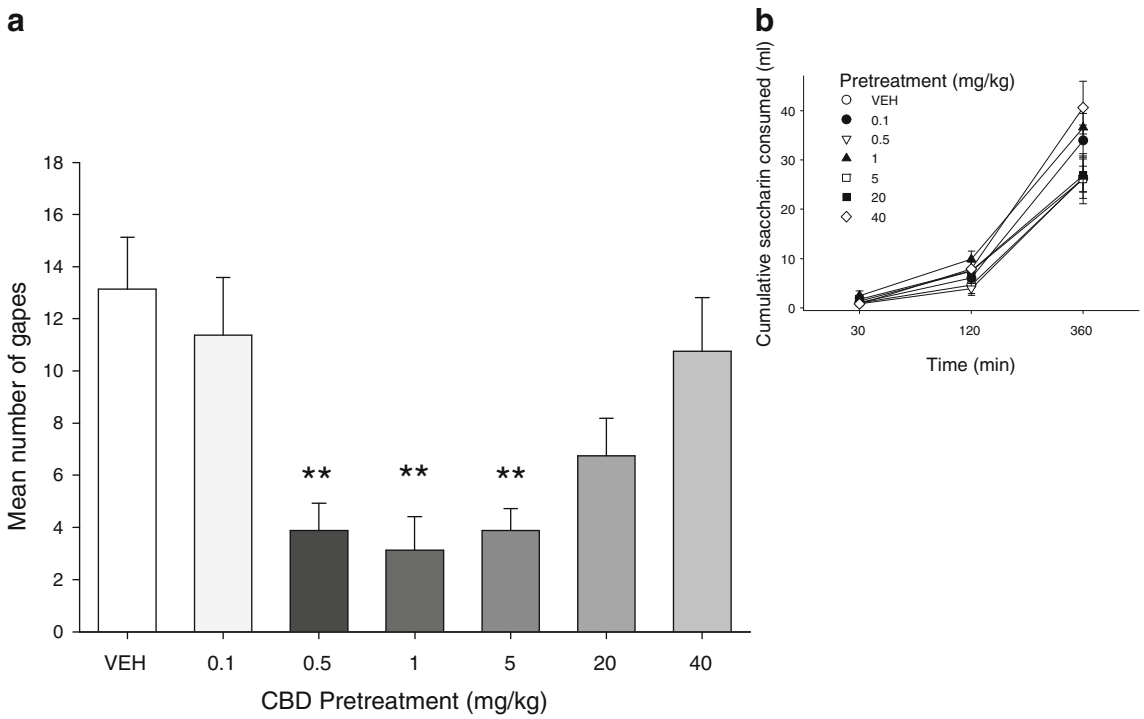

Fig. 1 The effect of CBD (0.1, 0.5, 1, 5, 20, 40 mg/kg, i.p.) or VEH administered 30 min prior to conditioning. (A) The mean number of conditioned gapes elicited by a LiCl-paired saccharin solution among rats ($n = 7–8$/group) was measured during the test trial. Each bar represents the mean ± SEM. The asterisks indicate a significant difference from the VEH-treated control animals (**$p < 0.01$). (B) The mean ± SEM cumulative amount of saccharin solution consumed (ml) during a one-bottle consumption test was measured at 30, 120 and 360 min after introduction of the bottle to fluid-restricted rats

ANOVAs at each timepoint revealed an effect only at 360 min $F(5, 41) = 2.9$, $p < 0.05$. Bonferroni post hoc comparison tests at the 360 min timepoint revealed that relative to VEH-pretreated controls no group significantly differed (p's > 0.05). These results indicate that no pretreatments interfered with CTA learning; therefore, administration of these compounds did not interfere with learning per se.

Experiment 3: CBDA dose-response in acute nausea

CBDA dose-dependently reduced LiCl-induced acute nausea. Figure 3A presents the mean number of gapes displayed by each pretreatment group. The one-way ANOVA revealed a significant main effect of pretreatment group, $F(2, 19) = 5.4$, $p < 0.02$. Bonferroni post hoc comparison tests revealed that relative to VEH-pretreated controls, the group pretreated with 0.0001 mg/kg (0.1 μg/kg) CBDA, but not 0.00001 (0.01 μg/kg) CBDA gaped significantly less ($p < 0.02$).

The mean amounts of saccharin consumed during the CTA test at 30, 120 and 360 min are presented in Fig. 3B. A 3 (time) × 3 (pretreatment group) mixed factors ANOVA revealed a significant main effect of time, $F(2, 38) = 67.3$, $p < 0.001$; a non-significant main effect of pretreatment group, $F(2, 19) = 0.3$, $p > 0.05$; and a non-significant time x group interaction, $F(4, 38) = 1.1$, $p > 0.05$.

Experiment 4: THCA dose-response in acute nausea

THCA dose-dependently reduced LiCl-induced acute nausea. Figure 4A presents the mean number of gapes displayed by each pretreatment group. The one-way ANOVA revealed a significant main effect of pretreatment group, $F(2, 21) = 8.6$, $p < 0.01$. Bonferroni post hoc comparison tests revealed that relative to VEH-pretreated controls, the group pretreated with 0.01 mg/kg, but not 0.001 mg/kg, THCA gaped significantly less ($p = 0.001$).

The mean amounts of saccharin consumed during the CTA test at 30, 120 and 360 min are presented in Fig. 4B. A 3 (time) × 3 (pretreatment group) mixed factors ANOVA revealed a significant main effect of time, $F(2, 42) = 169.4$,

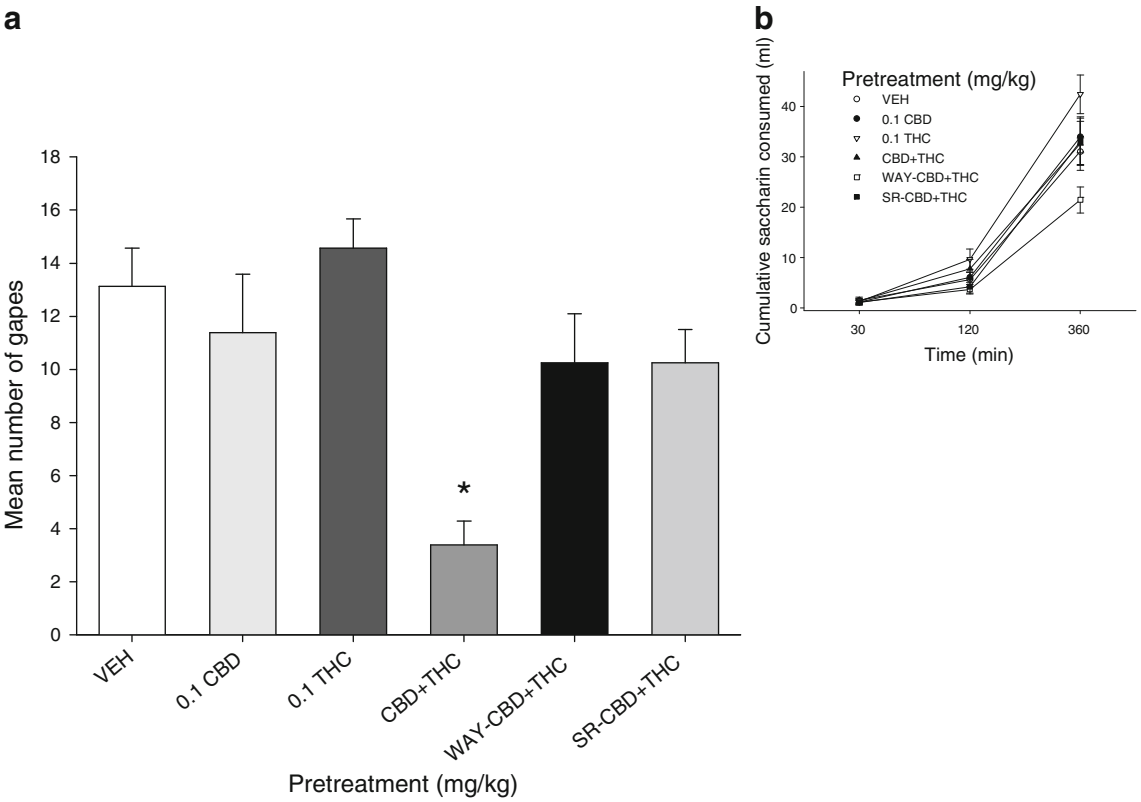

Fig. 2 The effect of subthreshold doses of CBD (0.1 mg/kg, i.p.), THC (0.1 mg/kg, i.p.), their combination, or VEH, administered 30 min prior to conditioning. Additionally, to investigate the mechanism of action for the combination, additional rats were injected with SR (1 mg/kg, i.p.) or WAY (0.1 mg/kg, i.p.) 45 min prior to conditioning. (A) The mean number of conditioned gapes elicited by a LiCl-paired saccharin solution among rats ($n = 7$–8/group) was measured during the test trial. Each bar represents the mean ± SEM. The asterisks indicate a significant difference from all other groups (*$p < 0.05$). (B) The mean ± SEM cumulative amount of saccharin solution consumed (ml) during a one-bottle consumption test was measured at 30, 120 and 360 min after introduction of the bottle to fluid-restricted rats

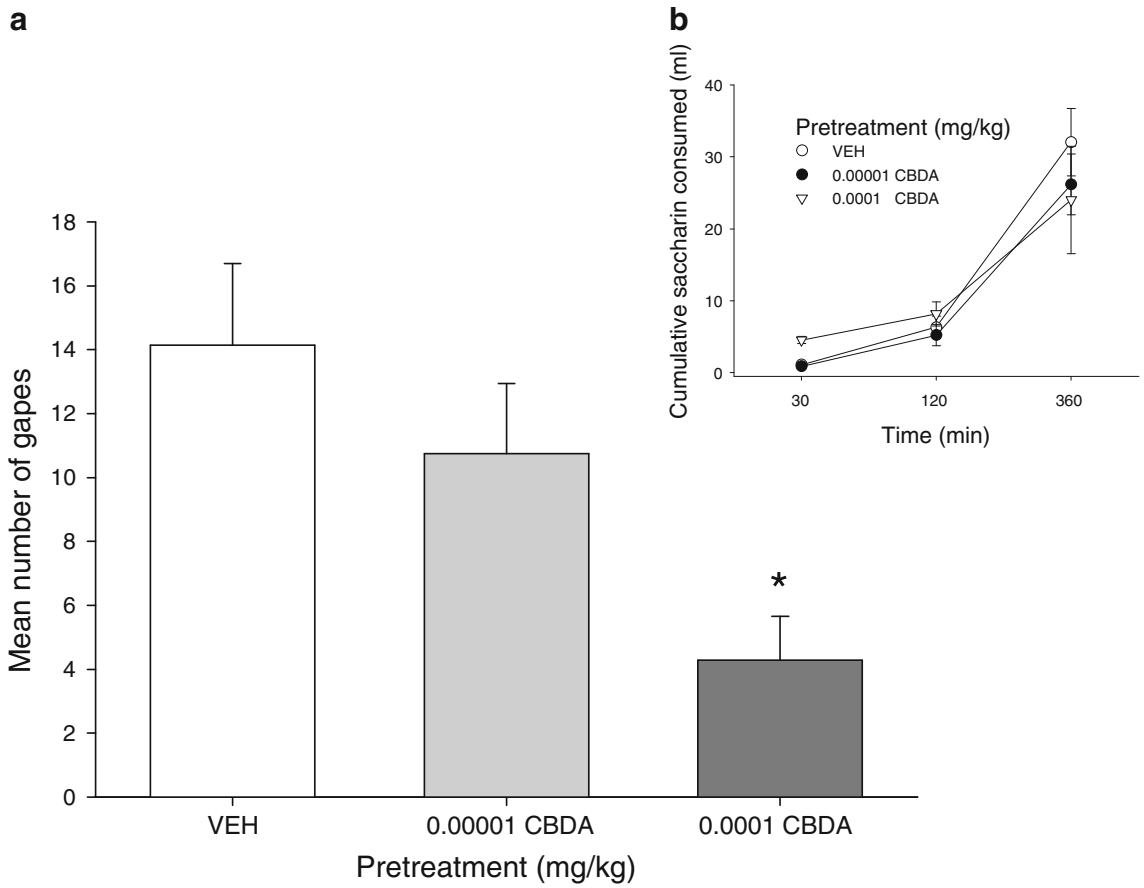

Fig. 3 The effect of CBDA (0.00001, 0.0001 mg/kg, i.p.) or VEH administered 30 min prior to conditioning. (A) The mean number of conditioned gapes elicited by a LiCl-paired saccharin solution among rats ($n = 7$–8/group) was measured during the test trial. Each bar represents the mean ± SEM. The asterisks indicate a significant difference from the VEH-treated control animals ($^*p < 0.05$). (B) The mean ± SEM cumulative amount of saccharin solution consumed (ml) during a one-bottle consumption test was measured at 30, 120 and 360 min after introduction of the bottle to fluid-restricted rats

$p < 0.001$; a non-significant main effect of pretreatment group, $F(2, 21) = 0.5$, $p > 0.05$; and a non-significant time x group interaction, $F(4, 42) = 1.1$, $p > 0.05$.

Experiment 5: THCA mechanism of action in acute nausea

Administration of MK886 (but not SR or AM630) blocked THCA's ability to reduce LiCl-induced acute nausea. Figure 5A presents the mean number of gapes displayed by each pretreatment group. The one-way ANOVA revealed a significant main effect of pretreatment group, $F(4, 31) = 16.5$, $p < 0.001$. Bonferroni post hoc comparison tests revealed that relative to VEH-pretreated controls, all pretreatment groups gaped significantly less (p 's < 0.001), except for those pretreated with MK886-THCA. In addition, those rats pretreated with THCA gaped significantly less than those pretreated with MK886-THCA ($p < 0.01$).

The mean amounts of saccharin consumed during the CTA test at 30, 120 and 360 min are presented in Fig. 5B. A 3 (time) × 5 (pretreatment group) mixed factors ANOVA revealed a significant main effect of time, $F(2, 68) = 226.3$, $p < 0.001$; a significant main effect of pretreatment group, $F(4, 34) = 2.9$, $p < 0.05$; and a significant time x group interaction, $F(8, 68) = 5.5$, $p < 0.001$. Subsequent one-way ANOVAs at each timepoint revealed an effect only at 360 min $F(4, 34) = 4.5$, $p < 0.01$. Bonferroni post hoc comparison tests at the 360 min timepoint revealed that relative to VEH-pretreated controls, only group SR-THCA differed ($p < 0.05$). In fact, group SR-THCA drank significantly less saccharin, suggesting that SR may have enhanced CTA learning.

Experiment 6: Combined subthreshold doses of THCA and CBDA and mechanism of action in acute nausea

Ineffective doses of CBDA (0.00001 mg/kg) or THCA (0.001 mg/kg) alone, when combined, reduced LiCl-induced acute nausea, and administration of either WAY or MK886 blocked the combined suppression of LiCl-induced

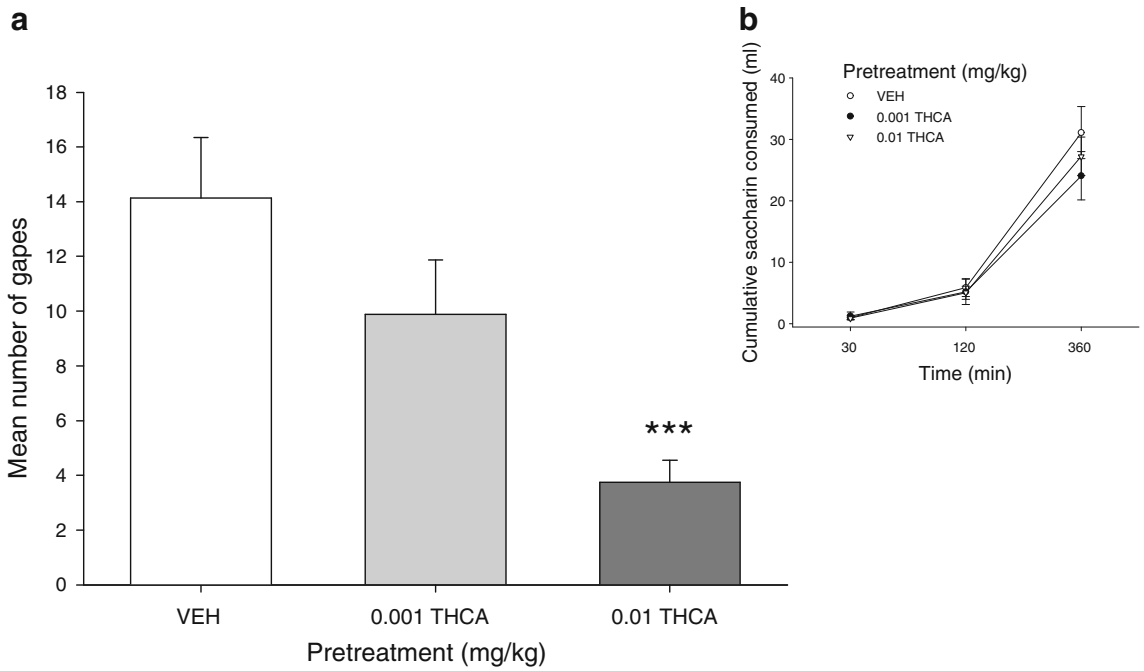

Fig. 4 The effect of THCA (0.001, 0.01 mg/kg, i.p.) or VEH administered 30 min prior to conditioning. (A) The mean number of conditioned gapes elicited by a LiCl-paired saccharin solution among rats ($n = 8$/group) was measured during the test trial. Each bar represents the mean ± SEM. The asterisks indicate a significant difference from the VEH-treated control animals (***$p = 0.001$). (B) The mean ± SEM cumulative amount of saccharin solution consumed (ml) during a one-bottle consumption test was measured at 30, 120 and 360 min after introduction of the bottle to fluid-restricted rats

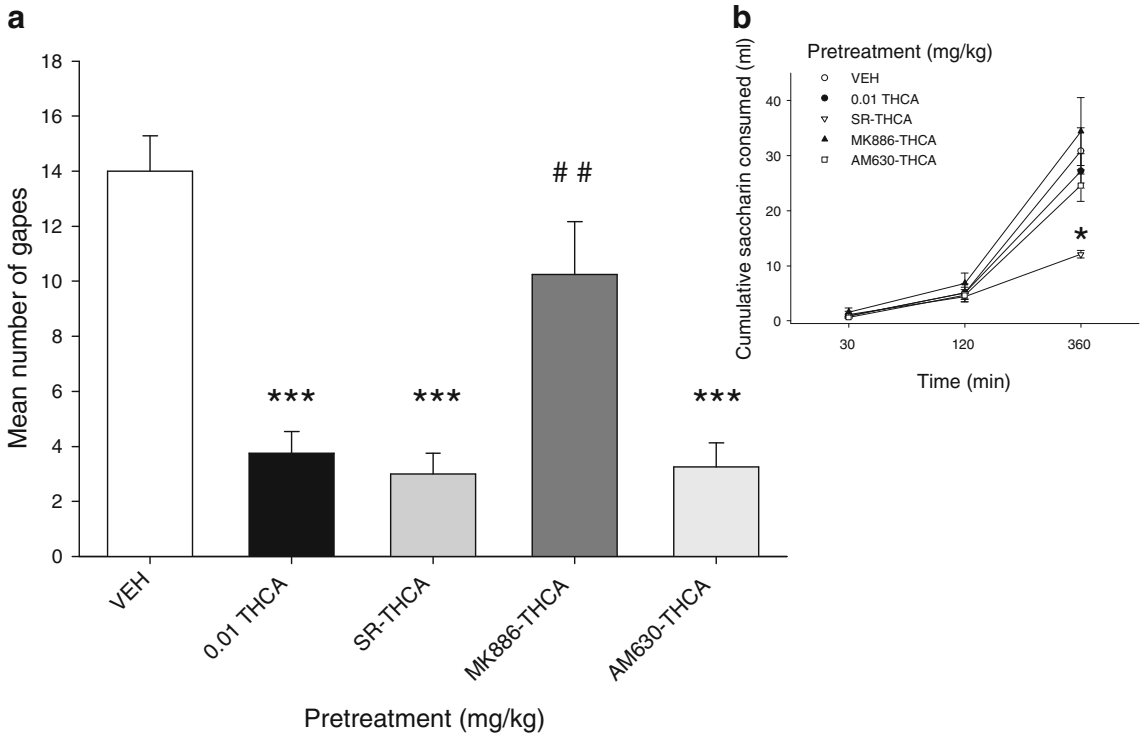

Fig. 5 The effect of THCA (0.01 mg/kg, i.p.) or VEH administered 30 min prior to conditioning. Additional groups were also given SR (1 mg/kg, i.p.), MK886 (1 mg/kg, i.p.) or AM630 (1 mg/kg, i.p.) 15 min prior to the THCA. (A) The mean number of conditioned gapes elicited by a LiCl-paired saccharin solution among rats ($n = 7$–8/group) was measured during the test trial. Each bar represents the mean ± SEM. (B) The mean ± SEM cumulative amount of saccharin solution consumed (ml) during a one-bottle consumption test was measured at 30, 120 and 360 min after introduction of the bottle to fluid-restricted rats. The asterisks indicate a significant difference from the VEH-treated control animals (***$p < 0.001$, *$p < 0.05$). The number sign represents a significant difference from the 0.01 THCA group ($^{\#\#}p < 0.01$)

conditioned gaping. Figure 6A presents the mean number of gapes displayed by each pretreatment group. The one-way ANOVA revealed a significant main effect of pretreatment group, $F(5, 42) = 5.7$, $p < 0.001$. Bonferroni post hoc comparison tests revealed that relative to VEH-pretreated controls, only group CBDA+THCA gaped significantly less ($p < 0.01$). Additionally, rats in group MK886 – CBDA + THCA or WAY – CBDA + THCA gaped significantly more ($p < 0.001$) or marginally more ($p = 0.06$), respectively, than group CBDA+THCA.

The mean amounts of saccharin consumed during the conditioned taste avoidance test at 30, 120 and 360 min are presented in Fig. 6B. A 3 (time) × 6 (pretreatment group) mixed factors ANOVA revealed a significant main effect of time, $F(2, 84) = 369.8$ $p < 0.001$; a non-significant main effect of pretreatment group, $F(5, 42) = 1.9$, $p > 0.05$; and a non-significant time x group interaction, $F(10, 84) = 1.6$, $p > 0.05$, suggesting that no pretreatment interfered with CTA learning.

Discussion

Here we show that CBD reduces LiCl-induced conditioned gaping at doses ranging from 0.5 to 5 mg/kg (i.p.). Interestingly, high doses of CBD were ineffective in reducing conditioned gaping, but also did not potentiate LiCl-induced conditioned gaping, suggesting an inverted U function as well as safety (but anti-nausea ineffectiveness) at higher doses. When we combined a subthreshold dose of CBD (0.1 mg/kg,

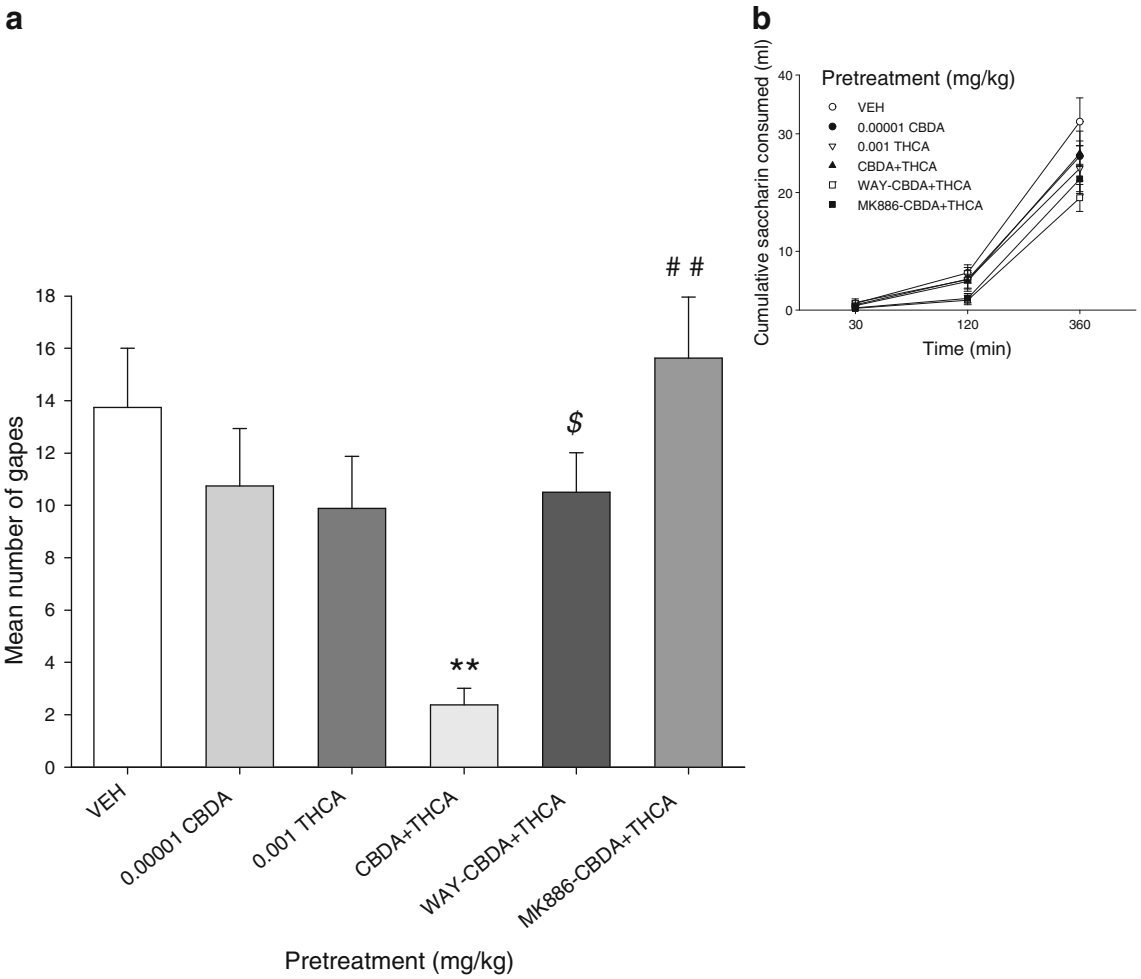

Fig. 6 The effect of subthreshold doses of CBDA (0.00001 mg/kg, i.p.), THCA (0.001 mg/kg, i.p.), their combination, or VEH, administered 30 min prior to conditioning. To investigate the mechanism of action for the combination, additional rats were injected with MK886 (1 mg/kg, i.p.) or WAY (0.1 mg/kg, i.p.) 45 min prior to conditioning. (A) The mean number of conditioned gapes elicited by a LiCl-paired saccharin solution among rats ($n = 8$/group) was measured during the test trial. Each bar represents the mean ± SEM. The asterisks indicate a significant difference from VEH-treated controls (**$p < 0.01$). The number sign represents a significant difference from the CBDA + THCA group ($^{\#\#\#}p < 0.001$). The dollar sign represents a marginal difference from the CBDA + THCA group ($^{\$}p = 0.06$). (B) The mean ± SEM cumulative amount of saccharin solution consumed (ml) during a one-bottle consumption test was measured at 30, 120 and 360 min after introduction of the bottle to fluid-restricted rats

i.p.) with a previously established subthreshold dose of THC (0.1 mg/kg, i.p.; Rock et al. 2015a), the combination effectively reduced conditioned gaping, suggesting a synergistic effect. Furthermore, administration of either WAY (the 5-HT_{1A} receptor antagonist) or SR (the CB_1 receptor antagonist) blocked the CBD + THC suppression of LiCl-induced conditioned gaping. This suggests that the combined suppressive effect of CBD + THC is likely due to their combined separate mechanisms of action. CBD is likely exerting its anti-nausea effect by activation of somatodendritic 5-HT_{1A} autoreceptors, resulting in a reduction in the firing rate of 5-HT afferents to terminal forebrain regions (Sotelo et al. 1990; Verge et al. 1985). Indeed, Limebeer et al. (2018) recently showed that LiCl-induced nausea is triggered by elevated 5-HT in the interoceptive insular cortex and is attenuated by CBD. THC is likely exerting its anti-nausea effect by activation of presynaptic CB_1 receptors, resulting in suppression of neurotransmitter release, presumably 5-HT (see Sharkey et al. 2014 for review). To produce the synergistic effect of subthreshold doses, both of these effects must occur simultaneously, as blocking either mechanism prevents the effectiveness of the compound.

Although we had determined a subthreshold dose of CBDA (0.0001 mg/kg, i.p.) in the cannabis extracts received from Prairie Plant Systems and GW Pharmaceuticals that were 97.7% and 97.9% pure, respectively (Rock and Parker 2013; Rock et al. 2015a), the subthreshold dose with the synthetic CBDA (98% pure) used here (provided by Toronto Research Chemicals) was 0.00001 mg/kg, i.p.. This suggests that the synthetic CBDA used here may be more potent than plant-derived CBDA. It is unlikely that the difference is the result of decarboxylation between batches of CBDA. It has recently been reported that hemp seed oil stored below 100 °C shows only 1–2% decarboxylation of CBDA, but storage above 100 °C results in losses of 20% (Citti et al. 2018). It is also necessary to note that the synthetic cannabinoids used in these studies were 95–98% pure; however, because these compounds are synthesized, the remaining content is unlikely to be other cannabinoids.

We also determined a threshold (0.01 mg/kg, i.p.) and subthreshold (0.001 mg/kg, i.p.) dose for synthetic THCA, as previous work from our laboratory had shown plant-derived THCA (0.05, 0.5 mg/kg, i.p.) reduces conditioned gaping, but no other doses had been tested (Rock et al. 2013). Furthermore, administration of the PPARα antagonist MK886 (but not the CB_1 receptor antagonist SR or the CB_2 receptor antagonist AM630) blocked THCA's suppression of LiCl-induced conditioned gaping. This is in agreement with findings that the anti-nausea effects of the fatty acid amide hydrolase (FAAH) inhibitor PF-3845 are mediated by activation of PPARα (Rock et al. 2017, 2019) and administration of a PPARα agonist similarly reduces LiCl-induced conditioned gaping (Rock et al. 2017, 2019). Together, these findings suggest a role for anti-nausea effects of THCA through activation of PPARα. Indeed in vitro studies suggest that THCA is a weak FAAH inhibitor (De Petrocellis et al. 2013); therefore, THCA may be elevating levels of the fatty acid ethanolamides *N*-oleoylethanolamide (OEA) and *N*-palmitoylethanolamide (PEA), which are high affinity PPARα agonists (Sun et al. 2007).

It is noteworthy that both SR and AM630 did not block the effects of THCA on acute nausea in this paradigm. We have previously demonstrated that the suppression of LiCl-induced contextually elicited conditioned gaping (a rodent model of anticipatory nausea) was reversed by a CB_1 receptor antagonist (Rock et al. 2013). These findings support growing evidence to suggest that differing mechanisms regulate anticipatory nausea and acute nausea (Rock et al. 2015b, 2017). Indeed, unlike acute nausea (Limebeer and Parker 2000), anticipatory nausea is resistant to treatment with the anti-emetic drug ondansetron in rats (Limebeer et al. 2006) and humans (e.g. Aapro et al. 2005; Morrow et al. 1998). A handful of studies have attempted to determine whether THCA binds to CB_1 and CB_2 receptors by testing the in vitro affinity of THCA, but contradictory results have been found (see Moreno-Sanz 2016 for review), which may be due to differing THCA purity (synthetic versus plant-derived) or the degree of decarboxylation into THC. Indeed, storage at 4 °C and even −18 °C results in THCA loss (Smith and Vaughan 1977). The stability of THCA can be improved when stored in olive oil (with 78% of THCA detectable after 10 days at 25°), over that of ethanol (with only 33% detectable; Citti et al. 2016). Further research that carefully monitors the stability of THCA is needed to fully understand the molecular mechanisms of THCA.

Finally, combined subthreshold doses of CBDA and THCA effectively reduce LiCl-induced conditioned gaping, suggesting a synergistic effect. Furthermore, administration of MK886 (the PPARα antagonist) significantly blocked, and WAY (the 5-HT_{1A} receptor antagonist) marginally blocked ($p = 0.06$), the CBDA + THCA suppression of LiCl-induced conditioned gaping. This suggests that the combined suppressive effect of CBDA + THCA is likely due to their separate mechanisms of action working in tandem. CBDA is likely exerting its anti-nausea effect by activation of somatodendritic 5-HT_{1A} autoreceptors, resulting in a reduction in the firing rate of 5-HT afferents to terminal forebrain regions (like CBD). THCA is likely exerting its anti-nausea effect by inhibiting FAAH, which elevates OEA and PEA (ligands of PPARα). Plasma (or brain tissue) analysis of fatty acid ethanolamides after THCA administration could shed light on this potential mechanism. Indeed, recent evidence from our laboratory suggests that FAAH inhibition's anti-nausea effects may occur in the ventral pallidum and involve PPARα activation (Rock et al. 2019). Recent in vitro work by D'Aniello et al. (2019) suggests that CBDA can act as a dual

PPARα/gamma agonist, so it is possible that this mechanism is also contributing to the combined CBDA + THCA effect, which was blocked by the PPARα antagonist. Future research could examine whether CBDA may also be exerting its anti-nausea effect through this mechanism(s).

As pretreatment with anti-nausea drugs does not interfere with CTA (suggesting that the animal still learns about the association between the taste and illness, even in the absence of experiencing nausea), this provides a measure of the potential of the putative anti-nausea drug to interfere with learning per se (see Parker 2014 for review). Interestingly, administration of the CB_1 receptor antagonist SR enhanced the strength of conditioning in the THCA pretreated rats, such that these rats drank significantly less saccharin than VEH-controls at the 360 min timepoint. In this paradigm, drinking *less* saccharin is indicative of an enhanced avoidance; however, as measured by conditioned gaping, there was actually a *reduced* aversion. This finding provides further evidence for the disparity between these two measures. It is unlikely that these drug manipulations interfered with learning per se. In fact, the co-administration of SR and THCA may have even enhanced learning, as displayed by a stronger CTA. This finding is consistent with the considerable body of literature showing that SR and CB_1 receptor antagonists may enhance memory (e.g. O'Brien et al. 2014; Takahashi et al. 2005; Terranova et al. 1996; Wolff and Leander 2003).

Taken together, our results suggest that very low doses of CBD or CBDA can be combined with very low doses of THC or THCA to robustly reduce LiCl-induced conditioned gaping. Taken at such low doses, the psychoactive effects of THC would not be clinically problematic, or could be completely avoided through the use of THCA, which is devoid of psychoactive side effects. As these phytocannabinoids have separate mechanisms of action, their combined suppressive effects are likely due to their separate mechanisms of action working simultaneously. Indeed, the suppressive effect of CBD + THC seems to be 5-HT_{1A} and CB_1 receptor mediated, respectively, and 5-HT_{1A} and PPARα mediated for CBDA + THCA, respectively. Clinical trials utilizing single or combined cannabinoids as adjunct treatments with existing anti-emetic regimens are needed to combat chemotherapy-induced nausea.

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Compliance with ethical standards

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

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